

THE ENZYMIC SYNTHESIS OF TREHALOSE PHOSPHATE¹

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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 activity³ 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 μ mole of glucose-6-phosphate, 0.6 μ mole of UDPG and 0.02 ml. of enzyme in 0.14 M/tris (hydroxymethyl)-aminomethane buffer of pH 7 during 100 minutes at 37°; total volume, 0.1 ml.; results expressed in μ moles. 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 <i>a</i>	Δ Reducing Power <i>b</i>	Δ Labile Glucose <i>c</i>	Δ UDP <i>d</i>
1	Glucose-6-phosphate	0	-0.04	+0.02
2	UDPG	0	0	0
3	None	-0.13	-0.14	+0.14

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

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

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 reaction: phosphopyruvate + UDP \rightarrow 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 buffer⁴ and the phosphate containing compounds were subsequently developed with a molybdate spray reagent⁵. 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 hydrolyzed 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.

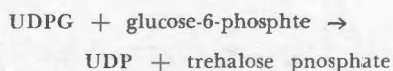
(4) R. Consden and W. M. Stainer, *Nature*, 169, 783 (1952).

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

The solvent used for paper chromatography was pyridine ethyl acetate water⁶ with which trehalose, saccharose, maltose and lactose can be separated and the developer was an alkaline silver reagent⁷ 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 reagent⁸. Thus the ester appears to be a phosphate of trehalose which is presumably identical to that isolated by Robinson and

Morgan⁹ 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.



(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).