Phytochemistry, 1976, Vol. 15, pp. 643–646. Pergamon Press. Printed in England.

D-XYLOSE, AN ANOMER-SPECIFIC INHIBITOR OF α-GALACTOSIDASE

T. N. SHARMA and C. B. SHARMA
Post Graduate Department of Chemistry, Biochemical Laboratories, D.A.V. College, (Meerut University), Muzaffarnagar, U.P. 251001, India

(*Revised received* 26 *November* 1975)

Key Word Index—*Cicer arietinum*; leguminosae; chick pea; α-galactosidase; β-galactosidase; D-xylose; anomer-specific; inhibitor.

Abstract—D-xylose is a highly anomer-specific, powerful and competitive inhibitor of plant α-galactosidases. The apparent inhibition constant (K_i) for the interaction of D-xylose with chick pea (*Cicer arietinum*) α-galactosidase and the apparent number of inhibitor molecules (n) bound per enzyme molecule, using *p*-nitrophenyl-α-D-galactopyranoside as substrate, were found to be 0.4×10^{-2} M and 0.8, respectively.

In general, free sugars [1–5], aldonolactones [6–8], polyols [9] and glycosides [5] are competitive inhibitors of the corresponding glycosidases. However, their inhibitory action lacks the anomeric specificity towards the anomeric glycosidases since these compounds inhibit both α- and β-glycosidases. Recently, Lai and Axelrod [10] have reported 1-aminoglycosides as a new class of specific inhibitors of glycosidases, but these compounds, too, lack the anomeric specificity of the inhibition. Myoinositol was earlier reported to be a stereospecific inhibitor of α-galactosidase without any effect either on β-galactosidase or any other glycosidases [11]. The mechanism of this absolute anomeric specificity is not fully understood. As a part of a programme to determine the mechanism of the anomeric specificity of an inhibitor, the effect of various sugars and sugar analogues on α- and β-galactosidases was investigated. In this communication we report that like myoinositol, D-xylose, too, is a stereospecific inhibitor for α-galactosidase without any apparent effect on β-galactosidase.

RESULTS

The results summarized in Table 1 permit a comparison of the relative effect of the D-xylose and other related sugars on α- and β-galactosidases from *Cicer arietinum*. These results clearly indicate that D-xylose is a powerful inhibitor of plant α-galactosidases with absolute anomeric specificity of inhibition. In this respect its action is similar to that of myoinositol [11], but is strikingly different from that of D-galactose and L-arabinose which were found to inhibit both α- and β-galactosidases. Furthermore, D-xylose was found to be a more powerful inhibitor of α-galactosidase than myoinositol and L-arabinose. It is also worth noting here that among the sugar alcohols tested only arabitol was found to be an effective inhibitor of α- and β-galactosidases. Even the relatively high concentrations of xylitol and galactitol produced no detectable effect either on α- or β-galactosidases. These results, as suggested by Goren *et al.* [12], indicated that alcohols bind at a different site on the enzyme than sugars.

The nature of the inhibition of α-galactosidase by D-xylose was evaluated according to the Lineweaver–Burk method [13] and found to be of the competitive type. In Dixon plots the intersection at a point above the ordinate was again indicative of the competitive inhibition. Cooperativity phenomena [14] were also not observed, as doubling or tripling the effective inhibitor concentration did not alter significantly the calculated values of the apparent inhibition constant (K_i) of the inhibitor. In addition, a Hill coefficient value of 0.8, as calculated from the standard procedure [15], also indicated the absence of cooperativity.

The relative effectiveness of the D-xylose inhibition of α-galactosidase was also evaluated by comparing the apparent K_i values with other sugar inhibitors. The K_i values for D-xylose, L-arabinose, D-galactose and myoinositol, as obtained from Dixon plots [16], were found to be 0.4×10^{-2} M, 0.4×10^{-1} M, 0.13×10^{-2} M and 0.25×10^{-1} M, respectively. If the apparent K_i values of the competitive inhibitors are assumed to represent the equilibrium constants for the inhibitor–enzyme interaction, these results then show that the affinity ($1/K_i$) of D-xylose for α-galactosidase is about one-third that of D-galactose, but it is about six and ten times higher than that of myoinositol and L-arabinose, respectively.

