EPIMERIZATION OF UDP-GLUCOSE AND UDP-XYLOSE IN ESCHERICHIA COLI: EVIDENCE FOR A SINGLE ENZYME

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In plant extracts UDP-D-xylose (UDPxyl) and UDP-L-arabinose (UDPara) are interconverted by an epimerase reaction involving carbon 4 of the pentosyl moieties (Neufeld et al., 1957, Feingold et al., 1960). We have recently observed that extracts of Escherichia coli B and K 12 catalyze the same reaction (Ankel and Maitra, 1968). It has been reported that highly purified preparations of yeast UDP-D-galactose 4-epimerase (UDPgal epimerase) also mediate epimerization of the mentioned UDP-pentoses (Salo et al., 1968). These authors assume from a lack of inhibition of UDPgal epimerase by UDPara or UDPxyl and from an apparent change of the ratios of the two epimerase activities during purification, that in yeast epimerization of the UDP-pentoses is carried out by an enzyme different from UDPgal epimerase. Our partially purified preparations of E. coli UDP-L-arabinose 4-epimerase (UDPara epimerase) contain UDPgal epimerase. In contrast to the situation in yeast no change of the ratios of both enzymatic activities during purification was observed (Ankel and Maitra, 1968). These observations led to the question whether in E. coli both epimerizations are mediated by the same enzyme, or whether two enzymes are involved that do not separate under the conditions of purification.

In an attempt to answer this question we are presenting our data obtained with E. coli mutants that have normal, decreased and non-detectable levels of UDPgal epimerase. In all three cases studied UDPara epimerase levels parallel those of UDPgal epimerase almost exactly, in the presence as well as in the

absence of the inducer D-fucose. To support our thesis that one enzyme catalyzes both reactions in E. coli, we include kinetic evidence obtained with "wild-type" extracts that shows competitive inhibition of UDPgal epimerase by UDPxyl and of UDPara epimerase by UDP-D-glucose (UDPG).

METHODS AND RESULTS

The following isogenic mutants of E. coli K 12 were kindly given to us by Dr. Sankar Adhya, University of Rochester, Department of Biology, Rochester, New York:

E. coli AM 3100, F^-, Sm^r, gal^+, his^-, having high UDPgal epimerase activity;

E. coli AM 53, F^-, Sm^r, gal^-_{SA89}, his^-, having defective UDPgal epimerase activity;

E. coli AM 61, F^-, Sm^r, gal^-_{UV211}, his^-, having low UDPgal epimerase activity.

The bacteria were grown on casamino acids, glycerol and salts (Wilson and Hogness, 1964) at 37 C with a New Brunswick thermoregulated culture shaker at 200 revolutions per min in one liter of medium contained in 2-liter Erlenmeyer flasks. One mM D-fucose (Sigma Biochemicals) was included where its effect was investigated. Fucose-free medium (100 ml) contained in a 250 ml Erlenmeyer flask was inoculated from a slant, the culture was allowed to grow overnight on the shaker and was subcultured once again by inoculating 100 ml of medium with 10 ml of the overnight culture. When the starter culture had reached a cell population of approximately 10^9 viable cells/ml, 30 ml were used for inoculation of each 2-liter flask. The cultures were grown until their density had reached a value corresponding to approximately 2×10^9 cells/ml. The cells were then harvested by centrifugation and washed once with 0.033 M sodium and potassium phosphate buffer, pH 6.8, containing 0.5 g/l disodium ethylenediaminetetracetate (EDTA). Cells from 3 liters of medium were resuspended to 18 ml in the same buffer and subjected to ultrasonic desintegration for 3 min in an ice-salt cooled container using the Branson Sonifier model 110 at 11 Amps output. All subsequent manipulations were carried out at 0 to 4 C. The cell debris was removed by centrifugation at 18,000 x g and the particulate material by centrifugation for 45 min at 105,000 x g. The supernate of the ultracentrifugation was dialyzed for 24 hours against 2 liters of 0.05 M Tris-HCl buffer, pH 7.5,

containing 0.5 ml/l mercaptoethanol and 0.5 g/l EDTA. The dialyzed extracts were stored at -20 C until they were assayed.

