Stimulation of Microsomal Uridine Diphosphate Glucuronyltransferase by Glucuronic Acid Derivatives

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The glucuronic acid adducts of 1-naphthol, 2-naphthol and 4-methylumbelliferone activate microsomal UDP-glucuronyltransferase (EC 2.4.1.17) when the enzyme is assayed with *p*-nitrophenol as aglycone. Phenyl glucuronide and oestriol 3 β -glucuronide also activate UDP-glucuronyltransferase, but to a lesser extent. Activation by glucuronides is not dependent on metal ions, but is blocked by prior treatment of microsomal fractions with *p*-chloromercuribenzoate. The kinetic mechanism of activation is concluded to be an increase in the affinity of the enzyme for UDP-glucuronic acid. Activation by 1-naphthyl glucuronide, at high concentrations of *p*-nitrophenol, is not affected by 1-naphthol. Apparently 1-naphthyl glucuronide activates the preparation by binding at a site that is separate from the site of glucuronidation of 1-naphthol. Further evidence for the existence of distinct effector sites for the glucuronides was provided by the finding that activation by glucuronides is inhibited competitively by aglycone glucosides. These glucosides do not inhibit the rate of glucuronidation of *p*-nitrophenol in the absence of glucuronide adducts, nor do they alter the rate of glucuronidation of 1-naphthol. When UDP-glucuronyltransferase is assayed with 1-naphthol as aglycone it is activated by *p*-nitrophenyl glucuronide, 4-methylumbelliferyl glucuronide and under appropriate conditions by its own glucuronide. These activations are similarly inhibited by aglycone glucosides. *p*-Nitrophenyl glucuronide also stimulates the rate of glucuronidation of *o*-aminophenol, *o*-amino-benzoate and bilirubin.

A number of enzyme systems located in the endoplasmic reticulum detoxify various non-polar compounds by means of oxidative or conjugative reactions (Fishman, 1970; Gillette *et al.*, 1969). These reactions metabolize a broad range of substrates. In spite of the interest in these enzyme systems the number of different proteins required for the recognition of a large number of structurally dissimilar substrates is unknown.

Recent studies of glucuronide formation in liver microsomal fractions from guinea pigs confirm, however, that there are a number of substrate-specific forms of UDP-glucuronyltransferase. For example, *o*-aminophenol and *p*-nitrophenol are not conjugated with glucuronic acid at identical enzyme sites (Zakim *et al.*, 1973*b*).

During the course of kinetic experiments designed to provide evidence about the multiplicity of conjugating enzymes that use UDP-glucuronic acid and UDP-galacturonic acid as substrates, an anomalous result was obtained. Addition of *p*-nitrophenyl glucuronide to reaction tubes containing *p*-nitrophenol and UDP-galacturonic acid increased the rate of conjugation of *p*-nitrophenol (Vessey & Zakim, 1973). It was subsequently found that a

number of other glucuronides also enhance the rate of conjugation of *p*-nitrophenol with UDP-glucuronic acid. Results reported in the present paper indicate that the stimulation of the rate of glucuronide conjugation of aglycones by glucuronide derivatives results from the binding of glucuronides at allosteric sites, and that this binding leads to an increase in the affinity of UDP-glucuronyltransferase for UDP-glucuronic acid.

Experimental

Material

4-Methylumbelliferyl β -D-glucuronide, 4-methylumbelliferyl β -D-glucoside, *p*-nitrophenyl β -D-glucuronide, *p*-nitrophenyl β -D-glucoside, 1-naphthyl β -D-glucuronide, oestriol 3 β -D-glucuronide, UDP-glucuronic acid (NH₄⁺ salt), UDP and saccharo-1,4-lactone were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. Phenyl β -D-glucuronide was obtained from Koch-Light Laboratories, Colnbrook, Bucks., U.K. 1-[1-¹⁴C]Naphthol was obtained from Amersham-Searle, Arlington Heights, Ill., U.S.A. UDP-[U-¹⁴C]glucuronic acid was obtained

from New England Nuclear Corp., Boston, Mass., U.S.A.

