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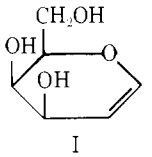
Slow Binding of D-Galactal, a "Reversible" Inhibitor of Bacterial β-Galactosidase†

David F. Wentworth and Richard Wolfenden*

ABSTRACT: The inhibition of bacterial β-galactosidase by D-galactal was found to be reversible and time dependent. Rate constants observed for binding (2.7×10^2 sec^{-1} M^{-1}) and release (4.6×10^{-3} sec^{-1}) of galactal were found to be consistent with an apparent K_i of 1.4×10^{-5} M for D-galactal as a competitive inhibitor in imidazole-HCl buffer (0.1 M, pH 7.0) at 25°. Efforts to trap and analyze the galactal–enzyme complex were unsuccessful. The rate of formation of this complex was, however, found to be markedly reduced in the presence of deuterium oxide; substitution of deuterium oxide for solvent water also raised the apparent K_i for galactal by a factor of 2.1. The conventional competitive inhibitor isopropyl β-D-thiogalactoside, in contrast, showed no detectable lag in binding or release, and its K_i was not appreciably affected by the substitution of D_2O for solvent water. The rate of release of galactal can be largely accounted for by the rate of its enzymatic conversion to 2-deoxygalactose.

The effectiveness of D-galactal (I) as an inhibitor of β-galactosidase from *Escherichia coli* has been attributed to its resemblance to a half-chair intermediate (II) which may be formed during substrate transformation (Lee, 1969). A



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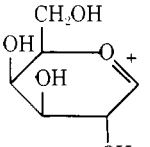
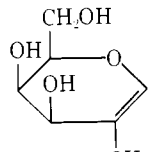


II

difficulty with this interpretation is that the position of unsaturation in galactal differs from that in the oxonium form of the carbonium ion, so that ring substituents are arranged differently with respect to coplanar atoms of the ring (Levvy and Snaith, 1972).

An alternative possibility would appear to be that substrate transformation may proceed by an elimination–addition mechanism, involving an unstable intermediate (III)



III

which resembles D-galactal in structure. To the extent that the enzyme stabilizes such an intermediate during catalysis, an analog of this intermediate might be expected to be tightly bound.

A third possibility is that a nucleophilic group on the enzyme may participate in a double displacement reaction, forming a covalent galactosyl–enzyme intermediate (Wallenfels and Malhotra, 1961; Viratelle *et al.*, 1969; Stokes and Wilson, 1972; Sinnott and Viratelle, 1973; Sinnott and Souchard, 1973). Galactal might form a covalent adduct resembling this intermediate.

The present studies were undertaken in order to provide further information about the mechanism of inhibition of β-galactosidase by D-galactal, and to attempt to distinguish between these alternatives if possible.

Materials and Methods

β-Galactosidase from *Escherichia coli* was purchased from Worthington as a crystalline suspension in 2.5 M ammonium sulfate. The enzyme was used after dialysis against a solution containing 0.1 M imidazole-HCl buffer (pH 7.0), 0.145 M NaCl, and 1 mM $MgSO_4$. D-Galactal, obtained from Raylo Chemicals Ltd., was recrystallized three times from ethyl acetate, mp 91–93° (lit. mp 104°, Overend *et al.* 1950). Isopropyl β-D-thiogalactoside (IPTG),[1] *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid, 2-deoxy-D-galactose, and β-galactose dehydrogenase (from *Pseudomonas fluorescens*) were obtained from Sigma Chemical

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[1] Abbreviations used are: IPTG, isopropyl β-D-thiogalactoside; ONPG, *o*-nitrophenyl β-D-galactoside.

