Strong Inhibitory Effect of Furanoses and Sugar Lactones on β-Galactosidase of *Escherichia coli*†

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ABSTRACT: Various sugars and their lactones were tested for their inhibition of β-galactosidase (*Escherichia coli*). L-Ribose, which in the furanose form has a hydroxyl configuration similar to that of D-galactose at positions equivalent to the 3- and 4-positions of D-galactose, was a very strong inhibitor, and D-lyxose, which in the furanose form also resembles D-galactose, was a much better inhibitor than expected. Structural comparisons preclude the pyranose forms of these sugars from being significant contributors to the inhibition, and inhibition at different temperatures (at which there are different furanose concentrations) strongly supported the conclusion that the furanose form is inhibitory. Studies with sugar derivatives that can only be in the furanose form also supported the conclusion. This is the first report of the inhibitory effect of furanoses on β-galactosidase. Lactones were also inhibitory. Every lactone tested was much more inhibitory than was its parent sugar. D-Galactonolactone was especially good. Experiments indicated that it was D-galactono-1,5-lactone rather than D-galactono-1,4-lactone which was inhibitory. Inhibition of β-galactosidases from mammalian sources by lactones has been reported previously, but this is the first report of the effect on β-galactosidase from *E. coli*. Since furanoses in the envelope form are analogous (in some ways) to half-chair or sofa conformations and since lactones with six-membered rings probably have half-chair or sofa conformations, the results indicate that β-galactosidase probably destabilizes its substrate into a planar conformation of some type and that the galactose in the transition state may, therefore, also be quite planar. The study also showed that the primary hydroxymethyl group of sugars can be either equatorial or axial without significantly affecting binding.

Planar sugar derivatives have been found to be inhibitors of some glycosidases. For example, glycals are compounds that are more planar than the more common pyranose ring forms of sugars, and these glycals are often inhibitors of glycosidases (Lee, 1969; Reese et al., 1971; Schwartz et al., 1970). One glycal, D-galactal, was initially thought to be an inhibitor of β-galactosidase (EC 3.2.1.23) of *Escherichia coli* (Lee, 1969) but was later shown to be a substrate (Lehmann & Schroter, 1972; Wentworth & Wolfenden, 1974). Regardless of whether it is an inhibitor or substrate, it was found to bind very tightly to β-galactosidase, and the reason for this tight binding may be its planar structure. Sugar lactones have also been found to be inhibitors of several glycosidases including some β-galactosidases (Leaback, 1968; Levvy et al., 1962; Levvy & Snaith, 1972). The effect has only previously been shown in mammalian β-galactosidases. It has been implied, but not experimentally shown, that the same inhibitory effect of lactones also holds for β-galactosidase of *E. coli* (Wallenfels & Weil, 1972).

The suggestion has been made that the inhibition of the mammalian β-galactosidases by glycals and lactones is due to stereochemical and conformational similarities between these compounds and the transition state in the enzyme-catalyzed pyranoside hydrolysis (Levvy & Snaith, 1972). The inhibitory effect might, therefore, be explained by a resemblance of the planar structures to some type of a destabilized form of the substrate. The destabilized substrate conformation could be formed upon binding of substrate to enzyme (Jencks, 1975) in the process of forming the transition state.

This study was begun because we found that L-ribose was a particularly good inhibitor of β-galactosidase. From structural considerations, only the furanose form of L-ribose can be inhibitory. Studies with lactones followed because, in some ways (as shall be explained later), furanoses resemble lactones and because of the reports in the literature that showed that lactones inhibit mammalian β-galactosidases. This paper reports the results of the studies on both the furanose and the lactone inhibition.

Experimental Procedures

Materials. PNPG[1] and most of the sugars, sugar acids, sugar lactones, and other derivatives were from Sigma. L-Arabonic acid and D-mannono-γ-lactone were from Terrochem (Canada). Other chemicals were from Fisher or similar sources. All chemicals used were of the purest grades available.

