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REVERSIBLE TRANSGLUCOSIDATION OF ISOMALTOSE*

by

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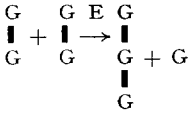
In a previous publication[1] it was shown that the transglucosidase of *Aspergillus oryzae* converted maltose to glucose, isomaltose (dextrobiose), panose and dextrotriose. The persistence of the α-1,6-glucosyl oligosaccharides in the reaction mixture indicated an equilibrium state and a reversibility of enzyme action. Experiments with radioactive tracers showed that the enzyme did not act reversibly on maltose. In this communication evidence is presented for the reversible transglucosidation of isomaltose by the *A. oryzae* transglucosidase.

Isomaltose (dextrobiose) and dextrotriose, the di- and trisaccharides of the dextran series of oligosaccharides, were isolated from a partial acid hydrolysate of dextran. Four mg of pure isomaltose (dextrobiose) was dissolved in 0.05 ml of water and mixed with 0.05 ml of a transglucosidase solution from *Aspergillus oryzae*[1]. An aliquot of 0.01 ml of this mixture was placed on a paper chromatogram and heated for 5 minutes at 100° C to arrest enzyme action. Subsequent samples were obtained at 12, 48 and 96 hours. The reducing compounds in these aliquots were separated and identified by paper chromatographic methods[2]. Initially, isomaltose was the only reducing compound in the reaction mixture; at 12 hours glucose and dextrotriose appeared in low concentrations; at 48 and 96 hours the concentrations of the glucose and dextrotriose increased while the concentration of isomaltose decreased. Since transglucosidase transfers glucose residues from a substrate to the 6-position of glucosyl cosubstrates[1,3] the action of the enzyme on isomaltose would proceed by the mechanism shown in the accompanying equation. (G, E and ▮ represent glucose unit, enzyme molecule and an α-1,6-glucosidic bond, respectively).



The reversibility of this reaction was tested with radioactive glucose and dextrotriose as substrates for the enzyme. One mg of ^{14}C-glucose (total activity 26,000 cpm) and 6 mg of dextrotriose

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in 0.05 ml of water were mixed with 0.05 ml of the enzyme solution. The products in the enzymolysate were separated and identified by the methods of the previous experiment. The radioactivities of the compounds were measured directly on the paper chromatogram and are recorded in Table I.

TABLE I

RADIOACTIVITIES OF THE COMPOUNDS IN AN ENZYMOLYSATE OF ^{14}C-GLUCOSE AND DEXTROTRIOSE

Compound	*Time*			
	0 h *cpm*	*12 h* *cpm*	*48 h* *cpm*	*96 h* *cpm*
Glucose	2610	2342	2085	1820
Isomaltose (dextrobiose)	13	135	406	648
Dextrotriose	8	29	64	102

The results show that radioactive isomaltose was produced in the digest and, therefore, verify the reversibility of the reaction. The radioactive isomaltose was, in turn, disproportionated to yield radioactive dextrotriose.

The transglucosidase of *Aspergillus oryzae* effects a reversible transglucosidation of isomaltose to glucose and dextrotriose. Quantitative analysis of the reaction mixture after attainment of equilibrium should provide values from which the free energy of formation of an α-1,6-glucosidic bond may be calculated.

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THE NATURE OF DIRECT AND INDIRECT BILIRUBIN

by

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It has been found that during electrophoretic experiments on filter paper the direct reacting pigment from bile migrates together with conjugated desoxycholate, as indicated by means of colour reactions for desoxycholic acid; this combination contributes to high polar character of this pigment. No cholate migrates together with it. On the contrary, the indirect form of bilirubin did not move at all during electrophoretic experiments on filter paper, thus behaving like free bilirubin. These experiments are in accordance with the findings of YAMAOKA AND KOSAKA[1], that the propionic acid residues in the direct bilirubin are bound, this suggesting the possibility of a compound of bilirubin with another substance, and with those of POLONOVSKI AND BOURRILLON[2] who suggest the complex of bilirubin with bile salts. Full details will be published elsewhere.

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