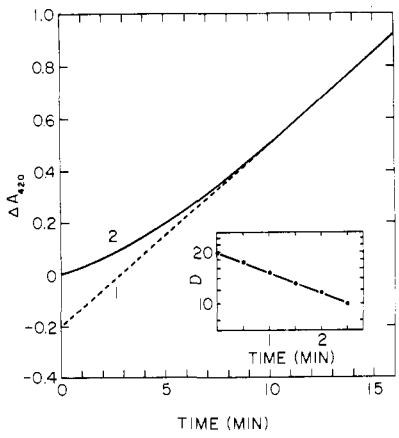


FIGURE 1: Time-dependent release of D-galactal. Enzyme (0.25 mg/ml) was preincubated with 1×10^{-4} M D-galactal in a standard salts solution (pH 7.0) and then diluted 1000-fold into assay solution. The lag phase is emphasized here. The dashed line (line 1) is extrapolated from much later parts of the time course of ONPG hydrolysis (not shown) which extended beyond 20 min and showed no detectable deviation from linearity after 10 min. The vertical distance between lines 1 and 2 is proportional to the amount of enzyme remaining in the inhibited state. k^{off} was calculated from a semilogarithmic plot (insert) of this vertical distance (D) *vs.* time.

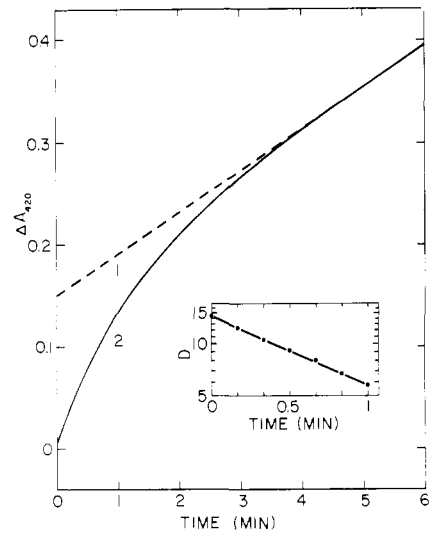


FIGURE 2: Time-dependent galactal inhibition of enzymic ONPG hydrolysis at 0.6 mM D-galactal and 1.0 μg/ml of enzyme. The burst phase is emphasized here. Line 1 is extrapolated back from much later parts of the time course (not shown) which extended beyond 10 min and showed no detectable deviation from linearity after 5 min. The vertical distance between lines 1 and 2 is proportional to the difference between the activity remaining at time t and the amount of activity remaining after establishment of the steady state. A semilogarithmic plot (insert) of this vertical distance (D) *vs.* time was used to determine k^{app}, the rate constant for the approach to the steady state, at the corresponding galactal concentration.

Co.; *o*-nitrophenyl β-D-galactoside (ONPG) was from Calbiochem; deuterium oxide (99.7%) was from Merck Sharp & Dohme of Canada Ltd.; and tritiated water was from International Chemical & Nuclear Corp.

ONPG hydrolysis was followed by measuring the appearance of *o*-nitrophenol at 420 nm with a Perkin-Elmer recording spectrophotometer. The assay solution contained, unless otherwise stated, 100 μmol of imidazole-HCl buffer (pH 7.0), 1 μmol of $MgSO_4$, 145 μmol of NaCl, 2 μmol of ONPG, and 0.5 μg of enzyme in a total volume of 1 ml. Imidazole-HCl was used in order to avoid the use of buffers containing hydroxyl groups which can act as galactosyl acceptors in the hydrolysis of ONPG (Wallenfels and Weil, 1972); 1.0 mM $MgSO_4$ and 0.145 M NaCl are saturating concentrations for the activating metal ions at pH 7.0 (Tenu *et al.*, 1971). Assay solution containing no ONPG is referred to as a *standard salts solution* in the text. Cuvets of 1-cm light path were used. Kinetic experiments were routinely performed at 25°. pH measurements were made with a Corning Model 7 meter with a glass electrode. pD measurements were made by adding 0.40 to the pH meter reading (Glasoe and Long, 1960).

The pre-steady-state kinetic curves (bursts or lags) were analyzed graphically by a method described by Fridovich (1968). Figure 1 shows the observed initial rate of reaction, using enzyme preincubated with inhibitor and then diluted into the assay solution. The final steady-state rate of ONPG hydrolysis is extrapolated to zero time (dashed line) whereas the solid line is the course of enzyme activation. If the enzyme is simply being converted from an inhibited to an active state, then the vertical distance between the solid and dashed lines at any time is proportional to the amount of enzyme remaining in the inhibited state. If this occurs with first-order kinetics, a plot of log vertical distance *vs.* time will be linear and the rate constant can be determined.