β-Galactosidase was purified from *E. coli* strain ML-308 by a procedure similar to that of Brake et al. (1978). After purification only one band was seen by both SDS–polyacrylamide and native polyacrylamide gel electrophoresis, and in the case of the native gel, the activity was found to comigrate with the protein band. The enzyme was stored in the presence of 0.4% sodium azide to prevent bacterial growth and subsequent breakdown of enzyme.

Enzyme Assay. Enzyme activity was determined by measuring the rate of hydrolysis of PNPG by β-galactosidase (obtained from rate of absorbance change at 420 nm). The

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[1] Abbreviations: PNPG, *p*-nitrophenyl β-D-galactopyranoside; SDS, sodium dodecyl sulfate; TES, 2-[[2-hydroxy-1,1-bis(hydroxymethyl)-ethyl]amino]ethanesulfonic acid.

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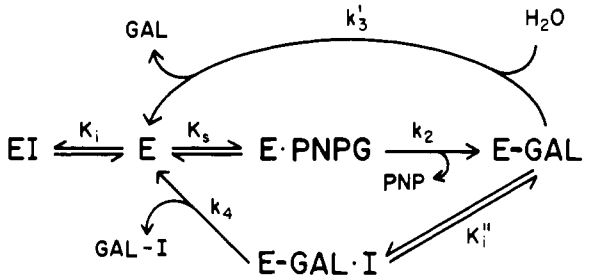
Scheme I: Probable Mechanism of Action of β-Galactosidase on PNPG in the Presence of Inhibitor[a]



[a] In this scheme, the dots between the symbols indicate that a complex of some kind, having a finite lifetime and made up of the constituents listed, probably exists. GAL, galactopyranose; I, inhibitor; PNP, *p*-nitrophenol; GAL–I, galactose–inhibitor product; E, β-galactosidase.

reaction was carried out at 25 °C in a 30 mM TES buffer, which contained 145 mM NaCl and 1 mM $MgSO_4$ at pH 7.0. One unit was defined as a rate of hydrolysis of 1 μmol of PNPG/min under the assay conditions described.

Inhibition Studies. The studies to be described concern binding to free enzyme. As reported earlier (Huber & Gaunt, 1983; Huber et al., 1984), this implies that binding is occurring with the "galactose" subsite since the "glucose" subsite is essentially inert to sugars in the free enzyme. We used the PNPG method described by Huber and Gaunt (1983) to determine K_i values. The K_i values represent dissociation constants of inhibitor complexes with free enzyme.

The kinetic mechanism shown in Scheme I (including transgalactosylic reactions) was assumed to hold. For PNPG, k_2 is rate limiting and thus

$$K_m = K_s \quad (1)$$

$$k_{cat} = k_2 \quad (2)$$

In the presence of inhibitors

$$\text{apparent } K_m = \frac{K_m\left(1 + \frac{[I]}{K_i}\right)\left(k'_3 + k_4\frac{[I]}{K''_i}\right)}{k'_3 + (k_2 + k_4)\frac{[I]}{K''_i}} \quad (3)$$

$$\text{apparent } k_{cat} = \frac{k_{cat}\left(k'_3 + k_4\frac{[I]}{K''_i}\right)}{k'_3 + (k_2 + k_4)\frac{[I]}{K''_i}} \quad (4)$$

and thus

$$\frac{\text{apparent } K_m}{\text{apparent } k_{cat}} = \frac{K_m}{k_{cat}}\left(1 + \frac{[I]}{K_i}\right) \quad (5)$$

Because the data necessary to calculate K_i from eq 5 can be generated by one experiment without inhibitor (which provides k_{cat} and K_m) and one with inhibitor (which provides apparent k_{cat} and apparent K_m), the determination is easily made from only two experiments. For accuracy, however, we used several inhibitor concentrations and averaged the resulting K_i values.

