SHORT COMMUNICATIONS

BBA 63311

Uridine diphosphate-D-glucose dehydrogenase of hen oviduct

UDPglucose and UDPglucuronate, as well as many other nucleotides, have been isolated from hen oviduct[1] In addition, the tissue contains enzymes which decarboxylate UDPglucuronate[2] and use the UDPxylose formed as donor of the D-xylosyl moiety for synthesis of glycoproteins[3].

NEUFELD AND HALL[4] have suggested that in tissues which contain glycosidically-bound D-xylose, UDPxylose functions as an allosteric feedback inhibitor of its own synthesis by exerting its action on UDPglucose dehydrogenase (UDPglucose: NAD oxidoreductase, EC 1 1 1.22) The latter enzyme mediates the formation of UDPglucuronate, the immediate precursor of UDPxylose. In this paper the partial purification of UDPglucose dehydrogenase of hen oviduct and some studies of its inhibition by UDPxylose are described.

Protein was estimated by the method of LOWRY *et al* [5] UDPglucose dehydrogenase was assayed essentially as described by STROMINGER *et al* [6] Reaction mixtures contained, in addition to the enzyme 1.25 μmole UDPglucose and 2 5 μmoles NAD in 1 ml of 0 1 M glycine buffer (pH 9 0) Absorbance at 340 mμ was determined with the Gilford Model 2000 multi-sample spectrophotometer The Model 215 Automatic Blank Compensator was used to correct readings for endogenous activity in the absence of UDPglucose. A unit of activity is defined as the amount of enzyme required to give an increase in absorbance of 0.001 per min in a cell with a 1-cm light path under the above conditions at 30°.

Young laying hens were killed by decapitation, and the complete oviduct was removed and used for enzyme purification. All subsequent operations were performed at 0–4° 80 g of tissue was homogenized in 220 ml of 0 15 M KCl in a Waring blender for 1 min After removal of large particles by centrifugation at 12 000 × *g* for 20 min, the turbid supernatant liquid was clarified by centrifugation at 80 000 × *g* for 45 min The clear supernatant fluid (crude extract) was brought to 40% satn with solid $(NH_4)_2SO_4$, the precipitate was discarded, and the supernatant fluid was brought to 58% satn. with more solid $(NH_4)_2SO_4$ The precipitated protein was taken up in sufficient 0.02 M sodium phosphate buffer (pH 7 0) (buffer), to yield 20 ml of solution ($(NH_4)_2SO_4$ fraction) 20 ml of the latter were applied to an 800-ml Sephadex G-100 column (3.5 cm diameter) (equilibrated with buffer) which was eluted with buffer. UDPglucose dehydrogenase emerged from the column immediately after 1 void vol of effluent (G-100 eluate), indicating that its molecular weight exceeds 100 000 G-100 eluate (110 ml) was concentrated by addition of solid $(NH_4)_2SO_4$ to 65% satn , and the resultant precipitate was dissolved in a volume of buffer sufficient to yield 6 ml of solution 3 ml of this solution were applied to a 200-ml column (2 5 cm diameter) of Sephadex G-200 equilibrated with buffer (4 mM in EDTA), the column was eluted with the same buffer Active fractions were pooled (G-200 eluate) Chromatography of the latter on CM-Sephadex yielded a preparation with 20-fold greater specific activity; however, since this procedure gave variable yields of highly unstable enzyme, it was not used routinely Table I summarizes the purification procedure Conc. G-100

TABLE I

SUMMARY OF PURIFICATION OF UDPGLUCOSE DEHYDROGENASE

Fraction	*Vol (ml)*	*Total units*	*Specific activity (units/mg protein)*	*Recovery (%)*
Crude extract	300	13 500	0 85	100
$(NH_4)_2SO_4$ fraction	30	11 500	2 0	85
G-100 eluate	120	8 000	30 0	59
G-200 eluate	70	6 000	45 0	44

eluate was used for kinetic studies after dialysis against 0 02 M phosphate buffer (pH 7.0) for 4 h. This fraction was stable for at least 6 months at −10°

Formation of UDPglucuronate from UDPglucose was shown as follows. 10 μl of conc G-100 eluate was incubated with 0.2 μmole of NAD and 0 2 μmole of UDP-glucose in 20 μl of glycine buffer (pH 9 0) at 30° for 30 min, the reaction was stopped by boiling UDPglucuronate was identified as one of the reaction products by co-chromatography with authentic [^{14}C]UDPglucuronate in 95% ethanol–1 M ammonium acetate (pH 7 0) (7 3, v/v)[7]