The conversion of D-galactal to 2-deoxy-β-D-galactose by β-galactosidase was followed in a coupled assay, in which β-galactose dehydrogenase was the auxiliary enzyme, by measuring the appearance of NADH at 340 nm (α_M = 6220) with a Zeiss PMQ II spectrophotometer equipped with a Sargent-Welch recorder and a Heath voltage reference source. The assay solution was the standard salts solution containing 1 μmol of NAD^+, 0.1 mg of β-galactose dehydrogenase, and 1.2 mg of β-galactosidase in a total volume of 1 ml. Under these conditions the assay solution was shown to contain 0.45 international unit of β-galactose dehydrogenase expressed with respect to its action on 2-deoxy-D-galactose, which showed an apparent K_m of 5.9×10^{-3} M under the above conditions. The concentration of auxiliary enzyme was shown to be in excess by varying its concentration above and below the concentration routinely employed. The galactal sample was found to contain an impurity (less than 1%) which served as a substrate for β-galactose dehydrogenase. Before the addition of β-galactosidase, galactal was therefore preincubated with β-galactose dehydrogenase in the assay solution until the appearance of NADH could no longer be detected.

Results

When β-galactosidase was preincubated with D-galactal, and the mixture was then diluted into an assay solution containing the substrate, ONPG hydrolysis was found to proceed at a constant rate after a pronounced lag period. Typical results are shown in Figure 1, where a sample of enzyme containing 1×10^{-4} M galactal (approximately $6.7 \times K_i$) was diluted 1000-fold upon addition to the assay solution, so that the eventual breakdown of the enzyme–inhibitor complex was virtually complete. The release from inhibition was consistently observed to proceed with first-order kinetics (Figure 1), indicating a rate constant (k^{off}) of galactal release of 4.6×10^{-3} sec^{-1}.

The onset of galactal inhibition was also observed to be slow. When enzyme was added to an assay mixture containing D-galactal, a linear rate of ONPG hydrolysis was achieved only after a burst of activity (a lag in galactal inhibition) as shown in Figure 2. Semilogarithmic plots of the

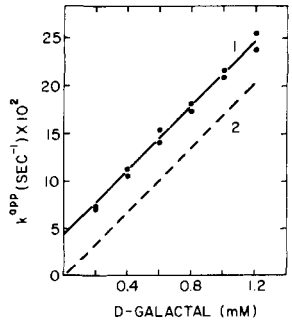


FIGURE 3: Determination of k^{on}, the galactal on-rate constant. Curve 1 is k^{app}, the pseudo-first-order rate constant for the approach to the steady state, as a function of galactal concentration. Curve 2 is curve 1 corrected for the contribution of k^{off}, the rate constant for galactal release. k^{on} was obtained by correcting the slope of curve 2 for substrate competition by use of eq 3.

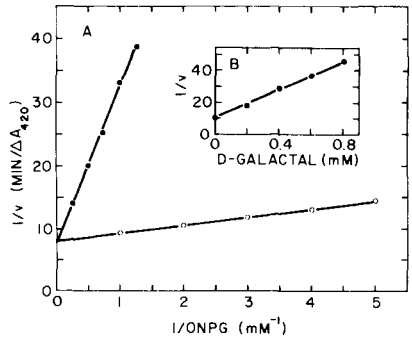


FIGURE 4: Steady-state galactal inhibition of ONPG hydrolysis. Linear rates achieved after an initial burst phase were used when the assay solution contained galactal. The reactions were initiated by the addition of enzyme. (A) Determination of K_i for D-galactal in H_2O (pH 7.0). Reciprocal plots in the absence (○) and in the presence of 0.25 mM D-galactal (●). (B) Test for nonlinear inhibition. $1/v$ is in units of min/ΔA_{420}. The fixed level of ONPG was 2 mM. Each point is the average of four determinations.

approach to steady state were linear (Figure 2), yielding apparent first-order rate constants (k^{app}). These apparent rate constants were found to vary with galactal concentration as shown in Figure 3.