NMR Studies. ^{13}C NMR spectra were obtained for the aldopentoses and the amount of furanose present was calculated from the areas of peaks representing the anomeric carbons of the pyranose and furanose form (Angyal, 1969). D-Galactonic acid and D-galactonolactone were also analyzed by ^{13}C NMR to determine the contents of the various structures present. These analyses were done under several differing conditions. The amount of D-galactono-1,5-lactone present was estimated by quantitating ^{13}C NMR peaks analogous to those found with D-glucono-1,5-lactone.

Table I: K_i Values of Various Inhibitors of β-Galactosidase Assayed at pH 7.0 Unless Otherwise Indicated

sugars	K_i (mM)
L-ribose	0.21
D-lyxose	80
D-galactose	34
L-lyxose	180
D-arabinose	240
D-talose	12
L-allose	31
L-gulose	80
D-galactonic acid	12
D-galactonolactone	1.1
D-galactonolactone (assayed at pH 5.5)	0.74
D-galactonolactone (treated at pH 2.0 for 10 min—assayed at pH 5.5)	0.25
L-galactonolactone	6.0
D-mannuronolactone	14
D-mannono-γ-lactone	7.0
D-gluconolactone	2.5
D-glucuronolactone	2.4
1,2-isopropylidene-D-glucofuranose	34
D-ribose	170
D-ribonolactone	26
L-arabinose	190
5-deoxy-L-arabinose	32
L-arabonic acid	20
L-arabonolactone	9.1
epiinosose	3.0

Table II: Percentage of a Solution (1.0 M) of Pentoses That Are Present as the Furanose Form[a]

compd	temp (°C)	% furanose	K_i (mM)	K_i (mM furanose)
L-ribose	5	15.8	0.24	0.029
L-ribose	22	18.5	0.21	0.031
L-ribose	50	22.2	0.16	0.030
D-ribose	22	18.5		
L-lyxose	22	0.1		
D-lyxose	5	0.06	207	0.097
D-lyxose	22	0.10	80	0.084
D-lyxose	50	0.12	59	0.124
L-arabinose	22	3.0		
D-arabinose	22	3.0		
L-xylose	22	<0.1		
D-xylose	22	<0.1		

[a] In the case of L-ribose and D-lyxose, this was done at several temperatures. These evaluations were done by ^{13}C NMR. In the case of L-ribose and D-lyxose, the K_i values are also given for the different temperatures. For L-ribose and D-lyxose, K_i values were also calculated on the assumption that all of the inhibition was due to the furanose forms of these sugars. It is seen that when this was done, the K_i values were almost identical for each of these two sugars.

RESULTS

Inhibition by Pentoses and Hexoses. The K_i values (competitive inhibitor constants) of various pentoses and hexoses are listed in Table I. Note that L-ribose was a very good competitive inhibitor. The following compounds were not inhibitors: D-xylose, L-xylose, D-glucose, D-mannose, L-mannose, D-glucose, D-galactose 1-phosphate, L-galactose, and inositol.

Concentration of Furanoses in Solution. The percentages of the furanose forms of the aldopentoses, as obtained from NMR, are listed in Table II. A significant portion of L-ribose was in the furanose form (18.5% at 22 °C), but only a very small amount of D-lyxose was (0.1% at 22 °C). The concentrations of the furanose form of L-ribose and D-lyxose

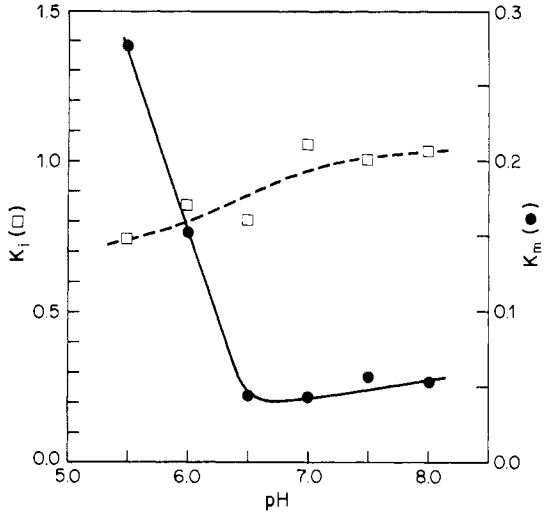


FIGURE 1: Inhibitory effect (K_i) of D-galactonolactone as a function of pH and K_m of PNPG as a function of pH.

