The Properties of Uridine Diphosphate Glucuronyltransferase(s) which Catalyse the Synthesis of Steroid Glucuronides in Microsomal Fractions from Guinea-Pig Liver

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The properties of the UDP-glucuronyltransferase(s) of guinea-pig liver that catalyse the synthesis of steroid glucuronides were examined. There are many similarities between apparently different substrate-specific forms of these enzymes in that all are activated by bivalent metal ions, and all contain at least 2 thiol groups important for enzyme activity. On the other hand, there are significant differences between the enzymes conjugating steroids and those conjugating non-steroids. Only the latter are activated by UDP-*N*-acetylglucosamine, which enhances their relatively poor affinity for UDP-glucuronic acid. The steroid-conjugating forms of UDP-glucuronyltransferase are not activated by UDP-*N*-acetylglucosamine and have relatively high apparent affinities for UDP-glucuronic acid. The rate of glucuronidation of testosterone was inhibited by treatment with phospholipase A. Treatment with cholate or Triton X-100 did not enhance the rates of glucuronidation of any steroid tested. The data indicate several similarities between different forms of UDP-glucuronyltransferase, suggesting that there is a large family of related proteins. At the same time there are important differences in the parameters that modulate the rates of different glucuronidation reactions.

Glucuronidation reactions are important for the detoxication of bilirubin and many other endogenous and exogenous compounds. Despite the importance of this mechanism of detoxication, the activity of microsomal UDP-glucuronyltransferase (EC 2.4.1.17) has been investigated thoroughly with respect to only one aglycone, namely *p*-nitrophenol. In these studies, catalytic rates, affinity and specificity for UDP-glucuronic acid and sensitivity to allosteric activators were all found to be subject to regulation (Eletr *et al.*, 1973; Leuders & Kuff, 1967; Vessey & Zakim, 1971, 1972, 1974; Vessey *et al.*, 1973; Winsnes, 1971, 1972; Zakim & Vessey, 1974, 1976; Zakim *et al.*, 1973*a,b*). Although the glucuronidation of some simple substituted phenols other than *p*-nitrophenol has been studied only to a limited extent, it is already clear that there are important differences in the regulatory properties of different glucuronidation reactions (Vessey *et al.*, 1973; Zakim *et al.*, 1973*a*; Zakim & Vessey, 1973). Understanding of the regulation of glucuronidation reactions is therefore limited because the properties of different glucuronidation reactions are variable. The basis for this variability is most likely that UDP-glucuronyltransferase activities are due to a family of closely related enzymes.

None of these have been purified, or even separated from their attachments to the microsomal membrane as active forms, but kinetic experiments support the idea of multiple enzymes with different specificities for aglycones (cf. Zakim & Vessey, 1976). Lack of progress in separating UDP-glucuronyltransferase(s) from microsomal membranes has impeded resolution of the questions of their number and specificities. Of critical importance with regard to the properties of these enzymes is the fact that the lipid environment of UDP-glucuronyltransferase is an important feature of the regulatory apparatus of this enzyme (Vessey & Zakim, 1971; Zakim *et al.*, 1973*b*; Zakim & Vessey, 1974, 1976) when the glucuronidation of simple phenolic compounds is studied. Studies limited to purified forms of UDP-glucuronyltransferase, separated from their native environments, could therefore lead to incorrect conclusions with regard to regulation *in vivo*.

Many additional glucuronidation reactions hence need to be studied before a general understanding of the regulation of UDP-glucuronyltransferases emerges. We present data in this paper on the properties of UDP-glucuronyltransferases catalysing the glucuronidation of steroid hormones.

Materials and Methods

Microsomal preparations, free of bivalent cations, were prepared from guinea-pig livers as described previously (Zakim *et al.*, 1973a). Purified phospholipase A was prepared from *Naja naja* venom (Cremona & Kearney, 1964). Nucleotides and nucleotide sugars were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Steroids were obtained from Sigma, and ^{14}C -labelled steroids from New England Nuclear Corp., Boston, MA, U.S.A. Mersalyl {*o*-[(3-hydroxymercuri-2-methoxypropyl)-carbamoyl]phenoxyacetic acid} was purchased from Sigma.

