A Comparison of Glucoside Formation by Liver Preparations from the Rabbit and the Mouse

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Liver homogenates from mice and from rabbits transfer glucose from UDP-[6-³H]glucose, at pH 7.0, to oestradiol-17 α , oestradiol-17 β , oestradiol-17 α 3-glucuronide, *p*-nitrophenol and diethylstilboestrol. In the rabbit the phenolic steroids were better substrates than *p*-nitrophenol for the glucosyltransferase, whereas the reverse was true in the mouse. At pH 8.0, rabbit liver, but not mouse liver, transferred glucose to oestradiol-17 α 3-glucuronide in better yield than that at pH 7.0. Evidence is presented for the presence of two glucosyltransferases in rabbit liver. One of these has a pH optimum at about 8.0, and is highly specific for oestradiol-17 α 3-glucuronide, whereas the other, which has a pH optimum at about 7.0, is similar in this respect to the transferase in mouse liver.

Studies have shown that microsomal preparations from mammalian liver can transfer glucose from UDP-glucose to hydroxyl groups on *p*-nitrophenol (Gessner & Vollmer, 1969; Gessner *et al.*, 1973), bilirubin (Wong, 1971; Fevery *et al.*, 1972), retinol (Rodriguez *et al.*, 1972) and steroids (Williamson *et al.*, 1969). Steroid glucosyltransferase activity has been found in the liver of rabbits (Collins *et al.*, 1970; Williamson *et al.*, 1971), sheep (Labow *et al.*, 1974) and humans (Williamson *et al.*, 1972). The enzymes in all three species have a very marked preference, and in some instances what appears to be an absolute requirement, that the steroid possess the phenolic ring A characteristic of the mammalian steroid oestrogens. The glucosyltransferase in human liver transfers glucose to the 17 α -hydroxyl group of oestradiol-17 α , and to a lesser extent to the 16 α -hydroxyl group of oestriol. In each case the free steroid is the substrate, and the prior addition of glucuronic acid to the 3-position of the steroid completely prevents the transfer of glucose to the hydroxyl group on ring D. In contrast, the enzyme in sheep liver, although also specific for the 17 α -hydroxyl group on oestradiol-17 α , requires the presence of glucuronic acid on the 3-hydroxyl group, and its substrate is thus oestradiol-17 α 3-glucuronide rather than the free steroid itself. No evidence has been found for the transfer of glucose to the phenolic 3-hydroxyl group of any of the steroid oestrogens by tissues from either the sheep or the human. In the rabbit, however, two distinct steroid glucosyltransferases have been identified (Labow *et al.*, 1973) one of which transfers glucose with equal facility to the 3-hydroxyl group of oestrone, oestradiol-17 α or

oestradiol-17 β , whereas the other enzyme, like that of sheep liver, has a high specificity for the 17 α -hydroxyl group of oestradiol-17 α 3-glucuronide.

Gessner & Vollmer (1969) reported the transfer of glucose to *p*-nitrophenol by mouse liver microsomal preparations fortified with UDP-glucose. Labow & Layne (1972) did not detect the formation of *p*-nitrophenyl glucoside by rabbit liver microsomal fractions which actively transferred glucose from UDP-glucose not only to phenolic steroids, but also to diethylstilboestrol, phenolphthalein and a series of isoflavones. Gessner *et al.* (1973) found that the *p*-nitrophenyl glucosyltransferase of mouse liver was inhibited by several steroids, some of which, in our experiments, did not form glucosides when incubated with rabbit liver preparations. The mouse *p*-nitrophenyl glucosyltransferase studied by Gessner *et al.* (1973) had a pH optimum of about 7.0, whereas Collins *et al.* (1970) found that the optimum activity of rabbit liver steroid glucosyltransferase, with oestradiol-17 α 3-glucuronide as substrate, occurred at pH 8.0. These results suggested that the steroid glucosyltransferases of mouse and of rabbit liver might have different specificities and physical characteristics.

The present paper provides further evidence for the presence of two separate steroid glucosyltransferases in rabbit liver, and compares their properties with those of the enzyme of mouse liver.

Experimental

Materials

Steroids, uridine nucleotides, chromatography

materials, solvents and other reagents were those used by Collins *et al.* (1970). *p*-Nitro[2,6-¹⁴C]phenol (16.7Ci/mol) was obtained from International Chemical and Nuclear Corp., Irvine, Calif., U.S.A. Rabbits were mature female New Zealand Whites, and mice were adult female Swiss-Webster strain.