increased with temperature. The effective K_i values of L-ribose and D-lyxose at the different temperature values are also listed in Table II. It is seen that the K_i values of L-ribose and D-lyxose decreased proportionally as the level of the furanose form increased. When K_i values were calculated assuming inhibition was from the furanose forms only, there was essentially no change in the inhibition constant as a function of temperature. These data are especially significant because the relative amount of change in furanose concentration as a function of temperature was quite different in the two sugars and yet the inhibition was still a function of the concentration of the furanose form in each case.

Inhibition by Lactones. Table I also shows the K_i values of various lactones and sugar acids. [L-Arabonic acid was converted to the lactone by heating in a boiling H_2O bath at pH 2.0 for 10 min according to the method of Levvy et al. (1962)]. D-Galactonolactone was an especially good inhibitor, but all lactones were much better inhibitors than were their parent sugars. Figure 1 shows that the inhibitory effect of D-galactonolactone increased as the pH dropped while there was a great decrease in the binding ability of PNPG (as gauged by the increased K_m values) at the lower end of the range.

Inhibition was also studied at pH 5.5 with D-galactonolactone that had been previously treated by heating at pH 2.0 for 2 min. The K_i value was about one-third of the value obtained without the treatment (Table I). Percentages of each of the lactone forms and of the acid form of D-galactonic acid present in solutions at several pH values (as calculated from ^{13}C NMR spectra in which the D-glucono-1,5-lactone ^{13}C NMR spectrum was used as a basis for identifying the D-galactono-1,5-lactone peaks) are listed in Table III.

Table III: Amounts of Two Types of Lactone and of Galactonic Acid Present in Various Solutions and Various pH As Determined by ^{13}C NMR

compd and pH	1,4-lactone (%)	1,5-lactone (%)	galactonic acid (%)
galactonic acid (pH 7.0)	undetectable	undetectable	100
galactonolactone (pH 7.0)	73	trace[a]	27
galactonolactone (pH 2.0)	>99.5	<0.5	undetectable
galactonolactone (boiled at pH 2.0 for 10 min)	91	9	undetectable
galactonolactone (boiled at pH 2.0 and then raised to pH 5.5)	90	trace[a]	10

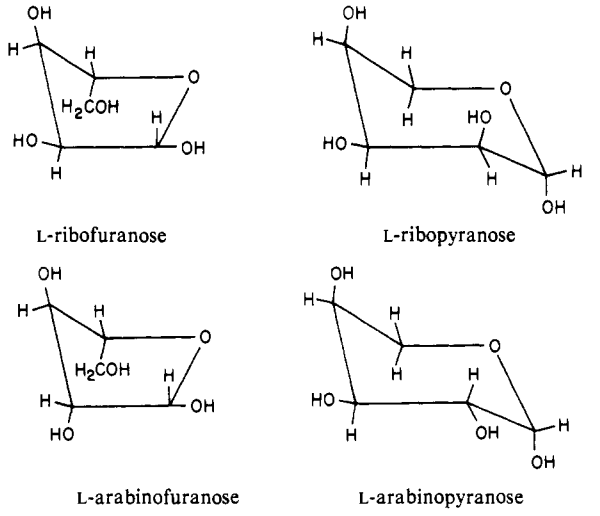
[a] There was about 3 times as much of the 1,5-lactone present in the galactonolactone boiled at pH 2 and then raised to pH 5.5 as there was in the pH 7.0 solution, but the amounts in both cases were so small that accurate quantitation was impossible.

