Stereochemistry of the Hydrolysis of Trehalose by the Enzyme Trehalase prepared from the Flesh Fly *Sarcophaga barbata*

By KENNETH H. CLIFFORD

(*Shell Research Limited, Shell Biosciences Laboratory, Sittingbourne Research Centre, Sittingbourne, Kent* ME9 8AG)

Summary Hydrolysis of αα-trehalose by the enzyme trehalase, prepared from *Sarcophaga barbata*, is shown to proceed with inversion of configuration at C-1 of one of the two glucose molecules released.

THE hydrolysis of sucrose by intestinal invertase has been determined as proceeding with retention of configuration.[1] However, the suggestion that trehalase (trehalose-1-glucohydrolase; EC 3.2.1.28) causes inversion of one of the two glucose molecules liberated from trehalose is based solely on the observation that the liberated glucose can be utilized as a substrate by glucose oxidase. Glucose oxidase is specific for β-glucose[2] but the rate of epimerization of glucose in solution could be sufficient to account for this observation. Work is presented here demonstrating that hydrolysis of trehalose by the enzyme trehalase,[3] prepared from the flesh fly *Sarcophaga barbata*, leads to an equimolar mixture of α- and β-glucose.

Glucose liberated by the enzyme was analysed for anomeric composition by g.l.c.[4] Samples of glucose solutions were immediately frozen in liquid nitrogen, freeze-dried, and derivatized by dissolving in Tri-Sil 'Z' (Pierce and Warriner Ltd., Chester). The solutions of pentatrimethylsilyl glucoses were analysed directly on a 3% SE-30 column at 210 °C. In this way the mutarotation of α-glucose was compared with the mutarotation of glucose released from an incubation of trehalose with trehalase. A typical incubation consisted of 0·4 ml trehalase (*ca.* 1·0 International Unit in 50 mM Tris-maleate buffer pH 6·0, 0·2 M NaCl), 0·6 ml 50 mM sodium acetate buffer pH 5·1, and 7·5 μmol trehalose in a final volume of 1·01 ml at 22 °C. 0·1 ml Samples were taken from 30 s to 10 min and immediately frozen in liquid nitrogen. The analysis of glucose formed by trehalase showed an α:β ratio of 49·9:50·1 after 30 s falling to 45·9:54·1 after 10 min (at 10 min 93% trehalose had been hydrolysed to glucose; see Table). An experiment using 15 μmol α-glucose to show the rate of mutarotation gave a value of 72:28 after 10 min.

TABLE. Hydrolysis of trehalose by the enzyme trehalase showing the percentage of α-glucose in the glucose released.

Time/min	Trehalose/%	α-Glucose/%
0·5	84·1	49·9
1·0	83·5	46·0
2·0	61·8	46·4
4·0	40·6	46·1
6·0	25·9	45·3
8·0	13·4	48·7
10·0	7·0	45·9

Owing to the ready mutarotation of glucose in solution, the rates of mutarotation of α-glucose, β-glucose, and an equimolar mixture of α- and β-glucose were determined (see Figure) using a Perkin-Elmer 241 polarimeter (589·3 nm, 10 cm light path). Superimposed on these is the optical rotation due to an incubation of trehalose and trehalase together with the glucose released. After 20 min, when all the trehalose had been converted into glucose (see Table), the rates of mutarotation were: α-glucose, −150 millidegrees h^{-1}; β-glucose, +80; equimolar α- and β-glucose, −3·5; and the glucose formed by enzymic hydrolysis of trehalose, −3·75. The rate of mutarotation of glucose formed from trehalose is almost equal to that of an equimolar mixture of α- and β-glucose.

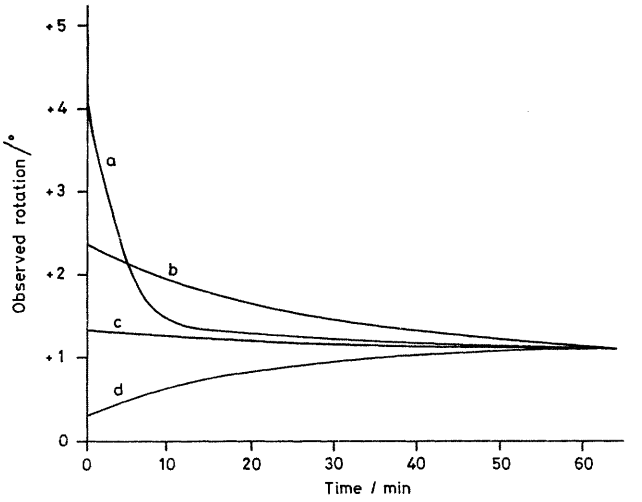

FIGURE. Comparison of the mutarotation of glucose from (a) trehalose–trehalase, (b) α-glucose, (c) α/β-glucose (1:1), (d) β-glucose.

The experiments were repeated using isotopically labelled water; $H_2{}^{18}O$ to a final concentration of 56% v/v was used in the incubation of trehalase and trehalose. The α- and β-glucose (Me_3Si derivatives) formed were separated and analysed by g.l.c.–mass spectroscopy. Although no parent ion was obtainable (*m/e* 540) the fragments at *m/e* 189—195, 345—350, 393—395, and 435—438 indicated incorporation of ^{18}O into β-glucose whereas there was no detectable ^{18}O in the α-glucose.

The results obtained indicate that, on hydrolysis of trehalose by trehalase, an equimolar mixture of α- and β-glucose is formed. This is interpreted as meaning that the configuration of one of the anomeric carbon atoms of the two glucose molecules is inverted on hydrolysis of trehalose by the enzyme and in turn this is consistent with a bimolecular nucleophilic substitution mechanism.

(*Received, 20th October* 1978; *Com.* 1129.)

[1] G. Semenza, C.-H. Curtis, J. Kolinska, and M. Muller, *Biochim. Biophys. Acta*, 1967, **146**, 196.
[2] D. Keilin and E. F. Hartree, *Biochem. J.*, 1952, **50**, 341.
[3] S. Friedman, *Arch. Biochem. Biophys.*, 1960, **87**, 252.
[4] R. Bentley and N. Botlock, *Analyt. Biochem.*, 1967, **20**, 312.