THE BIOSYNTHESIS OF SUCROSE*

By C. E. CARDINI, LUIS F. LELOIR, AND J. CHIRIBOGA

(From the Instituto de Investigaciones Bioquímicas, Fundación Campomar,† Buenos Aires, Argentina)

(Received for publication, October 18, 1954)

Hassid, Doudoroff, and Putman (1–3) discovered an enzyme in certain bacteria (*Pseudomonas saccharophila*) which catalyzes the following reaction:

Glucose-1-phosphate + fructose ⇄ sucrose + inorganic phosphate

The enzyme, which was named sucrose phosphorylase, has not been found in plant tissues (4), and thus the mechanism of sucrose synthesis remains obscure. Evidence obtained from tracer experiments led Buchanan *et al.* (5, 6) to assume that in plants sucrose phosphate is formed from UDPG[1] and fructose-1-phosphate. This hypothesis stimulated work which led to the discovery of an enzyme which catalyzes sucrose synthesis according to the following reaction:

$$\text{UDPG} + \text{fructose} \rightleftarrows \text{sucrose} + \text{UDP} \qquad (1)$$

A brief note (7) reported the preparation of this enzyme from wheat germ and its presence in some other plant materials. Furthermore, the validity of Reaction 1 was proved by the equivalence between the disappearance of UDPG and the formation of UDP and sucrose. A more detailed study is reported in this paper.

Methods

Analytical—Sucrose was estimated by the resorcinol method of Roe (8). The volumes taken were reduced to one-fourth, and the color was measured at 490 mμ. In order to avoid the interference of fructose, the samples were heated 10 minutes at 100° after adding sufficient sodium hydroxide to make the concentration 0.2 N. While 2 μmoles of fructose were found to give no color with resorcinol after this treatment, sucrose remained unaffected even when the concentration of alkali was 0.5 N. High concentrations of monosaccharides yield some color, even after alkaline treatment, but this can be corrected by the use of suitable blanks.

* This investigation was supported in part by a research grant (No. G-3442) from the National Institutes of Health, United States Public Health Service, and by the Rockefeller Foundation.

† J. Alvarez 1719.

[1] The following abbreviations are used: UDP for uridine diphosphate, UDPG for uridine diphosphate glucose, and Tris for tris(hydroxymethyl)aminomethane.

The method of Kunitz and McDonald (9) was followed for protein estimation.

Substrates—UDPG and UDP were prepared as described previously (10). The sugars were commercial samples.

Estimation of Enzyme—The following components were mixed: 0.5 μmole of UDPG, 2 μmoles of fructose, 0.01 ml. of 2 M Tris buffer of pH 7.2, and variable amounts of enzyme; total volume, 0.15 ml. After 30 minutes at 37°, water was added to 0.5 ml., followed by 0.02 ml. of 5 N sodium hydroxide. After careful mixing, the tubes were heated 10 minutes at 100° and sucrose was estimated. An equal sample, in which UDPG was added after the incubation, and sucrose standards (0.1 to 0.2 μmole) were run at the same time.

TABLE I

Purification of Enzyme

Fraction	Volume	Units*	Purity†
	ml.		
I. Crude extract	70		~0.05
II. 1st ammonium sulfate	35	672	0.24
III. Manganous chloride	40	132	0.66
IV. 2nd ammonium sulfate	20	108	1.16
V. Alumina, 1st supernatant	20	104	1.80
VI. " 2nd "	20	72	2.40

* 1 unit is defined as the amount of enzyme catalyzing the formation of 1 μmole of sucrose in 30 minutes under the conditions described in the text.

† Expressed in units per mg. of protein.

Plant Material—Beet, sweet sorghum, or pea seeds were allowed to germinate 4 to 5 days at 30° under light on wetted cotton. The shoots were then ground, and the solids were removed by filtration through muslin. The liquid was then treated with ammonium sulfate, and the fraction obtained between 0.25 and 0.60 saturation was redissolved and dialyzed overnight in the refrigerator against distilled water. The protein content of the extracts was found to decrease with the age of the plantules.

Sugar cane shoots (2 to 3 cm. long) and roots were obtained from stem cuttings which had been kept in the laboratory on wetted cotton at 30°. The extracts were prepared as described above.

Purification of Wheat Germ Enzyme—30 gm. of commercial wheat germ and 100 ml. of 0.05 M phosphate buffer of pH 7.2 were mixed in a blender. The suspension was centrifuged 15 minutes at 16,000 r.p.m., and the supernatant fluid was dialyzed with constant stirring at 5° during 4 to 5 hours. The liquid was centrifuged again as before (Fraction I, crude extract (Table I)).

