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Transfer of Xylose to Steroids by Rabbit Liver Microsomes†

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ABSTRACT: Rabbit liver microsomal preparations can transfer xylose from UDP-xylose to estrone, 17α-estradiol, and 17β-estradiol, and, in poorer yield, to diethylstilbestrol and *p*-nitrophenol. No transfer of xylose to estriol, testosterone, epitestosterone or 17α-estradiol 3-glucuronide could be demonstrated. The xyloside of [6,7-^{3}H]estrone which was formed by liver microsomes crystallized to constant specific activity with estrone β-D-xylopyranoside, the chemical preparation of which is described.

The transfer by liver microsomal preparations of nonacidic hexoses from their respective uridine nucleotides to specific hydroxyls on the estrogen molecule has been demonstrated in several species, including the human (Collins et al., 1968, 1970; Jirku and Levitz, 1972; Williamson et al., 1972; Labow et al., 1974; Labow and Layne, 1974). Rabbit liver microsomes can effect the transfer of *N*-acetylglucosamine or glucose to the 17α-hydroxyl of 17α-estradiol, provided that the 3-hydroxyl group of the steroid has previously been conjugated with glucuronic acid (Collins et al., 1968, 1970). Williamson et al. (1971) have shown that glucose or galactose, but not *N*-acetylglucosamine, can be transferred by washed rabbit liver microsomes to the phenolic 3-hydroxyl of estrone, 17α-estradiol, and 17β-estradiol, but not of estriol. These workers obtained evidence that this transfer was effected by an enzyme which was distinct from that which synthesized the 17-glycosides of 17α-estradiol 3-glucuronide, and that the formation of the 3-glucoside and the 3-galactoside was not totally dependent on the presence of UDP-glucose or UDP-galactose.

During an investigation of this reaction we obtained evidence for the formation of a conjugate of estrone when UDP-xylose was included in the incubation mixture, and the present paper presents the identification of this compound as 17-oxoestra-1,3,5(10)-trien-3-yl β-D-xylopyranoside by comparison with synthetic material, and an investigation of the specificity and characteristics of the xylosyl transferase involved.

Experimental Procedure

Materials. UDP-xylose was purchased from Sigma Chemical Co., St. Louis, Missouri. Steroids and other reagents were obtained and purified as previously described (Collins et al., 1968, 1970; Williamson et al., 1972; Labow et al., 1974). All labeled steroids contained tritium in the (6,7) positions and were obtained from New England Nuclear Corp., Boston, Mass. The materials were checked for purity by thin-layer chromatography. The specific activities used in all assays were between 30 and 40 Ci/mol.

General Methods. The procedures for the preparation of homogenates and of microsomes, thin-layer chromatography of steroid glycosides and their aglycones, the determination of protein and the assay of radioactivity in incubates and on thin-layer plates were all carried out as previously detailed (Collins et al., 1970; Williamson et al., 1972; Labow et al., 1974).

Assay of Transferase Activity. A methanol solution of 0.05 μmol of labeled steroid was evaporated to dryness in a 15-ml conical centrifuge tube fitted with a ground-glass stopper. A solution of 0.5 μmol of UDP-xylose and a microsomal suspension equivalent to 0.5 g of rabbit liver (approximately 10 mg of protein), each in 1.0 ml of 0.15 M Tris-HCl buffer, pH 7.0, were added. The total volume of buffer was brought to 3 ml, and the tubes were incubated for 30 min at 37 °C. The incubations and extraction of the media were done as described by Labow and Layne (1972), with the following modifications necessitated by the fact that the estrogen xylosides were appreciably soluble in benzene: the incubations were stopped by shaking with 5 ml of ethyl acetate. This extract was then evaporated to dryness and chromatographed in $CHCl_3$–MeOH (9:1) on silica gel H plates after the addition of standard estrone xyloside to each sample. After spraying with 2% H_2SO_4 in ethanol and heating at 110 °C for 5 min, the estrone xyloside area was scraped and counted and the percent conversion of the tritiated steroid to conjugate was calculated. When compounds other than estrone were tested as substrates, the entire plate was scraped in 1-cm sections and the radioactivity in the xyloside area was located by comparison with a control reaction incubated without UDP-xylose. The percent conversion was calculated as described above. Values were expressed either as picomoles of xyloside formed per minute per gram of liver, or per milligram of protein as determined by the method of Lowry et al. (1951).

