ESTROGEN GLUCOSIDES AND GALACTOSIDES: FORMATION BY RABBIT LIVER MICROSOMES IN VITRO

D.G. Williamson, A. Polakova and D.S. Layne

Department of Biochemistry, University of Ottawa, Ottawa 2, Ontario, Canada.

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SUMMARY

Triton-solubilized preparations of rabbit liver microsomes form glucosides and galactosides of phenolic steroids, but only if fortified with UDP-glucose or UDP-galactose. Formation of these steroid glycosides by washed microsomes can, however, take place in the absence of detectable amounts of the uridine nucleotides. The results indicate the presence in the microsomes of a water-insoluble sugar donor, which may be an intermediate in sugar transfer from the uridine nucleotides. The formation of steroid galactosides by animal tissues has not previously been demonstrated.

Rabbit liver microsomal preparations can effect the transfer of glucose from uridine diphosphoglucose (UDPG) to the 3-hydroxyl of estrone, 17α-estradiol or 17β-estradiol (1). The glucosyltransferase is solubilized by the detergent Triton X-100 and the solubilized enzyme shows a specific requirement for UDPG. We have now found that the substitution of uridine diphosphogalactose (UDPGal) for UDPG leads to the formation of the 3-galactosides of the phenolic steroids. However, washed microsomal preparations which contain no detectable UDPG or UDPGal can form appreciable quantities of estrogen glucosides and galactosides without the addition of the uridine nucleotides.

EXPERIMENTAL PROCEDURE AND RESULTS

Materials and Methods. Nucleotides were purchased from Sigma

Chemical Co. Steroids and other reagents were obtained and purified as previously described (1). The preparation of liver microsomes, treatment of these with Triton X-100, assays for conjugate formation, and thin-layer chromatography of steroid conjugates were all carried out as described by Collins et al (1).

Formation of Steroid Glycosides. Estrone-6,7-^{3}H was incubated with a Triton-solubilized preparation of microsomes from a female New Zealand rabbit in the presence of UDPG and UDPGal respectively. Extraction of the incubate with ethyl acetate yielded radioactive steroid conjugates in both cases, and the presumptive galactoside was more polar than the presumptive glucoside on thin layer silica gel chromatography in chloroform-ethanol (2). No conjugate formation could be detected in the absence of exogenous UDPG or UDPGal. The radioactive conjugates were crystallized to constant specific activity with authentic samples of estrone-3-β-D-glucoside and estrone-3-β-D-galactoside which were prepared in this laboratory by a modified Helferich synthesis (3).

When estrone-6,7-^{3}H was incubated with microsomal preparations which had not been treated with Triton X-100, and to which no nucleotides were added, two conjugates were formed which corresponded chromatographically to estrone-3-β-D-glucoside and estrone-3-β-D-galactoside. Their identity was again confirmed by recrystallization to constant specific activity with the authentic compounds. While the amounts of these conjugates formed could be respectively enhanced by the addition of UDPG or UDPGal, the ability to form them could not be abolished by repeated re-suspension and re-centrifugation of the microsomal pellet. In this respect the formation of these two

glycosides differed from that of estrone-3-glucuronide, since the ability of the microsomes to form this latter compound was almost entirely removed by washing, and was restored by the addition of uridine diphosphoglucuronic acid (UDPGA).

The above results with estrone were the same as those obtained when 17α-estradiol was used as the substrate in these reactions. Only the 3-hydroxyl group of 17α-estradiol is involved in glycosidations with rabbit liver preparations (1), and since this steroid is the major estrogen metabolite in the rabbit, it was used in further experiments. Table I illustrates the effect of washing on the ability of unfortified microsomes to form 17α-estradiol-3-glucoside and galactoside as compared to their ability to form 17α-estradiol-3-glucuronide.

Indications of Absence of Nucleotides from Washed Microsomes. In order to determine whether the synthesis of glycosides in the unfortified microsomes might be due to retention of uridine nucleotides in the preparations in spite of the washing pro-

TABLE 1. Effect of Repeated Washing on the Ability of Rabbit Liver Microsomes to form Glycosides of 17α-Estradiol. Figures are the Amounts of Conjugate formed per 1 mg of Protein in the Assay of Collins et al (1).

Fraction	pmoles of conjugate formed	
	Glucoside + Galactoside	Glucuronide
10,000 g supernatant	59	454
Microsomes (unwashed)	237	30
Microsomes (1st wash)	179	15
Microsomes (2nd wash)	154	11
Microsomes (3rd wash)	155	13
Microsomes (4th wash)	209	19

TABLE 2. Effects of UDPG Pyrophosphorylase and UDPG Dehydrogenase on the Synthesis of Glycosides of 17α-Estradiol by Rabbit Liver Microsomes *in vitro*. Assay as Described by Collins *et al* (1).

