Steroid Estrogen Glycosides

FORMATION OF GLUCOSIDES AND GALACTOSIDES BY HUMAN LIVER AND KIDNEY*

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SUMMARY
Homogenates of human liver can transfer both glucose and galactose from their respective uridine nucleotides to the 17α-hydroxyl of 17α-[6,7-3H]estradiol. Glucose was also transferred, but less readily, to the 16α-hydroxyl of [6,7-3H]-estradiol. Kidney homogenates effect these same transfers. The radioactive steroid glycosides formed, namely 17α-estradiol-17β-D-glucopyranoside, 17α-estradiol-17β-D-galactopyranoside, and estriol-16α-β-D-glucopyranoside, were rigorously identified by crystallization to constant specific activity with authentic glycosides. The chemical preparation of the latter two glycosides is described for the first time. The results are the first demonstration of steroid glucoside formation by human kidney, and of steroid galactoside formation by any human tissue.

Homogenates of human liver were recently shown to effect the transfer of glucose from UDP-glucose to the 17-hydroxyl group of 17α-estradiol (1). This was the first demonstration, with human tissue, of the formation of a steroid glucoside. In these experiments no transfer of glucose to the 17α-hydroxyl of 17β-estradiol, or to the phenolic 3-hydroxyls of estrone, or of either epimer of estradiol could be shown. This suggested that the microsomal steroid glucosyltransferase had a high specificity for the α-oriented 17α-hydroxyl group. The present paper describes the finding that human kidney, as well as liver, exhibits this glucosyltransferase activity, and that both liver and kidney can also effect the in vitro formation of 17α-estradiol-17β-D-galactopyranoside. The specificity of these glycosyl transfer reactions has been further investigated with a limited series of steroids as substrates.

EXPERIMENTAL PROCEDURE

Materials—Nucleotides were purchased from Sigma. Steroids and other reagents were obtained and purified as previously described (2, 3).

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Tissues—Healthy human liver tissue from a 26-year-old woman was the preparation obtained and described by Williamson and Layne (1). This tissue was homogenized immediately after surgery in 4 volumes of 0.15 M KCl at 4°. The homogenate was stored at -15°. Incubation experiments with this homogenate were performed within 48 hours after its preparation. Kidney tissue for preliminary experiments was obtained by excising random areas, containing both cortex and medulla, from the kidney of a 56-year-old man, which had been removed because of hypertensive renal failure. This tissue was obtained at surgery and homogenized at once. Incubations were carried out immediately with portions of the homogenate and the remainder was stored at -15°. Another small sample of kidney tissue was obtained from the macroscopically normal area, containing both cortex and medulla, of the kidney of a 37-year-old man who was undergoing surgery for removal of a renal calculus. This tissue was homogenized and incubations were carried out immediately after surgery.

Preparation of 17α-Estradiol-17β-D-galactopyranoside—This compound was prepared by the Koenigs-Knoevenagel reaction using the procedure previously applied (4) to the preparation of 17α-estradiol-17β-D-glucopyranoside. The product was recrystallized from ethanol (m.p. 254-256°, [α]D 20 = -2° (C = 0.28, methanol)). These constants remained unchanged by further crystallization. Infrared (Unicam SP-200 instrument); vmax 2800 to 3500 (OH); 1500 (aromatic); 1200 to 1270 (ester). Nuclear magnetic resonance (dimethylsulfoxide, Varian HA-100 instrument); δ 6.54 (d, 1, J1,2 = 6 Hz, H-1), 6.44 (m, 2, H-2,4), 4.71 (m, 1, H-4'), 4.37 (m, 1, H-2'), 5.84 to 3.54 (all OH groups, and covering signal for H-17 and 1 major proton), 0.66 (s, C-18 methyl, H-3). These spectra are in agreement with the assigned structure of 17α-estradiol-17β-D-galactopyranoside. As a final check on the synthesis, 500 μg of the product were hydrolyzed with hydrochloric acid and the sugar residue was examined on thin layer chromatography on Silica Gel H by the procedure described in detail by Williamson et al. (4). The systems used were n-propyl alcohol-water (7:1) and methyl acetate-isopropyl alcohol-water (18:1:1). In both systems the sugar residue had the same RF value as reference galactose, and was separated from reference glucose and mannose. The relative RF values of the three sugars were essentially as recorded by Williamson et al. (4). Elemental analysis of the synthetic material gave the following:
The crystallization of steroid glycosides with fractional molecular equivalents of water, which is not removed by prolonged drying under vacuum, has been well documented (4–6).