Synthesis of Estrone β-D-Xylopyranoside. The improved Koenigs–Knorr procedure described by Conrow and Bernstein (1971) for the preparation of estrogen hexopyranosides was used to couple estrone with xylose. Estrone (2.08 g, 0.77 mmol) was reacted with 2,3,4-tri-*O*-acetyl-α-D-xylo-

† From the Department of Biochemistry, University of Ottawa, Ottawa, Canada K1N 9A9. *Received July 8, 1975.* This work was supported by Grant MT-3287 from the Medical Research Council of Canada.

pyranosyl bromide (5.2 g, 1.54 mmol) in toluene in the presence of cadmium carbonate (Fisher certified, recrystallized). The resulting triacetyl glycoside was crystallized from ether–hexane in a yield of 31% (1.25 g): mp 185 °C (softening), 218–220 °C (melt); $[\alpha]^{23}D$ + 65° (*c* 0.51, chloroform); ir 1200–1250, 1600, 1720–1765 cm^{-1}; NMR ($CDCl_3$, Varian HA-100) δ 7.1 and 6.6 (three aromatic protons), broad signal from 5.2 to 4.8 (H1′, 2′, 3′, 4′; 4H), 4.18 (H-5′e, 1 H, m over 16 Hz), 3.48 (H-5′a, 1 H, m over 18 Hz), 2.84 (Hs6, 2 H, m 20 Hz), 2.04, 2.02, 2.01 (3 acetates, 9 H, 3 s), 0.88 (C-18 methyl). The relatively large difference between the chemical shifts of the 5α (a) and 5β (e) hydrogen atoms is probably caused by the presence of a bulky aglycone.

The acetylated glycoside was deblocked by means of dry ammonia in 30% chloroform–methanol solution. The reaction was carried out overnight at 0 °C. Solvent was removed and the product precipitated by the addition of a small amount of water. Recrystallization from ethanol–water gave 430 mg (74%) of 17-oxoestra-3,5(10)-triene-3-β-D-xylopyranoside: mp 116–120 °C (softening), 230–240 °C (melt); $[\alpha]^{23}D$ +102° (*c* 0.355, methanol); ir (Nujol) 3200–3400, 1700–1710, 1600 cm^{-1}; NMR (Me_2SO; Varian 60 MHz) 7.2 and 6.75 (3 H, aromatic), broad signal from δ 5.3 to 4.66 (H-1′, 2′, 3′, 4′; 4 H), 0.80 (C-18 methyl, 3 H, s). Anal. Calcd for $C_{23}H_{30}O_6$ (402.48): C, 68.63; H, 7.51. Found: C, 69.14; H, 7.66. The β-D-glycosidic linkage was assigned by Klyne's rule (Klyne, 1950) on the basis of the following data: [M]D calcd: β-D-xyloside, +330°; α-D-xyloside, +690°. [M]D found: +402°.

Characterization of Estrone Xyloside. The material (310 000 dpm) formed by the incubation of [6,7-^{3}H]estrone with liver microsomes in the presence of UDP-xylose was mixed with 31.7 mg of synthetic 17-oxoestra-1,3,5(10)-triene-3-β-D-xylopyranoside and was crystallized to constant specific activity from aqueous methanol.

Results

[6,7-^{3}H]Estrone, 17α-[6,7-^{3}H]estradiol, and 17β-[6,7-^{3}H]estradiol were found to serve as acceptors for xylose, and the xylosides of these three steroids were formed at the rate of 79, 400, and 80 pmol per gram of tissue, respectively. [6,7-^{3}H]Estriol showed no evidence of the formation of xyloside, nor did 17α-[6,7-^{3}H]estradiol 3-glucuronide, 17β-[6,7-^{3}H]estradiol 3-glucuronide, [1,2-^{3}H]epitestosterone, or [1,2-^{3}H]testosterone. Diethylstilbestrol and *p*-nitrophenol formed xylosides, but in poor yield as compared with estrone and the estradiols. No formation of xylosides was observed when substrates were incubated with washed microsomes in the absence of added UDP-xylose. Four successive recrystallizations of the tritiated conjugate formed from [6,7-^{3}H]estrone with synthetically prepared estrone xyloside resulted in constant specific activity with no appreciable loss of total radioactivity.

The pH optimum for the formation of estrone xyloside was close to pH 7.0. The transferase activity toward estrone in the supernatant obtained by centrifugation at 10 000*g* was similar to that in liver homogenate but was concentrated by a factor of 5 in the pellet obtained by further centrifugation at 100 000*g*. When this pellet was treated with Triton X-100 (Labow et al., 1973), no evidence was obtained for activation of the enzyme or its solubilization from the microsomal pellet.

The presence of UDP-xylose effectively inhibited (Figure 1) the transfer of glucose from UDP-[6-^{3}H]glucose to estrone in the assay for rabbit liver microsomal glucosyl transferase described by Labow and Layne (1972). The inhibition was proportional to the concentration of UDP-xylose over the range from 0.15 to 2.0 mM and was greater than 40% at a concentration of UDP-xylose (0.5 mM) equal to that of UDP-glucose in the incubation medium.

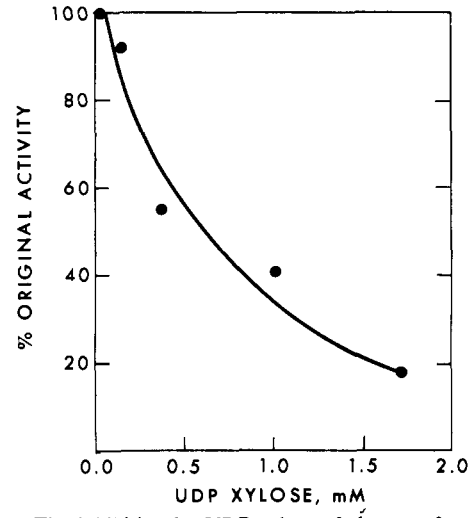

FIGURE 1: The inhibition by UDP-xylose of the transfer of glucose from UDP-[6-^{3}H]glucose to estrone by rabbit liver microsomes. Glucosyl transfer was assayed as described by Labow and Layne (1972).