Assays for both UDPgal epimerase and UDPara epimerase were performed as follows: 0.2 ml extract or a suitable fraction diluted to 0.2 ml were incubated for varying lengths of time with 0.02 ml of a 0.02 M solution of either UDPxyl or UDPG (P. L. Biochemicals) at pH 7.5 and 30 C. The reaction was stopped by addition of 0.02 ml 1 N hydrochloric acid and the reaction mixtures were hydrolyzed at 100 C for 10 min. To each of the reaction mixtures was added 1.0 ml of 0.5 M Tris buffer, pH 9.0, and the precipitated protein was removed by centrifugation. One ml of the supernate was placed in a cuvette with 1 cm light path and 0.02 ml of a 0.05 M DPN solution was added. After addition of an enzyme preparation (0.02 ml) from Pseudomonas saccharophila (A.T.C.C. 15946) that contained L-arabinose and D-galactose dehydrogenase (230 units/ml; Doudoroff, 1962) the optical density change at 340 mμ was followed with a Hitachi Spectrophotometer until the reaction had come to completion. Authentic D-xylose, D-arabinose and D-glucose do not react in this system, nor do L-glucose, D-mannose, D-lyxose, L-xylose and D-ribose (Doudoroff, 1962). Zero time controls were run to adjust for the small optical density change that is due to the enzyme solution itself. Samples of identically treated authentic D-galactose and L-arabinose were used as standards. Table I shows the results obtained. Whereas AM 3100 showed UDPara- and UDPgal epimerase activity, both activities were absent in the UDPgal epimerase-less mutant. The addition of D-fucose to the growth medium increased both specific activities 12- to 13-fold in AM 3100, but failed to show any effect on the mutant AM 53. In the mutant AM 61 both activities were lower by a factor of 10 to 12 as compared to AM 3100. Again addition of D-fucose to the growth medium increased both activities 12- to 14-fold.

That extracts of E. coli AM 3100 and AM 61 are indeed capable of forming UDPgal from UDPG, and UDPara from UDPxyl, whereas those of AM 53 are not, was ascertained in the following manner: 0.02 ml samples of the extracts were in-

Table I

Specific Activities of UDPgal- and UDPara Epimerase in E. coli Mutants

E. coli Mutant	Addition to Medium	Epimerase Activities* UDPgal	UDPara
AM 3100	none	8.75	0.43
	D-fucose	100	5.75
AM 61	none	0.7	0.04
	D-fucose	10.0	0.50
AM 53	none	0	0
	D-fucose	0	0

*Activities are expressed as mμmoles of product formed/mg protein x min.; protein was determined according to Lowry et al., 1951.

cubated with 0.1 μc of UDP-D-^{14}C-glucose (50 mc/mmole; New England Nuclear) or UDP-D-^{14}C-xylose (40 mc/mmole; Ankel and Feingold, 1966) for 3 hours at room temperature. The reaction mixtures were then subjected to paper chromatography on EDTA-washed Whatman 1 paper in ethanol - methyl ethyl ketone- 0.5 M morpholinium tetraborate, pH 8.6, in 0.01 M EDTA, (7 : 2 : 3; v/v) for 48 hrs (Carminatti et al., 1966). The radioactive spots on the paper were visualized by radioautography. Reaction mixtures containing extracts of E. coli AM 3100 and AM 61 revealed the formation of radioactive products with mobilities of authentic UDPgal and UDPara, whereas reaction mixtures containing extracts of E. coli AM 53 did not show the formation of such products from UDPG or UDPxyl. The spots corresponding in mobility to UDPgal and UDPara were eluted from the paper, hydrolyzed in 0.1 N hydrochloric acid at 100 C for 10 min. and sub-

jected to ascending chromatography on Whatman 1 paper in water-saturated phenol. Spots that had the mobility of arabinose were found in the samples originating from reaction mixtures with AM 3100 and AM 63 extracts containing UDPxyl, and of galactose from reaction mixtures that originally contained the same extracts and UDPG.