Methods

The preparation of the microsomal fractions and the assay of UDP-glucuronyltransferase with *p*-nitrophenol, *o*-aminophenol and *o*-aminobenzoate as aglycones have been described (Vessey & Zakim, 1971; Zakim & Vessey, 1973). Assays with all aglycones contained 10mM-saccharo-1,4-lactone to inhibit β -glucuronidase (Levy, 1952). Glucuronidation of naphthol was followed by an adaptation of the radioassay procedure described by Zakim & Vessey (1973). Samples (0.1ml) were removed from the assay tubes at timed intervals and added to 0.2ml of 0.5M-trichloroacetic acid–0.5M-sodium phosphate, pH3.5. The samples were diluted to 1 ml with water, and centrifuged to remove denatured protein. The supernatants were extracted with 3 $\times$ 1 vol. of ether to remove unchanged naphthol, and a sample (0.5 ml) of the aqueous phase was removed for radioactivity counting (Horgan *et al.*, 1968) in a liquid-scintillation counter.

Because of the need to use relatively fresh microsomal preparations (Zakim & Vessey, 1973) and owing to the fact that there are specific activity variations between different preparations, we were not able to do extensive statistical analysis for each data point. However, all differences alluded to in the text are significant in that they have been reproducible over three or more microsomal preparations. As discussed by Vessey *et al.* (1973), it has been established that under the assay conditions described reaction rates are true initial rates and do not reflect contributions from the metabolism of substrates or products in side reactions. There are also no detectable blank reactions. All rates are expressed as nmol of aglycone conjugated or released/min per mg of microsomal protein. Protein was determined by the biuret method (Gornall *et al.*, 1949).

The rate of transfer of radioactive label from UDP-[14 C]glucuronic acid to *p*-nitrophenol was determined with the same general procedure previously used to measure rates of isotope exchange (Vessey & Zakim, 1972). Assays were stopped by boiling, and 0.2mM-*p*-nitrophenyl glucuronide was added to each as unlabelled carrier. *p*-Nitrophenyl glucuronide was then separated from the other components of the deproteinized assay mixture by paper chromatography. A sample of deproteinized supernatant was counted for radioactivity before spotting, and an equivalent of 7030c.p.m. of each sample was applied. The *p*-nitrophenyl glucuronide spot was cut out and counted for radioactivity in a liquid-scintillation counter. A control incubation lacking only *p*-nitrophenol did not have significant

radioactivity in the *p*-nitrophenyl glucuronide region of the chromatogram.

Results

Activation of the rate of synthesis of p-nitrophenyl glucuronide by glucuronides

We have previously shown that a number of glucuronides, of which 4-methylumbelliferyl glucuronide was the most effective, enhanced the rate of conjugation of *p*-nitrophenol with UDP-galacturonic acid (Vessey & Zakim, 1973). We did not then try to determine whether the additional conjugate formed was *p*-nitrophenyl galacturonide, or to determine the nature of the activation. We decided for technical reasons to investigate the apparent activating effect of glucuronides by using UDP-glucuronic acid as the sugar donor instead of UDP-galacturonic acid. Rates of conjugation of *p*-nitrophenol as a function of the concentration of UDP-glucuronic acid were determined in the presence and absence of 4-methylumbelliferyl glucuronide and 1-naphthyl glucuronide. The results (Fig. 1) indicate that these glucuronides

Fig. 1. Effect of 1-naphthyl glucuronide and 4-methylumbelliferyl glucuronide on the rate of conjugation of *p*-nitrophenol as a function of the concentration of UDP-glucuronic acid

Initial rates of glucuronidation of *p*-nitrophenol were determined in assays containing 0.3 mM-*p*-nitrophenol and various concentrations of UDP-glucuronic acid. No addition ($\circ$); plus 5mM-1-naphthyl glucuronide (Δ); plus 5mM-4-methylumbelliferyl glucuronide ($\bullet$). Velocity is expressed as nmol of *p*-nitrophenol conjugated/min per mg of microsomal protein.

apparently stimulate the rate of synthesis of *p*-nitrophenyl glucuronide, and that the extent of activation depends on the concentration of UDP-glucuronic acid. Thus neither glucuronide appears to stimulate at infinite concentrations of UDP-glucuronic acid. In fact, 5 mM-1-naphthyl glucuronide becomes an inhibitor of the rate of conjugation of *p*-nitrophenol at concentrations of UDP-glucuronic acid greater than 2–4 mM.