Methods

Liver homogenates and microsomal fractions were prepared as detailed by Collins *et al.* (1968, 1970). The assays were performed by the procedure using UDP-[6-³H]glucose as described by Labow & Layne (1972). A methanol solution of 0.5 μ mol of the substrate was evaporated to dryness in a 15ml conical centrifuge tube fitted with a ground-glass stopper, and then incubated with 0.5 μ mol of UDP-[6-³H]glucose (2.0Ci/mol) and 0.2ml of liver microsomal preparation. The incubation was carried out at either pH 7.0 or 8.0 at 37°C for 1 h in 0.05M-Tris-HCl buffer. The total volume of each incubation mixture was 0.5ml. The extraction of the medium, assay of radioactivity and determination of transferase activity was as detailed by Labow & Layne (1972). The assay of glucosyltransferase activity when ³H-labelled steroids were used as substrates was carried out as described previously (Collins *et al.*, 1970; Labow & Layne, 1972). *N*-Acetylglucosaminyl-transferase activity was measured by the method of Collins *et al.* (1968). The assay of *p*-nitrophenyl glucoside formation was carried out by using *p*-nitro[2,6-¹⁴C]phenol (1.85Ci/mol). The material was dissolved in methanol and a portion containing 0.5 μ mol was evaporated to dryness and incubated with UDP-glucose as described above. To test the ability of steroids to inhibit the formation of *p*-nitrophenyl glucoside, 0.5 μ mol of the steroid was transferred in methanol to the incubation tube containing *p*-nitro[2,6-¹⁴C]phenol, and the mixture was evaporated to dryness before the addition of the other constituents of the incubation mixture. When *p*-nitrophenol was tested as an inhibitor of glucose transfer to steroids, samples of *p*-nitrophenol and ³H-labelled steroid were evaporated to dryness together and then incubated as described above.

Results

The relative ability of mouse and of rabbit liver homogenates to transfer glucose from UDP-[6-³H]glucose to a variety of substrates, at both pH 7.0 and 8.0, is shown in Table 1. The substrate specificity of the transferase activity in the livers of the two animals was qualitatively similar, in that glucose transfer could be demonstrated to *p*-nitrophenol, both oestradiols, oestradiol-17 α 3-glucuronide, and diethylstilboestrol. No evidence was obtained for the glucosylation of oestriol, non-phenolic steroids, or oestradiol-17 β 3-glucuronide by either rabbit or mouse liver.

Table 1. *Transfer of glucose from UDP-[6-³H]glucose to various substrates by homogenates of mouse and rabbit liver*

For experimental detail see the text. Results are expressed as nmol of glucoside formed/min per g of liver in parallel incubations performed at pH 7.0 and pH 8.0.

Substrate	Rabbit		Mouse	
	pH 7.0	pH 8.0	pH 7.0	pH 8.0
<i>p</i> -Nitrophenol	1.00	0.02	1.21	0.27
Oestradiol-17 α	1.83	0.75	0.08	0.02
Oestradiol-17 β	1.18	0.11	0.11	0.05
Oestriol	0	0	0	0
Testosterone	0	0	0	0
Deoxycorticosterone	0	0	0	0
Oestradiol-17 α 3-glucuronide	0.36	0.50	0.11	0
Oestradiol-17 β 3-glucuronide	0	0	0	0
Pregnan-3 α -ol-20-one	0	0	0	0
Diethylstilboestrol	0.62	0.38	0.15	0.14

Fig. 1. *Effect of pH on the glucosyl transferase activity of rabbit liver towards oestradiol-17 α (■) and oestradiol-17 α 3-glucuronide (●)*

Activity was assayed as described in the text by using ³H-labelled steroids as substrates.

Table 1 shows that, quantitatively, oestradiol-17 α and oestradiol-17 β are better substrates for rabbit liver glucosyltransferase than *p*-nitrophenol. In mouse liver, in contrast, *p*-nitrophenol is glucosylated readily, whereas the phenolic steroids and diethylstilboestrol are relatively poor substrates. Liver tissue from either animal carried out the transfer of glucose to all the substrates tested more effectively at pH 7.0, except for oestradiol-17 α 3-glucuronide, which was converted into its 17-glucoside by rabbit liver in better yield at pH 8.0 than at pH 7.0.

Fig. 1 shows the effect of pH on the glucosyltrans-

Table 2. *Inhibition by steroids of the transfer of glucose from UDP-[6-³H]glucose to p-nitrophenol by liver homogenate at pH7.0*

Concentration of inhibitors was 1 μ mol/ml of incubation mixture.

Percent inhibition =

$$\frac{\text{glucoside formed in presence of inhibitor}}{\text{glucoside formed in absence of inhibitor}} \times 100$$

Glucoside formation was measured as pmol of *p*-nitrophenyl glucoside formed/g of liver per min as described in the text.

Inhibitor	Inhibition (%)	
	Rabbit	Mouse
Oestradiol-17 α	78	35
Oestradiol-17 β	77	19
Oestriol	45	0
Oestradiol-17 α 3-glucuronide	2	28
Oestradiol-17 β 3-glucuronide	15	32
Deoxycorticosterone	8	40
Pregnan-3 α -ol-20-one	6	38
Diethylstilboestrol	39	39

ferase activity of rabbit liver towards oestradiol-17 α and oestradiol-17 α 3-glucuronide. Tris - maleate buffer, 0.2M (Delory & King, 1945), was used in the range pH5.5 to 8.9, and sodium carbonate-bicarbonate buffer, 0.2M (Gomori, 1948), from pH8.9 to 11.0. The pH-activity curve obtained when *p*-nitrophenol was used as substrate was similar to that for oestradiol-17 α .

