Product Inhibition of *o*-Aminophenol Glucuronidation by *p*-Nitrophenyl Glucuronide in Detergent-Treated Microsomal Fractions

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The glucuronidation of *o*-aminophenol is unaffected by *p*-nitrophenyl glucuronide when native microsomal fractions are the source of UDP-glucuronyltransferase. When microsomal fractions treated with Lubrol detergent are the source of the enzyme, however, *p*-nitrophenyl glucuronide exhibits competitive inhibition of *o*-aminophenol glucuronidation. In addition, the apparent K_i for *p*-nitrophenyl glucuronide is the same whether *o*-aminophenol or *p*-nitrophenol is the acceptor substrate. The data suggest that UDP-glucuronyltransferase has one binding site for the two phenols and that the absence of inhibition observed in native microsomal fractions is dependent on an intact microsomal membrane.

Hepatic microsomal UDP-glucuronyltransferase (EC 2.4.1.17) has been proposed to have at least two separate binding sites for the glucuronidation of *p*-nitrophenol and *o*-aminophenol in both the guinea pig (Zakim *et al.*, 1973) and the rat (Howland & Bohm, 1977) on the basis of the observations that *p*-nitrophenyl glucuronide does not inhibit *o*-aminophenol glucuronidation, although the glucuronide adducts of both phenols are competitive inhibitors of the glucuronidation of their respective aglycones (Zakim *et al.*, 1973). Evidence from the purification of UDP-glucuronyltransferase, however, indicates that no separation of activities towards *o*-aminophenol and *p*-nitrophenol occurs even when the enzyme is purified to apparent homogeneity (Burchell, 1977*a,b*). To resolve this question the effect of *p*-nitrophenyl glucuronide on *o*-aminophenol glucuronidation was studied in native and detergent-treated microsomal fractions. As shown in this paper the kinetic evidence for separate binding sites for *o*-aminophenol and *p*-nitrophenol appears to be dependent on an intact microsomal membrane.

Materials and Methods

Chemicals

Lubrol WX, a non-ionic detergent, was obtained from General Biochemicals, Chagrin Falls, OH, U.S.A. *o*-Aminophenol was purchased from Aldrich Chemical Co., Milwaukee, WI., U.S.A., and *p*-nitrophenol was purchased from Calbiochem, Los Angeles, CA., U.S.A. D-Glucaric acid 1,4-lactone (glucarolactone), UDP-glucuronic acid and *p*-nitrophenyl glucuronide were purchased from Sigma

Chemical Co., St. Louis, MO., U.S.A. Authentic *o*-aminophenyl glucuronide was obtained from Koch-Light Laboratories, Colnbrook, Bucks., U.K. All other chemicals were reagent grade and obtained from local suppliers.

Animals and tissue preparation

Male Wistar rats (200–250 g) were purchased from Marland Breeding Farms, Hewitt, NJ, U.S.A. Animals were allowed free access to food and water before being killed.

Animals were decapitated, exsanguinated and microsomal fractions were obtained from homogenates of liver in 0.32 M-sucrose/1 mM-Tris/HCl/1 mM-EDTA, pH 7.0 (buffered sucrose), as previously described by Howland *et al.* (1971). The microsomal pellets were resuspended in buffered sucrose such that 1 ml contained the microsomal fraction derived from 1 g of liver. One half of the microsomal suspension was diluted with an equal volume of buffered sucrose (native microsomal fraction). The remaining microsomal suspension was mixed with an equal volume of 0.4% (w/v) Lubrol in buffered sucrose (0.2% Lubrol final concentration) and served as the detergent-treated microsomal fraction.

Enzyme assays

Typical incubation mixtures contained the following final concentrations of reactants in a final volume of 0.5 ml: 0.5–2 mg of protein; 15.0 mM-MgCl₂; 5.0 mM-glucarolactone to inhibit β -glucuronidase; 50 mM-Tris/HCl buffer, pH 7.2; 2.0 mM-UDP-glucuronic acid. The concentrations of acceptor phenols and *p*-nitrophenyl glucuronide are given in the text

and legends. All reactants except UDP-glucuronic acid were preincubated together for 3 min and the reaction was allowed to proceed at 37°C for 10 min in a Dubnoff metabolic shaking incubator.

When *o*-aminophenol was the substrate, the reaction was terminated by the addition of an equal volume of 1.0M-trichloroacetic acid/1.0M-KH₂PO₄, pH 2.25. After pelleting the precipitated protein, the formation of *o*-aminophenyl glucuronide was quantified by the method of Dutton & Storey (1962).

When *p*-nitrophenol was the substrate the reaction was stopped by the addition of 1 vol. of 5% (w/v) trichloroacetic acid. After centrifuging the precipitated protein, the synthesis of *p*-nitrophenyl glucuronide was estimated by determining the disappearance of *p*-nitrophenol as previously described (Howland & Bohm, 1977).

