Competitive Inhibition and Substrate Activity of Uridine Diphosphate 6-Deoxygalactose for *Escherichia coli* Uridine Diphosphate Galactose 4-Epimerase

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UDP-6-deoxygalactose inhibits the UDP-galactose 4-epimerase (EC 5.1.3.2) from *Escherichia coli* in a competitive manner with respect to the substrate UDP-galactose, giving K_i 1.3×10^{-3} M. As a substrate for the enzyme, it is transformed into UDP-6-deoxyglucose, although the reaction stops before equilibrium is attained. Possible causes of this behaviour are discussed.

UDP-2-, -3-, -4- and -6-deoxyglucoses are all competitive inhibitors of the epimerization of UDP-glucose by UDP-galactose 4-epimerase (EC 5.1.3.2) as obtained from both calf liver and from *Escherichia coli* (Drushinina *et al.*, 1969). The magnitudes of the K_i values determined show that the hydroxyl groups at C-3 and C-6 of the hexose are important for substrate binding to the active site of the enzyme. UDP-6-deoxyglucose exhibits only 4% conversion into UDP-6-deoxygalactose (UDP-fucose) and was the only member of the family of inhibitors found to exhibit substrate activity. Although UDP-6-deoxyglucose is only a weak inhibitor, Jordan *et al.* (1967) suggested that UDP-6-deoxygalactose might be a stoichiometric and irreversible inhibitor of the action of the epimerase from *E. coli*.

We here report weak competitive inhibition of the enzyme by UDP-fucose, its partial conversion into UDP-6-deoxyglucose, its inactivation of the enzyme and isotope studies on the fate of H-4 during the epimerization.

Methods and materials

D(+)-6-Deoxygalactose was obtained from Serva Ltd. (London W.11, U.K.), tri-*n*-octylamine was from Koch-Light Laboratories Ltd. (Colnbrook, Bucks., U.K.), NAD⁺ (disodium salt), UDP-galactose (disodium salt) and UDP-glucose 6-dehydrogenase (430 units/mg) were purchased from Sigma (London) Chemical Co. Ltd. (London S.W.6, U.K.). D(+)-Deoxyglucose was a generous gift from Professor A. W. Szarek, Queen's University, Kingston, Ont., Canada. UDP-galactose 4-epimerase was purified from *E. coli* K12 HfrH by the method of Wilson & Hogness (1964).

UDP-6-deoxygalactose was synthesized by the method of Nordin *et al.* (1965) and converted into the disodium salt by ion exchange. The conversion of UDP-galactose into UDP-glucose was followed kinetically by the method of Kalckar *et al.* (1959).

Inhibition kinetics were investigated with concentrations of inhibitor in the range 1.0–3.0 mM

(Fig. 1). The substrate activity was established by the incubation of UDP-6-deoxygalactose (0.33 mM) with 224 units of the epimerase for 98 h at 30°C at pH 7.2. (Bacterial contamination was inhibited by the addition of a trace of toluene to the solution of the components in boiled glass-distilled water.) The mixture of sugar nucleotides was hydrolysed in acid and deionized to permit separation and identification of the hexoses by t.l.c. on silica gel with 2-methylpropan-1-ol–pyridine–water (6:4:3, by vol.) and detection of the sugars both qualitatively and quantitatively with an aniline–phthalate spray (Partridge, 1949).

Inactivation studies involved preincubation of UDP-6-deoxygalactose (0.25 mM) with UDP-galactose 4-epimerase (4 units/ml) in potassium phosphate (10 mM)–EDTA (1 mM) buffer, pH 7.0, at 27°C. Portions (10 μ l) of the preincubation reaction mixture were removed at intervals and assayed directly. Concomitant non-specific inactivation was determined in controls lacking UDP-6-deoxygalactose and due correction was made.

A sample of UDP-galactose was incubated with the epimerase in ²H₂O at neutral pH at 30°C. The equilibrium product of UDP-glucose and UDP-galactose was hydrolysed in acid and the mixture of hexoses was separated by preparative t.l.c. to give glucose and galactose in the ratio 2.2:1. The galactose component was further converted into methyl 2,3,6-tri-*O*-benzoyl- α -D-galactopyranoside and examined by high-resolution n.m.r. spectroscopy at 100 and 220 MHz.

