THE INHIBITION OF YEAST α-GLUCOSIDASE BY L-1,2-ANHYDRO-*myo*-INOSITOL

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1. Introduction

DL-1,2-Anhydro-*myo*-inositol has been shown to combine with the active site of β-glucopyranosidases to form an inositol ester with a carboxylate residue of the enzyme protein. Hydrolysis of the ester led to lD-*chiro*-inositol, suggesting that it is the D-isomer which is the inactivator [1, 2]. Study of the stereochemistry of the anhydro-inositols (fig. 1), suggests that the lL-form might behave in a similar way with α-glucosidases. We have therefore synthesized the lL-isomer [3] and now report an investigation of its interaction with yeast α-glucosidase.

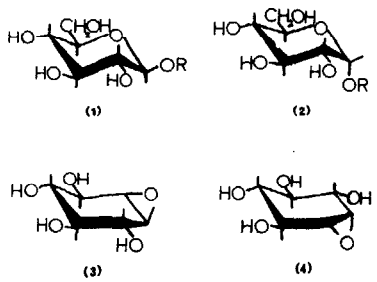

Fig. 1. Comparison of the structures of 1,2-anhydro-*myo*-inositols with those of α_1 and β-D-glucopyranosides. (1) β-D-glucopyranoside, (2) α-D-glucopyranoside, (3) lD-1,2-anhydro-*myo*-inositol, (4) lL-1,2-anhydro-*myo*-inositol.

2. Experimental

Preliminary tests showed that lL-1,2-anhydro-*myo*-inositol [4] was inhibitory and the nature of this inhibition was investigated. The K_m of the substrate *p*-nitrophenyl α-D-glucopyranoside was measured in the presence and absence of inhibitor. Yeast α-D-glucopyranoside (Sigma Ltd., Lettice Street, London, S.W.6.) (0.5 mg) was dissolved in 0.5 N sodium acetate/chloride buffer, pH 6.0 (4 ml). The enzyme is unstable in distilled water, but is stable in this buffer. 0.1 ml was added to *p*-nitrophenyl α-D-glucopyranoside (0.5 ml, final concentration 0.05–0.5 mM) 0.5 N sodium acetate/chloride buffer pH 6.0 (0.2 ml) and either inhibitor or water (0.2 ml). The solution was incubated at 25° for 15 min and the reaction stopped by addition of 1 M sodium carbonate, diluted with water (2 ml) and the colour read at 400 nm. lL-1,2-anhydro-*myo*-inositol and D-glucose were both competitive inhibitors, K_i 6.9 mM and 2.0 mM respectively (fig. 2).

lL-1,2-Anhydro-*myo*-inositol was also tested as a progressive inactivator of yeast α-glucosidase and almond emulsin (B.D.H. Ltd., Poole, Dorset, U.K.) β-glucopyranosidase. α-Glucopyranosidase (0.3 mg) was dissolved in 0.2 N sodium acetate/chloride buffer pH 6.0 (0.5 ml) containing lL-1,2-anhydro-*myo*-inositol (final concentration 14 mM) and incubated at 25°. The control lacked the inhibitor. Duplicate portions (25 μl) were removed at intervals up to 5 hr and added to preincubated tubes containing 1 mM *p*-nitrophenyl α-D-glucopyranoside in 0.05 N sodium acetate/chloride buffer, pH 6.0 (3 ml)

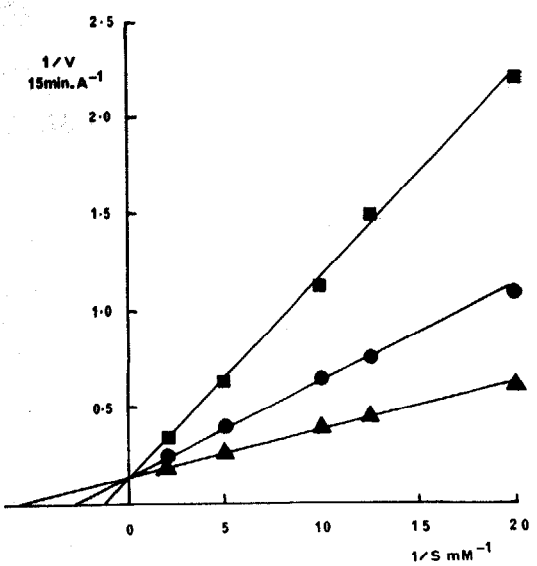

Fig. 2. Lineweaver-Burk plots of α-D-glucosidase activity in the presence and absence of inhibitors. ▲—▲, *p*-nitrophenyl α-D-glucopyranoside alone; ■—■, with 6.5 mM D-glucose; ●—●, with 9.0 mM lL-1,2-anhydro-*myo*-inositol.

and incubated for 15 min at 25°. 1 M Sodium carbonate was added to stop the reaction and the colours read immediately. No inactivation was detected. The inactivation of almond emulsin β-glucopyranosidase was tested in the same way using 3 mM inhibitor and *o*-nitrophenyl β-D-glucopyranoside as substrate. Again no inactivation was observed.

3. Discussion

As predicted almond emulsion β-glucosidase, which is inactivated by DL-1,2-anhydro-*myo*-inositol [2], was unaffected by the lL-isomer, confirming that the D-isomer is the form which inactivates.

The tests of lL-1,2-anhydro-*myo*-inositol as an inactivator of α-glucosidase revealed that it was an ordinary competitive inhibitor (fig. 2). It therefore interacts with the active site of the enzyme to form an enzyme–inhibitor complex, but since it did not inactivate the enzyme, it did not form a stable covalent bond of the type required for the identification of active site groups.

Although the binding of the anhydro-inositol [4] was much weaker than that of the substrate used, it was comparable to that of D-glucose, whereas *myo* and lL-*chiro*-inositol did not detectably inhibit the enzyme. The strong interaction may in part be due to the planar nature of the anhydro-inositol in the region where the bond-breaking occurs in the normal substrate (fig. 1) which has been suggested by Lee [4] as the explanation for the tight binding of D-galactal to β-galactosidases. Part of the binding might also be supplied by the epoxide oxygen which might form a hydrogen bond with an enzyme group involved in binding to C-2 of a normal substrate.

Acknowledgement

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References

[1] G. Legler, Z. Physiol. Chem. 349 (1968) 767.
[2] G. Legler and S.N. Hasnain, Z. Physiol. Chem. 351 (1970) 25.
[3] D. Mercier, A. Olesker, S.D. Gero and J.E.G. Barnett, Carbohydrate Res. (1971) in press.
[4] Y.C. Lee, Biochem. Biophys. Res. Commun. 35 (1969) 161.