Photosynthesis Research **79:** 1–24, 2004.

Review

ADP-glucose pyrophosphorylase: a regulatory enzyme for plant starch synthesis

Miguel A. Ballicora[1], Alberto A. Iglesias[2] & Jack Preiss[1,*]
[1]*Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824, USA;* [2]*Bioquímica Básica de Macromoléculas, GEM, FBCB, UNL, Santa Fe, Argentina;* **Author for correspondence (e-mail: preiss@msu.edu; fax: +1-517-353-9334)*

Received 11 April 2002; accepted in revised form 10 August 2003

Key words: ADP-glucose pyrophosphorylase, glucose-1 phosphate adenylyl transferase, glycogen, polysaccharide synthesis, starch

Abstract

In plants, the synthesis of starch occurs by utilizing ADP-glucose as the glucosyl donor for the elongation of α-1,4-glucosidic chains. In photosynthetic bacteria the synthesis of glycogen follows a similar pathway. The first committed step in these pathways is the synthesis of ADP-glucose in a reaction catalyzed by ADP-glucose pyrophosphorylase (ADPGlc PPase). Generally, this enzyme is allosterically regulated by intermediates of the major carbon assimilatory pathway in the respective organism. In oxygenic photosynthesizers, ADPGlc PPase is mainly regulated by 3-phosphoglycerate (activator) and inorganic orthophosphate (inhibitor), interacting in four different patterns. Recent reports have shown that in higher plants, some of the enzymes could also be redox regulated. In eukaryotes, the enzyme is a heterotetramer comprised of two distinct subunits, a catalytic and a modulatory subunit. The latter has been proposed as related to variations in regulation of the enzyme in different plant tissues. Random and site-directed mutagenesis experiments of conserved amino acids revealed important residues for catalysis and regulation. Prediction of the ADPGlc PPase secondary structure suggests that it shares a common folding pattern to other sugar-nucleotide pyrophosphorylases, and they evolved from a common ancestor.

Occurrence and function of starch

The biosynthesis of α-1,4-polyglucans is a main strategy developed by living organism for the intracellular storage of carbon and energy. Animals, fungi, bacteria including the cyanobacteria, and archaebacteria accumulate glycogen; whereas in algae and in plants starch is the polymer synthesized as a carbon and energy reserve. Starch is deposited as granules in almost all green plants and in various types of plant tissues and organs; for example, leaves, roots, shoots, fruits, grains, and stems. Two polymers can be clearly distinguished within the starch granule: amylose and amylopectin. Both are mainly composed by glucosyl units linked by α-1,4-bonds. Amylose is an essentially linear chain with few branches linked by α-1,6-bonds, whereas amylopectin is highly branched by α-1,6-bonds. Amylopectin molecules are large flattened disks consisting of α-1,4-glucan chains joined by frequent α-1,6-branch points. Many models for the amylopectin structure have been proposed and the ones that best fit the experimental data available are those proposed by Manners and Matheson (1981) and Hizukuri (1995). The chemical and physical aspects of the starch granule and its components amylose and amylopectin have been discussed in some excellent reviews (Morrison and Karkalas 1990; Hizukuri 1995).

Leaf starch

Illumination of the leaf in bright light causes the formation of starch granules in the chloroplast organelle and this was demonstrated in the 19th century (Sachs 1887). Disappearance of the starch occurs either by exposure of the leaf to low light or by extended exposure in the dark (24–48 h). This is readily observed

by iodine staining of the tissue (Edwards and Walker 1983) or by light or electron microscopy (Badenhuizen 1969). Starch accumulates due to carbon fixation during photosynthesis and it is then degraded, in the dark, to products that are in most cases utilized for sucrose synthesis. The reason for this is that the accumulated starch is required to synthesize sucrose, which serves as a carbon supply for sink tissues. Mutants of *Arabidopsis thaliana* unable to synthesize starch grow at the same rate as the wild type in a continuous light regime because they are able to synthesize sucrose, but their growth rate is drastically reduced if grown in a day-night regime (Caspar et al. 1985). Biosynthesis and degradation of starch in the leaf is therefore a dynamic process having diurnal fluctuations in its stored levels.

Starch also plays an important role in the operation of stomatal guard cells (Outlaw and Manchester 1979; Zeiger et al. 2002; Ritte and Raschke 2003). There, it is degraded during the day while the stomata are open, and it is resynthesized in the late afternoon or evening. Leaf starch is lower in amylose content than what is observed in storage tissues (Matheson 1996). The amylose structure is also of a smaller molecular size (Morrison and Karkalas 1990).

Starch in storage tissues

In storage organs, fruit or seed, during the development and maturation of the tissue, synthesis of starch occurs (Sivak and Preiss 1998). At the time of sprouting or germination of the seed or tuber, or ripening of the fruit, starch is degraded and the derived metabolites are used as a source of both carbon, and energy. The degradative and biosynthetic processes in the storage tissues may therefore be temporally separated. However, there is some possibility that during each phase of starch metabolism some turnover of the starch molecule does occur.

The main site of starch synthesis and accumulation in the cereals is the endosperm, with starch granules being located within the amyloplasts. Starch content in potato tuber, maize endosperm, and in roots of yam, cassava and sweet potato ranges between 65% and 90% of the total dry matter (Sivak and Preiss 1998). Patterns of starch accumulation during development of the tissue are specific to the species and are related to the unique pattern of differentiation of the organ.

Starch granules in storage tissues can vary in shape, size and composition depending on the plant source. In addition, in each tissue of a plant differences in size and shape are observed within a range. The diameter of the starch granule changes during the development of the reserve tissue. There are also some fine features, characteristic of each species, for example, the 'growth rings', spaced 4–7 μm apart, and the fibrillar organization seen in potato starch, which allows one to identify the botanical source of the starch by its microscopic examination (Morrison and Karkalas 1990; Hizukuri 1995; Sivak and Preiss 1998).

The biosynthetic reactions of starch synthesis

The metabolic routes for polyglucan accumulation were elucidated after the discovery of nucleoside-diphosphate-sugars by L.F. Leloir and coworkers in the 1950s (Leloir 1971). Leloir's group clearly established that biosynthesis and degradation of glycogen in mammalian systems occur by dissimilar pathways. It was shown that the synthetic route utilizes an activated form of glucose, specifically UDPGlc in mammalian and eukaryotic microorganisms (Sivak and Preiss 1998).

The process for the synthesis of storage polysaccharides, glycogen and starch, respectively, in bacteria and plants, takes place with ADPGlc as the glucosyl donor for the elongation of the α-1,4-glucosidic chain. In these organisms, ADPGlc is synthesized from ATP and Glc 1-P in a reaction catalyzed by ADPGlc pyrophosphorylase (ADPGlc PPase; glucose 1-P adenylyl transferase; reaction 1; E.C. 2.7.7.27).

$$\text{ATP} + \text{Glc 1-P} \Leftrightarrow \text{ADPGlc} + \text{PPi} \qquad (1)$$

This reaction was first described to occur in soybean extracts by Espada (1962). Thereafter, the enzyme was also found in many plant tissues and in bacterial extracts (Preiss and Sivak 1998a, b; Preiss 1999). ADPGlc PPase is the first committed step in the route leading to glycogen and starch synthesis in bacteria and plants, respectively. The ADPGlc PPase reaction takes place in the presence of a divalent metal ion, Mg^{2+}, and it is freely reversible *in vitro*. The hydrolysis of PPi by inorganic pyrophosphatase and the use of the sugar-nucleotide for polysaccharide synthesis cause the ADPGlc synthetic reaction to be essentially irreversible *in vivo* (Iglesias and Preiss 1992).

Then, synthesis of the polysaccharide follows by the steps shown in Reactions 2 and 3. The first is

catalyzed by starch synthase (plants, E.C. 2.4.1.21) or glycogen synthase (bacteria, also E.C. 2.4.1.21) (Preiss 1984, 1991; Iglesias and Preiss 1992; Sivak and Preiss 1998; Preiss and Sivak 1998a). Reaction 3 is catalyzed by branching enzyme (E.C. 2.4.1.18; 1,4-α-D-glucan: 1,4-α-D-glucan 6-glycosyl-transferase).

$$\text{ADPGlc} + \alpha\text{-1,4-glucan} \Rightarrow \alpha\text{-1,4-glucosyl-}\alpha\text{-1,4-glucan} + \text{ADP} \quad (2)$$

$$\text{Elongated } \alpha\text{-1,4-oligosaccharide chain} \Rightarrow \alpha\text{-1,4-}\alpha\text{-1,6 branched glucan} \quad (3)$$

The branch chains in amylopectin are longer (about 20–24 glucose units long) and there is less branching in amylopectin (~5% of the glucosidic linkages are α-1,6) as seen in bacterial glycogen (10–13 glucose units long and 10% of linkages are α-1,6) (Sivak and Preiss 1998). Thus, the starch branching enzymes may have different properties with respect to size of chain transferred, or placement of branch point, than the bacterial enzyme that branches glycogen. Alternatively, the interaction of the starch branching enzymes with the starch synthases may be different from the interaction of the bacterial branching enzymes with their respective glycogen synthases. The chain elongating properties of the starch synthases could be different from those observed for the bacterial glycogen synthases and may account for some of the differences observed in the amylopectin structure. The diversity of catalytic properties of the starch synthases and branching enzymes isolated from different plant sources may also account for the differences observed in the various plant starch structures (Sivak and Preiss 1998).

Isozymic forms of plant starch synthases (Preiss and Levi 1980; Preiss 1988, 1991; Denyer et al. 1993, 1995; Hylton et al. 1996) and branching enzymes (Preiss and Levi 1980; Preiss 1988, 1991; Mizuno et al. 1992; Burton et al. 1995) have been reported. They seem to play different roles in the synthesis of amylose and amylopectin and are products from different genes. In many different plants (Nelson et al. 1978; Shure et al. 1983; Van der Leij et al. 1991; Visser et al. 1991; Dry et al. 1992) as well in *Chlamydomonas reinhardtii* (Delrue et al. 1992) a granule-bound starch synthase has been shown to be involved in the synthesis of amylose. Mutants of many different plants defective in this enzyme, are known as *waxy* mutants and give rise to starch granules having only amylopectin.

The starch synthase reaction was first described by Leloir et al. (1961) with UDPGlc as the glucosyl donor, but it was later shown that ADPGlc was more efficient in terms of V_{max} and K_m values (Recondo and Leloir 1961). Soluble starch synthases from leaf and reserve tissues are specific for ADPGlc. In contrast, the starch synthases bound to the starch granule in reserve tissues do have some low activity with UDPGlc as compared to activity seen with ADPGlc (MacDonald and Preiss 1983).

ADPGlc PPases from photosynthetic systems

ADPGlc PPases from bacteria and plants are allosteric enzymes, whose activities are regulated by key metabolites from the main carbon assimilation pathway occurring in the organism (Iglesias and Preiss 1992; Sivak and Preiss 1998). These ADPGlc PPase effectors are key metabolites representing signals of high carbon and energy contents within the cell. The regulatory properties of this enzyme, together with the fact that ATP is one of the substrates, have the rationale that synthesis of reserve polysaccharide in bacteria and plants will be maximal when cellular carbon and energy are in excess, and *vice versa*. According to the affinity for allosteric regulators, ADPGlc PPases have been distributed in nine different classes (Ballicora et al. 2003). Among these, the enzymes from photosynthetic organisms are included in classes IV, V, VI, VIII, and IX (Table 1).

Ghosh and Preiss (1966) showed that the reaction catalyzed by ADPGlc PPase is an important step for regulation of starch synthesis in higher plants as well as in the cyanobacteria (Preiss 1999; Preiss and Sivak 1998a, b). Most of the plant ADPGlc PPases so far characterized are allosterically regulated by 3-PGA and inorganic orthophosphate (Pi) (Iglesias and Preiss 1992; Preiss and Sivak 1998a, b; Preiss 1999) (Table 1). Recently, it has been suggested that in higher plants the enzyme activity can also be regulated by its reductive state (Fu et al. 1998b; Ballicora et al. 2000; Tiessen et al. 2002).

Numerous studies have shown the relevance of mechanisms regulating ADPGlc PPase for carbon metabolism in photosynthetic organisms. The overall picture clearly determines the importance in establishing structure–function relationships for the better understanding of the different allosteric regulatory

Table 1. Regulatory and structural properties of ADPGlc PPase in organisms performing different metabolisms

	Class	ADPGlc PPase			Carbon metabolism
		Allosteric regulators		Quaternary structure	
		Activator	Inhibitor		
Accumulating glycogen					
Prokaryotes					Heterotrophic
Escherichia coli	I	Fru 1,6-bisP	AMP	Homotetramer (α_4)	Utilizing the Embden–Meyerhof (glycolysis) pathway
Aeromonas formicans	II	Fru 1,6-bisP, Fru 6-P	AMP, ADP		
Serratia marcescens	III	None	AMP		
Agrobacterium tumefaciens	IV	Pyruvate, Fru 6-P	AMP, ADP, Pi	Homotetramer (α_4)	Utilizing the Entner–Doudoroff pathway
					Performing anoxygenic photosynthesis
Rhodobacter capsulata, *Chromatium vinosum*	IV	Pyruvate, Fru 6-P	AMP, ADP		Utilizing the Entner–Doudoroff pathway
Rhodobacter gelatinosa, *Rhodobacter globiformis*, *Rhodobacter sphaeroides*, *Rhodocyclus purpureus*	V	Pyruvate, Fru 6-P, Fru 1,6-bisP	AMP, Pi	Homotetramer (α_4)	Utilizing glycolysis and the Entner–Doudoroff pathways
Rhodospipillum rubrum, *Rhodospirillum tenue*	VI	Pyruvate	None		Utilizing the TCA and the reductive carboxylic acid cycles
					Heterotrophic
Bacillus subtilis *Bacillus stearothermophillus*	VII	None	None	Heterotetramer ($\alpha_2\delta_2$)	Utilizing the TCA cycle during sporulation
Cyanobacteria					Performing oxygenic photosynthesis
Synechococcus PCC 6301, *Synechocystis* PCC 6803, *Anabaena* PCC 7120	VIII	3-PGA	Pi	Homotetramer (α_4)	Fixing CO_2 through the Calvin cycle
Accumulating starch					
Eukaryotes					
Green algae					
Chlamydomonas reinhardtii, *Chlorella fusca*, *Chlorella vulgaris*	VIII	3-PGA	Pi	Heterotetramer ($\alpha_2\beta_2$)	
Higher plants					
Photosynthetic tissues: leaves of spinach, wheat *Arabidopsis*, maize, rice	VIII	3-PGA	Pi	Heterotetramer ($\alpha_2\beta_2$)	

Table 1. Continued

	Class	ADPGlc PPase			Carbon metabolism
		Allosteric regulators		Quaternary structure	
		Activator	Inhibitor		
Non-photosynthetic tissues					Heterotrophic cells
Potato tubers	VIII	3-PGA	Pi	Heterotetramer ($\alpha_2\beta_2$)	Metabolizing sucrose imported from photosynthetic tissues
Endosperm of maize, barley and wheat	IX	None directly, 3-PGA and Fru 6-P reverse inhibitors effect	Pi, ADP, Fru 1,6-bisP	Heterotetramer ($\alpha_2\beta_2$)	

properties of a key enzyme in bacteria and plant metabolism.