Enzyme assays

Activities of UDP-glucuronyltransferase were assayed by using ^{14}C -labelled steroids as aglycones. In each instance reactions were stopped by the addition of a mixture of 0.5M-trichloroacetic acid and 0.25M-potassium phosphate, pH4.0. Unconjugated [^{14}C]aglycone was removed by several extractions with diethyl ether. Steroid glucuronides remained in the aqueous layer, and were counted for radioactivity in a liquid-scintillation counter. The scintillant was 5% 2,5-diphenyloxazole in dioxan. The extraction methods were tested by determining the amounts of [^{14}C]steroid that remained in the aqueous phase and the amounts of [^{14}C]steroid glucuronide extracted into the ether layer. In all instances only trace amounts of unconjugated [^{14}C]steroid remained in the aqueous layer after three extractions with ether. Inclusion of a separate zero-time sample in each assay allowed for correction for the unextracted ^{14}C -labelled substrates. No detectable amounts of steroid glucuronides were extracted into the ether phase. The products of the reaction were identified as glucuronides on paper chromatography (Millburn *et al.*, 1967a,b) by typical colour reactions (Millburn *et al.*, 1967b), comparisons of R_F values with known standards, and the fact that no compounds with the characteristics of glucuronides were formed in the absence of UDP-glucuronic acid. In addition, radioactivity from UDP-[^{14}C]glucuronic acid was incorporated into the appropriate region of chromatograms when incubated with microsomal fractions and each steroid.

All assays were carried out at 37°C in 50mM-Tris/HCl, pH7.4. The concentration of UDP-glucuronic acid was 1.0mM, unless stated otherwise. The concentrations of aglycones varied depending on the aglycone and the experiment. These concentrations are given in the legends to the Figures and Tables. The concentrations of added UDP-*N*-acetylglucosamine and Mn^{2+} , used in some experiments, are given in the legends. Assays were carried out with approx. 1.0–2.0mg of microsomal protein per assay. The concentrations of substrates are given in the text and

legends. All activities are initial rates determined from serial measurements of the formation of glucuronides at several successive intervals of 5–15s. The reproducibility of assays of UDP-glucuronyltransferase was 5–10% with the above method.

Technique of addition of steroids to assay system

The aglycones used in these experiments are sparingly soluble in water. There is uncertainty in fact as to their true solubility since nitroxide spin labels detect the formation of micelle-like structures (D. A. Vessey & D. Zakim, unpublished work) at concentrations of steroids (in water) below their reported limits of solubility. In addition, microsomal preparations remove large amounts of organic compounds from aqueous solutions, presumably into the lipid phase of the membrane (Vessey & Zakim, 1975). Concentrations of aglycone calculated from the known amount added and the volume of the assay system hence have no significance. For these reasons all [^{14}C]steroids were added by allowing microsomal fractions to take them up from a thin layer of steroid dried on the side of a tube. Nearly complete uptake of [^{14}C]steroids by microsomal preparations was achieved after 15min of mixing of microsomal fractions with the substrate at 37°C. Microsomal aglycones loaded in this manner with [^{14}C]steroid aglycones were kept at 0°C until activity was measured. Assays were always started by the addition of 0.3–0.5mg of microsomal protein (with aglycone). Protein was measured by the biuret method (Gornall *et al.*, 1949).

Results

UDP-glucuronyltransferase activity is regulated at two different levels. When assays are carried out with simple phenolic aglycones, several effectors appear to alter activity as a result of binding to the enzyme, as, for example, activation by bivalent metal ions (Zakim *et al.*, 1973a), glucuronides (Vessey & Zakim, 1974) and UDP-*N*-acetylglucosamine (Winsnes, 1972; Vessey *et al.*, 1973). The activity of UDP-glucuronyltransferase also is altered indirectly by treatment of microsomal fractions with phospholipases and detergents. These latter treatments primarily modify the lipid environment of UDP-glucuronyltransferase within the microsomal membranes. Both aspects of regulation, direct interaction with ligands and the indirect effects of altering the membrane lipids, have been studied.

