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REVIEW

ULTRASENSITIVITY IN (SUPRA)MOLECULARLY ORGANIZED AND CROWDED ENVIRONMENTS

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The ultrasensitive response of biological systems is a more sensitive one than that expected from the classical hyperbola of Michaelis-Menten kinetics, and whose physiological relevance depends upon the range of variation of substrate or effector for which ultrasensitivity is observed. Triggering and modulation of the ultrasensitive response in enzymatic and cellular systems are reviewed. Several demonstrations of ultrasensitive behavior in cellular systems and its impact on the amplification properties in signalling cascades and metabolic pathways are also highlighted. It is shown that ubiquitous cytoskeletal proteins may up- or downmodulate ultrasensitivity under physico-chemical conditions resembling those predominant in cells.

KEYWORDS: ultrasensitivity; signalling cascades; metabolic pathways; cytoskeleton; crowding.

INTRODUCTION

Supramolecular organization and crowding are two main features that characterize the cytoplasm of living cells. The supramolecular organization is mainly provided by the meshwork of polymers of cytoskeletal proteins (e.g. tubulin, actin, intermediate filaments) whereas its crowded nature is reflected by the high total concentration of macromolecules (Zimmerman, 1993; Aon and Cortassa, 1997; Minton, 1997; Waterman-Storer and Salmon, 1999; Gundersen and Cook, 1999; Luby-Phelps, 2000).

It is becoming increasingly evident that (supra)molecularly organized and crowded environments may provide an additional level of regulation of protein dynamics, such as their potentiality to function as switch-like devices. The powerful combination of co-operativity and sensitivity amplification allows sharp, digital-like responses of target enzymes (Koshland, 1987; Bray, 1995; Aon and Cortassa, 1997).

Formerly, Koshland *et al.* (1982) defined two types of amplification of a signal in a biological system: per magnitude and per sensitivity. The former occurs when the output molecules are produced in far greater numbers than the stimulus molecules. Sensitivity amplification mainly deals with the percentage change in a response compared to the percentage change in the stimulus. Ultrasensitivity belongs to this latter type of signal amplification and has been defined as the response in a biological system that is more sensitive than that to be expected from the classical hyperbola of Michaelis-Menten kinetics (Koshland *et al.*, 1982; Goldbeter and Koshland, 1982; Koshland, 1987, 1998). The 'normal' hyperbolic response requires an 81-fold change in ligand (e.g. substrate, effector) to increase the reaction rate from 10% to 90% of the maximal velocity. Thus, ultrasensitive systems are those that need less than 81-fold change whereas subsensitive ones demand more.

Sensitivity amplification is necessary when one changes from some finite non-zero background

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level to another finite level (Koshland, 1987). One of the best known mechanisms for sensitivity amplification is allosteric co-operativity. Notwithstanding, not every allosteric response is automatically at the origin of a physiologically relevant ultrasensitive behavior. This depends upon the range of variation of substrate or effector for which the specific ultrasensitive response is observed (Koshland *et al.*, 1982).

Sensitivity amplification has been quantified through the amplification factor, *As*, as defined by Koshland *et al.* (1982), and applied by Gomez-Casati *et al.* (1999, 2000*a*) as follows:

$$As = \frac{\dfrac{V_E(f) - Vi}{Vi}}{\dfrac{S_{(f)} - S_i}{Si}} \qquad (1)$$

where $V_E(f)$ is the reaction rate displayed by an enzyme, E, at a substrate or effector concentration corresponding to $S_{(f)}$ and V_i is the rate of the reaction catalyzed by E with respect to a concentration S_i corresponding to a reference state, e.g. 1% or 10%, of the maximal velocity of the enzyme. Alternative definitions arising from metabolic control analysis belong to the 'control strength' (Higgins, 1965) or 'response coefficient' introduced by Kacser and Burns (1973) and recently named 'sensitivity' to describe the amplification in protein kinase cascades (Ferrell, 1998; Kholodenko, 2000). The latter definition is a differential form of Eq. 1, useful in the analysis of very small (infinitesimal) changes in stimulus and also in dealing with decreasing functions where the integral definition (Eq. 1) is not appropriate. We have adopted the original definition of Goldbeter and Koshland (1982) since it allows us to deal with experimental data with finite changes in the effector or substrate concentration (LaPorte *et al.*, 1984).

Recent findings show the occurrence of ultrasensitive behavioral responses in several enzymatic systems and signalling cascades operating under intracellular conditions (Ferrell and Machleder, 1998; Gomez-Casati *et al.*, 1999, 2000*a*; Hardie *et al.*, 1999; Cluzel *et al.*, 2000). In this respect, two interesting findings described in this work have shown that polyethylenglycol (PEG)-induced molecular crowding may itself trigger or modulate ultrasensitivity in two enzymatic systems (Aon and Cortassa, 1997; Aon *et al.*, 2000*a*). These results are of great importance since they show, for the first time, that physico-chemical conditions resembling those prevailing in the cell may either induce or modulate the ultrasensitive response of enzymes. The importance of this link can be fully appreciated when it is realized that one of the main advantages of ultrasensitivity is to allow the increase of the flux by several-fold over a narrow range of variation in substrate or effector concentration above the background. This is a relevant and widespread biological phenomenon in signalling cascades (e.g. protein kinase cascades) involved in oocyte cell cycle response (Ferrell and Machleder, 1998) and changes in cellular energy charge (Hardie *et al.*, 1999), synthesis of storage polysaccharides during light–dark transitions in photosynthetic cells (Iglesias *et al.*, 2000; Gomez-Casati *et al.*, 2000*b*), pheromone detection in mammalian neurons (Leinders-Zufall, 2000), and the response of bacterial flagellar motors to chemotactic signalling proteins (Cluzel *et al.*, 2000; Stock, 1999).