Supporting data for the physiological importance of regulation of ADP-glucose pyrophosphorylase

Experimental evidence is available supporting the view that ADPGlc PPase is an important regulatory enzyme on the pathway for plant starch biosynthesis. In *C. reinhardtii* starch deficient mutants have been isolated and one class of mutant was shown to have an ADPGlc PPase that could not be activated by 3-PGA (Ball et al. 1991). Support for the importance of the allosteric regulation by ADPGlc PPase has also been obtained in *A. thaliana* (Lin et al. 1988a, b). One mutant, TL25, lacked both subunits and accumulated only 2% of the starch seen in the normal plant (Lin et al. 1988a), which would indicate that starch synthesis is almost completely dependent on the synthesis of ADPGlc. The other mutant, TL 46, was starch-deficient and lacked the regulatory 54 kDa subunit (Lin et al. 1988b). The mutant had only 7% of the wild-type activity and a subsequent study (Neuhaus and Stitt 1990) showed that in high light (photosynthesis) the rate of starch synthesis of TL 46 was only at 9% and at low light, only 26% of the rate of the wild type. This is supporting evidence that the regulation of ADPGlc PPase is of *in vivo* importance.

A maize mutant has also been isolated where the ADPGlc PPase was less sensitive to the inhibition by Pi than the wild type enzyme; the mutant endosperm had 15% more dry weight and more starch than the normal endosperm (Giroux et al. 1996). In potato tuber (Stark et al. 1992) and wheat endosperm (Smidansky et al. 2002), genetic manipulation of ADPGlc PPase activity led to an increase in starch production. Thus, regulation of ADPGlc synthesis in plants agrees with the concept that biosynthetic pathways are effectively regulated at its first unique step and that the synthesis of ADPGlc is rate-limiting. Allosteric mutant ADPGlc PPases from maize endosperm and *C. reinhardtii* and the resultant effects on starch synthesis provide strong evidence that the allosteric effects observed *in vitro* are operative in the *in vivo* situation (Lin et al. 1988a, b; Ball et al. 1991; Stark et al. 1992; Giroux et al. 1996; Van den Koornhuyse et al. 1996; Preiss and Sivak 1998a, b; Sivak and Preiss 1998).

Regulatory properties and quaternary structure of ADPGlc PPase from different photosynthetic sources

Regulatory properties: overview

ADPGlc PPases from bacteria performing anoxygenic photosynthesis are included in three different classes after their specificities for allosteric regulators, as shown in Table 1 (Iglesias and Preiss 1992; Preiss and Romeo 1994; Sivak and Preiss 1998; Ballicora et al. in press). Enzymes from classes IV and V are regulated by intermediates of glycolytic pathways;

Table 2. Different patterns in the interaction between 3-PGA activation and Pi inhibition of ADPGlc PPases from plants

Group	Principal effector	Secondary effector	Main effect on	Regulatory effect	ADPGlc PPase from
A	3PGA and Pi	Pi and 3-PGA	V_{max}	Ultrasensitive interaction between effectors	Cyanobacteria, green algae, spinach leaf, potato tuber
B	Pi	3-PGA	V_{max}	3-PGA reverses inhibition caused by Pi	Wheat endosperm
C	3PGA	Pi	V_{max}	Pi only inhibits the enzyme activated by 3-PGA	Leaf of CAM plants, maize endosperm
D	3-PGA	Pi	K_m	3-PGA increases affinity for the substrate, ATP, and Pi reverses the effect	Barley endosperm

whereas ADPGlc PPase from class VI is activated by pyruvate, a key product of the carbon fixing reductive carboxylic acid cycle that is channeled to make carbohydrates (Buchanan and Arnon 1990). On the other hand, the bacterial ADPGlc PPases activated by 3-PGA and inhibited by Pi are those from prokaryotes that perform oxygenic photosynthesis (cyanobacteria) (Iglesias et al. 1991; Charng et al. 1992; Kakefuda et al. 1992), and exhibit identical specificity for allosteric regulators than the enzyme from eukaryotic photosynthesizers, such as green algae and cells of higher plants (Table 1) (Preiss 1991, 1999; Sivak and Preiss 1998; Preiss and Sivak 1998b). These organisms utilize the reductive pentose phosphate pathway to photoassimilate atmospheric CO_2, producing 3-PGA as the first photosynthetic product; whereas under light conditions Pi is the substrate for ATP regeneration through photophosphorylation (Iglesias and Podestá 1996). Thus, photosynthetic ADPGlc PPases are typically regulated by the 3-PGA/Pi ratio under physiological conditions, allowing the enzyme to be mainly activated during the day and inhibited at night (Preiss 1991; Iglesias and Preiss 1992; Sivak and Preiss 1998; Preiss and Sivak 1998b).

Variation of 3-PGA interaction with Pi in ADPGlc PPases from different plant systems

As indicated above, most plant ADPGlc PPase activities are affected by 3-PGA and Pi. However, the pattern of regulation by these allosteric effectors may differ for various ADPGlc PPases. In fact, four patterns of interactions between 3-PGA and Pi in the plant enzymes can be distinguished (Table 2). One, observed for most enzymes is where Pi and 3-PGA affect the enzyme separately and where increasing concentrations of 3-PGA can reverse or overcome the Pi inhibition.

A second pattern is found in ADPGlc PPases from reserve tissues of some cereals exhibiting distinctive regulatory properties (Table 1). The enzymes from pea embryos (Hylton and Smith 1992), barley endosperm (Kleczkowski et al. 1993c; Rudi et al. 1997), bean cotyledon (Weber et al. 1995), and wheat endosperm (Gomez-Casati and Iglesias 2002) may be considered as relatively insensitive to regulation, mainly to activation by 3-PGA. However, a complete characterization of the ADPGlc PPase purified from wheat endosperm has shown that the enzyme is under regulation by the coordinate action of a series of metabolites (Gomez-Casati and Iglesias 2002). The wheat endosperm enzyme is allosterically inhibited by Pi, ADP, and Fru 1,6-bisP (Table 1). In all cases, inhibition is reversed by 3-PGA and Fru 6-P, which individually (in the absence of the inhibitors) have no effect on the enzyme activity (Gomez-Casati and Iglesias 2002). Consequently, the activity of this enzyme is affected by the 3-PGA/Pi ratio in a particular manner (Table 2). In fact, rather than being an unregulated ADPGlc PPase, the wheat endosperm enzyme seems to have distinctive regulatory properties (Table 1) that have Pi inhibition as a key signal (Table 2). The relevance of Pi inhibition on ADPGlc PPase from wheat endosperm and its consequence on the *in vivo* starch accumulation and seed yield was very recently shown through plant genetic transformation (Smidansky et al. 2002). Results on the characterization of the wheat

endosperm enzyme (Gomez-Casati and Iglesias 2002) not only agrees with reports showing that Pi limits starch biosynthesis in crop plants (Gomez-Casati and Iglesias 2002; Smidansky et al. 2002), but also suggests that the levels of several metabolites will also alter the biosynthetic pathway in the endosperm tissue.

Another variation of 3-PGA activation interaction with Pi inhibition is observed in CAM plant leaf ADPGlc PPases of *Hoya carnosa* and *Xerosicyos danguyi* (Singh et al. 1984) and of maize endosperm (Plaxton and Preiss 1987) (Table 2). The enzymes are activated by 2 mM 3-PGA about 10- to 25-fold but in the absence of 3-PGA are insensitive to Pi inhibition. The maize endosperm enzyme is only inhibited about 20% by 10 mM Pi and the CAM plant leaf enzymes 50% by 2 mM Pi. Further addition of Pi does not increase their inhibitions. However, at subsaturating concentrations of 3-PGA (~0.15–0.25 mM) the enzyme becomes more sensitive to Pi inhibition and becomes totally inhibited at 0.5–2 mM Pi. Higher 3-PGA concentrations reverse the Pi inhibition and decreases the affinity of the enzymes for Pi.

A fourth pattern of interaction between allosteric activator and inhibitor is seen with barley endosperm ADPGlc PPase, which is poorly activated by 3-PGA or inhibited by Pi (Table 2). However, 3-PGA lowers up to 3-fold the $S_{0.5}$ for ATP (i.e., increased the apparent affinity of ATP) and the Hill coefficient (Kleczkowski et al. 1993c). At 0.1 mM ATP the activation by 3-PGA is around 4-fold, and Pi 2.5 mM reverses the effect. Thus, in barley endosperm the important effect of 3-PGA or Pi may be in either increasing or decreasing the apparent affinity of the substrate, ATP.

Ultrasensitive behavior of ADPGlc PPase from cyanobacteria and photosynthetic organisms

A crosstalk between main allosteric regulators (activator vs. inhibitor) of ADPGlc PPase has been found for the enzyme from different sources (Sivak and Preiss 1998). Indeed, numerous studies have shown that the enzyme from photosynthetic organisms is strictly regulated by the ratio of 3-PGA/Pi (Sivak and Preiss 1998; Preiss and Sivak 1998b). Ultrasensitivity is a type of amplification by which the response of a biological system increases several fold after a narrow variation range of the stimulus (Goldbeter and Koshland 1982; Koshland 1987). Studies performed with ADPGlc PPase from the cyanobacterium *Anabaena* PCC 7120 have shown that under 'molecular crowding conditions' (those that presumably simulate cellular conditions, where there is a large concentration of macromolecules, such as other proteins), the allosteric inhibitor Pi elicits ultrasensitivity in the response of the enzyme to the activator 3-PGA (Gomez-Casati et al. 1999, 2000). A concentration of 9% or 15% polyethylene-glycol was used to induce molecular crowding (Gomez-Casati et al. 2000). Thus, activation of cyanobacterial ADPGlc PPase by 3-PGA exhibits a cooperative behavior in the presence of Pi, with a sharp increase in the enzyme activity in response to relatively small changes of the activator.

The cooperativity in the saturation curve for 3-PGA increases in crowded conditions and/or with higher concentration of Pi (Gomez-Casati et al. 2000). In fact, in the absence of Pi and molecular crowding a near 200-fold increase in the level of 3-PGA is necessary to increase the enzyme activity from 10% to 90% of V_{max}. However, in crowded media containing 5 mM Pi an increase of only 7-fold in the activator concentration produces the same kinetic response of the enzyme (Gomez-Casati et al. 1999, 2000, 2003). The ultrasensitive behavior adds a level of complexity in the interplay between both effectors that results in a highly sensitive regulation of ADPGlc PPase. A recent work demonstrates that such an ultrasensitive behavior of the enzyme is operative, *in situ* and that it propagates to the synthesis of reserve polysaccharide, under intracellular conditions occurring in cyanobacteria and probably in plastids of higher plant cells (Gomez-Casati et al. 2003). This is also in agreement with the regulatory properties reported for the potato tuber ADPGlc PPase; as relatively small changes in the 3-PGA and Pi concentrations can greatly affect the rate of ADPGlc synthesis, particularly at low 3-PGA levels, where the activation is minimal (Sivak and Preiss 1998).

Activation of plant ADPGlc PPases by thioredoxin

The ADPGlc PPase from potato tuber has an intermolecular disulfide bridge that links the two small subunits by the Cys^{12} residue. This enzyme was activated by reduction of the Cys^{12} disulfide linkage (Fu et al. 1998b). Both, reduced thioredoxin *f* and *m* from spinach (*Spinacia oleracea*) leaves reduce and activate the enzyme at low concentrations (10 μM) of 3-PGA. Fifty percent activation was obtained at

4.5 and 8.7 μM for reduced thioredoxin *f* and *m*, respectively, which is two orders of magnitude lower than for dithiothreitol. The activation was reversed by oxidized thioredoxin. Cys^{12} is conserved in the ADPGlc PPases from plant leaves and other tissues except for the monocot endosperm enzymes (Ballicora et al. 1999). It is very possible that in photosynthetic tissues, reduction could play a role in the fine regulation of the ADPGlc PPase mediated by the ferredoxin–thioredoxin system. This is the only reported mechanism of regulation in the synthesis of starch that is mediated by covalent modification.

Regarding the physiological relevance of the regulation by thioredoxin, it is worth noting that the same small subunit gene of the potato ADPGlc PPase is expressed both, in tuber and leaf (Nakata et al. 1994). Both enzymes are located in plastids; the leaf form is in the chloroplast and the tuber enzyme is in the amyloplast (Kim et al. 1989). In chloroplasts, the physiological interpretation of the regulation by thioredoxin *in vitro* is straightforward: the ferredoxin–thioredoxin system is located in the chloroplast and it has been thoroughly characterized (Wolosiuk et al. 1993). Upon illumination, this system produces reduced thioredoxin and at night, it generates oxidized thioredoxin. Thioredoxin could reduce or oxidize the chloroplastic ADPGlc PPase during the light/dark cycle, thus providing a fine tune regulation of starch synthesis in chloroplasts. Interestingly, a recombinant small subunit from *A. thaliana* ADPGlc PPase is also activated by reducing agents (B. Smith-White and J. Preiss, unpublished results).