Effects of UDP-*N*-acetylglucosamine and Mn^{2+} on rates of glucuronidation of steroids

Of the many effectors of UDP-glucuronyltransferase, UDP-*N*-acetylglucosamine seems to be the most important. This compound, which is a physiological intermediate, enhances the affinity

of UDP-glucuronyltransferase (assayed with *p*-nitrophenol or other substituted phenolic compound) for UDP-glucuronic acid (Vessey *et al.*, 1973). The concentration of UDP-glucuronic acid for half-maximal rates of glucuronidation ($K_{0.5}$) is 16mM in assays with *p*-nitrophenol, whereas the concentration of UDP-glucuronic acid in liver *in vivo* is about 0.3mM (Keppler *et al.*, 1970). Untreated UDP-glucuronyltransferase hence functions poorly under conditions presumed to exist *in vivo*. On the other hand, addition of UDP-*N*-acetylglucosamine decreases the $K_{0.5}$ for UDP-glucuronic acid to values approaching those *in vivo* (Vessey *et al.*, 1973). It was therefore surprising to find that UDP-*N*-acetylglucosamine had no effect on the activity of UDP-glucuronyltransferase when activity was measured with oestrone, oestradiol and testosterone (Table 1). Although Table 1 reports activities for only a single concentration of UDP-*N*-acetylglucosamine, no concentration produced significant activation of UDP-glucuronyltransferase in assays with steroids as aglycones. UDP-*N*-acetylglucosamine activation is metal-dependent with some aglycones (Zakim *et al.*, 1973a). Combined treatment with up to 3mM- MnCl_2 did not lead, however, to UDP-*N*-acetylglucosamine-induced effects on the rates of synthesis of steroid glucuronides. These results indicate that the regulatory properties of the form of UDP-glucuronyltransferase(s) catalysing the synthesis of at least some steroid glucuronides are different from those of glucuronidating enzymes studied previously.

The apparent importance of UDP-*N*-acetylglucos-

amine as an effector of some glucuronidating reactions makes it difficult to understand the lack of an effect on others. This question was explored in more detail by examining the rate of glucuronidation of oestrone and oestradiol as a function of the concentration of UDP-glucuronic acid (Fig. 1). With oestrone as aglycone the $K_{0.5}$ for UDP-glucuronic acid is approx. 0.4mM, nearly as low as the presumed physiological concentration of this substrate. The $K_{0.5}$ for UDP-glucuronic acid is larger (about 2.0mM) for assays with oestradiol, but this value is still substantially below that for unstimulated UDP-glucuronyltransferase assayed with *p*-nitrophenol as aglycone. The interesting conclusion to be drawn from these studies is that the UDP-glucuronyltransferases that metabolize steroids already possess properties at the UDP-glucuronic acid site that are produced by treatment of other forms of the enzyme with UDP-*N*-acetylglucosamine.

Although UDP-glucuronyltransferases differ in their response to UDP-*N*-acetylglucosamine, all forms of the enzyme tested were activated by bivalent metal ions. Mn^{2+} enhanced the rate of glucuronidation of oestrone, oestradiol and testosterone (Table 2). The kinetic mechanism of this activation was investigated with testosterone and oestrone as aglycones. Mn^{2+} increased the rate of glucuronidation of these compounds at infinite concentrations of UDP-glucuronic acid. This is similar to results with simple phenolic aglycones (Zakim *et al.*, 1973a). No studies were made of the influence of Mn^{2+} on

Table 1. Effect of UDP-*N*-acetylglucosamine on the rates of glucuronidation of steroids and *p*-nitrophenol

Microsomal fractions from guinea-pig liver were pre-treated with the indicated steroids as described in the Materials and Methods section. The activity of UDP-glucuronyltransferase was measured at 37°C in 50mM-Tris/HCl, pH7.4. The concentration of UDP-glucuronic acid was 1mM, and UDP-*N*-acetylglucosamine was 5.0mM, when added. The concentration of *p*-nitrophenol was 0.02mM. The final volume of each assay tube was 0.10ml and contained 0.3mg of microsomal protein. Each mg of microsomal protein contained 5.4nmol of oestradiol, 5.5nmol of oestrone, 4.5nmol of testosterone. Activities are nmol of aglycone glucuronidated/min per mg of protein.