The modulation of ultrasensitivity phenomena by cytoskeletal proteins (actin, microtubular protein) is also of great physiological significance since cytoskeleton dynamics may be a crucial link between cells sensing stress associated with changes in volume due to osmotic regulation (Haussinger *et al.*, 1994; Aon and Cortassa, 1997; Aon *et al.*, 2000*a*). Reorganization of cytoplasmic macromolecular assemblies by providing different spatial organization or orientational cues may in turn induce differential gene expression (Cook, 1989, 1991; Zimmerman, 1993; Zimmerman and Minton, 1993), signal transduction pathways (Grabski *et al.*, 1994; Gundersen and Cook, 1999), stress-sensing properties (Aon *et al.*, 2000*a*), and metabolic fluxes (Cortassa *et al.*, 1994; Haussinger *et al.*, 1994; Marmillot *et al.*, 1994; Bereiter-Hahn *et al.*, 1995, 1997; Rohwer *et al.*, 1998; Liliom *et al.*, 1999; Aon *et al.*, 2000*a*,*b*; Ovádi and Srere, 2000; Lloyd *et al.*, 2001).

EFFECTS OF CROWDING ON ENZYME KINETICS

The cytoplasm of a cell contains (macro)molecules at a concentration such that they occupy a large fraction of its total volume. Such media are referred to as 'crowded' since no individual (macro)molecular species is present at a high concentration *per se* (Minton, 1997). 'Background' species concern (macro)molecules that do not interact specifically with either the reactants or products of a particular reaction. The non-specific interactions (e.g. steric repulsion) contributed by 'background' species, e.g. PEG, may be very large in crowded environments (Minton, 2000). Due to

steric repulsion given essentially by molecular impenetrability, (macro)molecules exclude volume from one another. Important consequences of volume exclusion in crowded media are reflected in (macro)molecules, association and reaction rates (Minton, 2000, 2001). In fact, volume exclusion effects provide a non-specific force for (macro)-molecular compaction and association such that equilibrium association constants may be increased by several orders of magnitude, and exert modulatory effects on reaction rates (Minton, 2001). Let us explain the latter in more specific kinetic terms.

Considering the reactions involved in the overall conversion of S into P, for a simple reaction involving one ligand, S, one catalytic site, E, and one enzyme-substrate complex, ES, we have:

$$S + E \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \overset{k_2}{\rightarrow} E + P \qquad (2)$$

k_{-1} and k_2 are monomolecular rate constants whereas k_1 is a bimolecular rate constant. In the derivation of the Henri-Michaelis-Menten equation from a quasi-steady-state assumption, the dynamics of the ES complex association–dissociation is considered to be so fast that its concentration can be treated as if it were in steady state. Where otherwise stated, the k_1 step is not considered to be limiting to the S→P conversion except at low S concentrations. However, in crowded media this may not be the case, so that the overall rate of an enzymatic reaction may be affected by crowding at two main levels: (i) the rate of encounter between enzyme and substrate e.g. by diffusional restriction, and (ii) the rate at which an E-S transition-state complex decays into products. By the first of these effects a rate decrease is expected, whereas for the second type an enhancement may happen; as the higher the fractional volume occupancy by (macro)molecules, the higher the rate limitation by crowding.

ULTRASENSITIVITY IN ENZYMATIC SYSTEMS OPERATING UNDER CROWDING CONDITIONS

The ultrasensitive behavior of a highly purified recombinant ADPGlcPPase from the cyanobacterium *Anabaena* PCC 7120 was previously demonstrated *in vitro* under molecular crowding induced by PEG and under non-crowding conditions. Fluorescence emission and fourth-derivative spectroscopy showed that a shift of chromophores from tryptophan residues of ADPGlcPPase toward non-polar environments with unaltered oligomerization of the enzyme takes place during ultrasensitivity in crowded media (Gomez-Casati *et al.*, 2000*a*). These conformational effects were more pronounced in the presence of the crowding agent, i.e. PEG, alone or with the additional presence of the effectors, than in its absence (Gomez-Casati *et al.*, 2000*a*).

Quantitatively, ultrasensitivity is characterized by the sensitivity amplification factor, *As* (see Eq. 1), which presents a maximum for a certain range of the stimulus. From a physiological point of view, it becomes very important to note that *As* depends on the range over which the stimulus-response ratio is observed.

The experimental results plotted in Figure 1 illustrate the main concepts associated with ultrasensitivity, and Figure 2 further shows that it can be elicited by PEG-induced molecular crowding alone or modulated synergically between Pi and PEG. Although 1 mM Pi was able to induce ultrasensitivity in non-crowded medium, this was only possible in the presence of PEG-induced crowding, when Pi was absent (Fig. 2) (Gomez-Casati *et al.*, 2000*a*).

The response of cyanobacterial ADPGlcPPase toward its allosteric effector 3 phosphoglycerate (3PGA) (Fig. 1A) is ultrasensitive and characterized by *As* that attain maximal values of 19.3-fold in the presence of 9% PEG 8000 and 1 mM Pi (Fig. 1B; Fig. 2). Significant amplifications (15- or 11-fold) were also determined in the presence of either 1 mM Pi or 9% PEG, respectively (Fig. 1B; Fig. 2) (Gomez-Casati *et al.*, 1999, 2000*a*). That similar effects could be shown to exist in the presence of high concentrations of inert molecules like PEG, glycerol or ethylenglycol (unpublished results), or proteins such as albumin (data submitted for publication) is in agreement with the involvement of crowding in the ultrasensitive behavior exhibited by ADPGlcPPase. Such behavior was evidenced by the enzyme operating under zero- or first-order conditions with respect to its substrates, G1P and ATP (Gomez-Casati *et al.*, 1999). Moreover, the range over which the effector 3PGA is operating within the chloroplast (Gomez-Casati *et al.*, 1999, 2000*a*) belongs to the same range in which the optimal *As* for ADPGlcPPase shown in Figure 1 is observed.

