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Intramolecular hydrogen transfer catalyzed by UDP-D-glucose 4'-epimerase from *Escherichia coli*

Evidence obtained in a number of laboratories (for review see ref. 1) has shown that the UDP-D-glucose 4'-epimerase (EC 5.1.3.2) from all sources requires NAD as a cofactor. Furthermore it has been shown that neither hydrogen nor oxygen from the solvent is incorporated into the product. With the enzyme from *Escherichia coli* spectral evidence has been obtained for the appearance of enzyme bound NADH during the reaction[2] and an increase in enzyme bound NADH on substrate addition has been observed with the yeast enzyme[3]. The observation of BEVILL *et al.*[4] that a ^3H isotope effect can be observed with UDP-D-[4-^{3}H]glucose as a substrate indicates that the carbon–hydrogen bond at C-4 of the hexose is broken during the reaction. These observations have been reviewed in detail by ROSE[1].

Two possible reaction mechanisms can be written from these observations:

(I) UDP-Glc + E–NAD$^+$ ⇌ E–NADH–UDP-4-dehydro-Glc + H$^+$ ⇌ E–NAD$^+$ + UDP-Gal

(II) E(XH, NAD$^+$) + UDP-Glc ⇌ E(X, NADH)–UDP-4-dehydroGlc + H$^+$ ⇌ E(X, NADH) + UDP-D-Gal

E(X, NADH) ⇌ E(XH, NAD$^+$)

It is essential in order to understand these mechanisms in detail to determine whether the hydrogen transfer from glucose to galactose is intramolecular.

Mechanism II requires an intermolecular hydrogen transfer in going from glucose to galactose. In Mechanism I inspection of models suggests that a relatively minor rearrangement of the 4-dehydro-D-glucose and NADH is required, if a different hydrogen on NADH is used for the reduction of the intermediate to glucose or galactose. In this case also hydrogen transfer from glucose to galactose would be intermolecular. Finally if the same hydrogen on NADH is used for reduction of the intermediate to either glucose or galactose, a drastic conformational change is required, probably involving a change in the sugar conformation of the 4-dehydro-D-glucose. In this last instance the hydrogen transfer would be intramolecular.

It is possible to determine whether the hydrogen transfer is intramolecular or intermolecular, by a method we have recently used to answer a similar question for the dTDP-D-glucose oxidoreduction reaction[5]. In Fig. 1 are shown the expected products for an intermolecular or intramolecular hydrogen transfer when the UDP-D-glucose 4'-epimerase is allowed to react with a mixture of UDP-D-glucose and fully deuterated UDP-D-glucose.

UDP-D-glucose 4'-epimerase from *E. coli* was prepared by the method of WILSON AND HOGNESS[2]. UDP-D-[$^2H_7$]glucose was prepared as described previously for the preparation of dTDP-D-[$^2H_7$]glucose (ref. 5). The sugars were removed from the

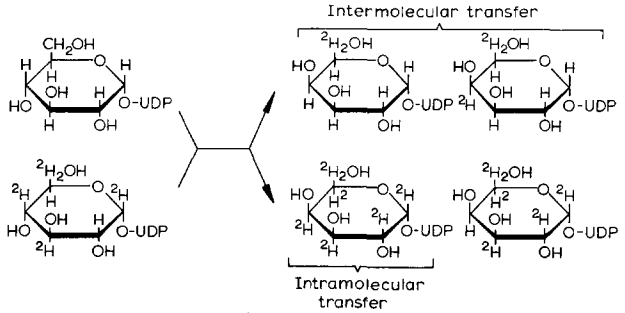

Fig. 1. Expected products for intramolecular and intermolecular hydrogen transfer in the UDP-D-glucose 4'-epimerase reaction. Since the reaction is reversible the same mixture of UDP-D-glucose derivatives would also be formed in each case.

nucleotide by mild acid hydrolysis, reduced with $NaBH_4$, acetylated and the mass spectrum of the acetylated sugar determined as described previously[5].