D-xylose also inhibited β-glucosidase (Table 2) which was not unexpected since it is an analogue of D-glucose. The K_i value for D-xylose for β-glucosidase inhibition was 0.8×10^{-1} M as compared to its K_i value of 0.4×10^{-2} M for α-galactosidase. Thus, the affinity of D-xylose for α-galactosidase was about 20 times higher than that for β-glucosidase. These results explain further why D-xylose is such a poor inhibitor for β-glucosidase compared to its effect on α-galactosidase.

The effect of the D-xylose on various glycosidases derived from a variety of plant sources is given in Table 2. From these results it is evident that the stereospecific inhibition of plant α-galactosidases is of a general type.

Table 1. Inhibition of α-galactosidases by sugars and related compounds*

Compound	Conc. (mM)†	Inhibition and K_i values‡			
		α-galactosidase		β-galactosidase	
		%	K_i (mM)	%	K_i (mM)
D(+) Xylose	5.0	35.0	4.0	0.0	
	15.0	52.2		0.0	
	30.0	64.6		0.0	
	52.5	77.7		0.0	
	100.0	89.8		0.0	
L(+) Arabinose	25.0	29.5	41.2	0.0	280.4
	50.0	40.0		12.6	
	100.0	50.2		23.3	
	125.0	52.0		25.0	
D(+) Galactose	1.0	56.5	1.3	26.9	4.3
	3.0	65.2		41.5	
	5.0	74.0		56.1	
D(+) Mannose	200.0	0.0		0.0	
D(+) Glucose	200.0	0.0		0.0	
D(+) Fructose	200.0	0.0		0.0	
Xylitol	300.0	0.0		0.0	
Arabitol	100.0	21.2	213.0	48.3	
	170.0	41.6		81.9	
	300.0	54.1		84.9	
Galactitol	170.0	0.0		0.0	
Sorbitol	170.0	0.0		0.0	
(+) Inositol	170.0	0.0		0.0	
Myoinositol-2-PO_4	170.0	0.0		0.0	
Myoinositol	50.0	46.8	25.1	0.0	
	100.0	60.7		0.0	
	170.0	81.2		0.0	

* The reaction mixture and assay conditions were same as described in "Experimental" except that substrate concentration was 0.2 mM.

† Compounds which produced no inhibition, only highest concentrations tested are reported.

‡ K_i values were obtained from Dixon plots [16].

DISCUSSION

The present results provide strong evidence that unlike most sugars, D-xylose is a powerful, competitive and anomer-specific inhibitor of α-galactosidase without any detectable inhibition of β-galactosidase. This effect was unexpected because D-xylose (**1a**, Fig. 1) has greater structural and configurational resemblance to D-glucose (**1b**, Fig. 1) rather than D-galactose (**2b**, Fig. 1) or L-arabinose (**2a**, Fig. 1). In addition, our results on the inhibition of α-galactosidase are contrary to those cited in a review article by Dey and Pridham [17] that D-xylose did not produce inhibition of the α-galactosidases. That our results do not represent a specific case is evident from the fact that α-galactosidases derived from a variety of sources were also strongly inhibited by D-xylose.

Glycosidase usually shows a very high specificity with respect to the sugar moiety of the substrate and the anomeric configuration of the glycosidic linkage [18]. According to Koshland [19], glycosidases have at least two binding sites: a specificity site and a catalytic site. The glycosyl residue of the substrate binds at the specificity site and the glycosidic linkage to the catalytic site to make an active enzyme–substrate complex. As

Table 2. The effect of D-xylose on glycosidases derived from a variety of sources*

Enzyme	Enzyme source and % inhibition			
	Cicer arietinum	*Arachis hypogaea*	*Phaseolus mungo*	*Phaseolus radiatus*
α-galactosidase	77.0	56.5	76.3	69.0
β-galactosidase	0.0	0.0	0.0	0.0
α-glucosidase	0.0	0.0	0.0	0.0
β-glucosidase	25.6	19.5	25.0	27.0
α-mannosidase	0.0	0.0	0.0	0.0
β-*N*-acetylglucosaminidase	0.0	0.0	0.0	0.0

* Assay conditions were same as described in "Experimental" except that the concentration of D-xylose (inhibitor) used in the case of α-galactosidase was 52.5 mM while for all other glycosidases tested it was 200 mM.