In crowded media, ultrasensitivity in ADPGlcPPase appears to result from the cross-talk characteristics between 3PGA and Pi, as revealed by the following evidence (Gomez-Casati *et al.*, 2000*a*): (i) the inhibitory action of Pi

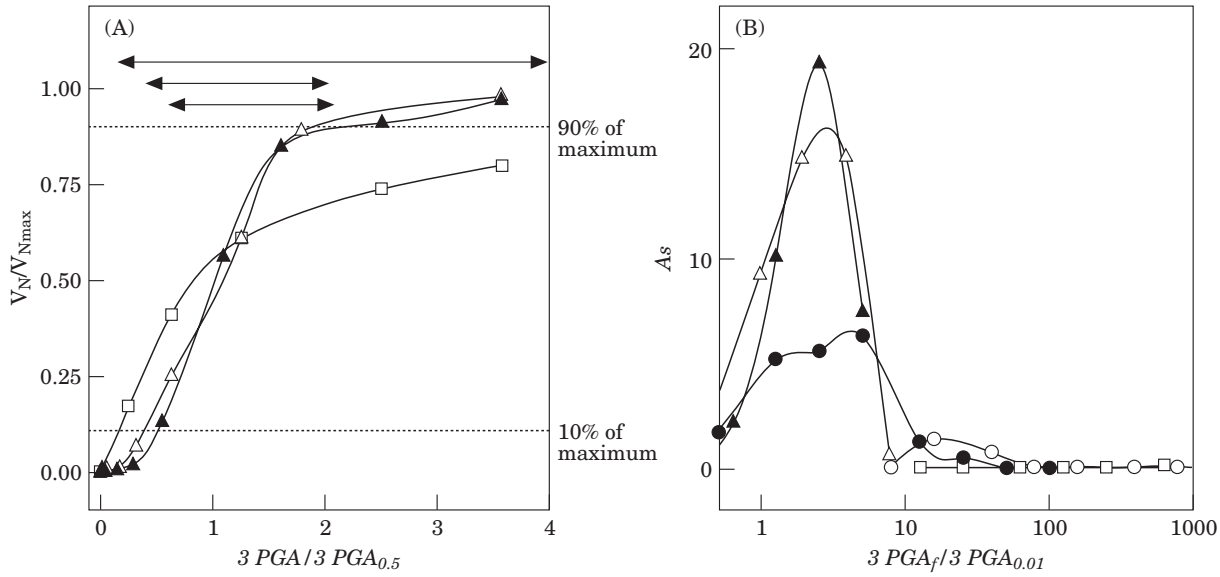

Fig. 1. Characterization of ADPGlcPPase ultrasensitive behavior in crowded medium. Stimulus-response behavior (A) and sensitivity amplification factor (*As*) (B) of ADPGlcPPase as a function of the allosteric effector 3PGA (A) or the ratio (logarithm) of the 3PGA concentration over the effector concentration corresponding to 1% of the maximal enzymatic rate (B). (A) Ratios of net velocities over the net maximal velocity were determined at different ratios of 3PGA over the effector concentration giving 50% of the maximal activation for assays of ADPGlc synthesis in media containing 9% PEG (w/w) without (—▲—) or with (—△—) 1 mM Pi. Controls run in the absence of Pi and PEG are illustrated by a square. Arrows indicate the amplitude in the 3PGA ratio producing an increase in the net velocity ratio from 10% to 90%. (B) The *As* was calculated as described in Gomez-Casati *et al.* (1999) (see also Eqs 1 and 5). Only small stimuli (given by the ratio $3PGA_{0.9}/3PGA_{0.01}$ close to one, i.e. small increases of 3PGA over its background level corresponding to 1% of the maximal enzyme activity) are necessary to obtain the highest amplifications achieved. Assays were carried out in buffered aqueous media without further additions (—□—), in the presence of 0.05 mM Pi alone (—○—) or plus 9% PEG (—●—), or in the presence of 1 mM Pi alone (—△—) or plus 9% PEG (—▲—). (Reproduced from Gomez-Casati *et al.*, 1999.)

interacting with the activating role of 3PGA; (ii) the half-maximal ADPGlcPPase activities obtained with higher concentrations of 3PGA in crowded as opposed to aqueous conditions; (iii) the relatively higher 3PGA concentrations needed in crowded medium to overcome the inhibitory power of Pi; (iv) the two-fold higher co-operativity of the enzyme under crowded conditions. The ultrasensitive behavior observed under crowding or non-crowding conditions *in vitro*, or *in situ*, is accounted for by a mathematical model simulating ultrasensitivity in ADPGlcPPase (data submitted for publication) (see Appendix).

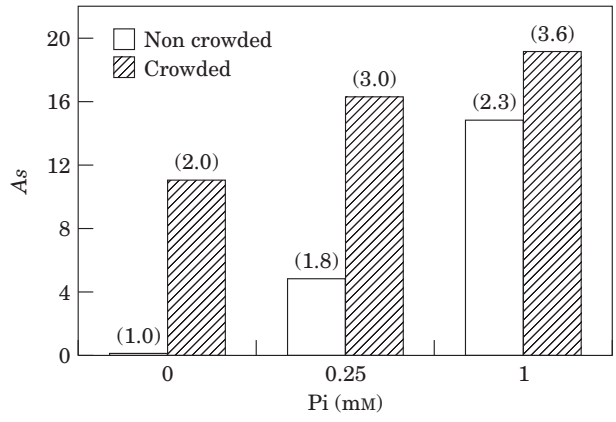

Fig. 2. Sensitivity amplification factor and Hill coefficient as a function of Pi concentration in crowded or non-crowded media. The amplification factor, *As*, at 1% of ADPGlcPPase maximal activity ($As_{0.01}$) was determined as described in Gomez-Casati *et al.* (1999, 2000*a*) (see Eqs 1 and 5). Between brackets are the Hill coefficients obtained under each experimental situation. The *As* of the control in the absence of Pi and PEG (first bar, Hill coefficient = 1.0) is barely noticeable above the *x*-axis (*As*<0.01).