Preparation of Estradiol-16α-β-D-glucopyranoside—A sample of estradiol glucuronide donated by Dr. Saul Cohen was used as starting material. This material was isolated in crystalline form from human pregnancy urine (5), and its structure has been established (8, 9) as estradiol-16α-β-D-glucopyranosiduronic acid. The compound was methylated and reduced to the corresponding glucoside (10, 11). The procedure followed was exactly as described by Collins et al. and Layne and Williamson (1). The final product melted sharply at 260° and gave an infrared spectrum entirely reconcilable with the structure of estradiol-16α-β-D-glucopyranoside; νmax 3550 (OH), 1600 and 1495 (aromatic), 1220 to 1280 (ester). Hydrolysis of the product with an acid emulsion and with hydrochloric acid yielded products which were identified by thin layer chromatography, by the procedures detailed previously (4), as estradiol and β-D-glucose.

Methods—The procedures for the preparation of homogenates and of microsomes, assays for conjugate formation, enzymatic hydrolysates, and thin layer chromatography of steroid conjugates and their aglycones were all carried out as previously detailed (2–4). Estrogen glycosides were well separated from the corresponding galactosides on Silica Gel II in chloroform-methanol (4:1). Specific activities of the steroids were adjusted to 5.6 mCi per pmole. The amounts of tritiated steroid, of buffer, and of homogenate, and the conditions of incubation, were those described by Williamson and Layne (1), as follows. Incubation mixtures contained radioactive steroid (4 × 10^-5 μmoles), UDP-glucose (0.5 μmole), 0.15 M Tris-HCl buffer, pH 8.0 (2.0 ml), 0.15 M KCl (0.5 ml), and tissue homogenate (0.5 ml). After incubation at 37° for 30 min, each incubation mixture was extracted three times with 5 ml of benzene and then three times with 5 ml of ethyl acetate. The extracts were assayed for radioactivity and examined for Estriol glucoside, Estriol galactoside, l7α-[6,7-3H]estradiol glucoside, and l7α-[6,7-3H]estradiol galactoside, as described previously (4) as estriol and β-D-glucose.

Results

Preliminary experiments indicated that the steroid glucosyl transferase of human liver was located in the microsomal fraction obtained at 105,000 × g. The activity in this fraction was, however, considerably enhanced by the addition of small amounts of the 105,000 × g supernatant, indicating the probable requirement for a soluble cofactor, which is a quite common situation with other steroid glucosyltransferases (12). Because of this finding, further experiments were carried out with whole homogenates. Table I shows the results of qualitative experiments to test the respective ability of liver and of kidney homogenates to transfer glucose and galactose from their respective uridine nucleotides to various steroids. No appreciable qualitative or quantitative difference in transferase activity was detected between the kidney tissue from the two donors. Loss of transferase activity on storage was negligible after 48 hours, but was about 50% after 2 weeks.

The results in Table I indicated that both liver and kidney tissue could effect the formation of both a glucoside and a galactoside of 17α-estradiol. Evidence was also obtained for the formation of a glucoside of estradiol by liver and by kidney donors. Loss of transferase activity on storage was negligible after 48 hours, but was about 50% after 2 weeks.

| Table I
| Transfer of sugars from uridine nucleotides to steroids by homogenates of human liver and kidney
| Substrate | Liver | Kidney |
|-----------|-------|--------|
| Glucosyl donors | UDPG  | UDPGal | UDPG  | UDPGal |
| UDP'glucose | 66 | 240 |
| UDP'galactose | 20 | 53 |
| Estradiol glucoside | 4 | 15 |
| Estradiol galactoside | 0 | 0 |

In a single experiment a very small amount of conjugation of epitestosterone by liver tissue to a presumptive glucoside was observed, but this finding could not be repeated in subsequent incubations with either liver or kidney homogenates.