The oviduct dehydrogenase has an optimum activity at about pH 9 0 The effect of UDPglucose and NAD concentrations on the reaction rate was studied under the conditions of assay with appropriate concentrations of each substrate The apparent K_m values determined from these data by the method of LINEWEAVER AND BURK[8] are 4 0 10^{-5} M for UDPglucose and 9 0 10^{-5} M for NAD

A study of the effect of UDPxylose on the reaction rate is presented in Fig 1 It can be seen that UDPxylose is a strong inhibitor of the hen oviduct dehydrogenase At low concentrations of UDPxylose, inhibition appears to be strictly competitive

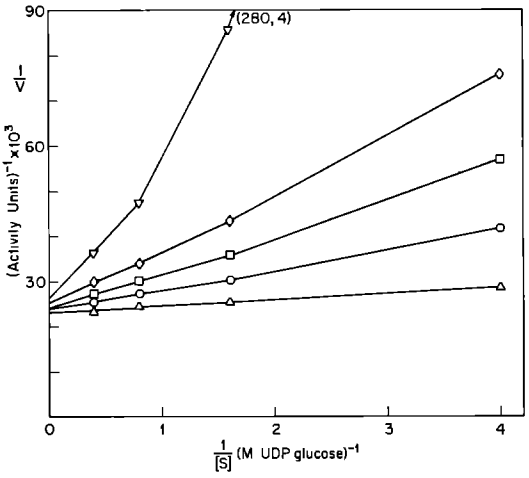

Fig 1. Dependence of reaction rate on UDPglucose concentration at different concentrations of UDPxylose The conditions of assay are those described in the text except that the concentration of UDPglucose was varied UDPxylose concentrations △—△, no UDPxylose added, ○—○, 0 05 mM, □—□, 0 1 mM, ◇—◇, 0 15 mM, ▽—▽, 0 3 mM

with UDPglucose (K_i = 1.4 10^{-5} M). However, at high inhibitor concentrations the plot shows an upward inflection with diminishing UDPglucose concentrations, similar curves have been reported for other UDPglucose dehydrogenases[4,9]. By plotting the logarithm of $v/(v_{max} - v)$ *versus* the logarithm of UDPxylose concentration (for discussion see ref. 10) at different concentrations of UDPglucose, parallel lines were obtained which yielded an average apparent number of inhibitory sites of 1.25. While this value is lower than that obtained for the UDPglucose dehydrogenase of liver, pea seedlings[4], or *Cryptococcus laurentii*[9], it is significantly greater than 1.0, indicating that UDPxylose exerts a cooperative inhibitory effect here also. These data suggest that the UDPglucose dehydrogenase of hen oviduct, like the other dehydrogenases mentioned, is allosterically inhibited by UDPxylose. This is consistent with the previous findings concerning the role that this sugar nucleotide plays in controlling its own intracellular concentration.

It is of interest that UDPxylose is a non-cooperative competitive inhibitor (average number of inhibitory sites equals 1.0) of UDPglucose dehydrogenase of *Aerobacter aerogenes*, an organism which lacks D-xylose and presumably does not form UDPxylose (A. BDOLAH AND D. S. FEINGOLD, unpublished data).

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Department of Microbiology,
University of Pittsburgh School of Medicine,
Pittsburgh, Pa. (U.S.A.)

AVNER BDOLAH*
DAVID SIDNEY FEINGOLD

1 Y. NAKANISHI, S. SHIMIZU, N. TAKAHASHI, M. SUGIYAMA AND S. SUZUKI, *J. Biol. Chem.*, 242 (1967) 967.
2 A. BDOLAH AND D. S. FEINGOLD, *Biochem. Biophys. Res. Commun.*, 21 (1965) 543.
3 E. E. GREBNER, C. W. HALL AND E. F. NEUFELD, *Biochem. Biophys. Res. Commun.*, 22 (1966) 672.
4 E. F. NEUFELD AND C. W. HALL, *Biochem. Biophys. Res. Commun.*, 19 (1965) 456.
5 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
6 J. L. STROMINGER, E. S. MAXWELL, J. AXELROD AND H. M. KALCKAR, *J. Biol. Chem.*, 224 (1957) 79.
7 A. C. PALADINI AND L. F. LELOIR, *Biochem. J.*, 51 (1952) 426.
8 H. LINEWEAVER AND D. BURK, *J. Am. Chem. Soc.*, 56 (1934) 658.
9 H. ANKEL, E. ANKEL AND D. S. FEINGOLD, *Biochemistry*, 5 (1966) 1864.
10 K. TAKETA AND B. M. POGELL, *J. Biol. Chem.*, 240 (1965) 651.

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* Present address: Department of Zoology, Tel Aviv University, Tel Aviv, Israel.