INHIBITION OF UDP-D-GLUCOSE DEHYDROGENASE BY UDP-D-XYLOSE: A POSSIBLE REGULATORY MECHANISM

Elizabeth F. Neufeld and Clara W. Hall

National Institute of Arthritis and Metabolic Diseases
National Institutes of Health
Bethesda, Maryland

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The formation of several sugar nucleotides has recently been shown to be subject to inhibition or activation by allosteric modifiers (Kornfeld et al, 1964; Melo and Glaser, 1965; Bernstein and Robbins, 1965; Preiss et al, 1965; Ghosh and Preiss, 1965; Mayer and Ginsburg, 1965; Kornfeld, 1965; Kornfeld and Ginsburg, 1965). Data presented in this paper show that UDP-D-glucose dehydrogenases from pea cotyledons, calf liver and chick cartilage are strongly and specifically inhibited by UDP-D-xylose. As this sugar nucleotide has been found to arise in some organisms by decarboxylation of UDP-D-glucuronic acid (Feingold et al, 1960; Ankel and Feingold, 1964) the inhibition of UDPG* dehydrogenases may represent another instance of regulation by feedback.

Preparation of Enzymes. Cotyledons were dissected from 3-day old etiolated pea seedlings, ground in a mortar with two volumes of 0.025 M PO_4 buffer, pH 6.8, and strained. The resulting homogenate was treated by a slight variation of the first steps described by Strominger and Mapson, 1957: acidification to pH 5.5; centrifugation, and neutralization; preparation of 0-40% ammonium sulfate fraction; dialysis against 10^{-2} M phosphate - 10^{-3} M EDTA, pH 6.6, and clarification at 100,000 x g. Typically, 120 g of cotyledons yielded 7 ml of dialyzed solution, of which 25-50 µl were used per assay.

*Abbreviations: UDPG, UDPGA, UDPXy represent, respectively, UDP-D-glucose, UDP-D-glucuronic acid and UDP-D-xylose.

Bovine liver dehydrogenase was purchased from Sigma Chemical Co. Extracts of cartilage were obtained from fused sacral vertebrae (synsacrum) of 14-day old chick embryos. Material from 18-24 chicks was ground in a mortar with sand and 5 volumes of 0.1 M Tris buffer, pH 7.6. The supernatant of 20,000 x g centrifugation was used for assays.

Chemicals. All nucleotides were of commercial origin. While the UDPXy used routinely was purchased from California Biochemicals, a sample was prepared from α-D-xylose 1-phosphate and UTP in the presence of crude mung bean pyrophosphorylase, and was purified by paper chromatography. UDP-L-arabinose was prepared in a similar manner from β-L-arabinose 1-phosphate. The α-D-xylose 1-P and β-L-arabinose 1-P were kindly given to us by Dr. W. Z. Hassid, as was UDP-D-glucose-C^{14}.

Results. As is apparent from Fig. 1, the dehydrogenases from both liver and peas can be almost completely inhibited by less than 0.05 mM UDPXy, particularly if the UDPG concentration is low. Reciprocal plots (Fig. 2) show that UDPG can reverse the inhibition, V_{max} remaining unchanged. At low concentrations of UDPXy, the inhibition appears to be competitive (K_i for pea enzyme, 0.005 mM; for liver enzyme, 0.004 mM).

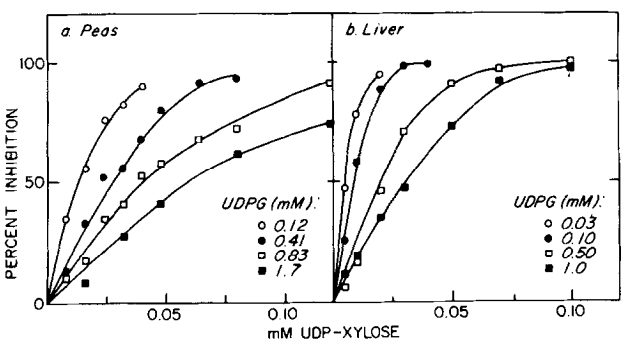

Figure 1. Inhibition of UDPG dehydrogenase activity by UDPXy. Assays were performed at 22° in 1.0 ml containing 0.1 M Tris, pH 8.5, 0.8 mM DPN, UDPG and UDPXy as indicated. Readings were taken at 340 mμ with the aid of a Gilford Multiple Sample Recorder. Thirty to fifty seconds were required to add enzyme, mix, and position cuvettes before the "initial" rate could be determined.