Because galactal binding is reversible (eq 1), k^{app}, the pseudo-first order rate constant for *approach to equilibrium* of the inhibited enzyme with free enzyme (eq 2), includes terms for both binding and release of the inhibitor (Gutfreund, 1955). When the observed rate constant (k^{off} = 4.6 × 10^{-3} sec^{-1}) for galactal release was subtracted from the values of curve 1 in Figure 3, curve 2 was obtained, passing nearly through the origin.

$$E + I \underset{k^{off}}{\overset{k^{on}}{\rightleftharpoons}} EI \quad (1)$$

$$k^{app} = k^{on}(I) + k^{off} \quad (2)$$

The second-order rate constant for galactal binding obtained from the slope of curve 2 (Figure 3) required further correction for competitive binding of substrate present in these experiments, approximately 1 order of magnitude above K_m. Assuming that equilibrium of substrate binding was established rapidly in comparison with galactal binding, the apparent second-order rate constant was multiplied by the factor $(1 + S)/K_m$ in order to obtain a second-order rate constant, 2.7 × 10^2 sec^{-1} M^{-1}, for galactal binding in the absence of substrate. Thus k^{on} can be calculated as

$$k^{on} = \frac{(k^{app} - k^{off})}{(I)}(1 + S/K_m) \quad (3)$$

K_i for galactal was determined under steady-state conditions by determining the rate of ONPG hydrolysis after an initial burst period in the presence of galactal. The final steady-state rates, shown in the double reciprocal plot of Figure 4A, indicated competitive inhibition, with K_i = 1.4 × 10^{-5} M, in reasonable agreement with the K_i (1.7 × 10^{-5} M) calculated from the ratio of the rate constants (k^{off}/k^{on}). When ONPG was maintained at a fixed level and the galactal concentration varied, the plot of $1/v$ *vs.* (I) (Figure 4B), where v is the final steady-state velocity, indicated linear inhibition (Cleland, 1970).

The inhibition of β-galactosidase by 2-deoxy-D-galactose could not be detected, even at a concentration of 0.1 M, when the fixed level of ONPG was 1 mM.

Covalent reaction of galactal with the enzyme was considered as a possible basis for slow binding and release. If the enzyme were to add at the position of unsaturation, tritium might be expected to be incorporated from solvent directly (or through prior addition to a nucleophilic group on the enzyme) into the adduct. In an effort to trap such a covalent derivative, enzyme (1 mg) was incubated for 30 min in a standard salts solution, 0.5 ml, pH 7.0, containing tritiated water (10 mCi) in the presence of 1 mM D-galactal and in its absence. The control and test samples were diluted twofold with 10 M urea and then dialyzed against 50 ml of an 8 M urea solution containing 0.1 M imidazole buffer (pH 7.0), 0.01 M NaCl, 1 mM EDTA, and 0.01 M β-mercaptoethanol. After nine changes of 8 M urea solution, practically no tritium (less than 0.1 equiv per active site) remained in the dialysis sac containing test solution.

In view of the possible lability of the covalent adduct in urea, evidence for its formation was sought by examining galactal inhibition in deuterium oxide. If a C–D bond were formed in the enzyme–galactal complex, one might expect to observe a deuterium isotope effect on the rate and equilibrium of its formation. Before testing for such effects, it was first established that k^{app} values showed little variation over the pH range 6.2–7.8 (Table I). It was therefore considered unlikely that a possible isotope effect observed at pD 7.0 would be due to the effect of a possible inequality between pD and pH, or to a shift in the state of ionization of enzyme residues involved in galactal binding. K_i values obtained in D_2O (pD 7.0) and in H_2O (pH 7.0) (Figure 4), are compared in Table II, and show a substantial isotope effect, with K_i (D_2O)/K_i (H_2O) = 2.07.