MODULATION OF THE ULTRASENSITIVE RESPONSE OF ENZYMATIC SYSTEMS IN THE PRESENCE OF CYTOSKELETAL PROTEINS

Ultrasensitivity induced by the presence of cytoskeletal proteins is also observed in enzymatic

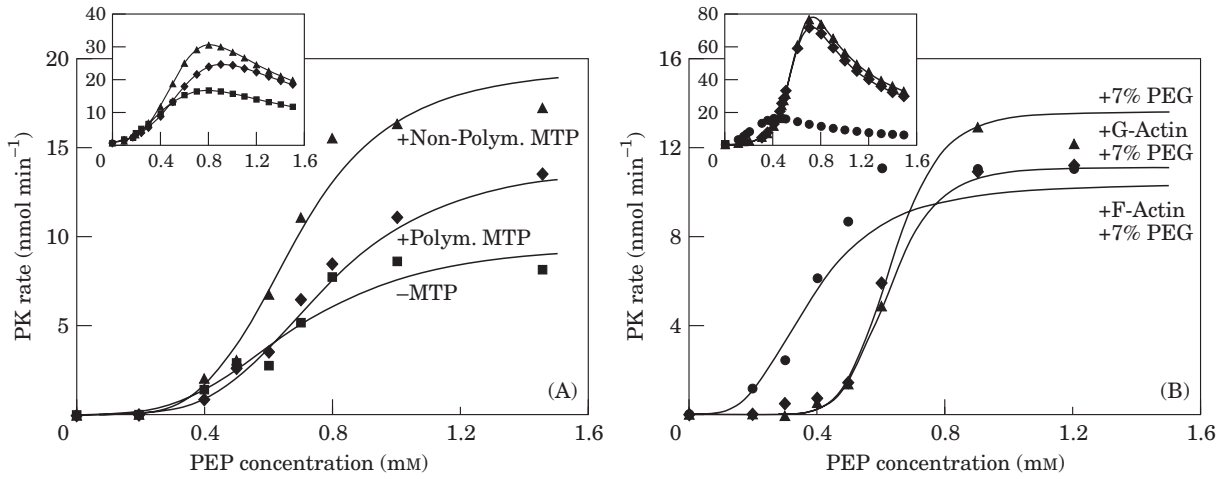

Fig. 3. Modulation of the ultrasensitive response of PK coupled to LDH in the presence of cytoskeletal proteins and PEG-induced crowding. (A) Modulation of PK activity and ultrasensitivity (inset: Sensitivity amplification factor (*As*, *y*-axis) versus PEP concentration (mM, *x*-axis)) as a function of its substrate and MTP. The symbols in the inset correspond to those shown in the main panel. The activity measurements in the presence of polymerized MTP (0.4 mg/ml) were referred to a control assayed in the presence of 2 mM GTP. Both polymerization and enzymatic activity were assayed and monitored as described in Cortassa *et al.* (1994). Lines drawn under the experimental points are the best-fit curves according to a generalized Hill equation:

$$V_{PK} = \frac{V_{max}[PEP]^{n_H}}{(K_{0.5})^{n_H} + [PEP]^{n_H}} \tag{4}$$

and the following kinetic parameters (Cortassa *et al.*, 1994): Aqueous: V_{max}=9.79 nmol min^{-1}; $K_{0.5}$=0.685 mM; n_H=3.31. +Polymerized MTP: V_{max}=14.14 nmol min^{-1}; $K_{0.5}$=0.773 mM; n_H=4.08. +Non-polymerized MTP: V_{max}=19.52 nmol min^{-1}; $K_{0.5}$=0.682 mM; n_H=4.48. (B) Modulation of PK activity and ultrasensitivity (inset: the same as panel A) as a function of its substrate in the presence of PEG or PEG plus actin (G or F). G-actin (0.4 mg/ml) was polymerized as described in Aon *et al.* (1996) in the presence of the enzymes except for NADH/PEP which were added after completion of the polymerization step. Both polymerization and enzymatic activity were assayed and monitored as described in Aon *et al.* (1996). Lines drawn under the experimental points are the best-fit curves according to Eq. 4 with the following kinetic parameters (Aon *et al.*, 1996): +7% PEG (w/w): V_{max}=13.7 nmol min^{-1}; $K_{0.5}$=0.63 mM; n_H=8.3. +G-Actin+7% PEG: V_{max}=11.2 nmol min^{-1}; $K_{0.5}$=0.62 mM; n_H=8.2. +F-Actin+7% PEG: V_{max}=10.5 nmol min^{-1}; $K_{0.5}$=0.38 mM; n_H=3.3. Amplification factors, *As* (insets of panels A, B), were obtained using the following expression (see also Eq. 1):

$$As = \frac{\dfrac{V_{PK}(f) - V_{PK}(1\%)}{V_{PK}(1\%)}}{\dfrac{PEP_{(f)} - PEP_{(1\%)}}{PEP_{(1\%)}}} \tag{5}$$

where V_{PK}(f) is the velocity in NADH consumption at a concentration corresponding to $PEP_{(f)}$ and V_{PK}(1%) is the rate at an amount of $PEP_{(1\%)}$ which corresponds to 1% of the maximal velocity of the enzyme.