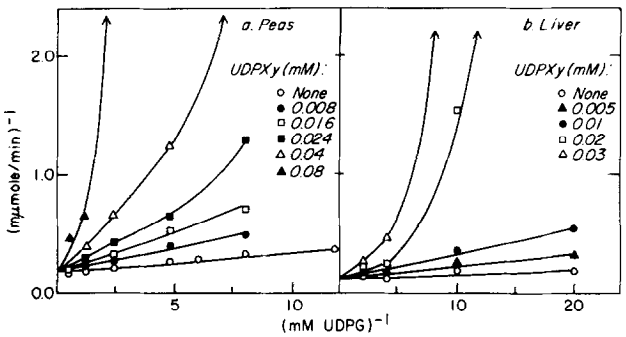

Figure 2. Reciprocal plots of velocity versus UDPG concentration at various levels of UDPXy. DPN concentration: 0.8 mM.

As the amount of UDPXy is increased, however, the reciprocal of velocity plot shows a marked upward inflection with diminishing UDPG. The second substrate of the reaction, DPN, can also overcome inhibition by UDPXy (Fig. 3). Since DPN and UDPXy are quite dissimilar in structure, these data can best be interpreted by postulating that UDPXy is an allosteric inhibitor, exerting its effect by modifying the conformation of the enzyme (Monod et al, 1963). The apparent number (n) of inhibitory sites was estimated by plotting the logarithm of $\frac{V-v}{v}$ against the logarithm of UDPXy concentration, at different concentrations of UDPG. A family of

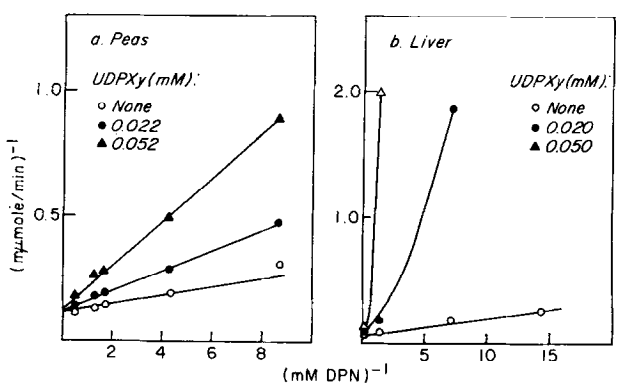

Figure 3. Reciprocal plots of velocity versus DPN concentration at various levels of UDPXy. UDPG concentration: a) 0.94 mM b) 0.37 mM.

parallel lines yielded an average "n" of 1.5 for the pea dehydrogenase, and 2.3 for the liver enzyme (for discussion, see Taketa and Pogell, 1965).

Both dehydrogenases are also inhibited by UDPGA, but at concentrations which are high relative to those of UDPXy. The inhibition is strictly competitive with UDPG (K_i: liver, 0.05 mM; peas, 0.3 mM), and non-competitive with respect to DPN (Fig. 4).

UDP-L-arabinose, the 4-epimer of UDPXy, has one-twentieth the inhibitory power of UDPXy for the liver enzyme, and one-third for the pea enzyme. The following compounds at a concentration of 1 mM exert no significant inhibition, nor do they reverse the inhibition by UDPXy: UTP, UDP, UMP, ATP, UDP-N-acetylglucosamine, CDP-D-glucose, ADP-D-glucose, GDP-D-glucose, dTDP-D-glucose, or α-D-xylose 1-phosphate. Heating

