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Preliminary communication

Studies of invertase (β-D-fructofuranosidase)

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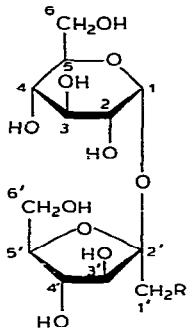
Invertase (EC 3.2.1.26, β-D-fructofuranosidase), which occurs widely in Nature, was one of the first substances to be identified as an enzyme and it was used to establish many of the principles of enzyme kinetics[1]. However, no systematic study of its mode of action has been made. The known facts on structure–activity of the yeast enzyme are that it hydrolyses not only sucrose (1), its well-known substrate, but also alkyl β-D-fructofuranosides (*e.g.*, 2). α-D-Fructofuranosides and β-D-fructopyranosides are not hydrolysed[1] The only compounds closely related to sucrose that have been tested as substrates are the 5′-epimer and the *nor*-6′-hydroxymethyl analogues, synthesised enzymatically by Hassid[2]. These compounds were not hydrolysed, but the structure of neither was rigorously established.

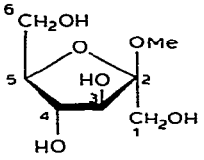
We have now examined the action of invertase* (from *Candida utilis*) on various derivatives of sucrose and methyl β-D-fructofuranoside, as well as on compounds with related structures. Evidence from our early studies, particularly the non-hydrolysis of methyl β-D-arabinofuranoside (3), suggested the importance of the 1′-CH_2OH group, and so 1′-deoxy (4) and 1′-chloro-1′-deoxysucrose (5) were examined. Compounds 4 and 5 were synthesised *via* 6,1′,6′-tri-*O*-(2,4,6-trimethylbenzenesulphonyl)sucrose 2,3,4,3′,4′-penta-acetate[4,5]. Displacement of the more reactive sulphonyloxy groups at the 6- and 6′-positions with benzoate ion left one such group at the 1′-position for displacement by chloride ion to give, after deacetylation, 5, which was then converted into 1′-deoxysucrose (4). The derivatives of methyl β-D-fructofuranoside were usually prepared *via* 2,3-*O*-isopropylidene-1,6-di-*O*-tosyl-D-fructofuranose[6], by modification at the 1- and/or 6-positions and then conversion into the appropriate methyl fructofuranosides. A few such compounds were made directly from methyl 1,6-di-*O*-tosyl-β-D-fructofuranoside or methyl 1,6-di-*O*-(2,4,6-trimethylbenzenesulphonyl)-β-D-fructofuranoside[4].

None of the compounds in Table I was hydrolysed by the enzyme, even though many of them are only slightly different structurally from sucrose or methyl β-D-fructofuranoside. Hassid's results[2] have been supported by the non-hydrolysis of the analogues

*The enzyme used was commercial β-D-fructofuranosidase (Siekago Lot AOBN 32008) purified on Ultrogel AcA 34. The fraction used was homogeneous by polyacrylamide gel electrophoresis. The enzyme had K_m of 5 mM for sucrose hydrolysis and a specific activity of 750 i.u. mg^{-1}. The assay method used was that of Somogyi–Nelson[3].



1 R = OH
4 R = H
5 R = Cl



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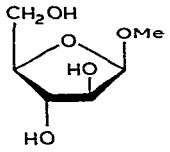

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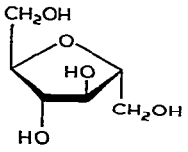

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TABLE I

COMPOUNDS NOT HYDROLYSED[a] BY β-D-FRUCTOFURANOSIDASE (FROM *Candida utilis*)

Derivatives of methyl β-D-*fructofuranoside* (2)
1-azido-1-deoxy
1-chloro-1-deoxy
1-deoxy-1-fluoro
1-deoxy
6-chloro-6-deoxy
6-deoxy
1,6-dichloro-1,6-dideoxy
1,6-dideoxy-1,6-difluoro