The relevance of the regulation by thioredoxin is not clear for an enzyme that is located in the amyloplast as the ADPGlc PPase from potato tuber. It is likely that a thioredoxin form is present in amyloplasts since thioredoxins are widely distributed and have been detected in many plant tissues and different subcellular locations, including cytosol, mitochondria, chloroplasts and nuclei (Jacquot et al. 1997). The other issue is what is the nature of the reducing power that would drive the regulation of a target protein (e.g., ADPGlc PPase) by thioredoxin. In the amyloplast, there is no photosynthetic electron transport, but like in other heterotrophic plastids; the oxidative pentose phosphate pathway generates reducing power yielding NADPH in a light-independent process (Emes and Neuhaus 1997). NADPH reduces a ferredoxin form that is present in heterotrophic plastids and serves as reducing power for ferredoxin-dependent enzymes (Jacquot et al. 1997). In heterotrophic plastids both electron donors are present, but it is not known whether either a ferredoxin–thioredoxin or NADP–thioredoxin system exists. More information about the biochemistry of the amyloplast is needed to make any conclusion about the role of reductive activation in the regulation of ADPGlc PPase from potato tuber *in vivo*.

The presence of a thioredoxin-like molecule has to be demonstrated before further speculation on the physiological relevance of reductive regulation of the ADPGlc PPase from amyloplasts. However a recent report (Tiessen et al. 2002) indicates that detachment of potato tubers from the growing plant causes inhibition of starch synthesis within 24 h after detachment despite *in vitro* ADPGlc PPase activity remaining high, as well as the substrates ATP and Glc 1-P, and the 3-PGA/Pi ratio increasing. Using non-reducing SDS-PAGE it was shown that the catalytic subunit in detached tubers was solely in the dimeric form and relatively inactive compared to the enzyme form in growing tubers. In growing tubers the enzyme was found as a mixture of monomers and dimers. The isolated enzyme from detached tubers had a marked decrease in affinity for the substrates as well as for the activator. Incubation of tuber slices with either DTT or sucrose reduced the dimerization of the ADPGlc PPase catalytic subunit and stimulated starch synthesis *in vivo*. These results strongly suggest that the reductive activation of the tuber ADPGlc PPase observed *in vitro* (Fu et al. 1998b) plays an *in vivo* role in regulating starch synthesis (Tiessen et al. 2002). The sucrose content in the tuber correlates strongly with the reductive activation of the ADPGlc PPase.

Subunit structure of plant ADPGlc PPases

Cyanobacterial ADPGlc PPases occupy a unique position with respect to structure–regulation relationships. They are immunologically related to, and regulated as the plant ADPGlc PPases; but they are homotetrameric in structure as observed for the enzymes from other bacteria (Table 1). Conversely, the higher plant ADPGlc PPase has been shown to consist of two subunits with different functions (Morell et al. 1987; Preiss et al. 1989; Okita et al. 1990). Other immunological studies in maize endosperm suggested that in both non-photosynthetic and photosynthetic tissues the ADPGlc PPase is comprised of two subunits that are products of two genes (Plaxton and Preiss 1987). ADPGlc PPases from all the eukaryotes characterized so far (starting with the green algae proteins, see Table 1) are composed of α and β subunits, to

form a heterotetrameric structure (Preiss 1991, 1999; Iglesias et al. 1992, 1994; Sivak and Preiss 1998; Preiss and Sivak 1998b). The recombinant potato tuber ADPGlc PPase was shown by N-terminal sequencing to have a structure of $\alpha_2\beta_2$ (Frueauf et al. 2003). For convenience, these subunits were named 'small' (α subunit, 50–54 kDa) and 'large' (β subunit, 51–60 kDa); even though the difference in mass between them in some cases is not more than 1 kDa (Nakata et al. 1994). The small subunit of the higher plant ADPGlc PPase is highly conserved (85%–95% identity), whereas the large subunit is less conserved (50%–60% identity) (Smith-White and Preiss 1992).

Role of the plant ADPGlc PPase subunits

Both subunits of ADPGlc PPases from eukaryotic organisms are required for optimal activity of the native enzyme. *In vivo* experiments in *A. thaliana* indicate that the lack of one of the subunits not only reduce the activity of the ADPGlc PPase but also the synthesis of starch (Lin et al. 1988a, b; Li and Preiss 1992). Also, a similar effect has been observed when either of the subunits are absent in maize endosperm (Preiss et al. 1990). To study the properties of the subunits, the cDNAs encoding the mature α and β subunits of the potato tuber enzyme were cloned and expressed in *Escherichia coli* (Iglesias et al. 1993; Ballicora et al. 1995). The small and large subunits could be expressed separately or together, which allowed studying their respective individual functions. The large subunit alone had no detectable activity (Iglesias et al. 1993; Ballicora et al. 1995). Conversely, when the small subunit was expressed by itself and purified to homogeneity, it was shown to form a homotetramer (α_4) that behaves as active as the native heterotetrameric enzyme when assayed at saturating concentrations of substrates and 3-PGA (Ballicora et al. 1995). The only difference was that the α_4 enzyme had about 15-fold lower affinity for 3-PGA than the heterotetramer and was more sensitive to Pi inhibition. Affinity for the substrates and Mg^{2+} were essentially the same for the α_4 or the $\alpha_2\beta_2$ native enzymes (Iglesias et al. 1993; Ballicora et al. 1995).

Further experiments involving mutagenesis of the Glc 1-P binding site of the α subunit confirmed the above hypothesis (Fu et al. 1998a). The affinity for this substrate decreased orders of magnitude in the mutants, whereas the same mutations on the homologous residue in the β subunit did not affect catalysis or the affinity for Glc 1-P. Mutations of the activator site showed that the most important lysines responsible for a high affinity binding of the 3-PGA were also located on the small subunit (Ballicora et al. 1998). However, the highest affinity for the activator was observed only when the large subunit was present forming a heterotetramer with the small subunit.

The above data strongly suggest that the small subunit of ADPGlc PPases from eukaryotic organisms is a fully catalytic subunit, with poor regulation; whereas the large subunit has a modulatory function. Thus, the large subunit would interact with the small subunit in order to increase the affinity for 3-PGA. Even though both seem to derive from the same ancestor, based on the homology of conserved regions, the higher homology between the small subunits indicates that there have been stronger evolutionary constraints for them than the large subunits. The higher heterogeneity seen in the large subunit sequence probably reflects different requirements in the modulation of the small subunit sensitivity to allosteric activation and inhibition posed by different demands of the tissue and species (Smith-White and Preiss 1992). Expression of large subunits would differ during development or in different plants and tissues (e.g., leaf, stem, guard cells, tuber, endosperm, root, embryo), providing the resulting ADPGlc PPases with different sensitivities to regulators (Smith-White and Preiss 1992; Sivak and Preiss 1998). The distinctive regulatory properties found for the wheat endosperm enzyme (Gomez-Casati and Iglesias 2002) (see also Table 1) seems to be a good example that agrees with this hypothesis.

Characterization of ADPGlc PPases from different sources

Table 3 summarizes the kinetic and regulatory properties of various ADPGlc PPases that have been either partially purified away from interfering reactions or purified to homogeneity. Below is a summary of the characterization of the properties of ADPGlc PPases from leaf, plant non-photosynthetic tissue, algae and cyanobacteria.

Spinach leaves

The characterization of the heterotetrameric ($\alpha_2\beta_2$) structure of ADPGlc PPases from eukaryotes was first determined for the purified (105 U/mg) spinach leaves enzyme (Morell et al. 1987). The velocity of synthesis

Table 3. Kinetic and regulatory parameters from ADPGlc PPases from cyanobacteria, green algae, and higher plants

Source	Effector	Constant (mM)	*n*	Activation-fold	Reference
Barley endosperm purified	ATP (−3PGA)	0.31	2.1		Kleczkowski et al. (1993b)
	ATP (+3PGA)	0.19	1.0		
	G1P (−3PGA)	0.12	NR[a]		
	G1P (+3PGA)	0.12	NR		
Barley leaves purified	3PGA	0.005	Hyperbolic	>20	Kleczkowski et al. (1993a)
	Pi	0.025	Hyperbolic		
	ATP (−3PGA)	1.0	Hyperbolic		
	ATP (+3PGA)	0.08	Hyperbolic		
	G1P (−3PGA)	0.33	Hyperbolic		
	G1P (+3PGA)	0.11	Hyperbolic		
Tomato fruit	3PGA	0.2	NR	Negligible activity in absence of 3-PGA	Chen and Janes (1997)
	ATP (+3PGA)	0.12	NR		
	G1P (+3PGA)	0.086	NR		
	Pi (3-PGA 0.5 mM)	0.7			
Maize endosperm purified	3-PGA	0.12	Hyperbolic		Plaxton and Preiss (1987)
	3-PGA (+Pi 1 mM)	1.2	1.5		
	Pi (+3PGA 1 mM)	0.44	1.0		
	Pi (+3PGA 10 mM)	9.8	1.2		
	ATP (−3PGA)	0.84	1.3		
	ATP (+3PGA)	0.11	1.0		
	G1P (−3PGA)	0.67	0.9		
	G1P (+3PGA)	0.03	1.0		
Chlamydomonas	3-PGA	0.23	1.3	15	Iglesias et al. (1994)
	Pi	0.054	1.0		
	Pi (+3PGA 2.5 mM)	0.53	1.7		
	G1P	0.22	1.7		
	G1P (+3PGA)	0.03	1.2		
	ATP	0.48	1.2		
	ATP (+3PGA)	0.08	1.3		
Wheat endosperm	Pi	0.7	1.3		Gomez-Casati and Iglesias (2002)
	3PGA	No effect			
	3PGA (+Pi 0.7 mM)	0.81	1.0		
	3PGA (+Pi 1.5 mM)	1.51	1.4		
	3PGA (+Pi 5 mM)	3.33	2.5		
	G1P	0.092	Hyperbolic		
	ATP	0.12	Hyperbolic		
	Fru 6-P	No effect			
	Fru 6-P (+Pi 0.7 mM)	2.5	NR		
Wheat leaf	Pi	0.2	1.2	11	Gomez-Casati and Iglesias (2002)
	3PGA	0.01	1.0		
	3PGA (+Pi 2.0 mM)	1.9	2.3		
	G1P	0.45	1.1		
	G1P (+3PGA)	0.08	1.0		
	ATP	0.73	1.2		
	ATP (+3PGA)	0.22	1.1		

Table 3. Continued

Source	Effector	Constant (mM)	*n*	Activation-fold	Reference
Rice endosperm	3PGA	0.65	NR	40	Sikka et al. (2001)
	Pi (+1 mM 3PGA)	0.40	NR		
	G1P (+3PGA)	0.17	NR		
	ATP (+3PGA)	0.18	NR		
Arabidopsis (recombinant APS1 + APL1)	3PGA	0.02	NR	NR	Kavakli et al. (2002)
	Pi (1 mM 3PGA)	1.2	NR		
	G1P	0.20	NR		
	ATP	0.30	NR		
Spinach leaf	3PGA	0.051	1.0	20	Copeland and Preiss (1981)
	Pi	0.045	1.1		
	Pi (+3PGA 1 mM)	0.97	3.7		
	ATP	0.38	0.9		
	ATP (+3PGA 1 mM)	0.062	0.9		
	G1P	0.12	0.9		
	G1P (+3PGA 1 mM)	0.035	1.0		
Potato tuber	3PGA	0.16	Hyperbolic	30	Ballicora et al. (1995); Fu et al. (1998)
	Pi (−3PGA)	0.04	NR		
	Pi (+3PGA 3 mM)	0.63	NR		
	ATP (+3PGA)	0.076	1.6		
	G1P (+3PGA)	0.057	1.1		
Anabaena	3PGA	0.12	1.0	17	Iglesias et al. (1991)
	Pi (−3PGA)	0.044	1.0		
	Pi (+3PGA 2.5 mM)	0.46	1.7		
	G1P (−3PGA)	0.13	1.2		
	G1P (+3PGA)	0.08	1.0		
	ATP (−3PGA)	1.55	1.2		
	ATP (+3PGA)	0.46	1.1		
Synechocystis	3PGA	0.81	2.0	126	Iglesias et al. (1991)
	Pi (−3PGA)	0.095	1.0		
	Pi (+3PGA 2.5 mM)	0.57	2.2		
	G1P (−3PGA)	0.18	1.1		
	G1P (+3PGA)	0.05	1.1		
	ATP (−3PGA)	3.2	2.2		
	ATP (+3PGA)	0.80	1.0		

[a]Not reported.

of ADPGlc was very sensitive to activation by 3-PGA and inhibition by Pi. The enzyme activity increased by 3-PGA from 9- to 60-fold at pH 7.5 and 8.5, respectively (Ghosh and Preiss 1966). In absence of 3-PGA, the $I_{0.5}$ for Pi is 64 and 45 μM at pH 7.3 and 8.0, respectively (Copeland and Preiss 1981). Inhibition by Pi is reversed by the activator 3-PGA and *vice versa*. The ratio 3-PGA/Pi controls the activity of the ADPGlc PPase and the synthesis of starch in chloroplasts (Sivak and Preiss 1998). It has been shown that the enzymes from several other plant leaves exhibit similar regulatory properties (Sanwal et al. 1968; Preiss 1982).

Potato tuber

The ADPGlc PPase from potato tuber behaves similarly to the enzyme from plant leaves. It has been

purified to apparent homogeneity (56.9 U/mg) and it has been the first plant heteromeric enzyme to be expressed fully active in *E. coli* (Sowokinos and Preiss 1982; Okita et al. 1990; Iglesias et al. 1993; Ballicora et al. 1995).