The supernatant solution was made 0.5 saturated with solid ammonium sulfate, and the precipitate obtained by centrifugation was dissolved in half the volume of Fraction I of water and dialyzed overnight at 5° against distilled water (Fraction II). 0.1 volume of 1 M manganous chloride was added, and the suspension was stirred during 30 minutes at 0°. After centrifugation the supernatant fluid (Fraction III) was made 0.3 saturated with ammonium sulfate, and the precipitate was discarded. Ammonium sulfate to 0.5 saturation was added, and the precipitate was dissolved in

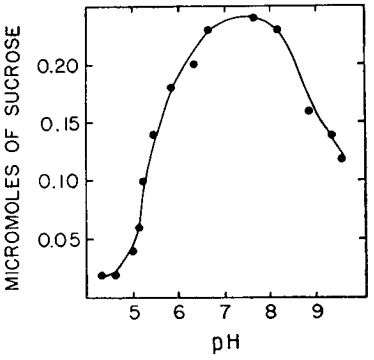

FIG. 1

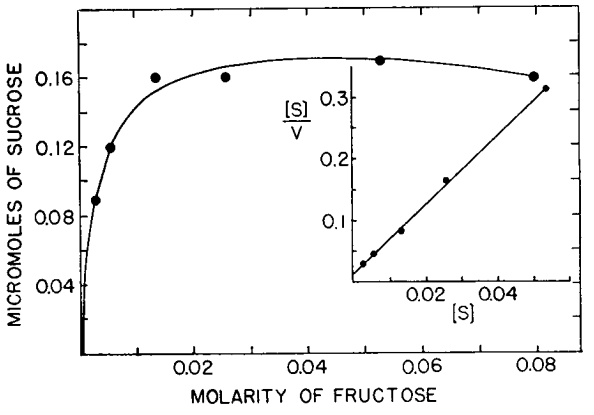

FIG. 2

FIG. 1. pH optimum curve. System as described in the text with Tris or acetate buffer at 0.15 M final concentration. Incubated 15 minutes at 37°. The pH was determined on aliquots with a glass electrode.

FIG. 2. Influence of fructose concentration. System as described in the text. The amount of purified enzyme corresponded to 0.18 mg. of protein. Fructose concentration as indicated. Section at right, a Lineweaver-Burk (22) plot. K_m = 2.3×10^{-3}.

half the volume of Fraction III of water and dialyzed during 1 to 2 hours in the cold with constant stirring (Fraction IV).

Alumina (Cγ) (usually 0.1 volume of a suspension containing 50 mg. of dry weight per ml.) was added to the liquid, and the precipitate was discarded. To the supernatant fluid more alumina was added (0.2 volume), and the precipitate was again discarded. The supernatant solution (Fraction VI) was the best preparation obtained (Table I).

Results

Properties of Enzyme—In crude extracts nearly all the activity could be recovered in the precipitate from adding acetic acid to pH 5. However, no appreciable purification was obtained by this procedure.

Heating 10 minutes to 60° led to nearly complete destruction of the

wheat germ enzyme. Considerable destruction was found to take place on precipitation with acetone, even at low temperature. The enzyme could be stored for months in the frozen state without much loss of activity.

Inhibitors—The following substances did not affect the activity: arsenate, arsenite, fluoride, iodoacetate, citrate, or pyrophosphate at 0.01 M concentration; calcium, barium, or magnesium ions at 0.05 M concentration; phlorizin, 8-hydroxyquinoline, or ethylenediaminetetraacetate at 0.2 saturation.

pH Optimum—As shown in Fig. 1, the highest activity was obtained at pH 7.2 with 0.15 M Tris buffer.

Substrate Concentration—The result of an experiment with different concentrations of fructose is shown in Fig. 2. If the amount of sucrose formed is taken as a measure of rate of reaction, calculation of the Michaelis constant was 2.3×10^{-3}. A 2-fold increase of UDPG concentration did not affect the rate of reaction.

Specificity—No substance reacting like sucrose was found to be formed when UDPG was replaced by glucose-1-phosphate, fructose-1- or 6-phosphate, UDP-acetylglucosamine (11), or guanosine diphosphate mannose (12), or when fructose was replaced by sorbose or by fructose-6-phosphate. However, the latter was true for only a few of the preparations obtained. This point will be dealt with in the following paper.