One interpretation of the data in Fig. 1 is that glucuronides enhance the conjugation of *p*-nitrophenol via a transfer of their glucuronic acid residue to *p*-nitrophenol. For example, there could be a direct transfer of sugar from glucuronide to aglycone such as in the reaction that is catalysed to a minor extent by β -glucuronidase (Fishman & Green, 1957). Since some UDP-glucuronyltransferases catalyse a UDP-dependent hydrolysis of glucuronides (back reaction) (Vessey & Zakim, 1972), it is also possible that a small amount of endogenous UDP present in the microsomal fractions could serve as an intermediary in a glucuronic acid 'shuttle' reaction between different aglycone sites. The rate of synthesis of *p*-nitrophenyl [14 C]glucuronide was therefore measured in the presence and the absence of 4-methylumbelliferyl glucuronide with UDP- 14 C]glucuronic acid as sugar donor. Any glucuronic acid transferred with 4-methylumbelliferyl glucuronide to *p*-nitrophenol, regardless of the mechanisms, would be devoid of radioactivity, and not contribute to the measured rate. For assays containing 0.3 mM-*p*-nitrophenol and 0.6 mM-UDP- 14 C]glucuronic acid (7030 c.p.m.) the microsomal fraction catalysed the transfer of 154 c.p.m. into *p*-nitrophenyl [14 C]glucuronide in 5 min at 37°C or a rate of 3.67 nmol transferred/5 min per mg of microsomal protein. In the presence of 5 mM-4-methylumbelliferyl glucuronide 314 c.p.m. of [14 C]glucuronic acid were transferred into *p*-nitrophenyl glucuronide in 5 min or a rate of 7.66 nmol transferred/5 min per mg of microsomal protein. 4-Methylumbelliferyl glucuronide more than doubled the rate of synthesis of *p*-nitrophenyl [14 C]glucuronide from UDP- 14 C]glucuronic acid. Stimulation by glucuronides of the rate of conjugation of *p*-nitrophenol results therefore from a direct effect of the glucuronide on the kinetic properties of the UDP-glucuronyltransferase preparation.

The lack of stimulation by glucuronides at high concentrations of UDP-glucuronic acid (Fig. 1) suggests that the glucuronides activate by enhancing the affinity of the enzyme for UDP-glucuronic acid. This possibility was investigated further in experiments designed to determine whether there is competition between glucuronide and UDP-glucuronic acid for binding at either the effector or active sites. Thus the effect of the concentration of UDP-glucuronic acid on the concentration of

4-methylumbelliferyl glucuronide needed for half-maximal activation was measured. This concentration was 2.5–3.0 mM-4-methylumbelliferyl glucuronide at concentrations of UDP-glucuronic acid of either 0.2 or 1.5 mM-UDP-glucuronic acid (Fig. 2), which indicates that UDP-glucuronic acid does not

Fig. 2. Activation by 4-methylumbelliferyl glucuronide at two concentrations of UDP-glucuronic acid

Initial rates of *p*-nitrophenol conjugation were determined as in Fig. 1 and plotted in double-reciprocal form to obtain the reaction rate at infinite concentrations of 4-methylumbelliferyl glucuronide. The plots were normalized by calculation of the percentage of maximum activation for each concentration of 4-methylumbelliferyl glucuronide at (□) 0.2 mM- and (●) 1.5 mM-UDP-glucuronic acid.

Fig. 3. Double-reciprocal plots of 1-naphthyl glucuronide-stimulated rates of glucuronidation of *p*-nitrophenol in the presence or the absence of 1-naphthol

Initial rates of glucuronidation of *p*-nitrophenol were determined in assays containing 0.4 mM-UDP-glucuronic acid, 5 mM-1-naphthyl glucuronide and various amounts of *p*-nitrophenol in either the presence (Δ) or the absence (○) of 0.04 mM-1-naphthol. Rates are expressed as in Fig. 1.

bind at the glucuronide effector site. It was also determined that glucuronides do not modify the apparent affinity of UDP-glucuronyltransferase for *p*-nitrophenol, and infinite concentrations of *p*-nitrophenol do not block activation by glucuronides. Hence it was concluded that prior binding of glucuronides enhances the affinity of UDP-glucuronyltransferase for UDP-glucuronic acid.