Derivatives of sucrose (1)
1′-deoxy (4)
1′-chloro-1′-deoxy (5)
4′-chloro-4′-deoxy[b]
6′-*O*-methyl[b]
1′,6′-di-*O*-methyl[b]
4,6′-di-*O*-methyl[b]
6,6′-di-*O*-methyl[b]
6,6′-diazido-6,6′-dideoxy[c]
6,6′-diamino-6,6′-dideoxy[c]
6,1′,6′-trideoxy[d]
6,1′,6′-trichloro-6,1′,6′-trideoxy[b]

Other compounds
6′-Chloro-6′-deoxyraffinose[d]
6′-Bromo-6′-deoxyraffinose[d]
6′-Deoxy-6′-iodoraffinose[d]
Methyl α-L-sorbofuranoside[e]
Methyl β-D-xylulofuranoside
Methyl β-D-arabinofuranoside (3)
2,6-Anhydro-β-D-fructofuranose[f]
1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside[b]
1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside[b]

[a] A 0.01M solution (~1 ml) of each compound in 0.04M acetate buffer (pH 4.7) was added to a jacketed polarimeter tube kept at 25°. The sample was left for 10–30 min to ensure that no change (*i.e.*, spontaneous hydrolysis) occurred. The solution was then removed from the cell and mixed with 30 μl of enzyme solution (0.45 mg/ml). The solution was returned to the cell and the rotation observed for 24 h. The reactions were also monitored by t.l.c. After 24 h, the whole of the remaining solution was assayed for reducing sugar. [b] Kindly provided by Dr. R. Khan (Tate and Lyle Ltd.). [c] Kindly provided by Professor E. Reist (Stanford Research Institute). [d] Kindly provided by Professor L. Hough (Queen Elizabeth College, London). [e] Kindly provided by Professor S. J. Angyal (University of New South Wales). [f] Kindly provided by Prof. A.J. Perlin (McGill University).

of his sucrose derivatives, namely methyl α-L-sorbofuranoside and methyl β-D-xylulofuranoside.

Where sufficient material was available, these compounds were studied as reversible inhibitors (see Table II); also in this group was 2,5-anhydro-D-mannitol (6). Only weak inhibitory properties were displayed.

TABLE II

COMPETITIVE INHIBITORS[a] OF β-D-FRUCTOFURANOSIDASE (FROM *Candida utilis*)

Substance	K_i *(mM)*
2,5-Anhydro-D-mannitol (6)	125
Methyl β-D-arabinofuranoside (3)	145
1′-Deoxysucrose (4)	140
1′-Chloro-1′-deoxysucrose (5)	50

[a] The inhibition was studied by measuring the rate of production of reducing sugar (Somogyi–Nelson[3]) at various inhibitor and substrate concentrations. A solution of inhibitor and substrate (1.0–5.0 ml, pH 4.7) was equilibrated at 30°. The reaction was initiated by the addition of enzyme (0.025–0.1 ml; concentration, 0.45 mg/ml). The resulting data were analysed by means of Dixon plots.

It is clear that minor changes at the 1′-, 4′-, or 6′-position cause loss of substrate capability, and we conclude that β-D-fructofuranosidase from *Candida utilis* is a highly specific enzyme. Further work is in progress on the significance of the 3′-position and on the design of potential inhibitors of this enzyme.

Although the majority of studies of invertase have been on the enzyme from the yeast *Saccharomyces cerevisiae*, it is known that enzymes with invertase-like activity have a wide occurrence. The Cavendish banana (*Musa cavendishii*) is a known source of invertase activity[7,8], but one from which reasonably pure enzyme has not previously been isolated. We have isolated a β-D-fructofuranosidase of apparent molecular weight 220,000 and of specific activity of the same order as that for the yeast enzyme. The banana enzyme hydrolysed both methyl β-D-fructofuranoside and sucrose.

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