In other tests, the disappearance of UDPG was measured by a method based on its activity as cogalactowaldenase (10). When fructose was added to the wheat germ enzyme and UDPG, the disappearance of the latter was increased. No increased disappearance was observed if fructose was replaced by D-glucose, D-galactose, D-mannose, D- or L-arabinose, D-ribose, or inositol.

Reaction Product—The isolation of crystalline sucrose was not attempted, since it would have required considerable amounts of UDPG. However, the tests which have been carried out make it reasonably certain that the product is sucrose.

Paper chromatography of the reaction products revealed the presence of a substance which gave the R_F value of sucrose (Table II). This substance was absent in controls in which the fructose or the UDPG was added at the end of the incubation period. The substance behaved like sucrose when the papers were developed with the alkaline silver (13), resorcinol (14), or benzidine-trichloroacetic acid reagents (15).

In other experiments, the reaction product was isolated by paper chromatography. The substance was found to have no reducing power and to behave like sucrose during paper chromatography. After mild acid hydrolysis (5 minutes, pH 2, 100°) or after treatment with yeast invertase, glucose and fructose could be detected chromatographically.

With the solvents used for paper chromatography (ethyl acetate-pyri-

dine-water (10:5:6) (16) and butanol-acetic acid-water (17)) and with the reagents used for revealing the spots, sucrose can be easily distinguished from maltose, trehalose, lactose, and raffinose.

TABLE II

Paper Chromatography of Reaction Product

	R_{Xylose}			
	Pyridine-ethyl acetate		Butanol-acetic acid	
Complete system	0.82	0.94	0.32	0.80
No UDPG*		0.93		0.82
" fructose*		0.93		0.83
Sucrose		0.82		0.32
Glucose		0.87		0.58
Fructose		0.93		0.82
Maltose		0.72		0.20
Trehalose		0.75		

* Added at the end of the incubation period.

TABLE III

Acid Hydrolysis of Reaction Product

The samples (0.18 μmole) were heated at 100° in 1 ml. of 0.1 M glycine-hydrogen chloride buffer of pH 2.25. Samples of sucrose were run at the same time under identical conditions. The reducing power was measured with ferricyanide (23). Results in per cent hydrolysis.

	Time		
	2 min.	4 min.	6 min.
Sucrose	36	70	82
Reaction product	32	73	85

The rate of acid hydrolysis of a known sample of sucrose was compared under identical conditions with a sample of the reaction product; both substances hydrolyzed at the same rate (Table III).

Reversibility—In order to detect the reversibility, the back-reaction was investigated by starting with 5 μmoles of sucrose, 2 μmoles of UDP, and enzyme. The mixture was deproteinized with trichloroacetic acid, and, after extracting the latter with ether, the samples were chromatographed on paper with an ethanol-ammonium acetate solvent of pH 7.5 (18) containing 0.01 M ethylenediaminetetraacetate. A spot having the same mobility as UDPG was visible under ultraviolet light. After extraction of the substance from the paper, UDPG was estimated by its cogalactowaldenase activity and absorption at 260 mμ. The amount obtained was

about 0.05 μmole. Control samples in which one of the reactants was omitted during the incubation gave no UDPG spot or cogalactowaldenase activity.

Many attempts to obtain a precise figure for the equilibrium constant were carried out by starting with known mixtures of reactants and products. The reducing power or the sucrose content of the samples was measured before and after enzyme action. A small correction had to be applied, owing to the liberation of reducing power from sucrose, which occurred even with the most purified enzyme preparations. Many experiments were carried out in this manner, but the results were not consistent and the value for K = (sucrose × UDP)/(UDPG × fructose) varied from 2 to 8 at 37° and pH 7.4 in different experiments.

Distribution—The detection of the enzyme in some plant tissues is difficult, owing to the presence of sucrase. However, it has been possible to obtain extracts from many sources which catalyze the formation of sucrose, and in general seeds were the best materials for the preparation of the enzyme. Quantitative measurements were carried out with some extracts, and the results were as follows (in micromoles of sucrose formed in 30 minutes per mg. of protein): beet shoots 0.6 to 1.0, sweet sorghum shoots 0.7 to 4.0, sugar cane shoots 0.25, sugar cane roots 2.4 to 3.0, pea shoots 0.35. Qualitative tests for the enzyme were positive with the following materials: pea, pine, and fenugreek seeds, corn germ, potato sprouts, and barley shoots. Negative or non-reproducible results which may be attributed to interfering enzymes were obtained with sugar beet and cane sugar leaves and with beet roots.

