INHIBITION OF β-D-GALACTOSIDASES BY D-GALACTAL[1)]

Y. C. Lee

Department of Biology and McCollum-Pratt Institute
The Johns Hopkins University, Baltimore, Md. 21218

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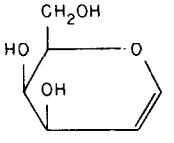
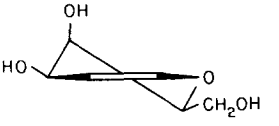
This report presents evidence indicating that D-galactal (1,2-dideoxy-D-lyxo-hex-1-enopyranose, (I)) is a potent inhibitor of β-D-galactopyranosidases derived from a variety of sources. D-Galactal represents a new type of inhibitor for glycosidases, and it is suggested that the planar half-chair conformation (II or IIa), preferred by D-galactal, is responsible for its inhibitory action.



(I)



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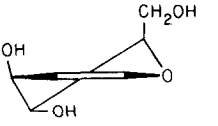
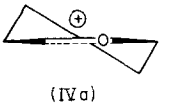


(IIa)

(III) (IV)



(IVa)

MATERIALS AND METHODS

The enzymes used in this study were D-glycopyranosidases. β-Galactosidases were obtained from the following sources as indicated: Aspergillus niger (prepared in our laboratory, by a method to be published elsewhere.), Clostridium perfringens[2)] (kindly provided by Dr. E. J. McGuire), Escherichia

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[1)] Contribution No. 548 from McCollum-Pratt Institute, The Johns Hopkins University.

[2)] Chipowsky, S., McGuire, E. J., and Roseman, S., Unpublished results.

coli (1) (a gift from Dr. E. Steers), sweet almond emulsin (partially purified from "β-glucosidase" of Worthington Biochem. Corp.), limpet (purchased from Pierce Chemical Co. under the name of "β-glucuronidase"), jack bean[3] (a gift from Dr. Y. T. Li), pinto bean (2) (a gift from Dr. O. P. Bahl). A homogeneous α-mannosidase from jack bean (3) was kindly provided by Dr. Y. T. Li. Almond emulsin α-mannosidases was partially purified in our laboratory by a method to be published elsewhere. β-N-Acetylglucosaminidase from C. perfringens[2] was a gift from Dr. E. J. McGuire. D-Galactal and p-nitrophenyl glycopyranosides were purchased from Pierce Chemical Co.

Activities of the glycosidases were assayed colorimetrically by measuring p-nitrophenol resulting from cleavage of the glycosides. The incubation mixtures (1.0-1.5 ml) from enzymatic assays usually contained 1-5 mM p-nitrophenyl β-D-galactopyranoside (or other suitable substrates), and 0-1 mM D-galactal in appropriate buffers (Table 1). After incubation at 37° for 5-15 min, depending on the enzyme activity, 2 ml of 0.2 M Na_2CO_3 were added, and absorbance at 410 mμ measured in a Bausch and Lomb Spectronic 20 colorimeter. The values of Km (Michaelis constant) and Ki (inhibition constant) were obtained by plotting 1/v versus 1/S, or 1/v versus I (4).

RESULTS AND DISCUSSION

The results obtained with β-galactosidases from A. niger, C. perfringens, E. coli, and pinto bean are shown in Figs. 1-3. In each case, the inhibitory action appeared to be competitive. In the 1/v versus I plot (Fig. 3a, b), the E. coli enzyme showed slight hyperbolicity. Similarly, β-galactosidases from sweet almond emulsin and jack bean showed hyperbolic competitive inhibition when the same plot was used. The reason for such hyperbolicity was not determined.

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[3] Li, Y. T., Unpublished results.