The possibility of an isotope effect on the rate constant for galactal release was next examined. Enzyme (0.25 mg/

TABLE I: Effect of pH on the Apparent Rate of Binding of Galactal.[a]

	pH				
	6.2	6.6	7.0	7.4	7.8
k^{app} (sec^{-1})	0.033	0.033	0.031	0.035	0.030
	0.031	0.036	0.033	0.033	0.029
	0.036	0.033	0.035	0.033	0.030
	0.035	0.033		0.031	0.029

[a] Assay solution contained 1 mM D-galactal and 1 μg/ml of enzyme.

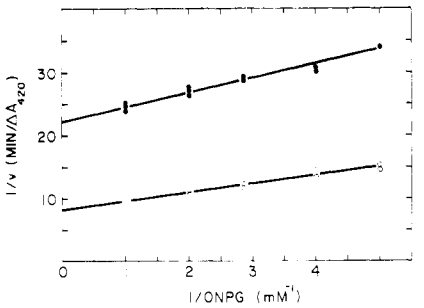


FIGURE 5: Observed kinetic behavior of ONPG in H_2O (pH 7.0) (○) and in D_2O (pD 7.0) (●). On the basis of the observed change in absorbance per min, $V_{max}(D_2O)/V_{max}(H_2O)$ was 0.37. The absorbance of *o*-nitrophenol at 420 nm, pH 7.0, arises primarily from its ionized form (Lederberg, 1950) and the pK_a of *o*-nitophenol, normally 7.17 (Kortum *et al.*, 1961), is expected to shift upward in D_2O (Bunton and Shiner, 1961). When hydrolysis of ONPG was carried to completion in the presence of enzyme at pD 7.0 and at pH 7.0 in assay solution under parallel conditions, the ratio $\Delta A_{420}(H_2O)/\Delta A_{420}(D_2O)$ was found to be 2.05. When this correction factor was applied to the observed rates of absorbance change shown here, corrected $V_{max}(D_2O)/V_{max}(H_2O)$ was found to be 0.75.

ml) was preincubated with D-galactal for 30 min in a standard salts solution, pD 7.0, containing D_2O as solvent, and then diluted 1000-fold into an assay solution containing H_2O as solvent, so that the final concentration of galactal (1×10^{-7} M) was less than $0.01 \times K_i$. A small isotope effect, $k(H_2O)/k(D_2O) = 1.14$, was observed when the rate of galactal release so obtained was compared with the rate of release after preincubation in water under parallel conditions (Table III).

In order to test for a possible isotope effect on the rate of galactal binding, it was necessary to determine the extent to which the K_m for ONPG changes in D_2O, to permit correction for substrate competition. $K_m(D_2O)/K_m(H_2O)$ was found to be 0.62 as indicated by double reciprocal plots of data such as those shown in Figure 5. The decrease of K_m in D_2O appears to be consistent with an earlier suggestion that the K_m for ONPG is a kinetic rather than an equilibrium constant (Viratelle *et al.*, 1969).

The possibility of an isotope effect on k^{on} was explored by determining k^{app}, the rate constant for the approach to the steady state, in H_2O and in D_2O. Values of k^{app} are compared in Table IV. Galactal on-rate constants were calculated by correcting the k^{app} values for the contribution of the off-rate constants and for substrate competition by use of eq 3. The ratio of $k^{on}(H_2O)/k^{on}(D_2O)$ was 2.47.

K_i values can be calculated from the ratio of rate constants, k^{off}/k^{on}. By this means $K_i(D_2O)/K_i(H_2O)$ was found to be 2.14, in good agreement with the isotope effect (2.07) calculated from the ratio of K_i values determined directly from results obtained in the steady state.

An enzyme conformation change, mediated by the exchange of deuterium into enzyme, was considered as a possible source of the isotope effect. The influence of slow deuterium exchange was examined by incubating enzyme for various time periods in a standard salts solution containing D_2O as solvent and then diluting this mixture into an assay solution containing H_2O as solvent. Over a period of 4 hr of incubation in D_2O, there was no change in initial velocities observed at 0.50 and 2.0 mM ONPG. The presence of deuterium in the enzyme, at positions where deuterium exchanges slowly, does not appear to affect the kinetic parameters K_m and V_{max}.