Location of effector sites for the binding of glucuronides

The demonstration that glucuronides increase the rate of synthesis of *p*-nitrophenyl glucuronide raises the question of the location of the sites at which these effectors bind. Does, for example, the enzyme that conjugates *p*-nitrophenol have an allosteric site for 1-naphthyl glucuronide or could this allosteric site be the site responsible for metabolizing 1-naphthol? We approached this problem by studying the effect of 1-naphthol on the 1-naphthyl glucuronide-activated conjugation of *p*-nitrophenol. 1-Naphthol obviously binds at its own active site. In addition, 1-naphthol is a competitive inhibitor of the conjugation of *p*-nitrophenol in unactivated preparations (Lucier *et al.*, 1971). We have found, however, that inhibition by 1-naphthol is competitive with respect to *p*-nitrophenol for assays in the presence of 1-naphthyl glucuronide (Fig. 3). As had been observed for non-activated microsomal fractions (Winsnes, 1969; Vessey & Zakim, 1972) the double-reciprocal plots in Fig. 3 are non-linear. The reason for the apparent activation at high concentrations of *p*-nitrophenol is still uncertain, but the fact that the plots can be extrapolated to a common intercept indicates that 1-naphthol does not displace 1-naphthyl glucuronide from the activator site. There would appear to be an effector site for 1-naphthyl glucuronide which is distinct from the site at which 1-naphthol is glucuronidated.

To delineate further the nature of the glucuronide effector site, we sought compounds that would inhibit binding at the effector site without inhibiting the rate of synthesis of the activating glucuronide. The glucosides of *p*-nitrophenol and 4-methylumbelliferone seem to satisfy this requirement. Thus *p*-nitrophenyl glucoside and 4-methylumbelliferyl glucoside have only a relatively small effect on the rate of glucuronidation of *p*-nitrophenol (Table 1), and a similarly small inhibitory effect on the rate of the UDP-dependent hydrolysis of *p*-nitrophenyl glucuronide. Also these glucosides are without effect on the rate of synthesis of naphthyl glucuronide, and therefore do not bind at the active site for the glucuronidation of 1-naphthol. On the other hand, these glucosides do inhibit the conjugation of *p*-nitrophenol in assays stimulated by 1-naphthyl glucuronide (Table 1). Similarly, these

Table 1. *Effect of glycosides on the glucuronidation of p-nitrophenol*

The assays contained 0.5mM-UDP-glucuronic acid and 0.3mM-*p*-nitrophenol. The concentration of glycoside added was 4mM in each case. The rates represent initial rates of reaction determined as described in the Experimental section.

Additions to Assay	<i>p</i> -Nitrophenol conjugated (nmol/min per mg of microsomal protein)
None	1.6
<i>p</i> -Nitrophenyl glucoside	1.8
4-Methylumbelliferyl glucoside	2.0
1-Naphthyl glucuronide	5.1
1-Naphthyl glucuronide+ <i>p</i> -nitrophenyl glucoside	2.4
1-Naphthyl glucuronide+ 4-methylumbelliferyl glucoside	1.7

glucosides inhibit when 4-methylumbelliferyl glucuronide is the activating glucuronide. Kinetic analysis of these inhibitions reveals that, as expected, the glucosides are competitive inhibitors with respect to the activating glucuronide (Fig. 4). Thus the glucosides are competing with the glucuronide for the activator site, and this activator site is indeed different from the site of synthesis of the activating glucuronide.

Glucuronide activation as a general property of UDP-glucuronyltransferases

Stimulation of the glucuronidation of *p*-nitrophenol seems to be a general property of glucuronide adducts. In addition to 4-methylumbelliferyl glucuronide and 1-naphthyl glucuronide, 2-naphthyl glucuronide, phenyl glucuronide and oestriol 3- β -glucuronide are activators. Moreover, activation by glucuronides occurs with aglycones other than *p*-nitrophenol. Table 2 demonstrates that the glucuronidation of 1-naphthol is stimulated by *p*-nitrophenyl glucuronide. This stimulation is decreased (Table 2) by the same glucosides that inhibit the glucuronide activation of the rate of conjugation of *p*-nitrophenol (Table 1). Conjugation of 1-naphthol is stimulated too by 4-methylumbelliferyl glucuronide. We have also found that *p*-nitrophenyl glucuronide stimulates the rates of glucuronidation of *o*-aminophenol, bilirubin and *o*-aminobenzoate at low concentrations of UDP-glucuronic acid.