Discussion

Although the sequence of β-galactosidase has been determined and some facts are known about groups at its active site (Fowler et al., 1978; Herrchen & Legler, 1984; Ring et al., 1985), its mechanism of action has not been firmly established. Fink and Angelides (1975) have shown that there is probably a transient intermediate galactosyl–enzyme form present at some time during the reaction, but evidence for both an S_N1 and an S_N2 reaction mechanism have been reported (Jones et al., 1977; Sinnott, 1978; Rosenberg & Kirsch, 1981). Our studies were initially concerned with the inhibition by L-ribose. The furanose forms of pentoses such as L-ribose seem to be predominantly of the envelope type with the 3-position carbon above the plane of the other four ring atoms (Brown & Levy, 1963; Groth & Hammer, 1968; Parthasarathy & Davis, 1967). This conformation, in some ways, resembles a half-chair or a sofa conformation. Because the 1,5 (δ) form of D-galactonolactone is probably a compound with a half-chair or a sofa conformation [inferred from crystallographic studies which show that D-glucono-1,5-lactone has a half-chair conformation (Hackert & Jacobson, 1969)], we also tested various lactones as inhibitors, and indeed, they were good inhibitors. The data presented in this paper suggest that a planar intermediate of some sort forms from the galactose moiety of the substrate molecule sometime during catalysis and that this planar form is tightly bound to the enzyme.

Furanose forms of monosaccharides rarely predominate (Shallenberger, 1982), but they do usually make up at least a small proportion of the molecules in solution. Two pentoses found to be of particular interest in this study were L-ribose and D-lyxose, because in both cases the K_i values were much lower than expected (Table I). With L-ribose, the furanose form makes up a significant proportion of the molecular population, but D-lyxose contains only very small amounts of the furanose form (Table II). The furanose forms of these sugars were most likely the inhibitory forms as shown by the following arguments.

(1) The pyranose form of L-ribose is similar in structure to

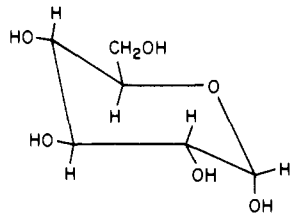


the pyranose form of L-arabinose, the only difference being the hydroxyl configuration at the 2-position [which does not significantly affect binding at the "galactose" site (Huber & Gaunt, 1983)]. However, L-arabinose (which is predominantly in the pyranose form) does not bind well (Table I), and

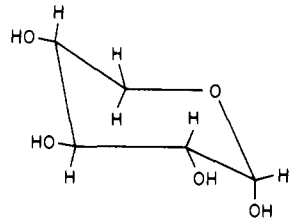
therefore, it follows that the pyranose form of L-ribose would also not bind well. The furanose form of L-ribose, however, looks more like D-galactose than does the furanose form of L-arabinose. This is especially true for hydroxyls 2 and 3 of L-ribose, which correspond to hydroxyls 3 and 4 of D-galactose [the important hydroxyls of D-galactose, in terms of binding (Huber & Gaunt, 1983)]. Thus, on this structural basis, the furanose form must be the inhibitory form.

(2) The inhibitory effect of L-ribose was found to increase with temperature to about the same extent as the percentage of the furanose form increased with temperature (Table II). When effective K_i values were determined on the basis of the amount of the furanose form present at the different temperatures, the effective K_i values were identical.

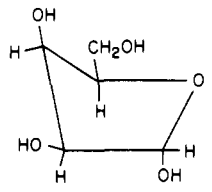
(3) The same arguments that have been made for L-ribose can also be made for D-lyxose. At first glance this may not



D-glucopyranose



D-lyxopyranose



D-lyxofuranose

seem to be the case because the inhibition by D-lyxose is not nearly as good as is the inhibition by L-ribose. However, a K_i of 80 mM is very low for a compound that in its very predominant pyranose form resembles D-glucose and that should thus bind very poorly to the "galactose" subsite (the K_i for glucose is <300 mM). In addition to having a pyranose structure that resembles D-glucose, D-lyxopyranose also lacks the 6-hydroxymethyl group that D-galactose has, a characteristic that also seriously decreases binding at the "galactose" site (Huber & Gaunt, 1983). The very small amount of the furanose form of D-lyxose (which resembles D-galactose) must, therefore, be responsible for the inhibition. Again, this was confirmed by the temperature studies. It is significant that the proportion change in the furanose form was much greater in the case of D-lyxose than for L-ribose and yet the effect as a function of the furanose concentration was the same.

