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MYO-INOSOSE-2 REACTIVATION OF REDUCED UDP-D-GALACTOSE 4-EPIMERASE FROM *ESCHERICHIA COLI*

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SUMMARY

myo-Inosose-2, a ketoinositol with the same stereochemistry around C-2 and C-3 as the proposed 4-keto intermediate in the epimerization reaction catalyzed by UDP-D-galactose 4-epimerase (EC 5.1.3.2), was found to reactivate the UMP and D-galactose-reduced enzyme. Cyclohexanone, dihydroxyacetone and acetone had no effect.

INTRODUCTION

Recent studies of the epimerizations catalyzed by UDPgalactose 4-epimerase (EC 5.1.3.2.) have indicated that the reaction proceeds through a 4-ketosugar or equivalent intermediate. Two types of substrates have been used with the enzyme, one being the natural nucleotide sugar, and the other being free sugar and UMP, a model reaction which results in the reductive inactivation of the enzyme and the net formation of tightly bound NADH[1].

The nucleotide sugar has been used in trapping experiments with NaB^3H_4 which resulted in label bound to the C-4 of the hexose moiety of UDPglucose and UDP-galactose, a result consistent with the formation of UDP-4-ketoglucose as an intermediate in the reaction[2]. Also, the epimerization of TDP[4-^{3}H]glucose has been shown to exhibit a small isotope effect in the presence of enzyme, and upon prolonged incubation to transfer the ^{3}H to form enzyme-bound [^{3}H]NADH.[3] The same result was observed utilizing UDP-6-[4-^{3}H]deoxyglucose. In addition, when the cofactor is reduced with NaB^3H_4 in the presence of UDPglucose, reoxidation and reactivation occurs when UDP-4-keto-6-deoxyglucose is added[4].

The model system, *i.e.* free sugar and UMP has been found to consist of competing reactions, that is, the transfer of hydride to bound NAD^+ from the 1 position of certain sugars[5], as well as transfer from the 4 position of galactose, but not glucose[6].

Since the model reaction is, in part, reflective of the normal mechanism, we have investigated the reactivation of reduced enzyme utilizing possible intermediate

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analogs and UMP. Reactivation of reduced epimerase by 2-ketoglucose preparations was observed by Bertland *et al.*[1], but it is not clear whether the 2-ketoglucose alone is the effective agent in this case.

MATERIALS AND METHODS

UDPgalactose 4-epimerase was purified by the procedure of Wilson and Hogness[7], with the omission of the hydroxyapatite step, from *Escherichia coli* strain D 96 (*proc*$^-$, *ade*$^-$, *thi*$^-$) *gal* R^c, λdg.φS, a culture containing a temperature-inducible λdg phage, and had a specific activity of $5 \cdot 10^3$–$7 \cdot 10^3$ μmoles per mg of protein per h at 27 °C[7]. Sodium dodecyl sulfate gel electrophoresis of these preparations revealed one major band comprising 95% or more of the protein present[8].

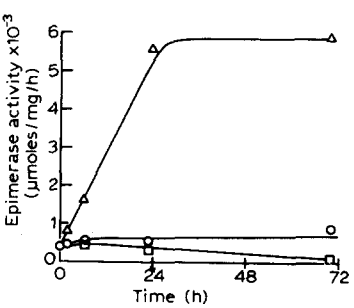
The reductive inactivation incubation consisted of between 0.03 and 0.10 mg of native UDPgalactose 4-epimerase; 60 mM sodium glycine buffer (pH 8.5); 1 mM 2-mercaptoethanol; 0.1 mM EDTA; 0.1 mM UMP; and 10 mM D-galactose in a total volume of 1 ml. The mixture was incubated 17 h at 4 °C after which time the enzymatic activity fell to 1–2% of that of the control in which D-galactose was omitted.

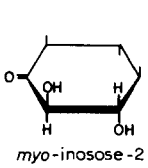
For the reactivation, 0.1 ml of the reduced enzyme was added to 0.5 ml of a 20 mM solution of each of the following compounds; *myo*-inosose-2 (Sigma Co.), cyclohexanone, dihydroxyacetone (Sigma Co.) and acetone; and incubated at 0 °C. At various intervals, aliquots were taken and assayed for epimerase activity.

RESULTS AND DISCUSSION

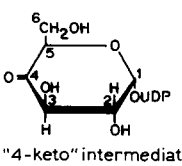
Fig. 1 shows the results of the reactivation experiments at 0 °C. Of the compounds tested, only *myo*-inosose-2 reactivated the reduced enzyme. When the reactivation mixture containing *myo*-inosose-2 was incubated at 27 °C return of enzymatic activity was complete within 6 h.

Attempts to inactivate the enzyme with inositol and UMP were, however, negative. This result is consistent with a conformational change occurring during the reduction of the enzyme as has been observed with yeast epimerase[9]. It suggests that the unreduced enzyme is capable of distinguishing between inositol and the normal





myo-inosose-2



"4-keto" intermediate

Fig. 1. Specific activity of epimerase in the reactivation incubation containing *myo*-inosose-2 (△—△); cyclohexanone or acetone (○—○) and dihydroxyacetone (□—□) as a function of time. Conditions were those described in the text.

Fig. 2. Structure of *myo*-inosose-2 and the proposed UDP-4-ketohexose intermediate.

substrate but after reduction the active site no longer discriminates between their keto derivatives. The similarity in structure between *myo*-inosose-2 and the 4-keto intermediate can be seen in Fig. 2. The ability of inosose-2 to function as an analog for the 4-keto intermediate is in agreement with our observation that hydride transfer occurs only from a sugar with the stereochemistry at C-2 and C-3 shown in Fig. 2.[6]

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