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THE ENZYMIC SYNTHESIS OF TREHALOSE PHOSPHATE[1]

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Uridine diphosphate glucose (UDPG)[2] has been found to disappear when incubated with a yeast extract and glucose monophosphate. This disappearance may be measured by estimating UDPG by its coenzymatic activity[3] and also as a decrease in acid-labile glucose. During the reaction UDP is formed and the reducing power of the mixture decreases. As shown in Table I, these changes are equivalent and do not take place when any one of the reactants is added at the end of the incubation period.

TABLE I

Analytical Changes Produced by the Enzyme

Incubation of 0.4 μmole of glucose-6-phosphate, 0.6 μmole of UDPG and 0.02 ml. of enzyme in 0.14 *M*/tris (hydrosymethyl)-aminomethane buffer of *p*H 7 during 100 minutes at 37°; total volume, 0.1 ml.; results expressed in μmoles. The enzyme was obtained by disintegrating brewer's yeast cells with sand in a 50 cycles per second oscillator. After centrifuging the supernatant was made 0.5 saturated with ammonium sulfate and the precipitate was dialyzed.

Sample	Substance omitted during incubation *a*	Δ Reducing Power *b*	Δ Labile Glucose *c*	Δ UDP *d*
1	Glucose-6-phosphate	0	−0.04	+0.02
2	UDPG	0	0	0
3	None	−0.13	−0.14	+0.14

a The substance omitted was added at the end of the incubation period. The Δ values represent the difference with sample 2. *b* Calculated as glucose. *c* Hydrolyzed 10 minutes at pH 2 followed by precipitation with zinc sulfate and barium hydroxide. Practically all the glucose liberated under these conditions is that of UDPG. *d* Estimated by a method based on the reacton: phosphopyruvate + UDP → pyruvate + UTP (A. Kornberg, in "Phosphorus Metabolism". The Johns Hopkins Press, Baltimore, Md., 1951, Vol. 1, p. 392). Pyruvate measured colorimetrically.

Samples equal to those shown in Table I were submitted to fractionation of the barium salts. The water soluble, alcohol-insoluble fractions were used for paper electrophoresis with borate buffer[4] and the phosphate containing compounds were subsequently developed with a molybdate spray reagent[5]. The experiment showed that sample 3, but not samples 1 or 2, contained a phosphate compound which migrated at 60 per cent the rate of glucose-6-phosphate. De phosphorylation of this compound with kidney phosphatase produced a substance which gave the same R_f value as trehalose when chromatographed on paper.

In other experiments the reaction products were deproteinized by heating, treated with charcoal in order to remove the nucleotides and submitted to the action of phosphatase. When chromatographed on paper a substance migrating like trehalose was found to be present in sample 3 but not in the others. The substance extracted from the paper was hydrolyed in 1 *N* acid during 3 hours at 100° and compared chromatographically with trehalose treated in the same manner. In both cases a glucose and a trehalose spot were obtained.

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(2) These abbreviations will be used: UDPG for uridine diphosphate glucose, UDP for urldine disphosphate, and UTP for uridine triphosphate.

(3) R. Caputto. L. F. Leloir, C. F. Cardini and A. C. Paladini. *Biol. Chem.*, 184, 333 1950).

(4) R. Consden and W. M. Stainer, *Nature*, 169, 783 (1952).

(5) R. S. Banduski and B. Axelrod, *J. Biol. Chem.*, 193, 405 (1951)

The solvent used for paper chromatography was pyridine ethyl acetate water [6] with which trehalose, saccharose, maltose and lactose can be separated and the developer was an alkaline silver reagent [7] which reacts slowly with non-reducing disaccharides. Furthermore, reducing from non-reducing sugars can be distinguished because only the latter give color with the aniline-phthalate spray reagent [8]. Thus the ester appears to be a phosphate of trehalose which is presumably identical to that isolated by Robinson and Morgan [9] from the products of yeast fermentation.

The enzyme has been only partially purified and still contains the enzymes which transform glucose-6-phosphate into glucose-1-phosphate and into fructose-6-phosphate, but the most simple explanation of the chemical changes observed is the equation.

UDPG + glucose-6-phosphte →
UDP + trehalose pnosphate

(6) M. A. Jermyn and F. A. Isherwood, *Biochem. J.* 44, 402 (1949).

(7) W. E. Trevelyan. D. P. Procter and J. S. Harrison, *Nature*, **166**, 444 (1950).

(8) S. M. Partridge, *ibid*., **164**, 443 (1949.

(9) R. Robison and W. T. J. Morgan, *Biochem. J.*, **22**, 1277 (1928).