(4) D-Ribose, L-arabinose, and D-arabinose all bind to the active site to a certain extent. In every case there are significant amounts of the furanose form present, but since the configuration of these furanose forms is not similar to that of D-galactose, the inhibitory effect was not nearly as dramatic as it was for L-ribose and D-lyxose. D-Xylose and L-xylose were very poor inhibitors. There is very little of the furanose form present for these two sugars (Table II), and the furanose forms do not resemble D-galactose. Thus, inhibition would be expected to be poor. L-Lyxose gave anomalous results in that the binding was somewhat better than expected for a compound with very little furanose present and with a structure that does not resemble D-galactose.

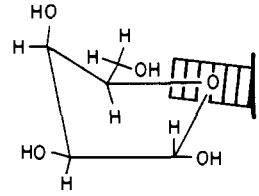
(5) 1,2-Isopropylidine-D-glucofuranose was a much better inhibitor than D-glucose itself despite its bulkiness and the fact that glucose binds very poorly to free β-galactosidase. The furanose conformation must be responsible for this.

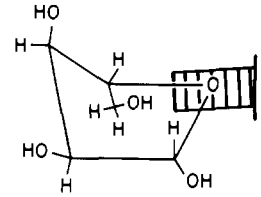
(6) 5-Deoxy-L-arabinose (which can only accommodate a furanose configuration) was a very good inhibitor relative to L-arabinose.

(7) Finally, L-glucose was a much better inhibitor than expected. In its furanose form it looks (in some ways) like the furanose form of D-lyxose.

All of these facts, taken together, show that the furanose forms of L-ribose and D-lyxose probably were the inhibitory forms. If indeed these arguments hold true and if one takes into account the proportion of L-ribose molecules that have the furanose form (~18%), a true K_i value for the furanose form of L-ribose would be about 0.03 mM and the true K_i value for the furanose form of D-lyxose (accounting for about 0.1% of the total population) would be about 0.10 mM.

If, as the evidence indicates, the furanose conformations of L-ribose and D-lyxose are indeed the inhibitory forms, then the axial as compared to the equatorial configuration of the 5-hydoxymethyl group must not greatly alter the binding. This may be reasonable because analysis of models shows that one





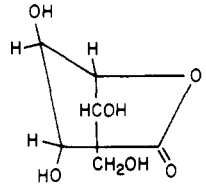
can align the hydroxyl group of either an axial or an equatorial hydroxymethyl group such that they could form the same H bond with a single group on the enzyme (with only a slight change of the angle of linkage to the complementary group on the enzyme).

The K_i values for L-allose and D-talose (31 and 12 mM, respectively) support the conclusion that the configuration of the primary hydroxymethyl group is not important for binding. The only difference in the pyranose configurations (the most likely inhibitory forms of L-allose and D-talose) is that the 6-hydroxymethyl group is axial for L-allose but equatorial for D-talose. Despite this significant difference, the binding, although changed, is not as dramatically altered as would be expected if this difference were important.

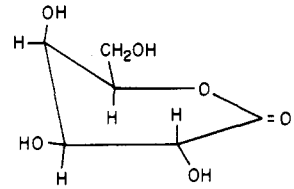
Although inhibition by lactones has been established in β-galactosidase from mammalian systems, this is the first time that it has been reported for β-galactosidase from *E. coli*. Table I shows that in every case tested the lactones of sugars were much better inhibitirs than were the parent sugars. In many cases when there was essentially no inhibition by the parent sugars, the lactones were quite good inhibitors [e.g., compare the K_i of D-gluconolactone to that of D-glucose (>300 mM)].