In Fig. 2, we compare the mass spectra of sugar alcohols obtained from glucose, [$^2H_7$]glucose, and from the mixture of hexoses from the UDP-glucose epimerase reaction. It is clear from examining these spectra that the epimerase reaction mixture appears to be a simple mixture of hexitol and [$^2H_7$]hexitol. The nature of the various

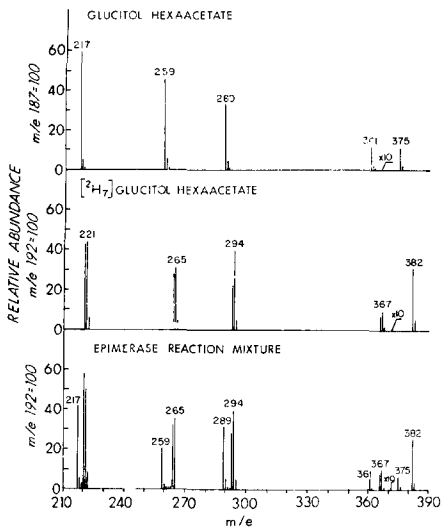

Fig. 2. Partial mass spectra of UDP-D-glucose 4'-epimerase reaction products. The reaction mixture contained 20 μmoles of potassium phosphate pH 7.0, 2 μmoles of EDTA, 1 μmole of β-mercaptoethanol, 5 μmoles of UDP-D-glucose, 5 μmoles of UDP-D-[$^2H_7$]glucose, and 1 unit of UDP-D-glucose 4'-epimerase and was incubated at 37° for 3 h. 1 unit of UDP-D-glucose 4'-epimerase catalyzes the conversion of 1 μmole of UDP-D-galactose to UDP-D-glucose per min. At the end of the incubation, the UDP-sugars were isolated by chromatography on DEAE-Sephadex and after mild acid hydrolysis, the hexoses were reduced to the sugar alcohols, acetylated and the mass spectrum determined as previously described[5], in this method glucose and galactose are not separated and we show here the spectrum of the combined hexitol acetates. Analysis of the reaction mixture indicated that UDP-D-glucose/UDP-galactose equilibrium had been reached after 40 min of incubation. Identical results were obtained with three different preparations of UDP-D-[$^2H_7$]glucose.

fragments of the mass spectrum have been discussed previously, for the present results we need only discuss one peak, for example, at *m/e* 361 in the glucitol spectrum. This peak corresponds to a fragment containing carbon atoms C-1 to C-5 or C-2 to C-6 of the original glucitol. If hydrogen transfer had been intermolecular, half of the molecules would have acquired 1 ^2H atom at C-4, and this peak should become a doublet with an approximate equal size peak at *m/e* 362. This is clearly not observed.

The corresponding peak in [$^2H_7$]glucitol is at *m/e* 366 and 367. This peak is already a doublet since in this case the fragment from C-1 to C-5 contains 5 ^2H atoms but the fragments from C-2 to C-6 contains 6 ^2H atoms. Intermolecular hydrogen transfer would have produced loss of one ^2H from this molecule, resulting in shift of part of the peak at *m/e* 367 to *m/e* 366 and the peak at *m/e* 366 to *m/e* 365, this again is not observed. Similar arguments apply to the other fragments.

We conclude from these observations that the hydrogen transfer in the UDP-glucose 4'-epimerase reaction is intramolecular. This excludes Mechanism II for this reaction and introduces an obligatory large conformation change in reaction Mechanism I which can now be written schematically, as indicated below, with the implication that glucose and galactose are bound with different conformations to the protein so that the hydrogen at C-4 of the hexose, is in the same spatial relationship to the nicotinamide portion of NAD^+ in each conformation. Such a situation could be achieved for example if one of the hexoses was bound in a boat conformation and the other in a chair, and that UDP-4-dehydro-D-glucose is bound in two different interconvertible conformations to the protein, corresponding to those of glucose and galactose, respectively.

$$E\text{–NAD}^+ + \text{UDP-Glc} \rightleftharpoons E\text{–NAD}^+\text{–UDP-Glc} \rightleftharpoons \{E\text{–NADH–UDP-4-dehydro-Glc} + H^+\}$$
$$\Downarrow$$
$$\{E\text{–NADH–UDP-4-dehydro-Glc} + H^+\}^*$$
$$\Downarrow$$
$$E\text{–NAD}^+\text{–UDP-Gal}^* \rightleftharpoons E\text{–NAD}^+ + \text{UDP-Gal}$$

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