An extremely noteworthy observation, consistent also with the above observation, is that the conjugation of 1-naphthol is end-product inhibited by 1-naphthyl glucuronide only at low concentrations of 1-naphthol (Fig. 5). As the concentration of 1-naphthol is increased, active-site-bound 1-naphthyl

Fig. 4. Inhibition by 4-methylumbelliferyl glucoside of 4-methylumbelliferyl glucuronide stimulation of the rate of conjugation of *p*-nitrophenol

Initial rates of glucuronidation of *p*-nitrophenol were determined in assays containing 0.3 mM-*p*-nitrophenol, 0.4 mM-UDP-glucuronic acid and various concentrations of 4-methylumbelliferyl glucuronide. The extent of activation by 4-methylumbelliferyl glucuronide in the presence (●) or the absence (□) of 5 mM-4-methylumbelliferyl glucoside was determined by subtracting the rate of conjugation in the absence of added glucuronide from all the rates. The adjusted rates are expressed as in Fig. 1, and are plotted in double-reciprocal form as a function of the concentration of 4-methylumbelliferyl glucuronide.

Table 2. Effect of glucosides on the *p*-nitrophenyl glucuronide-stimulated glucuronidation of 1-naphthol

The assays contained 0.5 mM-UDP-glucuronic acid and 0.1 mM of radioactive 1-naphthol. The initial rate of formation of 1-naphthyl glucuronide was determined as described in the Experimental section.

Additions to assay	1-Naphthol conjugated (nmol/min per mg of microsomal protein)
None	1.3
<i>p</i> -Nitrophenyl glucuronide	6.3
<i>p</i> -Nitrophenyl glucuronide+ 4-methylumbelliferyl glucoside	3.9
<i>p</i> -Nitrophenyl glucuronide+ <i>p</i> -nitrophenyl glucoside	4.9

glucuronide is presumably displaced so that at relatively high concentrations of 1-naphthol one observes only an activation by 1-naphthyl glucuronide of its own rate of synthesis. 1-Naphthyl glucuronide seems to bind at two distinct sites; one site which it has in common with 1-naphthol (the active site) and

Fig. 5. Double-reciprocal plot of initial rates of 1-naphthol conjugation as a function of the concentration of 1-naphthol in the presence and the absence of 1-naphthyl glucuronide

Initial rates of glucuronidation of 1-naphthol were determined as described in the Experimental section for assays containing 1 mM-UDP-glucuronic acid, various concentrations of 1-naphthol and either no further addition (■) or 4 mM-1-naphthyl glucuronic acid (●). Velocities are expressed as nmol of 1-naphthol conjugated/min per mg of microsomal protein.

Table 3. Effect of aglycone glucosides on the UDP-*N*-acetylglucosamine-stimulated rate of glucuronidation of *p*-nitrophenol

The assays contained 1 mM-UDP-glucuronic acid and 0.3 mM-*p*-nitrophenol. The UDP-*N*-acetylglucosamine was added at 2 mM and the glucosides were each added at 5 mM. Initial rates of reaction were determined in each case.

Additions to assay	<i>p</i> -Nitrophenol conjugated (nmol/min per mg of microsomal protein)
None	2.3
2 mM-UDP- <i>N</i> -acetylglucosamine	6.5
5 mM-4-Methylumbelliferyl glucuronide	6.3
2 mM-UDP- <i>N</i> -acetylglucosamine+5 mM- <i>p</i> -nitrophenyl glucoside	6.6

another which is responsible for the activation and does not bind 1-naphthol (the effector site).

The stimulation of UDP-glucuronyltransferase by glucuronide adducts is similar in many ways to the activation of the enzyme by UDP-*N*-acetylglucosamine (Vessey *et al.*, 1973). In both types of activation the effectors bind at site(s) other than the active site and thereby increase the affinity of the enzyme for UDP-glucuronic acid. Neither activator increases the rate of UDP-dependent hydrolysis of *p*-nitrophenyl glucuronide (i.e. the reverse reaction). An additional similarity is that *p*-chloromercuri-

benzoate, which activates the enzyme (Winsnes, 1971; Zakim & Vessey, 1972), blocks the activating effects of both UDP-*N*-acetylglucosamine (Winsnes, 1971) and glucuronides. On the other hand, there are differences between the two types of activations. Aglycone glucosides are without effect on the stimulation by UDP-*N*-acetylglucosamine (Table 3). Also, none of the glucuronide activations are sensitive to bivalent cations, on the basis of studies with EDTA or added Mn^{2+} . However, most of the UDP-*N*-acetylglucosamine activations require bivalent cation (Zakim *et al.*, 1973c). Although complete binding studies have not yet been conducted, this preliminary evidence suggests the distinctness of the glucuronide and UDP-*N*-acetylglucosamine sites.