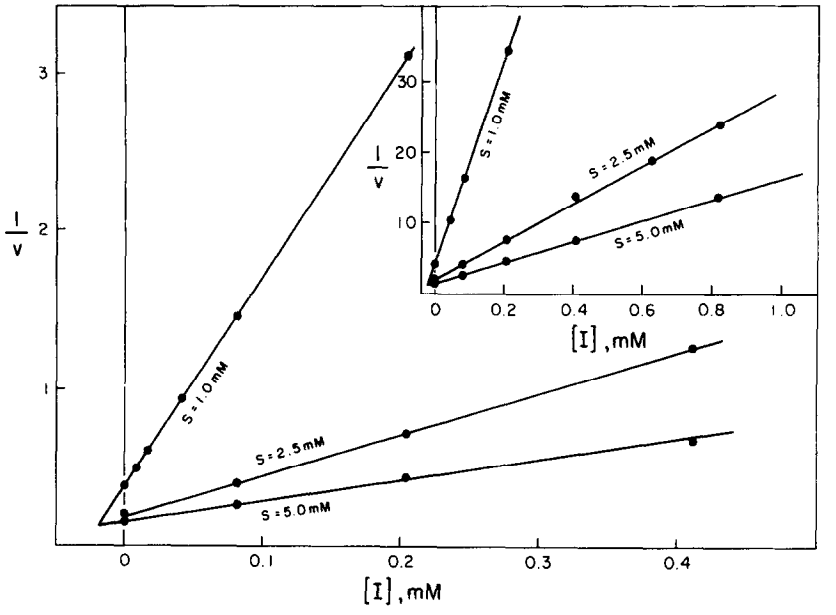

Fig. 1. Inhibition of A. niger β-D-galactosidase by D-galactal.

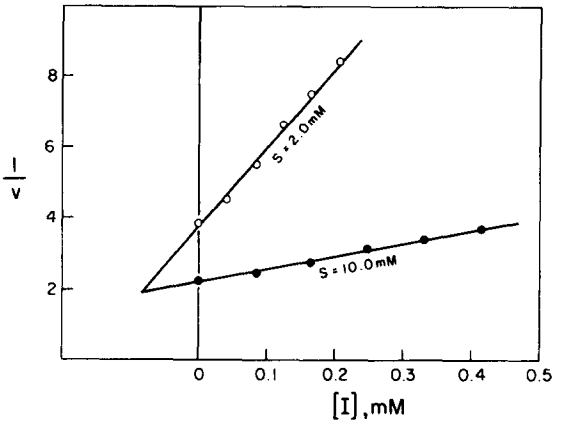

Fig. 2. Inhibition of C. perfringens β-D-galactosidase by D-galactal.

The values of Km and Ki for these enzymes are summarized in Table 1. Clearly, D-galactal is an extremely potent inhibitor of β-galactosidases. Under comparable conditions, α-mannosidase, β-glucosidase, and β-N-

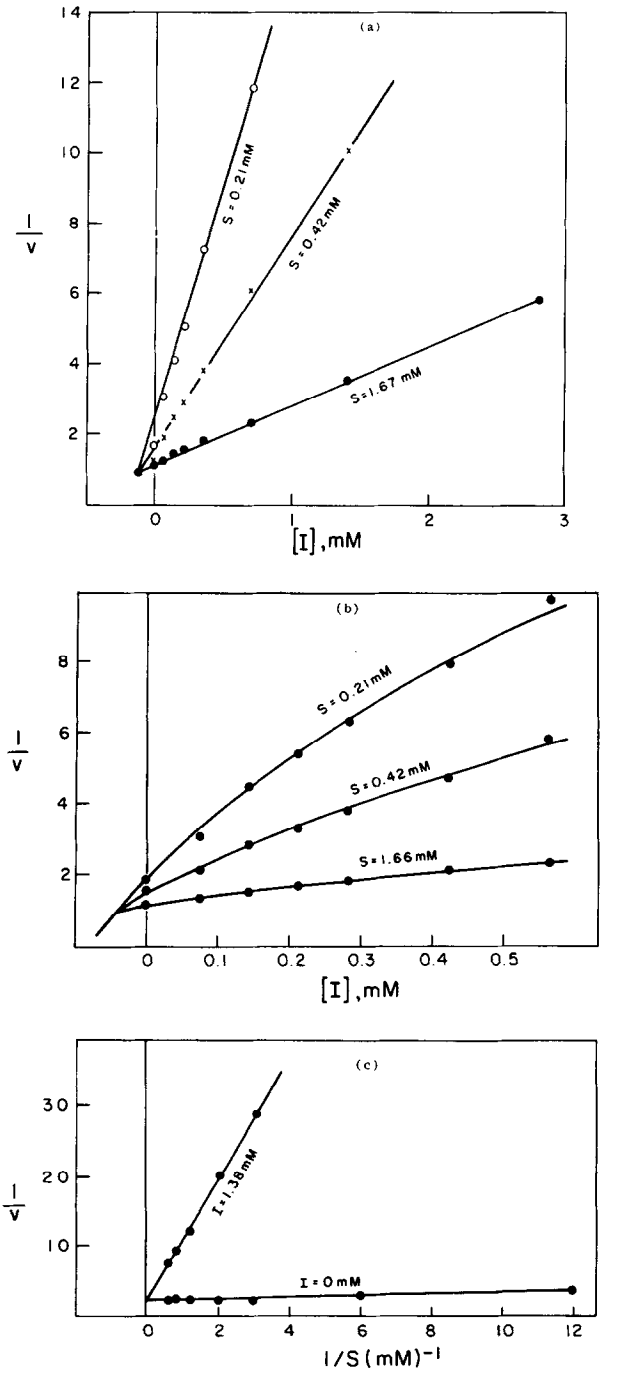

Fig. 3. Inhibition of E. coli β-D-galactosidase by D-galactal. (a) Plot of 1/v versus I. (b) Same as (a). The low inhibitor concentration region is expanded to show hyperbolicity. (c) Plot of 1/v versus 1/S.