Barley

The ADPGlc PPase from barley leaves was purified to near homogeneity (69.3 U/mg) and it shows high sensitivity toward activation by 3-PGA and inhibition by phosphate (Kleczkowski et al. 1993b). Substrate kinetics and product inhibition studies in the synthesis direction suggested a sequential Iso Ordered Bi-kinetic mechanism. ATP or ADPGlc bind first to the enzyme in the synthesis or pyrophosphorolysis direction, respectively, similar to the *E. coli* enzyme (Haugen and Preiss 1979).

The ADPGlc PPase from barley endosperm was partially purified (68-fold, 29.1 U/mg) (Kleczkowski et al. 1993c) and shows low sensitivity to the regulators 3-PGA and Pi. However, 3-PGA lowered up to 3-fold the $S_{0.5}$ for ATP and the Hill coefficient as seen in Table 3 (Kleczkowski et al. 1993a, c). At 0.1 mM ATP the activation by 3-PGA was around 4-fold (Kleczkowski et al. 1993c) and phosphate 2.5 mM reversed the effect. Moreover, the degree of the activation by 3-PGA was found dependent on the condition (mainly temperature and the presence of Mg^{2+}) at which the enzyme is maintained before assaying it for activity (Kleczkowski et al. 1993a).

A recombinant enzyme with a $(His)_6$-tag from barley endosperm was expressed using the baculovirus insect cell system (Rudi et al. 1997). It shows no sensitivity to the regulation by 3-PGA and Pi but it was assayed at saturating concentration of substrates only in the pyrophosphorolysis direction using the spectrophotometric method. For all ADPGlc PPases studied the magnitude of activation by the allosteric effectors is much greater in the synthesis reaction than what is observed in the pyrophosporolysis reaction. The ADPGlc synthesis direction and not the pyrophosphorolysis direction is considered to be the physiological reaction. When the recombinant enzyme without the $(His)_6$-tag was expressed in insect cells, the heterotetrameric form remained insensitive to 3-PGA activation and to Pi inhibition at saturated concentrations of substrates (Doan et al. 1999). It has not been reported if the 3-PGA had any effect on the affinity for the substrates as shown in the enzyme purified from the endosperm. Conversely, the small subunit expressed alone was very responsive to the allosteric effectors (Doan et al. 1999).

The small subunit from barley seems to be encoded by a single gene that gives rise to two different transcripts. One of them was found abundantly expressed in starchy endosperm but not in leaves, while the other was isolated from both tissues (Thorbjornsen et al. 1996a).

Pea embryos

The enzyme from pea developing embryos was purified to apparent homogeneity (56.5 U/mg) and it was found to be activated up to 2.4-fold by 1 mM 3-PGA in the synthesis direction. In pyrophosphorolysis assay, 1 mM Pi inhibited the enzyme 50% and 3-PGA reversed this effect (Hylton and Smith 1992). The effect of 3-PGA or Pi on the $S_{0.5}$ for ATP was not analyzed.

Three ADPGlc PPases cDNA clones have been isolated from a cotyledon cDNA library. Two of them encode small subunits and the third one a large subunit. The latter showed a greater selectivity in expression than the other two and it was highly expressed in sink organs and undetectable in leaves (Burgess et al. 1997).

Tomato

Three clones encoding different ADPGlc PPase isoforms were isolated from a cDNA library from tomato fruit. Sequence comparison and phylogenetic analysis revealed that all of them represent different types of the enzyme large subunits. It was proposed that the three isoforms are organ-specific in their expressions (Park and Chung 1998). In parallel, four clones were isolated by PCR; three corresponding to large subunits and one to a small subunit (Chen et al. 1998b). Concurrently, when the enzyme from tomato fruit was purified to apparent homogeneity (45 U/mg), multiple forms were detected by two-dimensional electrophoresis and immunological criteria. At least three polypeptides corresponded to large subunits and two to small subunits. The purified tomato fruit enzyme was highly sensitive to 3-PGA/Pi regulation as the enzyme from potato tuber (Table 3) (Chen and Janes 1997; Chen et al. 1998a).

Maize endosperm

ADPGlc PPase partially purified (34 U/mg) from maize endosperm was found to be activated by 3-PGA and Fru 6-P (25- and 17-fold, respectively)

and inhibited by Pi (Plaxton and Preiss 1987). The heterotetrameric endosperm enzyme was cloned and expressed in *E. coli* and some of its regulatory properties were compared to the isolated allosteric mutant less sensitive to Pi inhibition (Giroux et al. 1996). As above stated, the increase of starch noted in the mutant maize endosperm ADPGlc PPase insensitive to Pi inhibition supports the relevance of the allosteric effects of 3-PGA and Pi *in vivo*.

C. reinhardtii

The ADPGlc PPase from different green algae was characterized as being highly regulated by 3-PGA/Pi (Sanwal and Preiss 1967; Nakamura and Imamura 1985; Iglesias et al. 1994). The enzyme from *C. reinhardtii* was purified to apparent homogeneity (81 U/mg) and characterized as a heterotetramer ($\alpha_2\beta_2$), which is typical of the ADPGlc PPase from eukaryotic organisms (Iglesias et al. 1994). A starch deficient mutant of *C. reinhardtii* was shown to contain an ADPGlc PPase that could not be activated by 3-PGA but exhibited similar sensitivity to Pi inhibition than the wild type enzyme (Ball et al. 1991).

Wheat

Two cDNA clones that encode a large and a small subunit from the wheat endosperm ADPGlc PPase were isolated (Ainsworth et al. 1993, 1995). The ADPGlc PPase from wheat endosperm has been highly purified (2.44 U/mg) and characterized showing a novel regulatory behavior (see Tables 1 and 2) (Gomez-Casati and Iglesias 2002). This form is either the major or the only one present in the endosperm at 28-day post-anthesis. On the other hand, it has been claimed that a cytosolic and a plastidic isoform of the enzyme in the endosperm have been separated, but no kinetic characterization of them has been performed (Burton et al. 2002). More recently, it was shown that crude extracts of wheat endosperm contained two isoforms of the enzyme (Tetlow et al. 2003). One extract from the amyloplastidial fraction, was activated 2-fold by 3-PGA and inhibited by Pi, while the extract from the whole endosperm exhibited sensitivity toward 3-PGA only when Pi was present as described for the purified enzyme (Gomez-Casati and Iglesias 2002; Tetlow et al. 2003). The ADPGlc PPase from wheat leaves was purified (59 U/mg) and characterized as a typical 3-PGA/Pi regulated enzyme (Gomez-Casati and Iglesias 2002). Some of the kinetic data for the wheat leaf and endosperm ADPGlc PPases are seen in Table 3.

Rice

SDS-PAGE and iso-electrofocusing analysis detected multiple isoforms of the purified ADPGlc PPase (43 U/mg) of rice endosperm (Nakamura and Kawaguchi 1992). No kinetic characterization was performed. A recent work reported that a purified enzyme was 40-fold activated by 3-PGA and inhibited by Pi (Sikka et al. 2001). Allosteric kinetic constants for the rice endosperm enzyme are seen in Table 3.

A. thaliana

The *A. thaliana* leaf ADPGlc PPase containing the mature forms of one small subunit clone isolated from a cDNA library (Kavakli et al. 2002) and one large subunit (adg-2) (Villand et al. 1993) was expressed in a heterologous system and characterized enzymatically and the recombinant enzyme exhibited kinetic properties similar to the enzyme purified from leaves (Li and Preiss 1992; Kavakli et al. 2002).

Vicia faba

Several cDNA clones encoding two different ADPGlc PPase polypeptides were isolated from a cotyledonary library of *V. faba* L. (Weber et al. 1995). Both sequences are closely related to ADPGlc PPase small subunit from other plants. One polypeptide is expressed in developing cotyledons and leaves, while the other is found only in cotyledons. Studies performed on crude extracts showed that the enzyme from cotyledons is insensitive to 3-PGA whereas the one from leaves can be activated more than 5-fold, and Pi inhibited both enzymes. These experiments were performed in the pyrophosphorolysis direction using the spectrophotometric assay and whether, the 3-PGA reversed the Pi inhibition was not analyzed (Weber et al. 1995).

Subcellular localization of ADPGlc PPase in plants

In green algae and in leaf cells of higher plants ADPGlc PPase is a chloroplastic enzyme (Preiss 1982). Evidence of the latter is the early discovered fact that isolated chloroplasts synthesize starch with only requirement for CO_2 and illumination (Heldt et al.

1977). Conversely, reports on the localization of ADPGlc PPase in non-photosynthetic cells of higher plants have been controversial. Immunolocalization of the enzyme in storage tissues of higher plants has been performed by Kim et al. (1989) in developing potato tuber cells and by Miller and Chourey (1995) in developing endosperm cells of maize. These works, in agreement with studies of intact plastids isolation in wheat endosperm (Entwistle and ap Rees 1988), pea embryo (Denyer and Smith 1988), maize endosperm (Echeverria et al. 1988), and oilseed rape embryos (Kang and Rawsthorne 1994) provided good evidence that the enzyme is exclusively plastidial. The *in situ* detection of the enzyme in amyloplasts of developing maize endosperm has shown that the labeling of the ADPGlc PPase associated to the organelles is predominantly peripheral (Brangeon et al. 1997).

More recently, using plastids isolated from maize and barley endosperm (Denyer et al. 1996; Thorbjornsen et al. 1996b; Johnson et al. 2003), it was reported the existence of two, a plastidial form and a major cytosolic form of ADPGlc PPase. From this, it was proposed that, in cereals, there are two isoenzymes with different intracellular location (Kleczkowski 1996). This is largely based on that multiple forms of both the small and large subunits found in several plants may give rise to different isoenzymes, which localize in different intracellular compartments and possess distinctive kinetic and regulatory properties. The latter view on the cytosolic localization of ADPGlc PPase proposes that starch synthesis in cereals endosperm occurs with the involvement of an ADPGlc carrier in the amyloplast envelope (Pozueta-Romero et al. 1991; Kleczkowski 1996). A main problem of the studies on localization of ADPGlc PPase through plastids isolation is the low integrity of the amyloplasts in such preparations, since amyloplasts intactness lower than 25% were reported (Denyer et al. 1996; Johnson et al. 2003). In this respect, it is worth to point out a detailed analysis performed by ap Rees (1995), stating that the use of plastid preparations with low integrity highly affect error propagation in the calculation of enzyme distribution between subcellular compartments.

Identification of important amino acid residues within the ADPGlc PPases

Amino acid residues playing important roles in the binding of substrates and allosteric regulators have been identified in the ADPGlc PPases mainly by chemical modification and site directed mutagenesis studies. Thus, photoaffinity analogs of ATP and ADPGlc, 8-azido-ATP and 8-azido-ADPGlc, were used to identify Tyr^{114} as an important residue in the enzyme from *E. coli* (Lee and Preiss 1986; Lee et al. 1986). Site directed mutagenesis of this residue rendered a mutant enzyme exhibiting a marked increase in $S_{0.5}$ for ATP, but also a lower affinity for Glc 1-P and the activator Fru 1,6-bisP (Lee et al. 1986). The Tyr residue must be close to the adenine ring of ATP or ADPGlc, but probably also near the Glc 1-P and the activator regulatory sites. It is not very clear if this residue plays a direct role in the binding of the nucleotide. Interestingly, the homologous Tyr^{114} in the enzyme from plants is a Phe residue (Frueauf et al. 2001), which suggests that the functionality is not given by the specific residue but by its hydrophobicity.

Early chemical modification studies on the *E. coli* ADPGlc PPase, showing the involvement of Lys^{195} in the binding of Glc 1-P (Parsons and Preiss 1978a, b), were later confirmed by site-directed mutagenesis (Hill et al. 1991). The latter methodology was used to determine the role of this conserved residue in the small (Lys^{198}) and large (Lys^{213}) subunits of the potato tuber ADPGlc PPase (Fu et al. 1998a). Mutation of Lys^{198} of the small subunit with Arg, Ala or Glu, decreased the apparent affinity for Glc 1-P 135- to 550-fold. Conversely, there was little effect on kinetic constants for ATP, Mg^{2+}, 3-PGA and Pi. These results indicate that the Lys^{198} in the small subunit is directly involved in the binding of Glc 1-P. On the other hand, the homologous counterpart in the large subunit does not seem to be involved since similar mutations on the Lys^{213} had little effect on the affinity for Glc 1-P (Fu et al. 1998a). This agrees with the idea that the large subunit is a modulatory subunit without a catalytic role (Ballicora et al. 1995).

Recently, Asp^{142} in the *E. coli* ADPGlc PPase was predicted to be close to the substrate site and the amino acid was identified as mainly involved in catalysis (Frueauf et al. 2001). Characterization of site-directed mutant enzymes D142A and D142N confirmed that the main role of Asp^{142} is catalytic as they showed a decrease in specific activity of 10,000-fold, whereas other kinetic parameters showed no significant changes (Frueauf et al. 2001). This residue is highly conserved throughout ADPGlc PPases from different sources, as well as throughout the superfamily of nucleotide-sugar pyrophosphorylases (NDP-

sugar PPases) (Frueauf et al. 2003). The role of this Asp residue was also investigated in the heterotetrameric ADPGlc PPase from potato tuber. By site-directed mutagenesis, the homologous residues of the small (Asp^{145}) and large (Asp^{160}) subunits were substituted by either Asn or Glu residues (Frueauf et al. 2003). Mutation of the Asp^{145} of the small subunit rendered enzymes exhibiting four (D145N) or two (D145E) orders of magnitude decreases in specific activity. Conversely, mutations in the large subunit alone did not alter the specific activity, but did affect the apparent affinity for 3-PGA (Frueauf et al. 2003). Results agree with the statement that each subunit plays a particular role: catalytic for the small subunit and modulatory for the large subunit.