Discussion

The specificity of the transfer of xylose to steroids by rabbit liver microsomes is not definitively established by the results on the limited number of steroids tested. However, it resembles that found (Collins et al., 1970; Labow and Layne, 1972) for the transfer of glucose and galactose in three respects. These are: (a) the apparent requirement for a phenolic ring A in the acceptor steroid; (b) the formation of glucosides of *p*-nitrophenol and of diethylstilbestrol in yields much lower than those obtained with estrone and the estradiols; and (c) the failure to form a conjugate with estriol. The glycoside formed when rabbit liver microsomes were incubated with [6,7-^{3}H]estrone in the presence of UDP-xylose was rigorously identified as estrone β-D-xylopyranoside by crystallization to constant specific activity with synthetically prepared material. It is therefore probable that the 3-hydroxyl group is also the site of attachment of xylose to 17α-estradiol and 17β-estradiol, as is the case for glucose under similar conditions (Collins et al., 1970; Williamson et al., 1971). The synthesis of estrone β-D-xylopyranoside is the first recorded preparation of a steroid pentoside and indicates that the modification of the Koenigs–Knorr synthesis developed by Conrow and Bernstein (1971), using cadmium carbonate as base, can be conveniently applied to the coupling of a steroid to a pentose sugar.

The fact that the steroid xylosyl transferase activity is concentrated in the pellet obtained by centrifugation of liver homogenate at 100 000*g* indicates that, like the other steroid glycosyl transferases so far studied (Labow et al., 1973), it is "microsomal", or tightly bound to cellular membranes. Schwartz and Roden (1974) have found that the polysaccharide chain-initiating xylosyl transferase of embryonic chick cartilage is, in contrast to most other glycosyl transferases, largely soluble, and located in the high-speed

supernatant. No evidence for such a location of the steroid xylosyl transferase of rabbit liver was found in the present work.

It has been shown (Labow and Layne, 1974), that there are two enzymes in rabbit liver which effect the transfer of glucose to estrogens. One of these, which may be identical with the steroid *N*-acetylglucosaminyl transferase described by Collins et al. (1968), is highly specific for the 17α-hydroxyl group of 17α-estradiol, and requires the prior conjugation of the 3-hydroxyl group of the steroid with glucuronic acid. This enzyme has a pH optimum of about 8.0 for glucose transfer from UDP-glucose. The second enzyme, which transfers glucose or galactose from their respective uridine nucleotides to the phenolic 3-hydroxyl group of estrone, 17α-estradiol and 17β-estradiol, has a pH optimum of about 7.0, which is very similar to that of the xylosyl transferase studied in the present work. This, coupled with the similarities in substrate specificity, suggests that the same enzyme may be responsible for the formation of the steroid xylosides and the glucosides. This possibility is strengthened by the results in Figure 1, which show that the transfer of glucose from UDP-glucose to estrone by rabbit liver microsomes is inhibited by UDP-xylose. On the other hand, Williamson et al. (1971) have shown that washed microsomes can effect the formation of small amounts of estrogen glucosides and galactosides in the absence of exogenous UDP-glucose or UDP-galactose and have suggested that this may be due to the presence of a water-insoluble sugar donor, which may be an intermediate in sugar transfer from the uridine nucleotides. No evidence was found in the present work for such an intermediate in the xylose transfer reaction so that resolution of the question as to the relationship of the xylosyl to the glucosyl galactosyl transferase will require further work on purification of the enzymes.

The formation in mammals of *N*-ribosides of imidazoleacetic acid, a metabolite of histamine (Karjala, 1955), of 2-hydroxynicotinic acid (Schwartz et al., 1973) and of several purines and pyrimidines (Mandel, 1959) has been demonstrated. Recent work has shown that a xyloside of bilirubin is excreted in dog bile (Fevery et al., 1971) and can be formed in vitro by rat liver by conjugation of the bilirubin molecule with D-xylose donated by UDP-xylose (Fevery et al., 1972). The present work provides evidence for the formation of a steroid pentoside by animal tissue in vitro, the significance of which under physiological conditions remains to be investigated. It is possible that the xylosides may play a role in steroid transport such as that suggested by Collins et al. (1970) for the glucosides and galactosides of the estrogens, or in the regulation of estrogen metabolism in the liver as indicated by the finding of Williamson and Layne (1975) that formation of glycosides may facilitate the passage of estradiol into liver cells. Schwartz et al. (1974) have recently demonstrated stimulation by exogenous β-D-xylosides of the synthesis of free chondroitin sulfate chains in cultured chick embryo limb-bud cells previously treated with 5-bromo-2′-deoxyuridine or puromycin. These results enhance the interest of the present findings on the formation by animal tissues of β-D-xylosides of endogenous compounds such as the steroids.

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