Results and discussion

The Lineweaver & Burk (1934) plots (Fig. 1) show strict competitive inhibition of UDP-galactose epimerization by UDP-6-deoxygalactose. The inhibition constant, K_i , was evaluated by the method of Dixon (1953) from a plot of the reciprocal of the velocity against inhibitor concentration (Fig. 2) and found to be 1.3×10^{-3} M. This is of similar magnitude to the inhibition constants for UDP-deoxyglucoses (Drushinina *et al.*, 1969) and establishes that the

Fig. 1. Effect of various concentrations of UDP-6-deoxygalactose on the rate of epimerization of UDP-galactose by *E. coli* UDP-galactose 4-epimerase

Experimental details are given in the text. ○, Uninhibited; inhibited by UDP-6-deoxygalactose: ●, 1.0 mM; ▲, 1.5 mM; ■, 2.0 mM.

Fig. 2. Inhibition of *E. coli* UDP-galactose 4-epimerase by UDP-6-deoxygalactose at various concentrations of UDP-galactose

Experimental details are given in the text. UDP-galactose concentrations were: ■, 0.05 mM; ▲, 0.10 mM; ●, 0.20 mM; ○, 0.35 mM.

6-deoxygalactose inhibitor is bound less strongly to the *E. coli* enzyme than is UDP-galactose, for which we find K_m 1.3×10^{-4} M.

Although it is clear that UDP-6-deoxygalactose is a substrate for the *E. coli* epimerase since it is transformed into UDP-6-deoxyglucose, the extent of this conversion was limited to 7% in an incubation lasting several days. Since the true equilibrium position between UDP-6-deoxygalactose and UDP-6-deoxyglucose must be anticipated to lie very close to that for the corresponding UDP-galactose and -glucose compounds, the most probable explanation of the failure of the enzyme to effect equilibrium

between the deoxy compounds seems likely to stem from the inactivation of the enzyme by UDP-fucose.

Inactivation studies indicate that after only 22 min preincubation of UDP-6-deoxygalactose with UDP-galactose 4-epimerase some 50% of the original enzyme activity was lost, this rising to 75% loss after 73 min. This indicates that UDP-6-deoxygalactose is an effective inactivator of UDP-galactose 4-epimerase, as proposed by Jordan *et al.* (1967), as well as being a substrate.

Although this inactivation requires further evaluation before it can be interpreted accurately, it seems reasonable to suppose that the fucose moiety is bound less strongly than the corresponding sugar component in UDP-galactose. It follows that any intermediate derived from it during the reaction will also be more weakly bound than the corresponding intermediate in galactose epimerization. If such an intermediate resulted from the transfer of a hydride ion from substrate to the enzyme, as would be the case for a 4-oxo compound, as proposed by Nelsestuen & Kirkwood (1971), its dissociation from the active site would leave the enzyme in a reduced inert form and account for the enhanced inactivation effected by UDP-6-deoxygalactose. Such a mechanism calls for the removal of a hydride ion from the α -face of the galactose and its return to the β -face of the 4-oxo sugar to complete the epimerization.

The galactose isolated from the enzymically equilibrated mixture of UDP-galactose and UDP-glucose in $^2\text{H}_2\text{O}$ solution was analysed by high-resolution n.m.r. spectroscopy, which, at 220 MHz, permitted a first-order analysis of the sugar hydrogen atoms in the methyl tri-*O*-benzoylgalactoside. Under conditions where 5% replacement of H-4 by ^2H from solvent could have been detected, none was observed. This result complements that of Beville *et al.* (1963) on the intramolecular transfer of ^3H -4 in the conversion of UDP-glucose into UDP-galactose, and shows that the oxidizing function that accepts this hydrogen atom does not equilibrate it with solvent hydrogen atoms during the course of the reaction. Although such a 'sticky hydride' transfer presents no problems in an isomerization involving suprafacial geometry, its mechanistic interpretation is fraught with considerable stereochemical problems in a case that, like the present, formally requires antarafacial hydrogen migration.

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