Pyridoxal-5-phosphate (PLP) is a Lys modifying reagent that was useful to identify residues involved in the binding of allosteric effectors and substrates in ADPGlc PPases. In fact, PLP can be considered as having some structural analogy to 3-PGA, as it activates the enzymes from spinach leaf and *Anabaena*. In the enzyme from spinach, PLP bound at Lys^{440}, very close to the C-terminal of the small subunit, as well as to three other Lys residues in the large subunit (Ball and Preiss 1994). The binding to these sites was prevented by the allosteric effector 3-PGA, which indicated that they are close or directly involved to the binding of this activator (Morell et al. 1988; Ball and Preiss 1994).

Similar results were obtained with the ADPGlc PPase from *Anabaena*, where PLP modified Lys^{419} and Lys^{382}. Identification of these residues as regulatory binding sites was confirmed by site-directed mutagenesis of the *Anabaena* ADPGlc PPase (Charng et al. 1994; Sheng et al. 1996). Mutation of the homologous Lys residues of the potato tuber enzyme (Lys^{441} and Lys^{404} in the small subunit) revealed that they are also part of the 3-PGA site of heterotetrameric ADPGlc PPases and that they contribute additively to the binding of the activator (Ballicora et al. 1998). However, mutations of the small subunit yielded enzymes with lesser affinity to 3-PGA than the homologous mutations of the large subunit. Results indicate that Lys^{404} and Lys^{441} of the potato tuber small subunit are more important for 3PGA activation than their homologous counterparts on the large subunit. On the other hand, the large subunit seems to contribute to the enzyme activation by making the activator sites already present in the small subunit more efficient rather than providing more effective allosteric sites (Ballicora et al. 1998).

Arginine residues in ADPGlc PPases from cyanobacteria were found to be functionally important as shown by chemical modification with phenylglyoxal (Iglesias et al. 1992; Sheng and Preiss 1997). Also, the role played by Arg^{294} in the inhibition by Pi of the enzyme from *Anabaena* PCC 7120 was shown by Ala scanning mutagenesis studies (Sheng and Preiss 1997). More recently, it was found that replacement of this residue with Ala or Gln produces mutant enzymes with a changed pattern of inhibitor specificity, as having NADPH rather than Pi as the main inhibitor (Frueauf et al. 2002). All of these results suggest that the positive charge of Arg^{294} may play a key role in determining inhibitor selectivity, rather than being specifically involved in Pi binding. On the other hand, studies of the role of Arg residues located in the N-terminal of the enzyme from *Agrobacterium tumefaciens* demonstrated the presence of separate subsites for the activators Fru 6-P and pyruvate as well as a desensitization of R33A and R45A mutants to Pi inhibition (Gomez-Casati et al. 2001).

Random mutagenesis experiments performed on the potato tuber ADPGlc PPase have been useful to identify residues that are important for the enzyme. Mutation Asp^{253} on the small subunit showed a specific effect on the K_m for Glc 1-P, which increased 10-fold with respect to the wild type enzyme (Laughlin et al. 1998b). The small magnitude in the increase (only one, rather than two to four orders of magnitude) would suggest that the Asp^{253} residue is not directly involved in Glc 1-P binding. Remarkably, this residue is conserved in the NDP-sugar PPases that have been crystallized and the structure solved (Rost and Sander 1993; Brown et al. 1999; Blankenfeldt et al. 2000; Frueauf et al. 2001). The alignment of Asp^{253} in the latter according to the secondary structure elements suggests that the residue is close to the substrate site without a direct interaction with Glc-1-P. The most interesting finding in the random mutagenesis studies was related with Asp^{416} (in the article is described as Asp^{413}) in the large subunit and its relevance for the normal activation by 3-PGA (Greene et al. 1996). This residue is adjacent to the Lys^{417} that was characterized as a site for PLP binding and 3-PGA activation (Ball and Preiss 1994).

The identification of Lys and Arg residues involved in allosteric activator binding and located at the C-terminus in ADPGlc PPases from plant and cyanobacteria constitutes a main difference to what was found for the *E. coli* and *A. tumefaciens* enzymes. In the latter, Lys^{39} (*E. coli*) and Arg residues in the

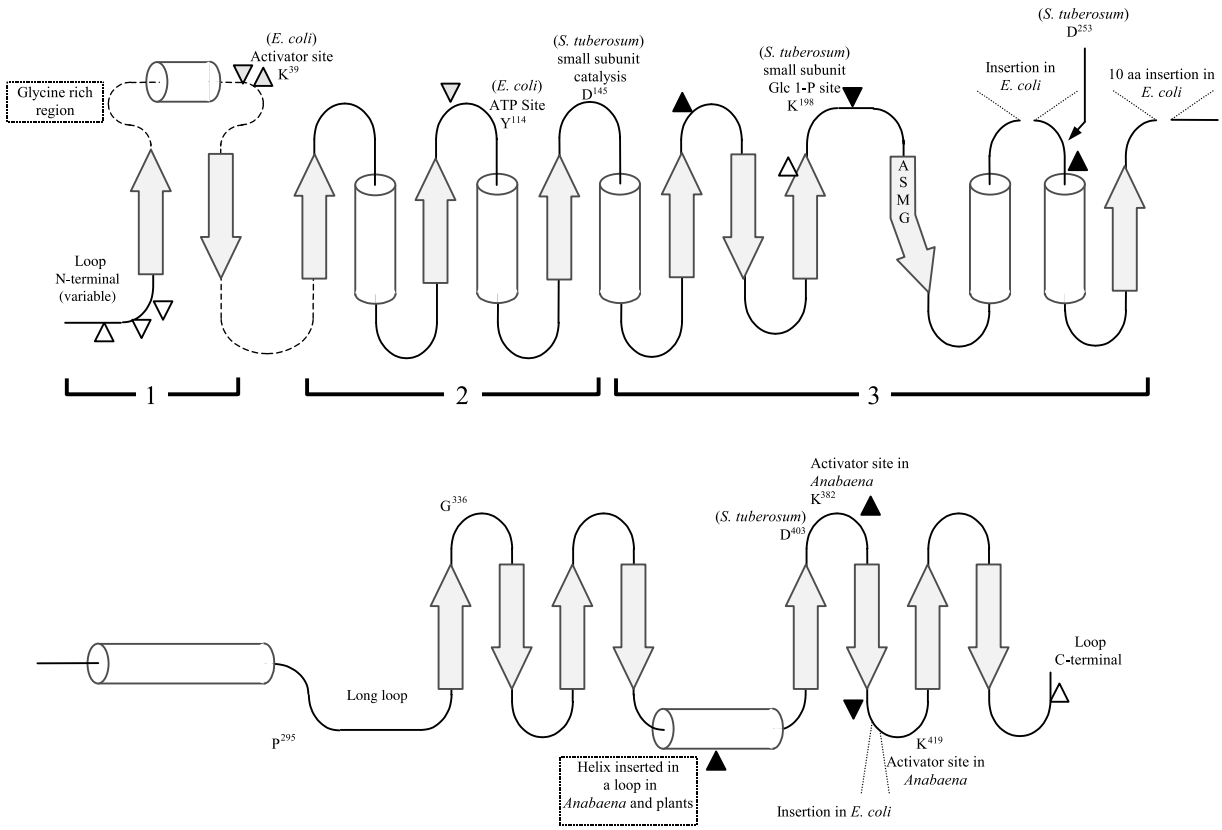

Figure 1. Prediction of the secondary structure of ADPGlc PPases. The secondary structure of various ADPGlc PPases from bacteria as well as plants was predicted by the PHD program (Rost and Sander 1993). Arrows are predicted β-pleated sheets and cylinders are predicted α-helices. These structures are interconnected with amino acid sequences indicated as neither α-helices or as β-pleated sheets. These possibly random structures or loops are shown as lines. White triangles indicate areas where proteinase K hydrolyzes the *E. coli* enzyme (Wu and Preiss 1998). Black triangles indicate where the *Anabaena* ADPGlc PPase is partially proteolyzed by trypsin and gray triangles indicates the partial hydrolysis of the *E. coli* enzyme by trypsin (unpublished). Residues P^{295} and G^{336} are amino acids that when mutated affect the allosteric properties of the ADPGlc PPase (Meyer et al. 1998a, b). Regions 1, 2, and 3 form the putative catalytic domain. The topology of regions 2 and 3 can be predicted with a high degree of accuracy since the secondary structure matches the crystallized NDP-sugar PPases. The topology of region 1, in dashed lines, could not be ascertained. Most probably, the glycine rich loop and the K^{39} loop are facing the same side of the loops that bind the substrates. Region 3 is possibly a domain that binds the Glc 1-P according to the secondary structure alignment with the crystal structures of NDP-sugar PPases. The motif ASMG is highly conserved in ADPGlc PPases and it is compatible with a β-pleated sheet, bent by glycine, present in the crystallized NDP-sugar PPases.

N-terminal (*A. tumefaciens*) were characterized as important for the interaction of the enzyme's activators and inhibitors (Parsons and Preiss 1978a, b; Gardiol and Preiss 1990; Gomez-Casati et al. 2001). These results suggest that regulatory domains may be located at different sites in the enzymes from bacteria and plant. However, recent studies performed with chimeric ADPGlc PPases from *E. coli* and *A. tumefaciens* have indicated that in the bacterial enzymes the C-terminus is also critical for determining regulator affinity and specificity (Ballicora et al. 2002). Thus, it is very possible that the regulation of ADPGlc PPases is determined by a combined arrangement and interaction between the N- and C-terminus in the respective protein.

Prediction of the structure of ADPGlc PPases

At the present, attempts to obtain ADPGlc PPase in a crystalline state allowing its structural characterization have failed. This strongly limits the complete understanding of structure–function relationships of the enzyme. A valid approach to solve this pitfall is the use of different methods to predict protein structure (Rost and Sander 1993). When a modified 'hydro-

phobic cluster analysis' (HCA) (Lemesle-Varloot et al. 1990) was applied to several ADPGlc PPases from different sources representing different classes according to homology of subunits and tissue, a high similarity in the distribution and pattern of the clusters was observed (Frueauf et al. 2001; Ballicora et al. 2003). This comparison also included bacterial and plant enzymes, thus strongly suggesting that ADPGlc PPases share a common folding pattern, despite a different quaternary structure ($\alpha_2\beta_2$ in plants and α_4 in bacteria) and specificity for allosteric regulators. Prediction of the secondary structure of ADPGlc PPases from different sources based on controlled proteolysis experiments, and other biochemical data allowed us to build a model for this enzyme that pictures a similar 3D-structure (Preiss and Sivak 1998b; Frueauf et al. 2001) (Figure 1).

Loops are prone to have insertions and deletions in homologous proteins, without altering the structure. In our model (Figure 1), all the insertions and deletions observed, mainly found between the enzymes from classes VIII and IX, and the rest (Table 1), fall in loops. Also located in loops are the conserved amino acids known to have specific roles in the binding of substrates (*E. coli* Tyr^{114}, potato tuber small subunit Lys^{198}), activators (*E. coli* Lys^{39}, *Anabaena* Lys^{382}, Lys^{419}), involved in catalysis (potato tuber small subunit Asp^{145}), or probably close to the substrate site (potato tuber small subunit Asp^{253}). The residue Asp^{403} that seems to be located in a region important for the regulation of the potato tuber enzyme (Greene et al. 1996) is also found in a loop.

The model also shows a domain that is typical of proteins that bind nucleotides (Figure 1). Close to the N-terminus, in region 1, there is a β-sheet followed by a Gly-rich loop that is similar to a 'P loop' present in protein kinases or nucleotide binding sites (Saraste et al. 1990). In addition, region 2 (Figure 1) is comprised by three β-sheets and helices that are compatible with the Rossman fold (Rossman et al. 1974). Interestingly, Tyr^{114}, which was shown to be reactive to the azido analog of ATP (Lee and Preiss 1986; Lee et al. 1986), is in this putative domain.

It is worth to point out in the model that all the amino acid residues characterized as playing a functional role in the substrate-binding site are facing the same side. Hence, this is compatible with the idea that the ATP would be facing the 'top' of the structure depicted in Figure 1. The opposite loops show low homology between ADPGlc PPases. This correlates with the topology observed in α/β structures that have functional loops on one side of the domain and only connector loops on the other (Branden 1980). In addition, the model fits very well with the recently crystallized NDP-sugar PPase domains (Brown et al. 1999; Blankenfeldt et al. 2000; Kostrewa et al. 2001; Olsen and Roderick 2001; Sivaraman et al. 2002). Most probably, despite the very low homology among some of them, all the NDP-sugar PPases share a similar catalytic domain. To support this hypothesis, it was demonstrated, by site directed mutagenesis, that Lys^{175} in the GDPMan PPase from *Pseudomonas aeruginosa* is responsible for the Man 1-P binding and it is homologous to the Glc 1-P binding site found in ADPGlc PPases (May et al. 1994).

Domain characterization

It has been proposed that the N- and C-terminus are responsible for the distinctive regulatory properties of the different classes of ADPGlc PPases (Ballicora et al. 2002). This is evident for the ADPGlc PPases from oxygenic photosynthetic organisms since key residues for the regulation have been found on the C-terminus of plant and cyanobacterial ADPGlc PPases (Charng et al. 1995; Greene et al. 1996; Sheng et al. 1996; Sheng and Preiss 1997; Ballicora et al. 1998; Frueauf et al. 2002). Also, several modifications on the C-terminus caused modifications on the regulation of plant enzymes (Giroux et al. 1996; Salamone et al. 2002). Residues that are critical for the binding of the activators have been found only on the N-terminus of enzymes from heterotrophic bacteria (Gardiol and Preiss 1990). However, recent experiments with chimeric constructs have shown that the C-terminal may be also important for the regulation of the bacterial enzyme (Ballicora et al. 2002). It is very possible combined arrangement and spatial interaction between the N- and C-terminus determines the regulation in all the ADPGlc PPases.