systems from yeast. Figure 3A shows the effect of the polymeric status of microtubular protein (MTP: tubulin+MAPs) on the kinetics of pyruvate kinase (PK) coupled to lactate dehydrogenase (LDH) in a concentration range in which MTP induced an increase in flux through the enzymatic couple (Cortassa *et al.*, 1994; Aon *et al.*, 1996; Aon and Cortassa, 1997; Aon *et al.*, 2000*a*). The enhanced flux is dependent upon several factors: (i) the presence, for some enzymatic reactions, of Microtubule Associated Proteins (MAPs) apart from tubulin; (ii) the concentration of microtubular protein; and (iii) the polymeric status. Very recently, we have shown that globality, coherence, and robustness are the main properties bestowed by cytoskeleton organization and dynamics to the spatio-temporal co-ordination of cellular metabolism and energetics (Lloyd *et al.*, 2001; Aon and Cortassa, unpublished observations).

The maximal *As* achieved *in vitro* by the PK's rate increased from 17-fold in the absence of MTP to 25-fold and 31-fold in the presence of

polymerized or non-polymerized MTP, respectively (Fig. 3A, inset). The remarkable aspect of this result is that the ultrasensitive response of PK towards its substrate is modulated by the presence of the cytoskeletal proteins. Furthermore, the extent of the ultrasensitive response depends upon the polymeric status of MTP (Fig. 3A).

Figure 3B depicts the *in vitro* sensitivity of PK rate response towards PEP, in the presence of a crowding agent such as PEG and polymerized (F) or non polymerized (G) forms of actin. In the presence of 7% PEG or PEG plus G-actin, the co-operativity of the enzyme, as could be judged through the Hill co-efficient, n_H, was high ($n_H \simeq 8$; see Fig. 3 legend). Under those conditions, amplification factors, *As*, of 72- to 77-fold were achieved at 0.7 mM PEP (Fig. 3B, inset). A decrease to a value of $n_H \simeq 3$ was observed in the presence of F-actin polymerized under conditions favoring bundling of filaments in 7% PEG (see Fig. 3B legend). A similar decrease in n_H was also observed in the presence of random filaments of F-actin (not shown). These kinetic effects of F-actin on PK activity (i.e. decrease in co-operativity and Vmax) are in agreement with the overall decrease in flux induced in the enzymatic couple PK/LDH (Aon *et al.*, 1996, 2000*a*).

Taken together, the results presented show that cytoskeletal proteins may exert key effects on the ultrasensitive response given by an enzymatic system through its nature (i.e. the sort of protein) and polymeric status, under crowded or non-crowded conditions. Moreover, the *in vitro* data presented further show that cytoskeletal proteins and/or crowding may not only trigger but also up- or downmodulate the ultrasensitive behavior (Figs 1–3).

ULTRASENSITIVITY IN CELLULAR SYSTEMS

Several cellular enzymatic systems have now been shown to behave in an ultrasensitive manner, in agreement with the ultrasensitivity displayed by enzymes *in vitro* under conditions resembling the (supra) molecular organized and crowding conditions of the intracellular milieu.

The AMP-activated protein kinase cascade is stimulated by elevation of AMP and ATP shortage during regulation of the cellular energy charge. In INS-1 cells, this cascade responds ultrasensitively over a critical range of nucleotide concentrations. The activity of the kinase progressed from 10% to 90% of its maximal velocity with only a 6-fold increase in activating nucleotide (Hardie *et al.*, 1999).

The turning on and off of the cell cycle in intact oocytes was shown to be exerted ultrasensitively by the mitogen-activated protein kinase (MAPK). The oocyte cell cycle response can be described by Hill coefficients as high as $n_H = 5.0$, with individual oocytes showing an even greater ultrasensitivity than the oocyte population (Ferrell and Machleder, 1998; Koshland, 1998). The flagellar motors in *Escherichia coli* exhibit steep, ultrasensitive, input–output relations as a function of the intracellular concentration of the chemotactic signalling protein fused to the green fluorescent protein (Cluzel *et al.*, 2000). A Hill plot of the data led to an apparent slope of $\sim 10.3 \pm 1.1$, with a dissociation rate constant of 3.1 μM s^{-1}. The authors explain the lower values of n_H ranging from 3.5 to 5.5 obtained in previous experiments as due to heterogeneity in protein concentrations existing in cell populations (Cluzel *et al.*, 2000). Similar observations made in *Xenopus* oocytes led to a similar explanation (Ferrell and Machleder, 1998).

Pheromone signal detection by vomeronasal neurons show a remarkably low (near 10^{-11} M) threshold placing these neurons among the most sensitive chemodetectors in mammals (Leinders-Zufall *et al.*, 2000). Six putative pheromones evoked excitatory responses in single vomeronasal neurons, leading to action potential generation and elevated calcium entry. Steep stimulus-response curves towards the ligands with pheromonal activity, farnesene, 2-heptanone or 2,5-dimethylpyrazine with n_H of 5, 7 or 5, respectively, were reported (Leinders-Zufall *et al.*, 2000).

AMPLIFICATION PROPERTIES DERIVED FROM ULTRASENSITIVITY IN SIGNALLING AND METABOLIC PATHWAYS

It has been emphasized that ultrasensitive responses in biological systems could be involved in the turning on or off of metabolic pathways, signal amplification in kinase cascades (Koshland *et al.*, 1982; Koshland, 1987) and protein translocation (Ferrell, 1998).