The possibility was also considered that the observed isotope effects might arise from an enzyme conformation change mediated by deuterium at positions where it exchanges rapidly. If either fast or slow deuterium exchange were responsible for the observed isotoped effects, it seems likely that if the enzyme were preincubated in D_2O solvent, this effect might be reflected in the K_i value of an inhibitor such as isopropyl β-D-thiogalactoside (IPTG). A comparison of K_i values determined in D_2O (pD 7.0) with enzyme preincubated over a period of 5 hr in a standard salts solution containing D_2O as solvent, and in H_2O (pH 7.0) with enzyme which had not been exposed to D_2O, showed no significant difference. A K_i value of 1.1×10^{-4} M was observed in both cases.

In contrast to D-galactal, IPTG was found to be a conventional competitive inhibitor, and showed no detectable lag in binding or release.

β-Galactosidase has recently been reported to *catalyze the addition of water to* D-*galactal,* although at a rate many orders of magnitude slower than galactoside hydrolysis (Lehmann and Schroter, 1972; Wallenfels and Weil, 1972). The rate constant, k^{off}, observed in our experiments might therefore be the sum of rate constants for release

TABLE III: Effect of Preincubation in D_2O on k^{off}.

k^{off} (sec^{-1})	
H_2O	D_2O
0.0045	0.0041
0.0045	0.0040
0.0046	0.0040
0.0046	0.0040

TABLE II: Effect of D_2O on K_i (D-Galactal).

K_i (D_2O)[a] (M)	K_i (H_2O) (M)
2.8×10^{-5} [b]	1.34×10^{-5} [c]
3.0×10^{-5} [c]	1.46×10^{-5} [c]

[a] Enzyme was preincubated in a standard salts solution containing D_2O as solvent. [b] 0.5 mM D-galactal. [c] 0.25 mM D-galactal.

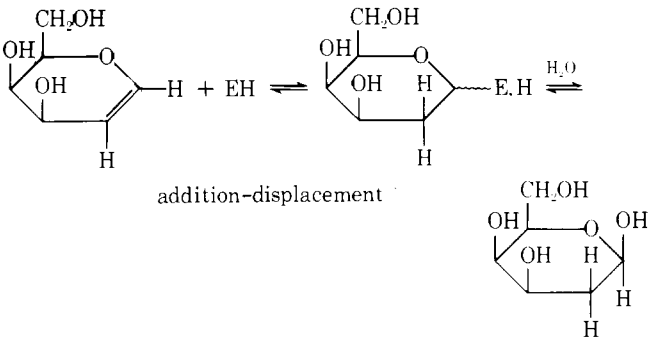
TABLE IV: Effect of D_2O on k^{app}.[a]

k^{app} (sec^{-1})	
H_2O[b]	D_2O[c,d]
0.020	0.0089
0.020	0.0076
0.020	0.0078
0.019	0.0082
0.020	0.0080
	0.0082
	0.0081
	0.0077

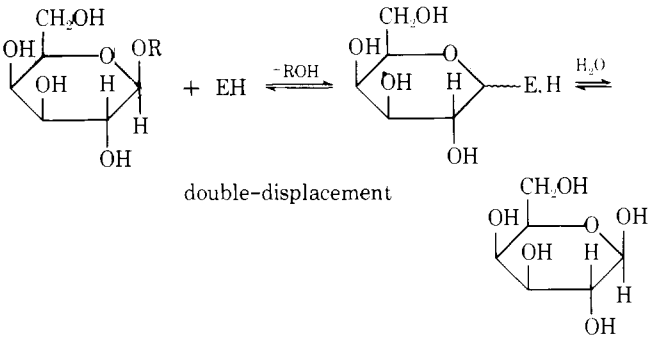
[a] Assay solution contained 1 mM D-galactal and 1 μg/ml of enzyme. [b] pH 7.0. [c] pD 7.0. [d] Enzyme was preincubated in a standard salts solution containing D_2O as solvent.