Compounds added to basic incubation medium of microsomes + steroid + buffer	pmoles of conjugate formed	
	Glucoside + Galactoside	Glucuronide
Control	166	66
UDPG pyrophosphorylase	152	47
UDPG dehydrogenase	146	51
UDPG	865	112
UDPG + UDPG pyrophosphorylase	137	72
UDPG + UDPG dehydrogenase	486	1500

cedures, experiments were done with enzymes which metabolize UDPG or UDPGal. Microsomal preparations were pre-incubated with UDPG pyrophosphorylase, which in the presence of pyrophosphate catalyses the formation of glucose-1-phosphate and UTP from UDPG (4), and with UDPG dehydrogenase, which converts UDPG to UDPGA in the presence of NAD (5). The ability of these preparations to form glycosides of 17α-estradiol was then assayed (Table 2). The results indicate that treatment with these enzymes does not significantly alter the ability of the unfortified microsomes to form glycosides. The presence of UDPG and UDPG dehydrogenase leads to a much increased formation of glucuronide, due to the production of UDPGA. In the experiment shown in Table 3, pre-incubation of the microsomes was carried out with UDP galactose-4-epimerase, which catalyses the conversion of UDPGal to UDPG (6). When UDPGal is added

TABLE 3. Effect of UDPGalactose-4-Epimerase on the synthesis of Glycosides of 17α-Estradiol Assayed as Described by Collins et al (1).

Incubation	pmoles glucoside + galactoside formed	Ratio glucoside / galactoside
Microsomes	90.5	2.84
Microsomes + epimerase	84.5	2.80
Microsomes + UDPG	581	12.9
Microsomes + UDPGal	355	0.065
Microsomes + UDPGal + epimerase	550	8.32

together with this enzyme, the production of steroid glucoside is increased, but in the absence of added nucleotide the epimerase does not alter either the ratio of steroid glucoside to galactoside, or the total amount of the two steroid glycosides formed by the microsomes.

DISCUSSION

These results establish that rabbit liver microsomes in vitro can form galactosides as well as glucosides of the phenolic hydroxyl of some steroid estrogens. This is the first demonstration of the formation of steroid galactosides by animal tissue. No evidence has, however, been found for the excretion of either the 3-glucosides or the 3-galactosides of the phenolic estrogens, and they may therefore function as metabolic intermediates. The addition of UDPG or UDPGal, or of other water soluble glycosyl donors, was not an absolute requirement for the formation of estrogen glucosides and galactosides by a rabbit liver microsomal pellet obtained by centri-

fugation at 105,000 x g. However, the addition of the nucleotides did increase the amount of the glycosides formed, and was, moreover, obligatory for their formation by a Triton-solubilized preparation of the microsomal pellet. It appears unlikely that endogenous UDPG or UDPGal could be retained on the microsomes in such a form as to survive repeated washing and to be protected from metabolism by such enzymes as UDPG pyrophosphorylase, UDPG dehydrogenase and UDPGal-4-epimerase. A more likely explanation of our results is that the transfer of glucose or galactose from the appropriate uridine nucleotide to the steroid can proceed by way of an intermediate acceptor, such as the lipid intermediates which have been shown to take part in some sugar transport reactions (7). The complex of this acceptor with the sugar would have to be water insoluble and to be retained in the microsomal pellet.

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REFERENCES

1. Collins, D.C., Williamson, D.G., and Layne, D.S., J. Biol. Chem. 245, 873 (1970).
2. Williamson, D.G., Collins, D.C., Layne, D.S., Conrow, R.B., and Bernstein, S., Biochemistry 8, 4299 (1969).
3. Stanek, J., Cerny, M., Kocourek, J., and Pacak, J., The Monosaccharides (Academic Press, New York, 1963) p. 265.
4. Munch-Petersen, A., and Kalckar, H.M., Methods in Enzymology, Vol. II, S.P. Colowick and N.O. Kaplan, Eds., Academic Press, New York, 1955, p. 675.
5. Strominger, J.L., Maxwell, E.S., and Kalckar, H.M., Methods in Enzymology, Vol. III, S.P. Colowick and N.O. Kaplan, Eds., Academic Press, New York, 1957, p. 974.
6. Darrow, R.A., and Rodstrom, R., Biochemistry 7, 1645 (1968).
7. Behrens, N.H., and Leloir, L.F., Proc. Nat. Acad. Sci. 66, 153 (1970).