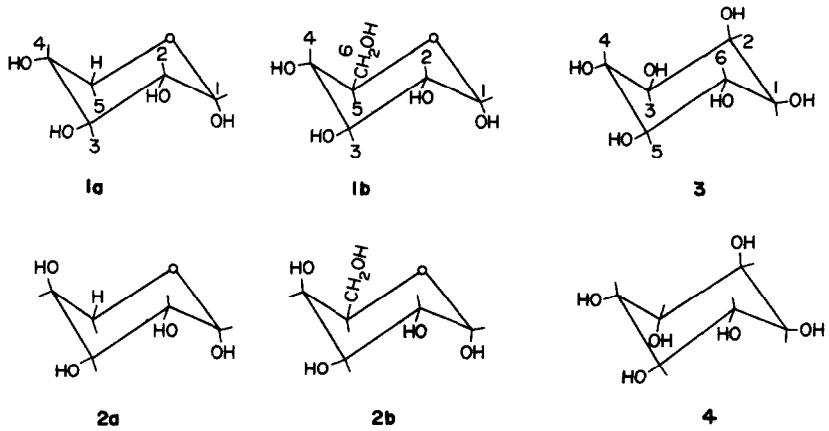

Fig. 1. Orientation of OH-groups in pyranoside ring of some monosaccharides and cyclohexane ring of inositols: 1a = α-D-xylose; 1b = α-D-glucose; 2a = α-L-arabinose; 2b = α-D-galactose; 3 = Myoinositol; 4 = (+) Inositol.

reported elsewhere [11], an anomer-specific competitive inhibitor of glycosidase should be able to compete with the substrate both for the specificity and catalytic sites as to prevent the formation of an active enzyme-substrate complex. D-xylose appears to satisfy this condition, although sugars in general do not interact at the catalytic site and, as such, lack the anomeric-specificity in the inhibition of corresponding glycosidases.

The fact that D-xylose shows normal mutarotation giving rise to an equilibrium mixture containing 29% α-form and 71% of β-form of D-xylose [20], the role of mutarotation in the anomeric-specificity of D-xylose interaction with α-galactosidase can definitely be ruled out. Furthermore, a solution of D-xylose in citrate buffer, pH 5.2, which was allowed to equilibrate for 48 hr neither produced any inhibition of β-galactosidase nor was there any change in the inhibition of α-galactosidase. It may, however, be important in case of D-galactose and L-arabinose which inhibit both α- and β-galactosidases.

The only difference between the configuration of D-xylose (**1a**) and L-arabinose (**2a**), analogue of D-galactose, is in the orientation of OH-group about the C_4-atom in the pyranoside ring, which is equatorial in D-xylose and axial in L-arabinose. This deviation in the configuration may be significant enough to prevent the interaction of D-xylose with β-galactosidase indicating that specificity sites in α- and β-galactosidases have different conformations and the latter is more specific for galactose configuration.

This view is in agreement with the results of Li and Shetlar [3] that for attachment of sugar to the enzyme, the galactose configuration is required and OH groups at C_1, C_2, C_4, and C_6 are involved in binding. An analogous situation was observed in case of myoinositol (**3**) and (+)-inositol (**4**). The former is an anomer-specific inhibitor of α-galactosidases [11] while the latter, which differs from myoinositol only in the orientation of one OH-group at C_2-atom, did not produce any effect either on α- or β-galactosidase (Table 1).

Though the information presented in this report is limited, nevertheless, it suggests that useful information can be obtained if a variety of sugar analogues are investigated with respect to their anomeric specificity of inhibition towards glycosidases.

EXPERIMENTAL

Chemicals. *p*-nitrophenyl-glycosides were obtained from Pierce Company (Rockford, Ill., U.S.A.). *p*-Nitrophenyl-*N*-acetyl-β-D-glucosaminide, myoinositol and D-galactal were obtained from the Sigma Chemical Comp. (St. Louis, Mo.). D-xylose (sp. rotation $[\alpha]_D^{20}$ +19° to 20°), L-arabinose (sp. rotation $[\alpha]_D^{20}$ +102 to 105°) and other sugar analogues were obtained from B.D.H. Ltd. All operations during the extraction and purification of enzymes were carried out at 4° unless otherwise specified. Glycosidases used in this study were obtained from the germinating seeds of *Cicer arietinum* by a procedure as described by Bahl and Agrawal [21]. The partially purified enzyme preparations from the germinating seeds of *Arachis hypogaea*, *Phaseolus mungo* and *Phaseolus radiatus*, obtained by the same method, were also used for studying the effect of various sugars on the activity of glycosidases. The partially purified enzyme preparation contained the following glycosidases: α-galactosidase, β-galactosidase, α-mannosidase, α-glucosidase, β-glucosidase, and β-*N*-acetyl-glucosaminidase.