It was observed that truncation of 10 amino acids in the small subunit of ADPGlc PPase from potato tuber modified the regulatory properties by increasing the apparent affinity for the activator 3-PGA and decreasing the one for the inhibitor Pi (Ballicora et al. 1995). Similar results have been observed when the large (modulatory) subunit was truncated 17 amino acids in the N-terminal (Laughlin et al. 1998a). Truncation of 11 amino acids in the *E. coli* enzyme also affects the regulatory properties (Wu and Preiss 1998, 2001). All data agree with the premise that the N-terminal region

of the ADPGlc PPase, which is predicted as a loop, may play a role as an 'allosteric switch' to regulate enzyme activity. This loop possibly interferes with the transition between two different conformations of the enzyme (activated and non-activated). A shorter N-terminus may favor a conformation of the enzyme that facilitates the activation. Interestingly, the residue responsible for the thioredoxin mediated regulation of the potato tuber ADPGlc PPase is located in the N-terminus of the protein (Ballicora et al. 2000; Fu et al. 1998b).

Evolution of the ADPGlc PPases

According to the model (Figure 1), it is very possible that the ADPGlc PPases and other NDP-sugar PPases derive from a common ancestor, since they seem to share a common pyrophosphorylase domain with a similar fold. The main difference is that ADPGlc PPases are allosteric enzymes, and are generally bigger because they have an extended C-terminus (120–150 amino acids) and a slightly longer N-terminus (10–40 amino acids). The other NDP-sugar PPases either lack this extended C-terminus or it is part of a completely different domain to form a bifunctional enzyme (Shinabarger et al. 1991; Brown et al. 1999). Probably, the ADPGlc PPase acquired the C-terminal fragment to become a regulated enzyme and/or to improve a rudimentary regulation already present. In plant and cyanobacterial ADPGlc PPases, the role of the C-terminus in the regulation is critical. In enzymes from other sources it is not known whether the regulatory sites are located in the same or distinct domains, but it seems that the C-terminus is playing an important role in all the ADPGlc PPase classes. Once acquired this C-terminal domain in the evolution, it is possible that a common ancestor evolved to other forms with different regulatory properties to accommodate to different metabolic environments, and developed into several classes of ADPGlc PPases (Table 1). Many ADPGlc PPases have a longer N-terminus than other NDP-sugar PPases. However, it is not clear at what point in evolution this was expanded, or if this has been more dynamic process.

The specificity for activators and inhibitors is not absolute in some of the ADPGlc PPases. Generally, besides the main activator, other effectors can activate the enzyme, but to a lesser extent and at higher concentrations. In addition, it was observed that minimal changes on the protein sequence could alter the specificity for the regulators. This suggests that the ADPGlc PPase was flexible to mutate and acquire the ability to be efficiently activated by different metabolites. In other words, the ADPGlc PPase could easily adapt to the regulatory needs imposed by metabolic changes along the evolution. With ADPGlc PPases from anoxygenic photosynthesizers there are data that support this view. The enzyme from *Anabaena* changed the preference for the activator from 3-PGA to Fru 1,6-bisP with a single change (K419Q) (Charng et al. 1995). Concurrently, few mutations on the small subunit from the potato tuber enzyme could alter the selectivity for activators of the homotetrameric form (α_4) (Salamone et al. 2002). In the *Anabaena* enzyme, mutants R294A, R294E and R294Q changed the specificity of inhibition from Pi to NADPH (Frueauf et al. 2002). This indicates that the inhibitory site also shares this ability for adaptation. In heterotrophic bacterial ADPGlc PPases, it was shown that a single 'crossover' between two genes renders two chimeric enzymes that would belong to different classes than their parents (Ballicora et al. 2002).

The origin of the ADPGlc PPases in eukaryotes is probably related to an endosymbiotic process with a prokaryote for plastid formation (Figure 2). Later, the gene must have transferred to the nucleus (Martin and Herrmann 1998). In fact, cyanobacterial homotetrameric enzymes share a higher homology with the small subunits from plants than with other heterotrophic bacteria. In eukaryotes, a β ('large') subunit appeared later in evolution, most probably, by gene duplication. Later, these genes diverged and specialized. This led to obtain different polypeptides, a catalytic and a modulatory subunit. Even though both seem to derive from the same ancestor, based on the homology of conserved regions, the higher homology between the small subunits indicates that there have been stronger evolutionary constraints for them than the large subunits (Figure 2). Also, the small subunits share a much higher homology with the cyanobacterial enzymes rather than with the large subunits. The small subunit kept the catalytic function but lost the ability to be activated efficiently in absence of the large subunit. Replacement of few amino acids showed that this process could be reversed *in vitro* (Salamone et al. 2002). The higher heterogeneity seen in the large subunit sequence probably reflects different requirements in the modulation of the small subunit sensitivity to allosteric activation and inhibition posed by different demands of the tissue and species (Smith-White and Preiss 1992). A further step in the evolution was that,

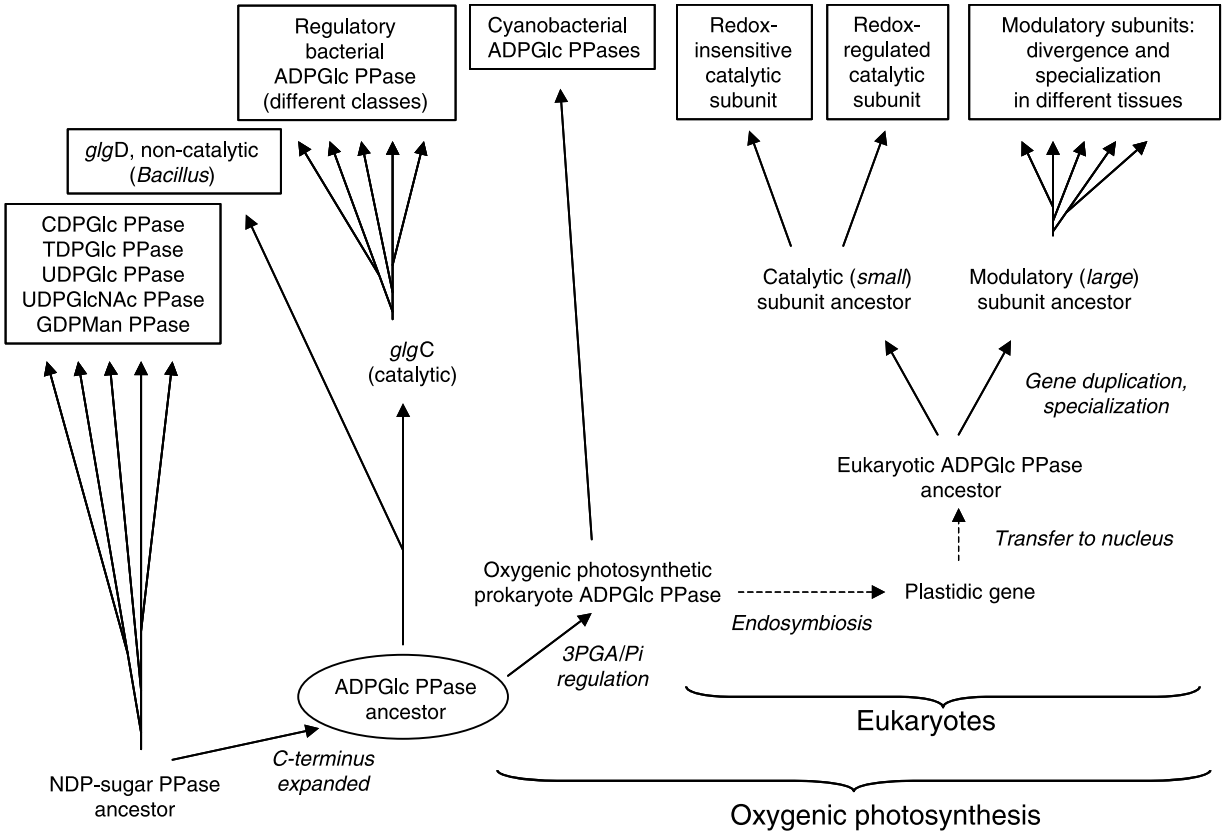

Figure 2. Evolution of ADPGlc PPases.

in certain tissues, the small subunit of the plant ADPGlc PPases gained the ability to be regulated by thioredoxin (Ballicora et al. 2000).

Acknowledgements

This work was supported in part by grants from Department of Energy DE-FG02-93ER20121 (JP), CONICET, Fundacion Antorchas, and ANPCyT (PICT'99 1-6074) (AAI).

References

Ainsworth C, Tarvis M and Clark J (1993) Isolation and analysis of a cDNA clone encoding the small subunit of ADP-glucose pyrophosphorylase from wheat. Plant Mol Biol 23: 23–33

Ainsworth C, Hosein F, Tarvis M, Weir F, Burrell M, Devos KM and Gale MD (1995) Adenosine diphosphate glucose pyrophosphorylase genes in wheat: differential expression and gene mapping. Planta 197: 1–10

ap Rees T (1995) Where do plants make ADP-Glc? In: Pontis H, Salerno G and Echeverria E (eds) Sucrose Metabolism, Biochemistry, Physiology and Molecular Biology, pp 49–62. American Society of Plant Physiologists, Rockville, Maryland

Badenhuizen IP (1969) The Biogenesis of Starch Granules in Higher Plants. Appleton-Century Crofts, New York

Ball K and Preiss J (1994) Allosteric sites of the large subunit of the spinach leaf ADPglucose pyrophosphorylase. J Biol Chem 269: 24706–24711

Ball S, Marianne T, Dirick L, Fresnoy M, Delrue B and Decq A (1991) A *Chlamydomonas reinhardtii* low starch mutant is defective for 3-phosphoglycerate activation and orthophosphate inhibition of ADPglucose pyrophosphorylase. Planta 185: 17–26

Ballicora MA, Laughlin MJ, Fu Y, Okita TW, Barry GF and Preiss J (1995) Adenosine 5′-diphosphate-glucose pyrophosphorylase from potato tuber. Significance of the N terminus of the small subunit for catalytic properties and heat stability. Plant Physiol 109: 245–251

Ballicora MA, Fu Y, Nesbitt NM and Preiss J (1998) ADP-Glucose pyrophosphorylase from potato tubers. Site-directed mutagenesis studies of the regulatory sites. Plant Physiol 118: 265–274

Ballicora MA, Fu Y, Frueauf JB and Preiss J (1999) Heat stability of the potato tuber ADP-glucose pyrophosphorylase: role of Cys residue 12 in the small subunit. Biochem Biophys Res Commun 257: 782–786

Ballicora MA, Frueauf JB, Fu Y, Schurmann P and Preiss J (2000) Activation of the potato tuber ADP-glucose pyrophosphorylase by thioredoxin. J Biol Chem 275: 1315–1320

Ballicora MA, Sesma JI, Iglesias AA and Preiss J (2002) Characterization of chimeric ADPglucose pyrophosphorylases of *Escherichia coli* and *Agrobacterium tumefaciens*. Importance of the C-terminus on the selectivity for allosteric regulators. Biochemistry 41: 9431–9437

Ballicora MA, Iglesias AA and Preiss J (2003) ADPglucose pyrophosphorylase; a regulatory enzyme for bacterial glycogen synthesis. Microbiol Mol Biol Rev 67: 213–225

Blankenfeldt W, Asuncion M, Lam JS and Naismith JH (2000) The structural basis of the catalytic mechanism and regulation of glucose-1-phosphate thymidylyltransferase (*RmlA*). EMBO J 19: 6652–6663

Branden CI (1980) Relation between structure and function of alpha/beta-proteins. Q Rev Biophys 13: 317–338

Brangeon J, Reyss A and Prioul JL (1997) *In situ* detection of ADP-glucose pyrophosphorylase expression during maize endosperm development. Plant Physiol Biochem 3: 847–858

Brown K, Pompeo F, Dixon S, Mengin-Lecreulx D, Cambillau C and Bourne Y (1999) Crystal structure of the bifunctional N-acetylglucosamine 1-phosphate uridyltransferase from *Escherichia coli*: a paradigm for the related pyrophosphorylase superfamily. EMBO J 18: 4096–4107

Buchanan BB and Arnon DI (1990) A reverse KREBS cycle in photosynthesis: consensus at last. Photosynth Res 24: 47–53

Burgess D, Penton A, Dunsmuir P and Dooner H (1997) Molecular cloning and characterization of ADP-glucose pyrophosphorylase cDNA clones isolated from pea cotyledons. Plant Mol Biol 33: 431–444

Burton RA, Bewley JD, Smith AM, Bhattacharyya MK, Tatge H, Ring S, Bull V, Hamilton WD and Martin C (1995) Starch branching enzymes belonging to distinct enzyme families are differentially expressed during pea embryo development. Plant J 7: 3–15

Burton RA, Johnson PE, Beckles DM, Fincher GB, Jenner HL, Naldrett MJ and Denyer K (2002) Characterization of the Genes Encoding the Cytosolic and Plastidial Forms of ADP-Glucose Pyrophosphorylase in Wheat Endosperm. Plant Physiol 130: 1464–1475

Caspar T, Huber SC and Somerville C (1985) Alterations in growth, photosynthesis and respiration in a starchless mutant of *Arabidopsis thaliana* (L.) deficient in chloroplast phosphoglucomutase activity. Plant Physiol 79: 11–17

Charng YY, Kakefuda G, Iglesias AA, Buikema WJ and Preiss J (1992) Molecular cloning and expression of the gene encoding ADPglucose pyrophosphorylase from the cyanobacterium *Anabaena* sp. strain PCC 7120. Plant Mol Biol 20: 37–47

Charng YY, Iglesias AA and Preiss J (1994) Structure–function relationships of cyanobacterial ADP-glucose pyrophosphorylase. Site-directed mutagenesis and chemical modification of the activator-binding sites of ADP-glucose pyrophosphorylase from *Anabaena* PCC 7120. J Biol Chem 269: 24107–24113

Charng YY, Sheng J and Preiss J (1995) Mutagenesis of an amino acid residue in the activator-binding site of cyanobacterial ADP-glucose pyrophosphorylase causes alteration in activator specificity. Arch Biochem Biophys 318: 476–480

Chen BY and Janes HW (1997) Multiple forms of ADP-glucose pyrophosphorylase from tomato fruit. Plant Physiol 113: 235–241