Aglycone	Additions to assay	Activity
Testosterone	None	0.080
	UDP- <i>N</i> -acetylglucosamine	0.080
Oestrone	None	3.1
	UDP- <i>N</i> -acetylglucosamine	3.1
Oestradiol	None	2.8
	UDP- <i>N</i> -acetylglucosamine	3.1
<i>p</i> -Nitrophenol	None	3.5
	UDP- <i>N</i> -acetylglucosamine	11.7

Fig. 1. Rates of glucuronidation of oestrone (○) and oestradiol (●) as a function of the concentration of UDP-glucuronic acid

Microsomal fractions were pre-loaded with steroid aglycones as described in the Materials and Methods section. Rates of glucuronidation were measured at 37°C in 50mM-Tris/HCl, pH7.4, and the indicated concentrations of UDP-glucuronic acid, as in the Materials and Methods section. The data are plotted in double-reciprocal form. Rates (*v*) are nmol of steroid glucuronidated/min per mg of microsomal protein. Each mg of microsomal protein contained 5.5nmol of oestrone or oestradiol.

Table 2. *Effect of Mn^{2+} on the rate of glucuronidation of steroids*

Activities of UDP-glucuronyltransferase were measured, as described in the Materials and Methods section, after pretreatment of microsomal fractions with the indicated steroids. The concentration of UDP-glucuronic acid was 2.0mM. Final assay volume was 0.1ml and contained 0.3mg of microsomal protein. The concentrations of steroid per mg of microsomal protein were the same as in Table 1. When added, the concentration of Mn^{2+} was 1.0mM. Activities are nmol of steroid glucuronidated/min per mg of protein.

Aglycone	Additions to assay	Activity
Testosterone	None	0.094
	Mn^{2+}	0.147
Oestrone	None	3.00
	Mn^{2+}	5.3
Oestradiol	None	6.0
	Mn^{2+}	10.3

rate of glucuronidation as a function of concentration of aglycones.

Effects of treatment with mersalyl on the rates of glucuronidation of steroids

One of the interesting characteristics of the *p*-nitrophenol-metabolizing form of UDP-glucuronyltransferase is activation by thiol-group reagents. Treatment with *N*-ethylmaleimide leads to a slight enhancement of activity, and treatment with organic mercurials produces a severalfold activation. Each of these reagents reacts with a specific type of thiol group (Winsnes, 1971; Zakim & Vessey, 1972). A third thiol group is titrated at very high concentrations of organic mercurials, leading to a decrease in activity (Zakim & Vessey, 1972). UDP-glucuronyltransferases catalysing the glucuronidation of oestrone, oestradiol and testosterone contain the two types of thiol groups that react with mersalyl. Thus treatment of microsomal preparations with mersalyl increases the rate of glucuronidation of oestrone (Fig. 2); maximal activation occurs at 1.0mM-mersalyl. The activity of UDP-glucuronyltransferase declines at higher concentrations of mersalyl, indicating that at least one additional type of thiol group is titrated as the concentration of mersalyl increases to 2.5mM. Similar results were obtained for assays of UDP-glucuronyltransferase by using oestradiol and testosterone as aglycones. Hence there are close similarities in some of the properties of UDP-glucuronyltransferases metabolizing steroids and smaller phenolic compounds.

Effects of treatment with phospholipase A and detergents on the rates of glucuronidation of steroids

Treatment of microsomal fractions with purified

phospholipase A activates UDP-glucuronyltransferase in assays with oestrone or oestradiol as aglycones (Table 3). This is observed too with all other aglycones studied to date. With the *p*-nitrophenol-metabolizing form of UDP-glucuronyltransferase, treatment with phospholipase A not only increases activity but alters the substrate specificity and potentiates end-product inhibition by UDP. Neither of these two effects was seen after treatment of the oestrone- and oestradiol-metabolizing forms of the enzyme with phospholipase A (Table 3).

Treatment with phospholipase A decreased the rate of glucuronidation of testosterone (Fig. 3). As shown in Fig. 3, the rate of glucuronidation of testosterone decreased after treatment of microsomal preparations with phospholipase A. Activity decreased to reach a nadir after 1 min of treatment with phospholipase A. Activity increased slightly between 1 and 2 min of treatment. This increase was not an isolated finding in the experiment reported in Fig. 3, but was observed repeatedly. Activity did not rise above the value observed at 2 min when treatment with phospholipase A was carried out for 15 min. So-called latency in the activity of UDP-glucuronyltransferase is thus not a general property of all glucuronidation reactions. Treatment with phospholipase A, however, did potentiate end-product inhibition by UDP of the testosterone-metabolizing form of the enzyme (Table 3). This latter form was also inhibited by UDP-glucose after treatment with phospholipase A, although UDP-glucose had no effect on the activity of the untreated enzyme.