Enzyme assay. Glycosidase activities with and without inhibitor were measured by the same method as described by Bahl and Agrawal [21]. Each enzyme was assayed at optimum pH in 0.05 M Na citrate buffer containing 0.2–1.0 μmol of *p*-nitrophenyl-glycosides as substrate, 0.2 ml of enzyme soln (0.5–1 mg protein) in a total vol. of 2 ml. The incubation was carried out for 15 min at 30°. The reaction was stopped by the addition of 2 ml of 4% Na_2CO_3 soln. The liberated *p*-nitrophenol, which produced a yellow colour at alkaline pH, was measured at 420 nm. The protein was determined by the method of ref. [22] using BSA as standard.

Kinetic studies. Measurements of enzyme activity for Lineweaver–Burk plots [13] both in the absence or presence of fixed level of inhibitor were made at optimum pH. Values of apparent inhibitor constant (K_i) were obtained from Dixon plots [16]. Interaction constants (*n* values) were determined by the method of Hill [15]. In all the experiments conditions of incubation were same as described under enzyme assay except that data for Dixon plots were obtained with 0.2 and 0.4 mM substrate concentrations.

REFERENCES

1. Dey, P. M. and Pridham, J. B. (1969) *Biochem. J.* **115**, 47.
2. Suzuki, H., Li, Su-Chen and Li, Yu-Teh (1970) *J. Biol. Chem.* **245**, 781.
3. Li, Yu-Teh and Shetlar, M. R. (1964) *Arch. Biochem. Biophys.* **108**, 523.

4. Lee, Y. C. and Wacek, V. (1970) *Arch. Biochem. Biophys.* **138,** 264.
5. French, D. (1954) *Advan. Carbohyd. Chem.* **9,** 158.
6. Conchie, J. and Levvy, G. A. (1957) *Biochem. J.* **65,** 389.
7. Levvy, G. A., McAllan, A. and Hay, A. J. (1962) *Biochem. J.* **82,** 225.
8. Levvy, G. A. and Snaith, S. M. (1972) *Adv. Enzymol.* **36,** 151.
9. Kelemen, M. V. and Whelan, W. J. (1966) *Arch. Biochem. Biophys.* **117,** 423.
10. Lai, H. L. and Axelrod, B. (1973) *Biochem. Biophys. Res. Commun.* **54,** 463.
11. Sharma, C. B. (1971) *Biochem. Biophys. Res. Commun.* **43,** 572.
12. Goren, G., Wouters-Leysen, J., Yde, M. and De Bruyne, C. K. (1973) *European J. Biochem.* **38,** 122.
13. Lineweaver, H. and Burk, D. J. (1934) *J. Am. Chem. Soc.* **56,** 658.
14. Rogers, K. S. and Yusko, S. C. (1969) *J. Biol. Chem.* **244,** 6690.
15. Hill, A. V. (1910) *J. Physiol. (London)* **40,** IV.
16. Dixon, M. (1953) *Biochem. J.* **55,** 170.
17. Dey, P. M. and Pridham, J. B. (1972) *Adv. Enzymol.* **36,** 91.
18. Malhotra, O. P. and Dey, P. M. (1967) *Biochem. J.* **103,** 739.
19. Koshland, D. E. (1959) *The Enzymes* (P. D. Boyer, H. Lardy and Myrback, eds.) Vol. 1, Academic Press, New York, p. 332.
20. Finnar, I. L. (1968) *Organic Chemistry* Vol. II, IV Ed. p. 257. Longman, London.
21. Bahl, O. P. and Agrawal, K. M. L. (1968) *J. Biol. Chem.* **243,** 98.
22. Lowry, O. H., Roseborough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265.