Chen BY, Wang Y and Janes HW (1998a) ADP-glucose pyrophosphorylase is localized to both the cytoplasm and plastids in developing pericarp of tomato fruit. Plant Physiol 116: 101–106

Chen BY, Janes HW and Gianfagna T (1998b) PCR cloning and characterization of multiple ADP-glucose pyrophosphorylase cDNAs from tomato. Plant Sci 136: 59–67

Copeland L and Preiss J (1981) Purification of spinach leaf ADP-glucose pyrophosphorylase. Plant Physiol 68: 996–1001

Delrue B, Fontaine T, Routier F, Decq A, Wieruszeski JM, Van Den Koornhuyse N, Maddelein ML, Fournet B and Ball S (1992) Waxy *Chlamydomonas reinhardtii*: monocellular algal mutants defective in amylose biosynthesis and granule-bound starch synthase activity accumulate a structurally modified amylopectin. J Bacteriol 174: 3612–3620

Denyer K and Smith A (1988) The capacity of plastids from developing pea cotyledons to synthesize acetyl CoA. Planta 173: 172–182

Denyer K, Sidebottom C, Hylton CM and Smith AM (1993) Soluble isoforms of starch synthase and starch-branching enzyme also occur within starch granules in developing pea embryos. Plant J 4: 191–198

Denyer K, Hylton C, Jenner CF and Smith AM (1995) Identification of multiple isoforms of soluble and granule-bound starch synthase in developing wheat endosperm. Planta 196: 256–265

Denyer K, Dunlap F, Thorbjornsen T, Keeling P and Smith AM (1996) The major form of ADP-glucose pyrophosphorylase in maize endosperm is extra-plastidial. Plant Physiol 112: 779–785

Doan DN, Rudi H and Olsen OA (1999) The allosterically unregulated isoform of ADP-glucose pyrophosphorylase from barley endosperm is the most likely source of ADP-glucose incorporated into endosperm starch. Plant Physiol 121: 965–975

Dry I, Smith A, Edwards A, Bhattacharyya M, Dunn P and Martin C (1992) Characterization of cDNAs encoding two isoforms of granule-bound starch synthase which show differential expression in developing storage organs of pea and potato. Plant J 2: 193–202

Echeverria E, Boyer CD, Thomas PA, Liu KC and Shannon J (1988) Enzyme activities associated with maize kernel amyloplasts. Plant Physiol 86: 786–792

Edwards GE and Walker DA (1983) C_3, C_4: Mechanisms of Cellular and Environmental Regulation of Photosynthesis. University of California Press, Berkeley

Emes MJ and Neuhaus HE (1997) Metabolism and transport in non-photosynthetic plastids. J Exp Bot 48: 1995–2005

Entwistle G and ap Rees T (1988) Enzymic capacities of amyloplasts from wheat (*Triticum aestivum*) endosperm. Biochem J 255: 391–396

Espada J (1962) Enzymic synthesis of adenosine diphosphate glucose from glucose-1-phosphate and adenosine triphosphate. J Biol Chem 237: 3577–3581

Frueauf JB, Ballicora MA and Preiss J (2001) Aspartate residue 142 is important for catalysis by ADP-glucose pyrophosphorylase from *Escherichia coli*. J Biol Chem 276: 46319–46325

Frueauf JB, Ballicora MA and Preiss J (2002) Alteration of inhibitor selectivity by site-directed mutagenesis of Arg(294) in the ADP-glucose pyrophosphorylase from *Anabaena* PCC 7120. Arch Biochem Biophys 400: 208–214

Frueauf JB, Ballicora MA and Preiss J (2003) ADP-glucose pyrophosphorylase from potato tuber: site-directed mutagenesis of homologous aspartic acid residues in the small and large subunits. Plant J 33: 503–511

Fu Y, Ballicora MA and Preiss J (1998a) Mutagenesis of the glucose-1-phosphate-binding site of potato tuber ADP-glucose pyrophosphorylase. Plant Physiol 117: 989–996

Fu Y, Ballicora MA, Leykam JF and Preiss J (1998b) Mechanism of reductive activation of potato tuber ADP-glucose pyrophosphorylase. J Biol Chem 273: 25045–25052

Gardiol A and Preiss J (1990) *Escherichia coli* E-39 ADPglucose synthetase has different activation kinetics from the wild-type allosteric enzyme. Arch Biochem Biophys 280: 175–180

Ghosh HP and Preiss J (1966) Adenosine diphosphate glucose pyrophosphorylase. A regulatory enzyme in the biosynthesis of starch in spinach leaf chloroplasts. J Biol Chem 241: 4491–4504

Giroux MJ, Shaw J, Barry G, Cobb BJ, Greene T, Okita T and Hannah LC (1996) A single gene mutation that increases maize seed weight. Proc Natl Acad Sci USA 93: 5824–5829

Goldbeter A and Koshland Jr DE (1982) Sensitivity amplification in biochemical systems. Q Rev Biophys 15: 555–591

Gomez-Casati DF and Iglesias AA (2002) ADP-glucose pyrophosphorylase from wheat endosperm. Purification and characterization of an enzyme with novel regulatory properties. Planta 214: 428–434

Gomez-Casati DF, Aon MA and Iglesias AA (1999) Ultrasensitive glycogen synthesis in Cyanobacteria. FEBS Lett 446: 117–121

Gomez-Casati DF, Aon MA and Iglesias AA (2000) Kinetic and structural analysis of the ultrasensitive behaviour of cyanobacterial ADP-glucose pyrophosphorylase. Biochem J 350: 139–147

Gomez-Casati DF, Igarashi RY, Berger CN, Brandt ME, Iglesias AA and Meyer CR (2001) Identification of functionally important amino-terminal arginines of *Agrobacterium tumefaciens* ADP-glucose pyrophosphorylase by alanine scanning mutagenesis. Biochemistry 40: 10169–10178

Gomez-Casati DF, Cortassa S, Aon MA and Iglesias AA (2003) Ultrasensitive behavior in the synthesis of storage polysaccharides in cyanobacteria. Planta 216: 969–975

Greene TW, Woodbury RL and Okita TW (1996) Aspartic acid 413 is important for the normal allosteric functioning of ADP-glucose pyrophosphorylase. Plant Physiol 112: 1315–1320

Haugen TH and Preiss J (1979) Biosynthesis of bacterial glycogen. The nature of the binding of substrates and effectors to ADP-glucose synthase. J Biol Chem 254: 127–136

Heldt HW, Chon CJ, Maronde D, Herold A, Stankovic ZS, Walker DA, Kraminer A, Kirk MR and Heber U (1977) Role of orthophosphate and other factors in the regulation of starch formation in leaves and isolated chloroplasts. Plant Physiol 59: 1146–1155

Hill MA, Kaufmann K, Otero J and Preiss J (1991) Biosynthesis of bacterial glycogen. Mutagenesis of a catalytic site residue of ADP-glucose pyrophosphorylase from *Escherichia coli*. J Biol Chem 266: 12455–12460

Hizukuri S (1995) Starch: analytical aspects. In: Eliansson AC (ed) Carbohydrates in Food, pp 347–429. Marcel Dekker, New York

Hylton C and Smith AM (1992) The *rb* mutation of peas causes structural and regulatory changes in ADP-Glc pyrophosphorylase from developing embryos. Plant Physiol 99: 1626–1634

Hylton C, Denyer K, Keeling PL, Chang MT and Smith A (1996) The effect of the waxy mutations on the granule-bound starch synthases of barley and maize endosperms. Planta 198: 230–237

Iglesias AA and Podestá FE (1996) Photosynthate formation and partitioning in crop plants. In: Pessarakli M (ed) Handbook of Photosynthesis, pp 681–698. Marcel Dekker, New York

Iglesias AA and Preiss J (1992) Bacterial glycogen and plant starch biosynthesis. Biochem Educ 20: 196–203

Iglesias AA, Kakefuda G and Preiss J (1991) Regulatory and structural properties of the cyanobacterial ADPglucose pyrophosphorylases. Plant Physiol 97: 1187–1195

Iglesias AA, Kakefuda G and Preiss J (1992) Involvement of arginine residues in the allosteric activation and inhibition of *Synechocystis* PCC 6803 ADPglucose pyrophosphorylase. J Protein Chem 11: 119–128

Iglesias AA, Barry GF, Meyer C, Bloksberg L, Nakata PA, Greene T, Laughlin MJ, Okita TW, Kishore GM and Preiss J (1993) Expression of the potato tuber ADP-glucose pyrophosphorylase in *Escherichia coli*. J Biol Chem 268: 1081–1086

Iglesias AA, Charng YY, Ball S and Preiss J (1994) Characterization of the kinetic, regulatory, and structural properties of ADP-glucose pyrophosphorylase from *Chlamydomonas reinhardtii*. Plant Physiol 104: 1287–1294

Jacquot JP, Lancelin JM and Meyer Y (1997) Thioredoxins: structure and function in plant cells. New Phytol 136: 543–570

Johnson PE, Patron NJ, Bottrill AR, Dinges JR, Fahy BF, Parker ML, Waite DN and Denyer K (2003) A low-starch barley mutant, Riso 16, lacking the cytosolic small subunit of ADP-glucose pyrophosphorylase, reveals the importance of the cytosolic isoform and the identity of the plastidial small subunit. Plant Physiol 131: 684–696

Kakefuda G, Charng YY, Iglesias AA and Preiss J (1992) Molecular cloning and sequencing of ADP-glucose pyrophosphorylase from *Synechocystis* PCC 6803. Plant Physiol 99: 344–347

Kang F and Rawsthorne S (1994) Starch and fatty acid synthesis in plastids from developing embryos of oilseed rape (*Brassica napus* L.). Plant J 6: 795–805

Kavakli IH, Kato C, Choi SB, Kim KH, Salamone PR, Ito H and Okita TW (2002) Generation, characterization, and heterologous expression of wild-type and up-regulated forms of *Arabidopsis thaliana* leaf ADP-glucose pyrophosphorylase. Planta 215: 430–439

Kim TW, Francheschi VR, Okita TW, Robinson NL, Morell M and Preiss J (1989) Immunocytochemical localization of ADPglucose pyrophosphorylase in developing potato tuber cells. Plant Physiol 91: 217–220

Kleczkowski LA (1996) Back to the drawing board: redefining starch synthesis in cereals. Trends Plant Sci 1: 363–364

Kleczkowski LA, Villand P and Olsen OA (1993a) Hysteresis and reversible cold-inactivation of ADPglucose pyrophosphorylase from barley seeds. Z Naturforsch 48c: 457–460

Kleczkowski LA, Villand P, Preiss J and Olsen OA (1993b) Kinetic mechanism and regulation of ADP-glucose pyrophosphorylase from barley (*Hordeum vulgare*) leaves. J Biol Chem 268: 6228–6233

Kleczkowski LA, Villand P, Lüthi E, Olsen OA and Preiss J (1993c) Insensitivity of barley endosperm ADP-Glc pyrophosphorylase to 3-phosphoglycerate and orthophosphate regulation. Plant Physiol 101: 179–186

Koshland DE (1987) Switches, thresholds and ultrasensitivity. Trends Biochem Sci 12: 225–229

Kostrewa D, D'Arcy A, Takacs B and Kamber M (2001) Crystal structures of *Streptococcus pneumoniae* N-acetylglucosamine-1-phosphate uridyltransferase, GlmU, in apo form at 2.33 A resolution and in complex with UDP-N-acetylglucosamine and Mg(2+) at 1.96 A resolution. J Mol Biol 305: 279–289

Laughlin MJ, Chantler SE and Okita TW (1998a) N- and C-terminal peptide sequences are essential for enzyme assembly, allosteric, and/or catalytic properties of ADP-glucose pyrophosphorylase. Plant J 14: 159–168

Laughlin MJ, Payne JW and Okita TW (1998b) Substrate binding mutants of the higher plant ADP-glucose pyrophosphorylase. Phytochemistry 47: 621–629

Lee YM and Preiss J (1986) Covalent modification of substrate-binding sites of *Escherichia coli* ADP-glucose synthetase. Isolation and structural characterization of 8-azido-ADP-glucose-incorporated peptides. J Biol Chem 261: 1058–1064

Lee YM, Mukherjee S and Preiss J (1986) Covalent modification of *Escherichia coli* ADPglucose synthetase with 8-azido substrate analogs. Arch Biochem Biophys 244: 585–595

Leloir LF (1971) Two decades of research on the biosynthesis of saccharides. Science 172: 1299–1303

Leloir LF, deFekete MAR and Cardini CE (1961) Starch and oligosaccharide synthesis from uridine diphosphate glucose. J Biol Chem 236: 636–641

Lemesle-Varloot L, Henrissat B, Gaboriaud C, Bissery V, Morgat A and Mornon JP (1990) Hydrophobic cluster analysis: procedures to derive structural and functional information from 2-D-representation of protein sequences. Biochimie 72: 555–574

Li L and Preiss J (1992) Characterization of ADP-glucose pyrophosphorylase from a starch-deficient mutant of *Arabidopsis thaliana* (L). Carbohydr Res 227: 227–239

Lin TP, Caspar T, Somerville C and Preiss J (1988a) Isolation and characterization of a starchless mutant of *Arabidopsis thaliana* L. Henyh lacking ADPglucose pyrophosphorylase activity. Plant Physiol 86: 1131–1135

Lin TP, Caspar T, Somerville C and Preiss J (1988b) A starch deficient mutant of *Arabidopsis thaliana* with low ADPglucose pyrophosphorylase activity lacks one of the two subunits of the enzyme. Plant Physiol 88: 1175–1181

MacDonald FD and Preiss J (1983) Solubilization of the starch-granule-bound starch synthase of normal maize kernels. Plant Physiol 73: 175–178

Manners DJ and Matheson NK (1981) The fine structure of amylopectin. Carbohydr Res 282: 247–262

Martin W and Herrmann RG (1998) Gene transfer from organelles to the nucleus: how much, what happens, and why? Plant Physiol 118: 9–17