A mathematical model of the mitogen-activated protein kinase (MAPK) cascade behaves ultrasensitively even though none of the enzymes in the cascade are regulated co-operatively (Huang and Ferrel, 1996). In agreement with model predictions, the MAPK activation was shown to be ultrasensitive in extracts (Huang and Ferrel, 1996) or intact

Xenopus oocytes (Ferrell and Machleder, 1998). The stimulus/response behavior of the MAPK was shown to be as steep as that of a co-operative enzyme with a n_H=4–5 that enables, in principle, the cascade to convert graded inputs into switch-like outputs. This behavior can be accounted for by the intrinsic ultrasensitivity of the oocyte's MAPK cascade and a positive feedback loop in which the cascade is embedded (Ferrell and Machleder, 1998). It has been shown that, theoretically, a negative feedback loop combined with the intrinsic ultrasensitivity of the MAPK cascade can bring about sustained oscillations in MAPK phosphorylation (Kholodenko, 2000).

One potential mechanism for ultrasensitivity is the multistep effect, a process in which a single effector acts at various steps in a pathway (Koshland *et al.*, 1982; Goldbeter and Koshland, 1982; Koshland, 1987). We have explored whether a simple metabolic pathway like glycogen synthesis in cyanobacteria might operate as a switch-like device during dark–light transitions. Based on the *in vitro* kinetic and regulatory properties of cyanobacterial ADPGlcPPase under PEG-induced crowded or non-crowded environments (Fig. 1), or *in situ* in permeabilized cyanobacterial cells (Gomez-Casati *et al.*, 2001) a mathematical model was formulated (see Appendix) (data submitted for publication). The mathematical model takes into account the ultrasensitive ADPGlc production by ADPGlcPPase as well as glycogen synthesis and degradation. Simulations performed with this model at the steady state allowed us to reproduce the ultrasensitive behavior of ADPGlcPPase and the *As* under all experimental situations analyzed. Indeed, the results obtained either *in vitro* or *in situ* were simulated, qualitatively or quantitatively, at three main levels: (i) the rate of ADPGlcPPase, and (ii) the amplification factor, *As*, as a function of the ratio 3PGA/Pi; (iii) the 3PGA/Pi ratio at which the ultrasensitive behavior of the enzyme is triggered (data submitted for publication).

Model results show that in addition to ADPGlc production, glycogen synthesis is also ultrasensitive, a fact that has been suggested (Gomez-Casati *et al.*, 1999) but not demonstrated (Fig. 4). The ultrasensitive response of ADPGlcPPase propagates from ADPGlc into glycogen levels as a function of 3PGA at varying concentrations of Pi (Fig. 4). Seemingly, sharper ultrasensitive responses were obtained in crowded than in non-crowded media with *As* at least 4-fold higher (data submitted for publication).

Although our results have been obtained in cyanobacteria (which accumulate glycogen), several lines of evidence suggest that they may be extended to starch synthesis by cells performing oxygenic photosynthesis (Iglesias *et al.*, 2000; Gomez-Casati *et al.*, 1999, 2000*a*,*b*). Experimental and theoretical results show that an amplification of up to 20-fold of storage polysaccharide levels can be obtained in a narrow 3PGA/Pi ratio (data submitted for publication). Filtering out small stimuli as well as quick deactivation of polysaccharide synthesis during the light→dark transition might be another distinct advantage of this mechanism for plant cells.

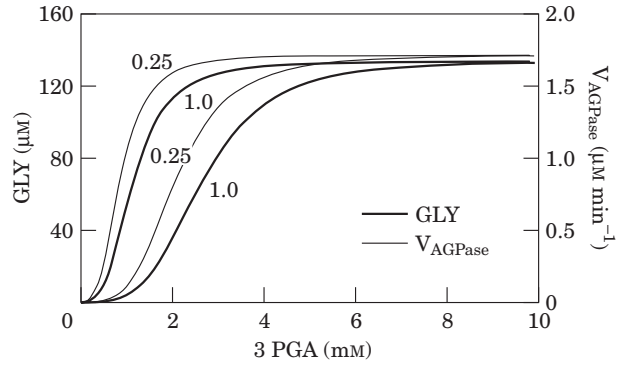

Fig. 4. Ultrasensitive glycogen synthesis in Cyanobacteria. The pathway of glycogen synthesis was modelled and simulated as described in the Appendix. Steady state values attained by ADPGlcPPase activity (V_{AGPase}) and glycogen (GLY) as a function of 3PGA at two different Pi concentrations (0.25 and 1 mM) are depicted.

CONCLUDING REMARKS

The relevance of the results reviewed must be seen in the framework of the crowded though organized nature of the cytoplasm of cells. Compelling experimental and theoretical evidence shows the highly structured and crowded nature of cellular cytoplasm (Luby-Phelps, 2000; Minton, 1997; Aon and Cortassa, 1997; Aon *et al.*, 2000*a*,*b*). Moreover, these conditions as well as their alteration are not without consequence for cell motility (Waterman-Storer and Salmon, 1999; Stock, 1999; Cluzel *et al.*, 2000), division (Ferrell and Machleder, 1998), and metastatic disease (Alonso *et al.*, 1999), signalling cascades (Huang and Ferrell, 1996; Ferrell and Machleder, 1998; Hardie *et al.*, 1999; Burack and Shaw, 2000), metabolic fluxes (Aon and Cortassa, 1997; Rohwer *et al.*, 1998; Ovádi and Srere, 2000; Aon *et al.*, 2000*a*), enzyme activity (Aon and Cortassa, 1997; Aon *et al.*, 2000*a*,*b*), protein folding and aggregation (van den Berg *et al.*, 1999; Minton, 2000), light-induced accumulation of

storage polysaccharides (Iglesias *et al.*, 2000; Gomez-Casati *et al.*, 2000*a*,*b*) and stress sensing stimuli (Aon *et al.*, 2000*a*; Lloyd *et al.*, 2001).