The inhibition of D-galactonolactone was dependent on the pH. As the pH was decreased to pH 5.5 (the lowest pH that did not cause irreversible denaturation of β-galactosidase), the binding increased (see the lowering of K_i in Figure 2) despite the fact that the binding of substrate to the "galactose" site (as reflected by increased K_m values for PNPG) decreased dramatically at this low pH. Lactones are more stable at low pH than at neutral pH (Levvy et al., 1962), and thus, the relatively increased inhibitory effect is probably due to the increased amount of lactone present at the lower pH. This suggests that the true inhibitory effect of the lactones is really greater than is implied from the studies at pH 7.0.

It would seem, from the structures shown, that the D-galactono-1,5-lactone form of D-galactonolactone is much more likely to be the inhibitory form than the 1,4 (γ) isomer, because



D-galactono-1,4-lactone



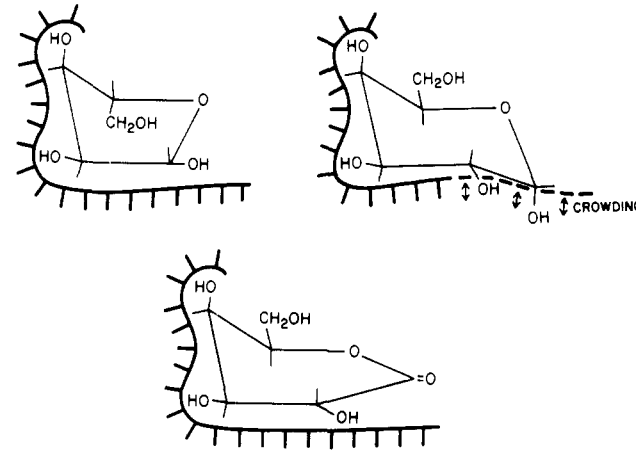
D-galactono-1,5-lactone

the important 2-position hydroxyl group is in an orientation that prevents binding (Huber & Gaunt, 1983), but in solution, at neutral or slightly acidic pHs, there are only trace amounts of it present [Table III and see Levvy et al. (1962)]. If it is the inhibitory form, it is obviously a very good inhibitor. Conchie et al. (1967) have proposed that in every case where the lactones are inhibitors it is caused by the 1,5-lactone and any apparent inhibition by a 1,4 (γ) form is only from the fact that some 1,5-lactone is in equilibrium with it. The data of this study support the contention that the 1,4 (γ) form of D-galactonolactone is not inhibitory for β-galactosidase from *E. coli* while the 1,5 (δ) form is. At pH 7.0 the 1,4 (γ) form predominated while in D-galactonic acid there was no trace of the 1,4 (γ) form. Yet D-galactonic acid was inhibitory [the inhibition was only decreased about 11-fold relative to when the 1,4 (γ) form was present in large amounts]. The 1,4 (γ) form cannot, therefore, be the strongly inhibitory form as the inhibition would have been totally eliminated if it were the inhibitory component. Because of the negative charge of D-galactonic acid (Huber & Gaunt (1982) (note also that galactose 1-phosphate has no inhibitory effect) and because of its linear structure (Huber & Gaunt, 1983), it is highly unlikely that D-galactonic acid could be inhibitory. It follows then that the 1,5 (δ) form must be the inhibitory form and that it must be a very strong inhibitor. The 1,5 (γ) form was undetectable in D-galactonic acid, but since it was present in D-galactonolactone at pH 7.0 in amounts that could (although just barely) be detected (see Table III) and yet presumably caused such good inhibition, it could be present in amounts that cannot be detected at all in D-galactonic acid and still bring about the inhibition (which in that case was 11-fold poorer). Other evidence for the fact that the 1,5-lactone was the inhibitory form comes from the inhibition studies using D-galactonolactone that was heated first in a boiling H_2O bath at pH 2.0 for 10 min. This treatment causes the production of more D-galactono-1,5-lactone (Levvy et al., 1962). This boiled solution was tested by NMR at pH 2.0 and at pH 5.5. At pH 2.0 there was a significant amount of the 1,5 (δ) form present (Table III), but because of instability, there was much less at pH 5.5. Nevertheless, the solution that was treated had more 1,5-lactone at pH 5.5 than the untreated lactone (from sizes of NMR peaks), and there was a significant increase (3-fold) in the inhibitory effect as a result of this (Table I). These studies, therefore, are in agreement with the suggestion that it is the 1,5 (δ) form of the lactone that is inhibitory.

epi-Inosose (2,3,4,6/5-pentahydroxycyclohexanone) was also a good inhibitor. This is especially true when compared to *myo*-inositol, its parent compound. Because of its carbonyl group, it is likely to have at least a partial planar (possibly half-chair or sofa) structure.