Matheson NK (1996) The chemical structure of amylose and amylopectin fractions of starch from tobacco leaves during development and diurnally-nocturnally. Carbohydr Res 282: 247–262

May TB, Shinabarger D, Boyd A and Chakrabarty AM (1994) Identification of amino acid residues involved in the activity of phosphomannose isomerase-guanosine 5′-diphospho-D-mannose pyrophosphorylase. A bifunctional enzyme in the alginate biosynthetic pathway of *Pseudomonas aeruginosa*. J Biol Chem 269: 4872–4877

Miller ME and Chourey PS (1995) Intracellular immunolocalization of adenosine 5′-diphosphoglucose pyrophosphorylase in developing endosperm cells of maize (*Zea mays* L.). Planta 197: 522–527

Mizuno K, Kimura K, Arai Y, Kawasaki T, Shimada H and Baba T (1992) Starch branching enzymes from immature rice seeds. J Biochem (Tokyo) 112: 643–651

Morell MK, Bloom M, Knowles V and Preiss J (1987) Subunit structure of spinach leaf ADPglucose pyrophosphorylase. Plant Physiol 85: 182–187

Morell M, Bloom M and Preiss J (1988) Affinity labeling of the allosteric activator site(s) of spinach leaf ADP-glucose pyrophosphorylase. J Biol Chem 263: 633–637

Morrison WR and Karkalas J (1990) Starch. In: Dey PM (ed) Methods in Plant Biochemistry, pp 323–352. Academic Press, London

Nakamura Y and Imamura M (1985) Regulation of ADP-glucose pyrophosphorylase from *Chlorella vulgaris*. Plant Physiol 78: 601–605

Nakamura Y and Kawaguchi K (1992) Multiple forms of ADP-glucose pyrophosphorylase of rice endosperm. Physiol Plant 84: 336–342

Nakata PA, Anderson JM and Okita TW (1994) Structure and expression of the potato ADP-glucose pyrophosphorylase small subunit. J Biol Chem 269: 30798–30807

Nelson OE, Chourey PS and Chang MT (1978) Nucleoside diphosphate sugar-starch glucosyl transferase activity of wx starch granules. Plant Physiol 62: 383–386

Neuhaus HE and Stitt M (1990) Control analysis of photosynthate partitioning: Impact of reduced activity of ADP-glucose pyrophosphorylase or plastid phosphoglucomutase on the fluxes to starch and sucrose in *Arabidopsis*. Planta 182: 445–454

Okita T, Nakata PA, Anderson JM, Sowokinos JR, Morell M and Preiss J (1990) The subunit structure of potato tuber ADP-glucose pyrophosphorylase. Plant Physiol 93: 785–790

Olsen LR and Roderick SL (2001) Structure of the *Escherichia coli* GlmU pyrophosphorylase and acetyltransferase active sites. Biochemistry 40: 1913–1921

Outlaw Jr WH and Manchester J (1979) Guard cells starch concentration quantitatively related to stomatal aperture. Plant Physiol 64: 79–82

Park SW and Chung WI (1998) Molecular cloning and organ-specific expression of three isoforms of tomato ADP-glucose pyrophosphorylase gene. Gene 206: 215–221

Parsons TF and Preiss J (1978a) Biosynthesis of bacterial glycogen. Incorporation of pyridoxal phosphate into the allosteric activator site and an ADP-glucose-protected pyridoxal phosphate binding site of *Escherichia coli* B ADP-glucose synthase. J Biol Chem 253: 6197–6202

Parsons TF and Preiss J (1978b) Biosynthesis of bacterial glycogen. Isolation and characterization of the pyridoxal-P allosteric activator site and the ADP-glucose-protected pyridoxal-P binding site of *Escherichia coli* B ADP-glucose synthase. J Biol Chem 253: 7638–7645

Plaxton WC and Preiss J (1987) Purification and properties of non-proteolytically degraded ADPglucose pyrophosphorylase from maize endosperm. Plant Physiol 83: 105–112

Pozueta-Romero J, Viale AM and Akazawa T (1991) Comparative analysis of mitochondrial and amyloplast adenylate translocators. FEBS Lett 287: 62–66

Preiss J (1982) Regulation of the biosynthesis and degradation of starch. Annu Rev Plant Physiol 54: 431–454

Preiss J (1984) Bacterial glycogen synthesis and its regulation. Annu Rev Microbiol 38: 419–458

Preiss J (1988) Biosynthesis of starch and its regulation. In: Preiss J (ed) The Biochemistry of Plants: Carbohydrates, Structure and Function, pp 184–254. Academic Press, New York

Preiss J (1991) Biology and molecular biology of starch synthesis and its regulation. In: Mifflin B (ed) Oxford Surveys of Plant Molecular and Cell Biology, pp 59–114. Oxford University Press, Oxford

Preiss J (1999) Biosynthesis of bacterial and mammalian glycogen and plant starch synthesis and their regulation. In: Hecht SM (ed) Biorganic Chemistry: carbohydrates, pp 489–554. Oxford University Press, Oxford

Preiss J and Levi C (1980) Starch biosynthesis and degradation. In: Preiss J (ed) The Biochemistry of Plants, pp 371–423. Academic Press, New York

Preiss J and Romeo T (1994) Molecular biology and regulatory aspects of glycogen biosynthesis in bacteria. Prog Nucleic Acid Res Mol Biol 47: 299–329

Preiss J and Sivak MN (1998a) Starch and glycogen biosynthesis. In: Pinto BM (ed) Comprehensive Natural Products Chemistry, pp 441–495. Pergamon Press, Oxford

Preiss J and Sivak MN (1998b) Biochemistry, molecular biology and regulation of starch synthesis. Genet Eng 20: 177–223

Preiss J, Cress D, Hutny J, Morell M, Bloom M, Okita T and Larsen R (1989) Regulation of starch synthesis. Biochemical and genetic studies. In: Somnet P and Whitaker J (eds) Biocatalysis in Agricultural Biotechnology, pp 84–92. American Chemical Society, Washington, DC

Preiss J, Danner S, Summers PS, Morell M, Barton CR, Yang L and Nieder M (1990) Molecular characterization of the brittle-2 gene effect on maize endosperm ADPglucose pyrophosphorylase subunits. Plant Physiol 92: 881–885

Recondo E and Leloir LF (1961) Adenosine diphosphate glucose and starch synthesis. Biochem Biophys Res Commun 6: 85–88

Ritte G and Raschke K (2003) Metabolite export of isolated guard cell chloroplasts of *Vicia faba*. New Phytologist 159: 195–202

Rossman MG, Moras D and Olsen KW (1974) Chemical and biological evolution of a nucleotide binding protein. Nature 250: 194–199

Rost B and Sander C (1993) Improved prediction of protein secondary structure by use of sequence profiles and neural networks. Proc Natl Acad Sci USA 90: 7558–7562

Rudi H, Doan DNP and Olsen OA (1997) A $(His)_6$-tagged recombinant barley (*Hordeum vulgare* L.) endosperm ADPglucose pyrophosphorylase expressed in the baculovirus-insect cell system is insensitive to regulation by 3-phosphoglycerate and inorganic phosphate. FEBS Lett 419: 124–130

Sachs J (1887) In: Ward HM (ed) Lectures of the Physiology of Plants, pp 304–325. Clarendon Press, Oxford

Salamone PR, Kavakli IH, Slattery CJ and Okita TW (2002) Directed molecular evolution of ADPglucose pyrophosphorylase. Proc Natl Acad Sci USA 99: 1070–1075

Sanwal GG and Preiss J (1967) Biosynthesis of starch in *Chlorella pyrenoidosa*. II. Regulation of ATP: alpha-D-glucose 1-phosphate adenyl transferase (ADP-glucose pyrophosphorylase) by inorganic phosphate and 3-phosphoglycerate. Arch Biochem Biophys 119: 454–469

Sanwal GG, Greenberg E, Hardie J, Cameron EC and Preiss J (1968) Regulation of starch biosynthesis in plant leaves: activation and inhibition of ADPglucose pyrophosphorylase. Plant Physiol 43: 417–427

Saraste M, Sibbald PR and Wittinghofer W (1990) The P-loop – a common motif in ATP-and GTP-binding proteins. Trends Biochem Sci 15: 430–434

Sheng J and Preiss J (1997) Arginine294 is essential for the inhibition of *Anabaena* PCC 7120 ADPglucose pyrophosphorylase by phosphate. Biochemistry 36: 13077–13084

Sheng J, Charng YY and Preiss J (1996) Site-directed mutagenesis of lysine382, the activator-binding site, of ADPglucose pyrophosphorylase from *Anabaena* PCC 7120. Biochemistry 35: 3115–3121

Shinabarger D, Berry A, May TB, Rothmel R, Fialho A and Chakrabarty AM (1991) Purification and characterization of phosphomannose isomerase-guanosine diphospho-D-mannose pyrophosphorylase. A bifunctional enzyme in the alginate biosynthetic pathway of *Pseudomonas aeruginosa*. J Biol Chem 266: 2080–2088

Shure M, Wessler S and Fedoroff N (1983) Molecular identification and isolation of the *Waxy* locus in maize. Cell 35: 225–233

Sikka VK, Choi S-B, Kavakli IH, Sakulsingharoj C, Gupta S, Ito H and Okita TW (2001) Subcellular compartmentation and allosteric regulation of the rice endosperm ADPglucose pyrophosphorylase. Plant Sci 161: 461–468

Singh BK, Greenberg E and Preiss J (1984) ADPglucose pyrophosphorylase from the CAM plants *Hoya carnosa* and *Xerosicyos danguyi*. Plant Physiol 74: 711–716

Sivak MN and Preiss J (1998) Starch: basic science to biotechnology. In: Taylor SL (ed) Advances in Food and Nutrition Research, pp 1–199. Academic Press, San Diego, California

Sivaraman J, Sauve V, Matte A and Cygler M (2002) Crystal structure of *Escherichia coli* glucose-1-phosphate thymidylyltransferase (RffH) complexed with dTTP and Mg^{2+}. J Biol Chem 277: 44214–44219

Smidansky ED, Clancy M, Meyer FD, Lanning SP, Blake NK, Talbert LE and Giroux MJ (2002) Enhanced ADPglucose pyrophosphorylase activity in wheat endosperm increases seed yield. Proc Natl Acad Sci USA 99: 1724–1729

Smith-White BJ and Preiss J (1992) Comparison of proteins of ADP-glucose pyrophosphorylase from diverse sources. J Mol Evol 34: 449–464

Sowokinos JR and Preiss J (1982) Pyrophosphorylases in *Solanum tuberosum* III. Purification, physical and catalytic properties of ADPglucose pyrophosphorylase in potatoes. Plant Physiol 69: 1459–1466

Stark DM, Timmerman KP, Barry GF, Preiss J and Kishore GM (1992) Role of ADPglucose pyrophosphorylase in regulating starch levels in plant tissues. Science 258: 287–292

Tetlow IJ, Davies EJ, Vardy KA, Bowsher CG, Burrell MM and Emes MJ (2003) Subcellular localization of ADPglucose pyrophosphorylase in developing wheat endosperm and analysis of the properties of a plastidial isoform. J Exp Bot 54: 715–725

Thorbjornsen T, Villand P, Kleczkowski LA and Olsen OA (1996a) A single gene encodes two different transcripts for the ADP-glucose pyrophosphorylase small subunit from barley (*Hordeum vulgare*). Biochem J 313: 149–154

Thorbjornsen T, Villand P, Denyer K, Olsen OA and Smith A (1996b) Distinct isoforms of ADPglucose pyrophosphorylase occur inside and outside the amyloplasts in barley endosperm. Plant J 10: 243–250

Tiessen A, Hendriks JH, Stitt M, Branscheid A, Gibon Y, Farre EM and Geigenberger P (2002) Starch synthesis in potato tubers is regulated by post-translational redox modification of ADP-glucose pyrophosphorylase: a novel regulatory mechanism linking starch synthesis to the sucrose supply. Plant Cell 14: 2191–2213

Van den Koornhuyse N, Libessart N, Delrue B, Zabawinski C, Decq A, Iglesias A, Carton A, Preiss J and Ball S (1996) Control of starch composition and structure supply in the monocellular alga *Chlamydomonas reinhardtii*. J Biol Chem 271: 16281–16287

Van der Leij FR, Visser RFG, Ponstein AS, Jacobsen E and Feenstra WJ (1991) Sequence or the structural gene for the granule-bound starch synthase of potato (*Solanum tuberosum* L.) and evidence of a single point deletion in the *amf* allele. Mol Gen Genetics 228: 1279–1284

Villand P, Olsen OA and Kleczkowski LA (1993) Molecular characterization of multiple cDNA clones for ADP-glucose pyrophosphorylase from Arabidopsis thaliana. Plant Mol Biol 23: 1279–1284

Visser RFG, Somhorst I, Kuipers GJ, Ruys NJ, Feenstra WJ and Jacobsen E (1991) Inhibition of the gene for granule-bound starch synthase in potato by antisense constructs. Mol Gen Genet 225: 289–296

Weber H, Heim U, Borisjuk L and Wobus U (1995) Cell-type specific, coordinate expression of two ADPglucose pyrophosphorylase genes in relation to starch biosynthesis during seed development in *Vicia faba* L. Planta 195: 352–361

Wolosiuk RA, Ballicora MA and Hagelin K (1993) The reductive pentose phosphate cycle for photosynthetic CO_2 assimilation: enzyme modulation. FASEB J 7: 622–637

Wu MX and Preiss J (1998) The N-terminal region is important for the allosteric activation and inhibition of the *Escherichia coli* ADP-glucose pyrophosphorylase. Arch Biochem Biophys 358: 182–188

Wu MX and Preiss J (2001) Truncated forms of the recombinant *Escherichia coli* ADP-glucose pyrophosphorylase: the importance of the N-terminal region for allosteric activation and inhibition. Arch Biochem Biophys 389: 159–165

Zeiger E, Talbott LD, Frechilla S, Srivastava A and Zhu J (2002) The guard cell chloroplast: a perspective for the twenty-first century. New Phytol 153: 415–424