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard.

Statistical significance between means was tested by Student's *t* test.

Results and Discussion

Product inhibition in native microsomal fractions

In native microsomal fractions, the rate of glucuronidation of *o*-aminophenol was unaffected by the addition of 10mM-*p*-nitrophenyl glucuronide to the reaction media (Fig. 1), which is in accord with previous reports (Zakim *et al.*, 1973; Howland & Bohm, 1977). In addition, no inhibition by 10mM-*p*-nitrophenyl glucuronide was observed when UDP-glucuronic acid was the varied substrate over a concentration range of 0.25–2.0mM (results not shown).

Product inhibition in detergent-treated microsomal fractions

In detergent-treated microsomal fractions, *p*-nitrophenyl glucuronide inhibited the glucuronidation of its own aglycone (Fig. 2a). Thus UDP-glucuronyltransferase can exhibit product inhibition even in the presence of a modified microsomal membrane as indicated by the activation of enzyme activity that occurs after Lubrol treatment (Howland *et al.*, 1971; Burchell, 1977a).

In contrast with native microsomal fractions, 10mM-*p*-nitrophenyl glucuronide competitively inhibited *o*-aminophenol glucuronidation when detergent-treated microsomal fractions were used as the source of UDP-glucuronyltransferase (Fig. 2b). In three such preparations the apparent *K_i* for *p*-nitrophenyl glucuronide was 7.4 ± 0.8 mM when *o*-aminophenol was the substrate and 8.3 ± 1.0 mM when *p*-nitrophenol was the acceptor (mean $\pm$ S.E.) which were not statistically different.

Fig. 1. Effect of *p*-nitrophenyl glucuronide on the synthesis of *o*-aminophenyl glucuronide in native microsomal fractions. Rates of activity were measured in native microsomal fractions as a function of variable concentrations of *o*-aminophenol in the presence (○) or absence (●) of 10mM-*p*-nitrophenyl glucuronide. Velocity (*v*) is expressed as nmol/min per mg of protein.

Fig. 2. Effect of *p*-nitrophenyl glucuronide on the synthesis of glucuronides in detergent-treated microsomal fractions. Rates of activity were measured in detergent-treated microsomal fractions as a function of variable concentrations of either *p*-nitrophenol (a) or *o*-aminophenol (b) in the presence (○) or absence (●) of 10mM-*p*-nitrophenyl glucuronide. Velocity (*v*) is expressed as nmol/min per mg of protein.

The results with detergent-treated microsomal fractions are consistent with there being one binding site on UDP-glucuronyltransferase for *o*-aminophenol and *p*-nitrophenol, as evidenced by the

competitive inhibition pattern and the close agreement of the apparent K_i values obtained when the glucuronidation of either aglycone was determined in the presence of *p*-nitrophenyl glucuronide. The kinetic evidence for the separate binding sites observed in native microsomal fractions would appear to be dependent on the microsomal membrane or some component thereof. Indeed, previous work from this laboratory demonstrated that when the microsomal membrane is exposed to high (pH 5.4) or low (pH 9.2) H^+ concentrations *o*-aminophenol glucuronidation is competitively inhibited by *p*-nitrophenyl glucuronide (Howland & Bohm, 1977). It has also been reported that alkaline pH can activate and partially solubilize UDP-glucuronyltransferase, indicating that H^+ concentrations can disrupt the integrity of the microsomal membrane (Marniemi & Vainio, 1976).

As was pointed out by Howland & Bohm (1977), the absence of inhibition by *p*-nitrophenyl glucuronide in native microsomal fractions is not related to the stimulatory effect of glucuronic acid derivatives on UDP-glucuronyltransferase (Vessey & Zakim, 1974), since the stimulation is only observed at UDP-glucuronic acid concentrations below 2.0 mM. Furthermore, as reported in the present paper, *p*-nitrophenyl glucuronide neither stimulated nor inhibited *o*-aminophenol glucuronidation when the UDP-glucuronic acid concentration was 0.25 mM. The only difference between the work of Vessey & Zakim (1974) and that in this laboratory is that microsomal fractions were obtained from guinea-pig

liver in the former and from rat liver in the latter, suggesting that this is a species-specific phenomenon.

In conclusion, the data presented here coupled with the evidence from purification (Burchell, 1977*a,b*) favour there being one binding site on UDP-glucuronyltransferase for *o*-aminophenol and *p*-nitrophenol. The apparent distinct binding sites observed in native microsomal preparations may reflect either conformational differences in the enzyme or differences in some other, as yet unidentified, membrane component that regulates UDP-glucuronyltransferase activity.

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