The ability of rabbit liver to transfer glucose to *p*-nitrophenol was effectively inhibited (Table 2) by oestradiol-17 α , oestradiol-17 β and diethylstilboestrol, which are themselves substrates of glucosyltransferase in this tissue (Table 1). Considerable inhibition was also obtained with oestriol, which does not form a glucoside, but non-aromatic steroids and oestradiol-17 α glucuronide were not effective inhibitors although the latter is readily glucosylated at the 17 position. In the case of mouse liver, no correlation was evident between the effect of various compounds as inhibitors of *p*-nitrophenyl glucosyltransferase (Table 2) and the ability of these compounds to serve as substrates for glucosyl transfer (Table 1). *p*-Nitrophenol produced demonstrable inhibition of the glucosylation of oestradiol-17 α and of oestradiol-17 β by rabbit liver at both pH7.0 and pH8.0. Inhibition of the 17-glucosylation of oestradiol-17 α 3-glucuronide by *p*-nitrophenol was observed at pH7.0, but not at pH8.0.

Discussion

Table 1 suggests a resolution for the discrepancy pointed out by Gessner *et al.* (1973) between the pH optimum of 7.0, which they found for the formation

of *p*-nitrophenyl glucoside in mouse liver, and that of 8.0 reported by Collins *et al.* (1970) for the transfer of glucose by rabbit liver from UDP-glucose to oestradiol-17 α 3-glucuronide. Since the assays in the present paper were performed by measuring the transfer to the steroid of [³H]glucose from UDP-[6-³H]glucose, any possible contribution from the UDP-glucose-independent steroid glucosyltransferase described by Williamson *et al.* (1971) can be eliminated. The results in Table 1 support the indication (Labow *et al.*, 1973) that two separate UDP-glucose-steroid glucosyltransferases exist in rabbit liver, one of which is highly specific for the 17 α -hydroxyl group of oestradiol-17 α 3-glucuronide, whereas the other transfers glucose to the 3-hydroxyl group of the steroid oestrogens, as well as to phenolic groups on some other compounds, including *p*-nitrophenol. The failure of Labow & Layne (1972) to obtain evidence of the glucosylation of *p*-nitrophenol by rabbit liver preparations is explained by the fact that their experiments were carried out at pH8.0, which, although it is the optimum pH for the transfer of glucose to oestradiol-17 α 3-glucuronide, almost completely inhibits the formation of *p*-nitrophenyl glucoside that is evident at pH7.0 (Table 1).

Fig. 1 confirms that the pH optimum for the transfer of glucose to *p*-nitrophenol and to oestradiol-17 α by rabbit liver is similar to that of the *p*-nitrophenyl glucosyltransferase studied by Gessner *et al.* (1973) in mouse liver. The pH-activity curve of the rabbit liver enzyme that forms oestradiol-17 α 3-glucuronide-17-glucoside is, as reported by Collins *et al.* (1970), indistinguishable from that of the enzyme that transfers *N*-acetylglucosamine to oestradiol-17 α 3-glucuronide. The behaviour of these enzyme activities during purification procedures and on treatment with proteases (Labow *et al.*, 1973) is also similar, and this makes it seem likely that the transfer of glucose from UDP-glucose, and of *N*-acetylglucosamine from UDP-*N*-acetylglucosamine to the 17 α -hydroxyl group of oestradiol-17 α 3-glucuronide may be effected by the same enzyme.

Gessner *et al.* (1973) found that in mouse liver the formation of *p*-nitrophenyl glucoside was inhibited by the addition of phenolic steroids, and members of the pregnane series to the incubation mixture. Pregnan-3 α -ol-20-one was a particularly effective inhibitor of *p*-nitrophenyl glucosyltransferase in their system, and they suggested that these steroids might also form glucosides and function as competitive inhibitors. The results in Table 1 confirm our previous findings that rabbit liver preparations do not transfer glucose from UDP-glucose to non-phenolic steroids, and similar results were found in mouse liver, where only the phenolic steroids were found to form glucosides. A comparison of the results in Tables 1 and 2 indicates no correlation in either rabbit or mouse preparations between the

efficacy of steroids as inhibitors of *p*-nitrophenyl glucoside formation, and their own ability to form glucosides. The inhibition of steroid glycoside formation by steroids and other compounds which themselves do not form the corresponding glycoside has been reported (Collins *et al.*, 1968). Labow *et al.* (1971) suggested that this effect may be due to the binding of these steroids to the enzyme without proceeding to the formation of product.

The results of the present work indicate that, as is the case with glucuronyltransferases, the glucosyl-transferase activity of mammalian liver is due to what may be a large family of enzymes, specific as to substrate and species.

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