Discussion

The data in the present paper indicate that a wide range of aglycone glucuronides increase UDP-glucuronyltransferase activity. The activation is not limited to any one homologous form of the enzyme (Zakim *et al.*, 1973a), but rather seems to be a general property of all the substrate-specific forms tested. The data establish that activation by glucuronide adducts is the result of their binding to effector sites which are distinct from the glucuronide's respective active site and that binding at this effector site can be specifically inhibited by aglycone glucosides.

The fact that a number of the aglycone glucuronides have two distinct binding sites presents a problem not only for kinetic studies with a single aglycone, but also for studies on the multiplicity of substrate-specific forms of UDP-glucuronyltransferase. The problem arises from the fact that the most useful approach in the study of enzyme multiplicity is through either alternate substrate inhibition or alternate product inhibition. The data in the present paper indicate that such studies must be planned very carefully, since some glucuronides may have dual effects, i.e. inhibition by binding at the active site but activation by binding at an allosteric site.

For the effect of 1-naphthyl glucuronide on the rate of conjugation of *p*-nitrophenol, the balance between binding of glucuronide at the effector site and the active site seems to be in favour of the effector site. At relatively low concentrations of UDP-glucuronic acid the activating effect predominates and one is unaware of the underlying competitive inhibition at the *p*-nitrophenol site. It is possible, however, to nullify the activating effect of 1-naphthyl glucuronide by using high concentrations of UDP-glucuronic acid, and under these conditions 1-naphthyl glucuronide acts as an inhibitor at the active site. A similar consideration explains the ability to detect the binding of 4-methylumbelliferyl glucuronide and phenyl glucuronide at the aglycone

site for *o*-aminophenol in studies using 10mM-UDP-glucuronic acid (Zakim *et al.*, 1973b). These inhibitions might have been missed at lower concentrations of UDP-glucuronic acid. A noteworthy result is that of Hänninen & Marniemi (1971), who found that the inhibition of the glucuronide conjugation of *p*-nitrophenol by 1-naphthyl glucuronide and phenolphthalein glucuronide was non-competitive with respect to *p*-nitrophenol. Glucuronides should function as product inhibitors by virtue of their ability to compete for the aglycone binding site (Vessey & Zakim, 1972; Zakim *et al.*, 1973b). Although it is not possible on the basis of our studies with guinea-pig liver microsomal fractions to directly predict what effect glucuronides would have on the rat liver preparation of Hänninen & Marniemi (1971), it is quite possible that their unexpected finding reflects the binding of glucuronide at an effector site as well as at the active site.

A similar problem can arise in studies of alternate substrate inhibition if true initial rates of reaction are not measured. Mulder (1971) has clearly shown this in a study of the effect of *p*-nitrophenol on the rate of conjugation of *o*-aminophenol. The time-course of the synthesis of *o*-aminophenyl glucuronide in the presence of *p*-nitrophenol is anomalous in that the rate continually increases over the initial 10–20min of the assay. This anomalous behaviour could, in part, be related to the fact that *p*-nitrophenyl glucuronide has been shown to stimulate the glucuronidation of *o*-aminophenol. A portion of the *p*-nitrophenol added as an alternate substrate inhibitor is conjugated with glucuronic acid to form *p*-nitrophenyl glucuronide, which has the opposing effect of increasing the rate of synthesis of *o*-aminophenyl glucuronide.

Activation of the conjugation of *p*-nitrophenol by glucuronide is half maximal at a glucuronide concentration ($K_{0.5}$) of about 2mM. It is doubtful whether under normal circumstances such concentrations of glucuronide are attained *in vivo*. The effector site might be of greatest use therefore in those disease states accompanied by impaired biliary secretion of glucuronides. In these states, the tendency of the accumulated glucuronides to act as end-product inhibitors of continuing conjugation could be overridden by the ability of the glucuronides to activate UDP-glucuronyltransferases. In this way there would be an accumulation of glucuronides which are generally less toxic and more easily excreted by alternate means than the free aglycones. Alternatively, the $K_{0.5}$ value for glucuronides activating the conjugation of aglycones other than *p*-nitrophenol might be in a more physiological range, and glucuronides such as bilirubin glucuronide, which predominate *in vivo*, will prove to be more effective activators of UDP-glucuronyltransferase than the glucuronides used in the present study.

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