The evidence reviewed suggests that ultrasensitivity may occur and be modulated under physicochemical conditions prevalent in the cellular cytoplasm and in the presence of the ubiquitous cytoskeletal proteins. Moreover, it shows that this particular type of signal amplification is widespread among prokaryotic and eukaryotic cells as well as in different cellular processes. A wide scenario is open for unravelling the occurrence and dynamics of this phenomenon in the mass–energy–information-carrying networks of cells (Lloyd *et al.*, 2001).

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REFERENCES

Alonso DF, Farina HG, Arregui C, Aon MA, Gomez DE, 1999. Modulation of urokinase-type plasminogen activator and metalloproteinase activities in cultured mouse mammary-carcinoma cells: Enhancement by paclitaxel and inhibition by nocodazole. *Int J Cancer* **83:** 242–246.

Aon MA, Cáceres A, Cortassa S, 1996. Heterogeneous distribution and organization of cytoskeletal proteins drive differential modulation of metabolic fluxes. *J Cell Biochem* **60:** 271–278.

Aon MA, Cortassa S, 1997. *Dynamic Biological Organization. Fundamentals as applied to cellular systems*. London, Chapman & Hall.

Aon MA, Cortassa S, Gomez-Casati DF, Iglesias AA, 2000*a*. Effects of stress on cellular infrastructure and metabolic organisation in plant cells. *Int Rev Cytol* **194:** 239–273.

Aon MA, Cortassa S, Lloyd D, 2000*b*. Chaotic dynamics and fractal space in biochemistry: Simplicity underlies complexity. *Cell Biol Int* **24:** 581–587.

Bereiter-Hahn J, Stubig C, Heymann V, 1995. Cell cycle-related changes in F-actin distribution are correlated with glycolytic activity. *Exp Cell Res* **218:** 551–560.

Bereiter-Hahn J, Airas J, Blum S, 1997. Supramolecular associations with the cytomatrix and their relevance in metabolic control: Protein synthesis and glycolysis. *Zool-Anal Complex Syst* **100:** 1–24.

Bray D, 1995. Protein molecules as computational elements in living cells. *Nature* **376:** 307–312.

Burack WR, Shaw AS, 2000. Signal transduction: hanging on a scaffold. *Curr Op Cell Biol* **12:** 211–216.

Cluzel P, Surette M, Leibler S, 2000. An ultrasensitive bacterial motor revealed by monitoring signaling proteins in single cells. *Science* **287:** 1652–1655.

Cook PR, 1989. The nucleoskeleton and the topology of transcription. *Eur J Biochem* **185:** 487–501.

Cook PR, 1991. The nucleoskeleton and the topology of replication. *Cell* **66:** 627–635.

Cortassa S, Cáceres A, Aon MA, 1994. Microtubular protein in its polymerized or non-polymerized states differentially modulates *in vitro* and intracellular fluxes catalyzed by enzymes related to carbon metabolism. *J Cell Biochem* **55:** 120–132.

Doedle E, 1986. *AUTO Manual*. Pasadena, California Institute of Technology.

Ferrell JE Jr, Machleder EM, 1998. The biochemical basis of an all-or-none cell fate switch in *Xenopus* oocytes. *Science* **280:** 895–898.

Ferrell JE Jr, 1998. How regulated protein translocation can produce switch-like responses. *TIBS* **23:** 461–465.

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

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

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

Gomez-Casati DF, Aon MA, Cortassa S, Iglesias AA, 2000*b*. Intracellular flux measurements of ADP-glucose pyrophosphorylase and glycogen in cyanobacteria. In: *Plant Biology 2000, Annual Meeting of the American Society of Plant Physiologists*. San Diego, California, American Society of Plant Physiologists, p. 128, 607 (Abstr.).

Gomez-Casati DF, Aon MA, Cortassa S, Iglesias AA, 2001. Measurement of the glycogen synthetic pathway in permeabilized cells of cyanobacteria. *FEMS Microbiol Lett* **194:** 7–11.

Grabski S, Xie XG, Holland JF, Schindler M, 1994. Lipids trigger changes in the elasticity of the cytoskeleton in plant cells: A cell optical displacement assay for live cell measurements. *J Cell Biol* **126:** 713–716.

Gundersen GG, Cook TA, 1999. Microtubules and signal transduction. *Curr Op Cell Biol* **11:** 81–94.

Hardie DG, Salt IP, Hawley SA, Davies SP, 1999. AMP-activated protein kinase: an ultrasensitive system for monitoring cellular energy charge. *Biochem J* **338:** 717–722.

Haussinger D, Stoll B, vom Dahl S, Theodoropoulos PA, Markogiannakis E, Gravanis A, Lang F, Stournaras Ch, 1994. Effect of hepatocyte swelling on microtubule stability and tubulin mRNA levels. *Biochem Cell Biol* **72:** 12–19.

Higgins J, 1965. Dynamics and control in cellular reactions. In: Chance B, Estabrook RK, Williamson JR, eds. *Control of Energy Metabolism*. New York, Academic Press. 13–46.

Huang Ch-YF, Ferrell JE Jr, 1996. Ultrasensitivity in the mitogen-activated protein kinase cascade. *Proc Natl Acad Sci USA* **93:** 10078–10083.

Iglesias AA, Gomez-Casati DF, Aon MA, 2000. Regulation of starch biosynthesis: An intracellular view. In: Paulson R, ed. *Science in an uncertain millennium. A74 (Abstr.)*. American Association for the Advancement of Science.

Kacser H, Burns JA, 1973. The control of flux. *Symp Soc Exp Biol* **27:** 65–104.

KAISER WM, BASSHAM JA, 1979. Light-dark regulation of starch metabolism in chloroplasts. II. Effect of chloroplastic metabolite levels on the formation of ADP-Glucose by chloroplast extracts. *Plant Physiol* **63:** 109–113.

KHOLODENKO BN, 2000. Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascade. *Eur J Biochem* **267:** 1583–1588.