Fig. 2. *Effect of mersalyl on the rate of glucuronidation of oestrone*

Microsomal fractions were pretreated with oestrone, as described in the Materials and Methods section, and then incubated at 23°C for 15 min in 50mM-Tris/HCl, pH 8.0, and the indicated concentrations of mersalyl (mM). UDP-glucuronyltransferase activity was assayed subsequently at 37°C in 50mM-Tris/HCl, pH 7.4, and 1.0mM-UDP-glucuronic acid. Activities (*v*) are nmol of oestrone glucuronidated/min per mg of protein. Each mg of microsomal protein contained 20nmol of oestrone.

Table 3. *Effect of treatment with phospholipase A on the properties of UDP-glucuronyltransferase*

Microsomal fractions pretreated with the indicated steroid were treated at 23°C with phospholipase A at a ratio of 50 mg of microsomal fraction/mg of phospholipase A in 50mM-Tris/HCl, pH 8.0. EDTA (final concentration 5mM) was added after 60s of this treatment to inhibit the further action of phospholipase A. UDP-glucuronyltransferase activities were measured as described in the Materials and Methods section at 37°C in the presence of 1.0mM-UDP-glucuronic acid. When added, the concentrations of UDP and UDP-glucose were 1.0mM and 5.0mM respectively. The final volume of each assay tube was 0.1 ml and contained 0.3mg of microsomal protein with steroids as in Table 1. Activities are nmol of steroid glucuronidated/min per mg of protein.

Aglycones	Treatment of microsomal fraction	Activity		
		No addition	+UDP	+UDP-glucose
Oestradiol	None	2.8	1.1	2.8
	Phospholipase A	9.7	1.5	7.4
Oestrone	None	2.0	1.2	2.0
	Phospholipase A	5.4	1.6	5.4
Testosterone	None	0.094	0.076	0.088
	Phospholipase A	0.048	0.011	0.014

Fig. 3. *Effect of treatment with phospholipase A on the rate of glucuronidation of testosterone*

Microsomal fractions were pretreated with testosterone as described in the Materials and Methods section and then treated with 1 mg of purified phospholipase A/100mg of microsomal protein at 23°C, in 50mM-Tris/HCl, pH 8.0. EDTA (final concn. 5mM) was added at the indicated times to inhibit the further action of phospholipase A. UDP-glucuronyltransferase was assayed as described in the Materials and Methods section in 50mM-Tris/HCl, pH 7.4, and 1.0mM-UDP-glucuronic acid. Rates (v) are nmol of testosterone glucuronidated/min per mg of protein. Each mg of microsomal protein contained 3.5 nmol of testosterone.

The above data indicate that the activities of steroid-metabolizing forms of UDP-glucuronyltransferase depend on interactions with their membrane environments. It was surprising, in view of these results, that detergents did not activate any of these forms of UDP-glucuronyltransferase. Representative data for Triton-treated enzyme, assayed with oestrone as aglycone, are shown in Fig. 4. Low concentrations of Triton X-100 had no effect on rates of glucuronidation, and high concentrations inhibited activity.

Fig. 4. *Effect of Triton X-100 on the rate of glucuronidation of oestrone*

Microsomal fractions were pretreated with oestrone as described in the Materials and Methods section. UDP-glucuronyltransferase activity was then measured at 37°C in 50mM-Tris/HCl, pH 7.4, 1.0mM-UDP-glucuronic acid, and the indicated amounts of Triton X-100 as given in the Materials and Methods section. Activities (v) are nmol of oestrone glucuronidated/min per mg of protein. Each mg of microsomal protein contained 3.2 nmol of oestrone.

Treatment with cholate also failed to enhance the rate of glucuronidation of steroids. The effects of detergents were similar for assays with any steroid as aglycone.