Table 1. Inhibition of β-D-galactopyranosidases by D-galactal at 37°

Sources	Km (mM)	Ki (μM)	pH	Buffer	Method of analysis	Mode of Inhibition
A. niger	3.1	18	4.0	A[a]	1/v vs. I	C[c]
	3.3		"	"	1/v vs. 1/s	C
C. perfringens	1.7	85	6.3	CP[b]	1/v vs. I	C
E. coli	-	-	7.1	"	"	HC[d]
	0.13	51	"	"	1/v vs. 1/s	C
Pinto bean	2.0	90	4.0	"	1/v vs. I	C
Jack bean	-	-	"	"	"	HC
Almond emulsin		58%[e]	4.2	A	"	HC
Limpet		83%[f]	"	"	-	-

a) Sodium acetate. b) Sodium citrate-sodium phosphate. c) Competitive. d) Hyperbolic competitive. e) Percentage of inhibition at 0.66 mM D-galactal and 0.83 mM substrate. f) Percentage of inhibition at 1.0 mM D-galactal and 1.6 mM substrate.

Table 2. Effect of D-Galactal on Other Glycosidases

Enzymes	Substrate (mM)	pH	Buffer	D-Galactal (mM)	Inhibition (%)
Jack bean α-D-Mannosidase	0.91	4.0	CP[a]	0.91	1.2
Almond Emulsin α-D-Mannosidase	0.91	4.8	A[b]	1.76	3.7
C. perfringens β-N-acetyl-D-glucosaminidase	1.43	6.0	CP	14.3	5

a) Sodium citrate-sodium phosphate. b) Sodium acetate.

acetylglucosaminidase were not inhibited by D-galactal (Table 2). Although the list of glycosidases tested here is by no means exhaustive, these results suggest that the inhibitory action of D-galactal on β-galactosidases is highly stereospecific, and not due to indiscriminate

reactions of a double bond in the ring with the glycosidases.

Acid hydrolysis of glycosides is generally believed to proceed via a cyclic carbonium ion intermediate, IV or IVa (5, 6), which is expected to assume a half-chair conformation. An analogous situation seems to exist in the enzymatic hydrolysis of amino sugar oligosaccharides by egg-white lysozyme (7, 8). Recently, evidence has been presented to suggest the occurrence of a carbonium ion intermediate in the catalytic process (9). It it important to note, however, the binding of substrate to lysozyme requires the sugar ring at which cleavage of the glycosidic bond occurs to be in a half-chair conformation. In other words, distortion of a puckered ring (III) to a planar ring (IV) even precedes the presumed formation of a glycosyl carbonium ion.

It follows, then, that sugar analogs that prefer planar to chair conformations will be strongly bound to a glycosidase, if the enzyme under consideration follows a reaction mechanism similar to the one delineated for lysozyme. In such an event, competitive inhibition may be observed. Efficacy of such a sugar derivative as an inhibitor for a glycosidase can be rationalized by the fact that the energy needed for ring distortion (chair to half-chair) is not required when a planar sugar derivative is bound to the enzyme. This concept is supported by a report (10) that appeared while this work was in progress, in which the inhibitory action of aldono-(1→5)-lactones on glycosidases was explained on the basis of conformational flexibility of the lactones, and their preference for a half-chair conformation.

D-Galactal is expected to be in a half-chair conformation (II or IIa), if it behaves similarly to tri-O-acetyl-D-glucal. Nuclear magnetic resonance studies (11) showed that the latter compound probably exists in H1 conformation (corresponding to II). Currently, no data is available to demonstrate the preference of one of the two conformers (II or IIa) by D-galactal.

Although none of the β-galactosidases tested here has been studied as carefully as egg-white lysozyme with respect to enzymatic reaction mechanism and three dimensional structure, the fact that they were all effectively inhibited by D-galactal suggests that they probably have a common reaction mechanism, similar to that of lysozyme.

Obviously neither II nor IIa has exactly the same shape as the carbonium ion (IV) which is expected from a C1 conformer (III). However it is possible that the enzymes require only the overall planarity of the ring for binding.

ACKNOWLEDGEMENTS

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