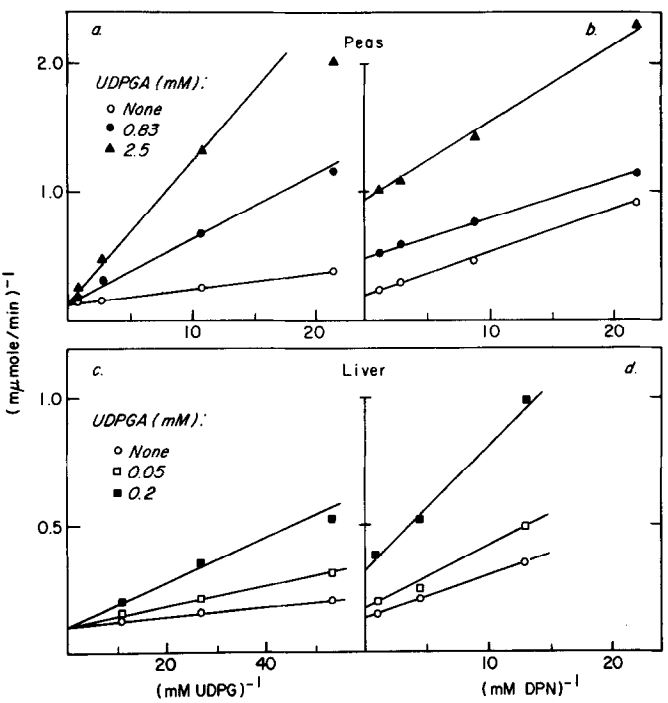

Figure 4. Reciprocal plots of velocity versus substrate concentration at various levels of UDPGA. Nucleotide concentrations other than indicated on graph: a) DPN, 1.2 mM; b) UDPG, 0.19 mM; c) DPN, 0.8 mM; d) UDPG, 0.024 mM.

UDPXy in 0.1 N HCl for 15 minutes at 100° completely destroys its ability to inhibit. UDPXy prepared enzymatically is as inhibitory as the chemically prepared commercial product.

The dehydrogenase activity of chick cartilage extract is likewise strongly and specifically inhibited by UDPXy (Table 1).

Table 1

UDPXy mM	cpm in UDPGA	Inhibition %
0	705	
0.01	666	5
0.04	382	46
0.09	18	97
0	520	
0.07	18	96

The reaction mixture contained 100 µl of cartilage extract, 0.4 mM UDPG (10,000 cpm) and 1 mM DPN, in total volume of 150 µl. After 1 hr at 37°, an aliquot (30 µl) was subjected to chromatography in ethanol-1 M ammonium acetate (75:30), pH 7.5, and the UDPGA area was eluted and counted. The radioactive material in this area moved as a single spot with the mobility of UDPGA in ethanol-1 M ammonium acetate (75:30), pH 3.6, and in isobutyric acid-1 M NH_4OH (10:6). The following compounds at 0.8 mM, did not inhibit by more than 6%: UTP, UDP, UMP, UDP-N-acetylglucosamine, UDPGA, α-D-xylose 1-phosphate. Only 12% inhibition was obtained by 0.16 mM UDP-L-arabinose.

Discussion. In plant tissues, UDPXy is known to be derived from UDPGA by decarboxylation (Feingold et al, 1960), and its effect on the dehydrogenase is therefore a clear case of feedback inhibition by an end product of a pathway on the first step.+ Since plants are able to form UDPGA from myo-inositol as well as from UDPG (Loewus et al, 1962), an interesting question is whether both pathways are equally subject to regulation by UDPXy.

Although neither UDPXy nor UDPGA decarboxylase have been detected in animal tissues, the identification of D-xylose as the residue connecting protein to carbohydrate in a number of mucopolysaccharides (Rodén et al, 1964; Anderson et al, 1965) strongly suggests that both

+A similar feedback inhibition occurs in Cryptococcus laurentii. UDPXy, formed in this organism by decarboxylation of UDPGA, strongly inhibits the UDPG dehydrogenase (Ankel and Feingold, 1964, and personal communication).

the nucleotide and the decarboxylase occur. If the sugars of the mucopolysaccharide are added sequentially to the protein, starting with D-xylose, then a reduction in the rate of synthesis of the protein moiety would cause an immediate surplus of UDPXy, which in turn would stop further production of UDPGA. Thus the inhibition of the dehydrogenase of cartilage by UDPXy can reasonably be expected to function as an effective regulatory device.

The inhibition of liver dehydrogenase is more difficult to interpret, since UDPGA is believed to serve in the liver primarily for the synthesis of glucuronides and ascorbic acid, and for the glucuronic cycle (Touster, 1962) — none of which are likely to be metabolically related to UDPXy. One might speculate that there exist isozymes of UDPG dehydrogenases, derived from different liver cell types, some of which are sensitive to UDPXy and others not. However, the dehydrogenase activity of a crude homogenate of rat liver was also found to be completely inhibitable by UDPXy. More data are needed before a regulatory function can be assigned to UDPXy in the liver.

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