Very little definitive information on the conformation of lactones is available. X-ray crystallography (Hackett & Jacobson, 1969) of D-glucono-1,5-lactone has shown that in crystals this lactone has a half-chair conformation. Fersht (1977) has suggested that the structure might be in a sofa conformation. Both of these ideas are reasonable on structural grounds, and one would also expect it for other 1,5-lactones (including D-galactono-1,5-lactone). Half-chair and sofa structures have similarities to the envelope structure of furanoses. In the half-chair, four adjoining ring atoms are coplanar while one of the other two ring atoms is above and the other is below the plane while for the sofa conformation five adjoining ring atoms are coplanar while one ring atom is either above or below the plane of the ring. In the envelope [which seems to be the preferred conformation for pentose furanoses (Brown & Levy, 1963; Groth & Hammer, 1968; Parthasarathy & Davis, 1967)] four atoms are coplanar while the other one is either above or below the plane (Schallenberger, 1982) of the four coplanar atoms.

Marshall et al. (1977) have shown that crowding of the primary hydroxymethyl group of sugars is not important in the mechanism of β-galactosidase (*E. coli*); our studies showing that the configuration of the primary hydroxymethyl group is not of great importance for binding (see above) support this. However, since furanoses, lactones, and, as was shown earlier, D-galactal (Lee, 1969; Wentworth & Wolfenden, 1974) do bind much better than do the equivalent pyranoses, there must be some steric hindrance on the substrate that is relieved for these inhibitory structures. The steric hindrance could be near to the anomeric carbon position of D-galactose, and molecules in which an equivalent group is missing (such as in the furanoses) or which are somewhat planar, such as the lactones and galactal, thus bind better. A possible model might be



The furanoses, lactones, and glycals may be transition-state analogues. Some enzymes destabilize their substrate so that the substrate takes on the conformation of the transition state (Jencks, 1975). In those cases binding of substrate is quite poor, but then, because of the destabilization, the reaction readily goes to a stabilized transition state and then to product (Jencks, 1975). Jones et al. (1977) have presented kinetic evidence suggesting that the destabilization of the substrate in the enzyme–substrate complex of β-galactosidase (*E. coli*) can account for energy differences of 14–16 kcal/mol for β-galactosidase and that this destabilization is the main source of the catalytic power of β-galactosidase. The destabilization could be as we have suggested. Lysozyme is another glycohydralase for which destabilization of this type has been proposed (Blake et al., 1967; Vernon, 1967; Secemski & Lienhard, 1973). However, some workers (Warshel & Levitt, 1976; Schindler et al., 1977) have concluded from their studies that the sugar bound at the active site of lysozyme is undistorted. In this context it must be remembered that β-galactosidase is a much different enzyme since it is much larger and acts on much smaller substrates than does lysozyme.

A cautionary statement should be made. The carbonyl group of the lactone ring probably has at least some positive

character (Ferscht, 1977). Thus, part of the good binding capacity of lactones to β-galactosidase could be due to an ionic effect, where a carboxyl group at the active site [such as that suggested by Herrchen and Legler (1984)] forms an ionic bond to the partially positively charged lactone. Positively charged groups at the anomeric end of sugars do indeed enhance binding to β-galactosidase (Huber & Gaunt, 1982). We feel, however, that since the lactone positive charge is only partial, the effect would not be too great. Also, a positive charge would not explain the furanose results. The significant inhibitory effect found here must, therefore, be mainly a result of the nature of the conformation of the structues of the inhibitors.

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