DECARBOXYLATION OF URIDINE DIPHOSPHATE-D-GLUCURONIC ACID BY AN ENZYME PREPARATION FROM HEN OVIDUCT*

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In higher plants (Feingold et al., 1960) and Cryptococcus laurentii (Ankel and Feingold, 1964) uridine diphosphate-D-xylose[1] is formed by decarboxylation of uridine diphosphate-D-glucuronic acid. Neufeld and Hall (1965) found that UDPXyl is a potent and specific inhibitor of UDPG dehydrogenase (EC 1.1.1.22) both in higher plants and rat liver and suggested that it is formed also in vertebrate tissues.

We have now demonstrated the conversion of UDPGA to UDPXyl by a crude enzyme preparation from hen oviduct.

Materials and Methods. UDPGA labeled with C^{14} in the D-glucuronosyl moiety was prepared as described previously (Feingold et al., 1964). It was converted to C^{14}-labeled UDPXyl with UDPGA carboxy-lyase from Cr. laurentii (Ankel and Feingold, 1964). Authentic unlabeled UDPGA was purchased from Sigma and UDPXyl from Calbiochem. Xyl-1-P was a gift from Dr. E. F. Neufeld, N.I.H. Nucleotides and Xyl-1-P were separated by electrophoresis on Whatman No. 1 filter paper at pH 3.6 or 5.8 (Feingold

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1. The following abbreviations are used: uridine diphosphate-D-glucose, UDPG; uridine diphosphate-D-xylose, UDPXyl; uridine diphosphate-D-glucuronic acid, UDPGA; α-D-xylopyranosyl phosphate, Xyl-1-P; ethylenediamine tetraacetic acid, EDTA.

et al., 1964), by chromatography on Whatman No. 1 filter paper in (Solvent 1) ethanol-1M ammonium acetate, pH 7.5 (7:3, v/v), (Paladini and Leloir, 1952), or by chromatography on a cellulose thin-layer, using (Solvent 2) n-butanol-acetone-acetic acid-5% aq. NH_3 - H_2O (3.5:2.5:1.5:1.5:1, v/v), (Randerath, 1962) or Solvent 1. Neutral sugars were chromatographed on paper using H_2O-saturated phenol in the first dimension and n-butanol-acetic acid-H_2O (5:1:2, v/v) in the second. C^{14}-labeled compounds were located by radioautography, nucleotides by visual inspection at 254 mμ, inorganic and organic phosphate with the molybdic acid reagent of Bandurski and Axelrod (1951), and xylose with alkaline silver nitrate (Trevelyan et al., 1950).

Experimental. The complete oviduct of a one-year old laying hen (30 g) was homogenized for 1 min in a chilled Waring Blendor in 100 ml of ice-cold 0.1 M Na phosphate buffer, pH 6.0, 0.005 M in mercaptoethanol. The crude homogenate was filtered through several thicknesses of cheese cloth and centrifuged in the cold at 35,000 x g for 30 min. The pellet was suspended in 15 ml of buffer and tested for enzyme activity.

Oviduct preparation (0.1 ml) was mixed with 0.05 ml of 0.5 mM C^{14}-labeled UDPGA (30 μc/umole) 0.03 M in EDTA and after 2 hrs at 37°C the mixture was held at 100°C for 2 min and reaction products were separated by paper chromatography with Solvent 1. In addition to residual UDPGA, the reaction mixture contained 5 percent of a compound with the typical chromatographic mobility of UDPXyl.

The newly-formed radioactive compound was shown to be UDPXyl by the following criteria. It was identical with authentic unlabeled UDPXyl upon co-chromatography on paper and upon two-dimensional co-chromatography on a cellulose thin-layer, using Solvent 2 in the first and Solvent 1 in the second dimension. It also was identical with UDPXyl upon co-electrophoresis on paper at pH 3.6 and 5.8. Hydrolysis with 0.1 N HCl for 15 min at 100 C gave a single radioactive compound with the mobility of xylose upon two-dimensional paper co-chromatography. Treatment of the newly-formed com-

pound with phosphodiesterase (EC 3.1.4.1) from snake venom released a radioactive compound with the thin-layer-chromatographic (Solvent 2) and paper-electrophoretic (pH 3.6) mobility of authentic Xyl-1-P. Treatment of this compound with acid phosphatase (EC 3.1.3.2) yielded a neutral radioactive compound with the mobility of xylose upon two-dimensional paper co-chromatography.

When the newly-formed compound was incubated with acceptor in the presence of UDPXyl:acceptor xylosyltransferase from Cr. laurentii the radioactive moiety was completely incorporated into polysaccharide. Identical results were obtained using authentic UDPXyl labeled with C^{14} in the D-xylosyl moiety; neither Xyl-1-P nor UDPGA are glycosyl donors with the enzyme (Cohen and Feingold, 1964).

In a separate experiment enzyme preparation (0.4 ml) was incubated with 1.2 μmole of UDPGA and 5 μmole EDTA in a total volume of 0.5 ml for 6 hrs at 37°C. At the end of this time the mixture was held at 100°C for 3 min, precipitated protein was removed by centrifugation, and the reaction products were separated by paper chromatography with Solvent 1. Approximately 0.02 μmole of ultraviolet-absorbing material with the typical paper-chromatographic mobility of UDPXyl was present in the reaction mixture. This substance in addition had the mobility of authentic UDPXyl upon co-electrophoresis at pH 3.6; its spectrum at pH 7.0 was identical with that of UDPXyl. Hydrolysis (0.1 N HCl, 100 C, 15 min) liberated three major fragments: UMP, identified by its ultraviolet absorption and electrophoretic mobility at pH 3.6; inorganic phosphate, identified by its reaction with molybdic acid spray and its electrophoretic mobility at pH 3.6; and xylose, identified by its chromatographic mobility in H_2O-saturated phenol. In addition a small quantity of an ultraviolet-absorbing substance with the electrophoretic mobility (pH 3.6) of UDP was present in the hydrolyzate.

UDPXyl is a D-xylosyl donor in reactions catalyzed by enzyme preparations from higher plants (Feingold et al., 1959) and Cr. laurentii (Cohen

and Feingold, 1964). Since D-xylose is present in bovine chondroitin 4-sulfate (Gregory et al., 1964) and heparin (Lindahl and Roden, 1964) as well as in human brain (Stary et al., 1964) it is likely that UDPXyl has a similar donor function in vertebrate tissues. This view is substantiated by the finding of Grebner, Hall and Neufeld (personal communication) that hen oviduct preparations catalyze the incorporation of the D-xylosyl moiety of UDPXyl into glycoprotein-like material.

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