from inhibition by two different routes: release of galactal *per se* and conversion to the product 2-deoxy-D-galactose. To what extent does the rate of conversion to product contribute to k^{off}? V_{max} for galactal hydration by β-galactosidase was determined in a coupled assay in which β-galactose dehydrogenase (EC 1.1.1.48) from *Pseudomonas fluorescens* was used to indicate the rate of formation of 2-deoxy-β-D-galactose. β-Galactose dehydrogenase has been shown to be specific for the β-D-galactopyranose homologs of galactose, the C-1 hydroxyl group being required for oxidation to the corresponding D-galactonolactone (Wallenfels and Kurz, 1962; Blachnitzky *et al.*, 1974). β-Galactose dehydrogenase, used in the coupled assay, was shown to be nonlimiting by varying its concentration, and a plot of reciprocal steady-state velocities *vs.* reciprocal galactal concentration was linear. The molar concentration of active β-galactosidase was estimated by dividing a V_{max} for ONPG hydrolysis determined under the conditions of Tenu *et al.*, 1971, by the k_{cat} for ONPG hydrolysis obtained by those workers. From these data, the k_{cat} for galactal hydration by β-galactosidase was estimated as 4×10^{-3} sec^{-1}.

SCHEME I



SCHEME II



Discussion

A striking characteristic of galactal inhibition is the rate of its onset, which is so slow that it can be followed by ordinary spectrophotometric procedures. The inhibitory combination of galactal with galactosidase occurs with a rate constant (2.7×10^2 sec^{-1} M^{-1}) some four orders of magnitude slower than k_{cat}/K_m for ONPG hydrolysis (Tenu *et al.*, 1971). Inhibition thus occurs with an activation barrier much higher than any which is encountered during the hydrolysis of galactosides, and, therefore, seems likely to involve a chemical reaction with the enzyme.

The value observed for the inhibition constant of galactal ($K_i = 1.4 \times 10^{-5}$ M) is in reasonable agreement with the ratio of the rate constants for release and binding of the inhibitor, but somewhat lower than the value ($K_i = 5.1 \times 10^{-5}$ M) reported by Lee (1969). Lee also reported evidence of "hyperbolicity" in contrast to the linear competitive inhibition observed under steady-state conditions in the present experiments. These discrepancies are likely to have arisen, at least in part, from the considerable time required for establishment of the steady state, unrecognized during the earlier experiments which were performed by discontinuous procedure with analysis after a fixed time interval (Lee, 1969).

Chemical precedent, in the addition of nucleophiles to 2,3-dihydropyran (Woods and Kramer, 1947; Parham *et al.*, 1952), suggests that β-galactosidase might combine chemically with D-galactal, to form a 2-deoxygalactosyl-enzyme (Scheme I) analogous in structure to simpler pyranylated nucleophiles. This derivative would also be analogous to galactosyl-galactosidase, a possible high-energy intermediate (see introduction) in the catalytic action of the enzyme on galactosides (Scheme II).

The marked sensitivity of the rate[2] of galactal binding to deuterium oxide is readily understood in terms of a process involving formation of a bond from carbon to hydrogen or to deuterium. The majority, and perhaps all, of the observed "off-rate" appears to describe the release of galactal, not as galactal itself but as 2-deoxygalactose. The slow rate of inhibitor binding and release suggests that both these processes involve a chemical reaction with the enzyme, presumably the formation and breakdown, respectively, of a 2-deoxygalactosyl-galactosidase intermediate.

This addition-displacement sequence for *hydration* of galactal supports a double-displacement mechanism for the *hydrolytic* action of the enzyme on its normal substrates. It is also of interest that the 2-hydroxyl group of substrates seems to exert an important influence in promoting the rate of breakdown of the presumed covalent intermediate.

[2] It is worth noting that there was no indication of "saturation" when the rate constant for galactal binding was studied as a function of galactal concentration. There is thus no evidence in support of a (presumably noncovalent) rapid preequilibrium of binding of galactal in the concentration range studied, and unmodified galactal is presumably poorly bound ($K_i > 10^{-3}$ M) like galactose (Kuby and Lardy, 1953) and 2-deoxygalactose.