An important technical question is whether pre-incubation with steroids at 37°C alters the latency of the steroid-metabolizing forms of UDP-glucuronyltransferase. Incubation under these conditions does not alter the latency of forms of the enzyme metabolizing simple phenols, such as *p*-nitrophenol in microsomal preparations from guinea-pig liver. This incubation also does not do away with the latency in

the glucuronidation of oestrone and oestradiol (cf. Table 3). Since the rate of glucuronidation of testosterone was not increased by treatment with either phospholipase A or detergent, it is possible that latency of this activity was destroyed by preincubation at 37°C. This problem was investigated by treating microsomal fractions with phospholipase A before incubation with testosterone. This procedure did not alter the results in Fig. 3. In addition, testosterone dissolved in a small volume of ethanol was added directly to microsomal fractions treated for various periods of time with phospholipase A. No activations of the rate of glucuronidation of testosterone was seen under these conditions. It was found in separate experiments that ethanol itself did not alter the rate of glucuronidation of testosterone or other steroids, and that rates of glucuronidation of steroids were similar when microsomal preparations were preincubated with the steroid, as described in the Materials and Methods section, or when steroids dissolved in ethanol were added directly to the microsomal preparations.

Discussion

There are many important similarities between the forms of UDP-glucuronyltransferase in guinea-pig liver catalysing the synthesis of steroid glucuronides and the glucuronides of simple substituted phenols. All these forms of UDP-glucuronyltransferase are activated by Mn^{2+} , and all contain at least two distinct types of thiol groups. On the other hand, there are significant differences between these forms. Thus UDP-*N*-acetylglucosamine appears to be an important modulator of the rates of glucuronidation of simple phenols and some carboxyl-containing compounds (Winsnes, 1972; Vessey *et al.*, 1973), but has no effect on the rates of glucuronidation of steroids. Of note is the fact that the different responses to UDP-*N*-acetylglucosamine of various glucuronidation reactions correlate with the apparent affinity for UDP-glucuronic acid of the different forms of UDP-glucuronyltransferase. Glucuronidation reactions that are stimulated by UDP-*N*-acetylglucosamine need relatively large amounts of UDP-glucuronic acid for half-maximal rates of glucuronidation in the absence of UDP-*N*-acetylglucosamine. The reactions (steroid glucuronidations) that are unresponsive to UDP-*N*-acetylglucosamine need relatively small amounts of UDP-glucuronic acid for half-maximal rates of glucuronidation. The untreated forms of steroid-glucuronidating enzymes seem to have a built-in UDP-*N*-acetylglucosamine effect. The significance of this difference between UDP-glucuronyltransferases, with regard to allosteric activation by UDP-*N*-acetylglucosamine, remains obscure. Of interest, however, is the evolutionary relationship between forms. The fundamental difference in the

way these enzymes bind UDP-glucuronic acid may be the result of their having different evolutionary origins.

Another difference between glucuronidation reactions is that the rate of at least one (for testosterone) is insensitive to treatment with phospholipase A, even though the latter treatment does modify substrate specificity and the extent of end-product inhibition. The total lack of detergent-induced activations of the steroid-conjugating forms of UDP-glucuronyltransferase is also different from what is observed with other aglycones studied to date by using guinea-pig liver as the source of enzyme. The significance of these latter findings is not limited to reflecting variable properties of different UDP-glucuronyltransferases but extends to the general question of the mechanism of activations of these enzymes induced by phospholipase A and detergent. It has been proposed, for example, that UDP-glucuronyltransferases are on the inside of the microsomal vesicle (Berry & Hallinan, 1974). Activations, in this model, occur because of mechanical breakdown of the membrane that allows substrates access to enzyme on the inside of the vesicles. The data for the effects of treatment with phospholipase A on the rate of glucuronidation of testosterone make that an unlikely model. The absence of detergent-induced activations of the rate of glucuronidation of oestrone and oestradiol, but its activation by treatment with phospholipase A, reinforces this conclusion. At the same time, these data add considerable force to the argument that the properties of UDP-glucuronyltransferase depend on lipid-protein interactions, because these interactions would determine the conformation and hence kinetic properties of UDP-glucuronyltransferases. Thus kinetic studies in the guinea pig of the *p*-nitrophenol-conjugating form of UDP-glucuronyltransferase indicate that treatment with phospholipase A increases activity at V_{max} , and decreases K_s for UDP-glucuronic acid. Triton-induced activation, on the other hand, is due to a decrease in K_s of UDP-glucuronic acid only (Zakim & Vessey, 1975). Since the $K_{0.5}$ for UDP-glucuronic acid already is relatively low for the glucuronidation of steroids as compared with *p*-nitrophenol, the lack of detergent-induced activation is not unexpected.

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