A quantitative estimate of the amounts of steroid glycosides formed in comparable incubations was provided by the per-
centage of the radioactive steroid which was converted to glycoside. The conversion of 17α-estradiol to glucoside and galactoside by liver tissue was 16% and 5%, respectively. The corresponding figures for kidney tissue were 83% and 13%. The formation of estriol glucoside amounted to 1.5% of the steroid incubated with liver and 6.6% of that incubated with kidney. These figures can be converted to picomoles of conjugate formed per g of wet tissue, and are shown in this form in Table II.

The identity of the 17α-estradiol 17β-D-glucopyranoside formed by human liver has been previously established (1) by crystallization to constant specific activity with material prepared synthetically (4). Similar recrystallizations were carried out in the present work to establish the identity of the glucoside of 17α-estradiol formed by kidney tissue and of the galactoside of this steroid formed both by liver and by kidney. As shown in Table III, the tritiated conjugate formed in the incubations crystallized in each case to constant specific activity with the corresponding 17α-estradiol-17β-D-glucopyranoside. In addition, the identity of the tritiated glucoside of estriol formed by liver homogenates was established by crystallization as estriol-16α-D-glucopyranoside. In these experiments the calculated specific activity (Table III) was obtained for each compound by dividing the number of disintegrations per minute of tritium in the presumptive glycoside by the number of milligrams of authentic compound which were mixed with it prior to crystallization. The weight of the radioactive material was negligible. The figures in the individual cases were, estriol-16α-glucoside, 135,390 dpm, 9.05 mg; 17α-estradiol-17galactoside (liver) 195,300 dpm, 15.41 mg; 17α-estradiol-17glycoside (kidney), 130,300 dpm, 8.8 mg; 17α-estradiol-17galactoside (kidney), 180,955 dpm, 15.44 mg.

**Discussion**

Williamson et al. (13) have detected the formation of galactosides of some phenolic steroids by rabbit liver, but the present work is the first demonstration of the formation of a steroid galactoside by human tissue. The finding that human kidney homogenates as well as those of liver had steroid glucosyl- and galactosyltransferase activities contrasts with the results obtained in the rabbit, where these glycosyltransferase activities were detected only in liver, and were apparently specific for the phenolic 3-hydroxyl group. Human kidney tissue was apparently more effective than liver in forming the steroid galactoside by human tissue. The finding that human kidney homogenates as well as those of liver had steroid glucosyl- and galactosyltransferase activities which might obtain in vivo could well differ, therefore, from that found in vitro.

No precautions were taken to exclude bacterial contamination of the homogenates used in this work. However, it was possible with kidney homogenates to carry out incubations within 2 hours of excision of the tissue, and these incubations were of 30-min duration. The possibility that the steroid glucosylations were caused by bacterial contamination is extremely remote, the more so because such contamination would have had to affect to the same extent three tissue samples obtained at different times from different hospitals. Prolonged storage of the homogenates at −15°C showed no evidence of any effect other than a slow loss of transferase activity. This would be expected in an endogenous enzyme activity, and militates against the possibility that the transferases were produced by contamination during storage.

The significance of the formation of these novel glycosides of 17α-estradiol is as yet unknown. This steroid, if it is formed in the human at all, is of minor quantitative importance (1). It is of interest that neither the 17β-hydroxy of 17β-estradiol nor the 3-hydroxy of any of the phenolic steroids tested appear to be acceptors of glucose or galactose. Estriol, which is a quantitatively major excretion product in the human, did form a 16α-glucoside. This reaction, however, took place in poor yield, which might explain the failure to detect the formation of the corresponding estriol galactoside.

The number of steroids tested as substrates (Table 1) is limited, but the glucosyltransferase or transferases, in addition to the requirements with regard to the hydroxyls in Ring D, appear to have a considerable specificity for the steroids with an aromatic A ring. That this may not be an absolute requirement is suggested by the evidence obtained in a single experiment for the formation of a glucoside by epitestosterone. The fact that the presence of glucuron acid at position 3 of 17α-estradiol prevents the addition of glucose to the 17α-position is at variance with the results obtained in rabbits (2, 4) where the glucuronidation of the 3-hydroxy is obligatory for the addition of glucose to the 17α-position.

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