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Purification and Characterization of Two Forms of Geranyl Transferase from *Ricinus communis*†

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ABSTRACT: Two forms of geranyl transferase (I and II) were purified 970- and 645-fold, respectively, from cell-free extracts of germinating castor bean (*Ricinus communis* L.) seedlings. Both enzymes catalyze the specific formation of *trans,trans*-farnesyl pyrophosphate from isopentenyl pyrophosphate and geranyl pyrophosphate and can utilize either dimethylallyl pyrophosphate or geranyl pyrophosphate, but not farnesyl pyrophosphate, as an initial allyl pyrophosphate substrate. Transferases I and II show similar pH dependencies with an optimum of 6.8 and metal ion requirements with Mg^{2+} (optimum 1–2 mM) in preference to Mn^{2+}. They are less sensitive to inhibition by common sulfhydryl reagents than the corresponding transferases from mammalian sources. At higher protein concentrations both transferases show nearly identical molecular weights of 72,500 ± 3000 on a calibrated G-100 Sephadex column. However, at lower enzyme concentrations, somewhat lower molecular weights of 56,000 ± 2000 and 60,000 ± 2000 were determined for transferases I and II, respectively, on the same column. The apparent K_m values for substrates were also seen to vary as a function of protein concentrations; the K_m values at protein concentrations of 42–56 μg ml^{-1} for purified preparations of transferases I and II were found to be in the range of 30–50 μM for iPe-PP and geranyl-PP and 4–6 μM for Me_2allyl-PP. However, the K_m values at 12–20 μg ml^{-1} of the same preparations for both enzymes were found to be in the range of 2–3 μM for iPe-PP, 4–6 μM for geranyl-PP, and 1–2 μM for Me_2allyl-PP. These and other observations indicate that transferases I and II are capable of undergoing reversible protein–protein interactions which serve to modulate their catalytic properties.

The prenyl transferases as a group catalyze the sequential condensations of iPe-PP(C_5)[1] with Me_2allyl-PP(C_5) and longer prenyl pyrophosphates to produce pools of geranyl-PP(C_{10}), farnesyl-PP(C_{15}), Ger_2PP(C_{20}), and in some cases cis isomers of these all-trans compounds. Longer chain polyprenyl pyrophosphates are also produced in some instances. Through sequences of enzyme-catalyzed cyclizations, alkylations, and other modifications, these prenyl pyrophosphates serve as precursors in the synthesis of a wide array of biologically significant molecules. Such isoprenoid end products include photosynthetic pigments (plastoquinones, chlorophylls, and carotenoids), mitochondrial electron transport components (ubiquinones and cytochrome oxidase), sterol and triterpene products implicated as membrane components and surfactants, growth and regulatory hormones (steroid hormones, ecdysone and juvenile hormone, gibberellins, and abscisic acid), phytoalexins (antibiotics) directed against common plant pathogens, polyprenols involved in the biosynthesis of cell walls and complex glycosides, and a wide array of other compounds, many of whose functions are obscure. The metabolic pathways leading to these diverse end products have been elucidated in many cases through isotopic tracer studies and in some instances studies of participating enzymes, but the nature of the regulation by modulation of enzyme activities and compartmentation of enzymes which must occur are still poorly understood. Hence the role of prenyl transferase in shunting the flow of carbon in this pathway between short and longer chain prenyl pyrophosphates would appear to be of considerable importance in determining the availability and distribution of starting material at branch points of the isoprenoid pathway from which such important end products arise. Our interest in prenyl transferases is in evaluating the regulatory role they may play in higher isoprenoid biosynthesis.

Lynen and coworkers (Lynen *et al.*, 1959; Grob *et al.*, 1961) first detected and partially purified prenyl transfer-

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[1] Abbreviations used are: iPe-PP, isopentenyl pyrophosphate; Me_2-allyl-PP, dimethylallyl pyrophosphate; Ger_2PP, geranylgeranyl pyrophosphate; PP_i, inorganic pyrophosphate.