The effect of UDPxyl on UDPgal epimerase and of UDPG on UDPara epimerase activity was studied using extracts of AM 3100 (grown in the presence of inducer). Since both l-arabinose and D-galactose react with the P. saccharophila dehydrogenase, the assay procedure had to be modified. In the case of UDPgal epimerase the amount of D-galactose liberated after hydrolysis of the reaction mixtures was determined with D-galactose oxidase (Galactostat, Worthington). In the case of UDPara epimerase after hydrolysis of the reaction mixtures the pentoses were separated from the hexoses by ascending paper chromatography on Whatman 3 MM paper in n-propanol-ethyl acetate-water (7 : 1 : 2; v/v). After elution of the pentoses the L-arabinose content in the eluates was determined with P. saccharophila dehydrogenase. In figures 1 and 2 the reciprocal reaction velocities at different substrate concentrations are plotted against inhibitor concentration (Dixon, 1953). In both figures the straight lines obtained meet at a common intercept typical of competitive inhibition. The K_i values calculated from these plots - 0.7 mM for UDPG and 1.8 mM for UDPxyl - are in good agreement with the Michaelis constants for UDPG (1 mM; Imae et al., 1964) and UDPxyl (1.2 mM; Ankel and Maitra, 1968).

DISCUSSION

Our findings clearly indicate that epimerization of UDPxyl to UDPara in E. coli is catalyzed by an enzyme that is inducible by D-fucose to the same extent as is UDPgal epimerase. In addition they demonstrate that mutations that affect UDPgal epimerase have the same effect on UDPara epimerase. While these data are consistent with the concept of one enzyme mediating both reactions, they do not rule out the possibility of a separate gene for UDPara epimerase on the galactose operon. However, the inhibition data strongly support the con-

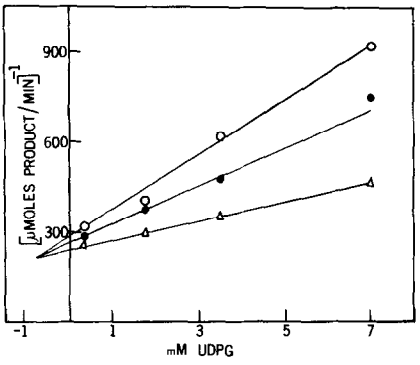

Figure 1. Inhibition of UDPxyl epimerization by UDPG: Plots of reciprocal reaction velocity versus inhibitor concentration. UDPxyl concentrations:

(o —— o) 1.75 mM
(● —— ●) 3.5 mM
(Δ —— Δ) 7.0 mM

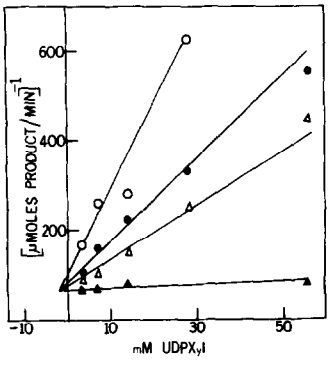

Figure 2. Inhibition of UDPG epimerization by UDPxyl: Plots of reciprocal reaction velocity versus inhibitor concentration. UDPG concentrations:

(o —— o) 2.5 mM
(● —— ●) 5.0 mM
(Δ —— Δ) 10.0 mM
(▲ —— ▲) 15.0 mM

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clusion that only one enzyme is responsible for both epimerization reactions, in contrast to the situation in yeast where no such inhibition was observed (Salo et al., 1968).

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