DISCUSSION

The equilibrium constant of the sucrose phosphorylase reaction has been found to be 0.053 at pH 6.6 and 30° (4). This displacement in favor of the monosaccharides makes the enzyme appropriate for the utilization of sucrose, and this is probably its main function in *P. saccharophila*. In contrast, the equilibrium of the reaction starting with UDPG and fructose is in favor of sucrose synthesis. Accurate values have not been obtained, but the $\Delta F°$ at 37° can be estimated to be about -1000 as compared to $+1770$ for sucrose phosphorylase.

The enzyme has been found in many plant materials, and hence we may conclude that it catalyzes a reaction which is fairly general in the plant kingdom. Thus, the enzyme is probably involved in the formation of sucrose by pea extracts detected by Turner (19). However, the synthesis of sucrose from fructose and UDPG is not the only pathway in plants since, as reported in the following paper, another enzyme catalyzes the synthesis of sucrose phosphate from fructose-6-phosphate and UDPG, and the reaction product can be transformed into sucrose by phosphatase action.

According to modern nomenclature (20), the enzyme might be named UDPG-fructose transglycosylase. Following the suggestion of von Euler (21) that enzymes might receive the name of the substrates which they synthesize, with the ending changed to *ese*, another possible name is saccharese. This shorter alternative is used currently in this laboratory.

SUMMARY

A wheat germ enzyme which catalyzes the reaction

$$\text{UDPG} + \text{fructose} \rightleftarrows \text{sucrose} + \text{UDP}$$

has been studied.

Methods for its estimation and purification are described, as well as the conditions for its maximal activity. The equilibrium of the reaction was found to be displaced in favor of sucrose synthesis, the value of K being about 5 at 37° and pH 7.4. The enzyme was detected in several plant tissues.

BIBLIOGRAPHY

1. Hassid, W. Z., and Doudoroff, M., *Advances in Enzymol.*, **10**, 123 (1950).
2. Hassid, W. Z., and Doudoroff, M., *Advances in Carbohydrate Chem.*, **5**, 29 (1950).
3. Hassid, W. Z., and Putman, E. W., *Ann. Rev. Plant Physiol.*, **1**, 109 (1950).
4. Hassid, W. Z., in McElroy, W. D., and Glass, B., Phosphorus metabolism, Baltimore, **1**, 11 (1951).
5. Buchanan, J. G., Bassham, J. A., Benson, A. A., Bradley, D. F., Calvin, M., Daus, L. L., Goodman, M., Hayes, P. M., Lynch, V. H., Norris, L. T., and Wilson, A. T., in McElroy, W. D., and Glass, B., Phosphorus metabolism, Baltimore, **2**, 440 (1952).
6. Buchanan, J. G., *Arch. Biochem. and Biophys.*, **44**, 140 (1953).
7. Leloir, L. F., and Cardini, C. E., *J. Am. Chem. Soc.*, **75**, 6084 (1953).
8. Roe, J. H., *J. Biol. Chem.*, **107**, 15 (1934).
9. Kunitz, M., and McDonald, M. R., *J. Gen. Physiol.*, **29**, 393 (1946).
10. Caputto, R., Leloir, L. F., Cardini, C. E., and Paladini, A. C., *J. Biol. Chem.*, **184**, 333 (1950).
11. Cabib, E., Leloir, L. F., and Cardini, C. E., *J. Biol. Chem.*, **203**, 1055 (1953).
12. Cabib, E., and Leloir, L. F., *J. Biol. Chem.*, **206**, 779 (1954).
13. Trevelyan, W. E., Procter, D. P., and Harrison, J. S., *Nature*, **166**, 444 (1950).
14. Bryson, J. L., and Mitchell, T. J., *Nature*, **167**, 864 (1951).
15. Bacon, J. S. D., and Edelman, J., *Biochem. J.*, **48**, 114 (1951).
16. Jermyn, M. A., and Isherwood, F. A., *Biochem. J.*, **44**, 402 (1949). Leloir, L. F., *Arch. Biochem. and Biophys.*, **33**, 186 (1951).
17. Partridge, S. M., *Biochem. J.*, **42**, 238 (1948).
18. Paladini, A. C., and Leloir, L. F., *Biochem. J.*, **51**, 426 (1952).
19. Turner, J. F., *Nature*, **172**, 1149 (1953).
20. Hoffmann-Ostenhof, O., *Advances in Enzymol.*, **14**, 219 (1953).
21. von Euler, H., *Z. physiol. Chem.*, **74**, 13 (1911).
22. Lineweaver, H., and Burk, D., *J. Am. Chem. Soc.*, **56**, 658 (1934).
23. Schales, O., and Schales, S. S., *Arch. Biochem.*, **8**, 285 (1945).