KOSHLAND DE JR, GOLDBETER A, STOCK JB, 1982. Amplification and adaptation in regulatory and sensory systems. *Science* **217:** 220–225.

KOSHLAND DR JR, 1987. Switches, thresholds and ultrasensitivity. *TIBS* **12:** 225–229.

KOSHLAND DR JR, 1998. The era of pathway quantification. *Science* **280:** 852–853.

LAPORTE DC, WALSH K, KOSHLAND DE, 1984. The branch point effect. Ultrasensitivity and subsensitivity to metabolic control. *J Biol Chem* **259:** 14068–14075.

LEINDERS-ZUFALL T, LANE AP, PUCHE AC, MA W, NOVOTNY MV, SHIPLEY MT, ZUFALL F, 2000. Ultrasensitive pheromone detection by mammalian vomeronasal neurons. *Nature* **405:** 792–796.

LILIOM K, WÁGNER G, KOVÁCS J, COMIN B, CASCANTE M, OROSZ F, OVÁDI J, 1999. Combined enhancement of microtubule assembly and glucose metabolism in neuronal systems *in vitro*: Decreased sensitivity to copper toxicity. *Biochim Biophys Res Commun* **264:** 605–610.

LLOYD D, AON MA, CORTASSA S, 2001. Why homeodynamics, not homeostasis? *The Scientific World* **1:** 133–145. (http://216.25.242.189/tsw/iPublish/pubs_list.html).

LUBY-PHELPS K, 2000. Cytoarchitecture and physical properties of cytoplasm: Volume, viscosity, diffusion, intracellular surface area. *Int Rev Cytol* **192:** 189–221.

MARMILLOT P, KEITH T, SRIVASTAVA DK, KNULL HR, 1994. Effect of tubulin on the activity of the muscle isoenzyme of lactate dehyrogenase. *Arch Biochem Biophys* **315:** 467–472.

MINTON AP, 1997. Influence of excluded volume upon macromolecular structure and associations in 'crowded' media. *Curr Op Biotechnol* **8:** 65–69.

MINTON AP, 2000. Implications of macromolecular crowding for protein assembly. *Curr Op Struct Biol* **10:** 34–39.

MINTON AP, 2001. The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media. *J Biol Chem* **276:** 10577–10580.

OVÁDI J, SRERE PA, 2000. Macromolecular compartmentation and channeling. *Int Rev Cytol* **192:** 255–280.

PREISS J, 1982. Regulation of the biosynthesis and degradation of starch. *Ann Rev Plant Physiol* **33:** 431–454.

ROHWER JM, POSTMA PW, KHOLODENKO BN, WESTERHOFF HV, 1998. Implications of macromolecular crowding for signal transduction and metabolite channeling. *Proc Natl Acad Sci USA* **95:** 10547–10552.

STOCK J, 1999. Sensitivity, cooperativity and gain in chemotaxis signal transduction. *Trends Microbiol* **7:** 1–4.

VAN DEN BERG B, ELLIS RJ, DOBSON CHM, 1999. Effects of macromolecular crowding on protein folding and aggregation. *EMBO J* **18:** 6927–6933.

WATERMAN-STORER CM, SALMON ED, 1999. Positive feedback interactions between microtubule and actin dynamics during cell motility. *Curr Op Cell Biol* **11:** 61–67.

ZIMMERMAN SB, 1993. Macromolecular crowding effects on macromolecular interactions: Some implications for genome structure and function. *Biochim Biophys Acta* **1216:** 175–185.

ZIMMERMAN SB, MINTON AP, 1993. Macromolecular crowding: Biochemical, and physiological consequences. *Annu Rev Biophys Biomol Struct* **22:** 27–65.

APPENDIX

Mathematical modelling of ADPGlcPPase ultrasensitivity and dynamics of storage polysaccharides in cyanobacteria

Carbon metabolism from G1P towards storage polysaccharides (cyanobacterial glycogen) synthesis and subsequent degradation, was modelled according to the following reaction scheme:

$$\mathrm{ATP} + \mathrm{G1P} \xrightarrow{\mathrm{ADPGlcPPase}} \mathrm{ADPGlcPPi}$$

$$\mathrm{ADPGlc} + \mathrm{GLY}_n \xrightarrow{\mathrm{GLYsy}} \mathrm{GLY}_{n+1} + \mathrm{ADP} \quad (3)$$

$$\mathrm{GLY} \xrightarrow{\mathrm{GLYdeg}}$$

A system of two ordinary differential equations (ODEs) representing the dynamics of ADPGlc and glycogen (GLY), describe reaction scheme 3 (data submitted for publication). The rate expression V_{AGPase} for ADPGlcPPase presented elsewhere (data submitted for publication) accounts for the ultrasensitive behavior exhibited by the enzyme (Gomez-Casati *et al.*, 1999, 2000*a*, 2001). The rate expression for glycogen synthesis (V_{GLYsy}) with respect to ADPGlc was shown to follow Michaelis-Menten kinetics in permeabilized cells of cyanobacteria (Gomez-Casati *et al.*, 2001). Glycogen degradation (V_{GLYdeg}) was assumed to follow Michaelis-Menten kinetics with respect to GLY. ATP and G1P concentration values utilized in the simulations were taken from *in vitro* (Gomez-Casati *et al.*, 1999, 2000*a*) or *in situ* experiments (Gomez-Casati *et al.*, 2001) or from measured intracellular or chloroplast concentrations (Preiss, 1982; Kaiser and Bassham, 1979).

The temporal evolution of the model was obtained by numerical integration with the ScoP package (Duke University) using the ADAMS' predictor–corrector method. The behavior of the model in terms of type and stability of steady states as a function of several model parameters was performed with AUTO (Doedle, 1986) running in a Linux environment on a desktop computer.