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KINETIC PROPERTIES OF MICROSOMAL UDP-GLUCURONYLTRANSFERASE
EVIDENCE FOR COOPERATIVE KINETICS AND ACTIVATION BY UDP-*N*-ACETYLGLUCOSAMINE

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SUMMARY

The kinetics of microsomal UDPglucuronyltransferase (EC 2.4.1.17) with *p*-nitrophenol and *o*-aminobenzoate as glucuronyl acceptors deviate from Michaelis–Menten at low concentrations of UDPglucuronic acid in that double reciprocal plots are concave. The deviation from linearity in these plots represents a real property of UDPglucuronyltransferases and does not result from artifacts in the assay due to metabolism of substrates or products in side pathways. Careful analysis of the data indicate that they also cannot be explained by postulating multiple enzymes for the synthesis of each glucuronide. The most reasonable and simplest mechanism which is compatible with all the data is that there is cooperativity in the sequential binding of UDPglucuronic acid to UDPglucuronyltransferase. Thus, the binding of the first molecule of UDPglucuronic acid to the enzyme makes the subsequent binding of UDPglucuronic acid more difficult.

Assay of UDPglucuronyltransferase in the presence of UDP-*N*-acetylglucosamine is associated with a reversible decrease in the apparent $K_{0.5}$ for UDPglucuronic acid with *p*-nitrophenol or *o*-aminobenzoate as aglycone. The binding of UDP-*N*-acetylglucosamine to UDPglucuronyltransferase also shows anomalous kinetics which appear to be most compatible with negative cooperativity. Despite similarities in their structures there is no overlap in the binding of UDPglucuronic acid and UDP-*N*-acetylglucosamine at the active site and regulatory site of the enzyme.

INTRODUCTION

Changes in the conformation of soluble enzymes and associated modification of kinetic properties as a result of alterations in their environments or on the binding of soluble ligands are well-known phenomena useful for the dynamic regulation of rates of substrate flux in various metabolic pathways[1–3]. Interest in this type of re-

gulatory mechanism has focused on the properties of soluble proteins and enzymes, but the same general principles are likely to apply also to proteins which are bound to membrane structures. As compared to a soluble enzyme, in fact, the number of parameters which potentially can alter protein structure and function is greater for a membrane-bound enzyme since the latter are in contact with portions of the membrane lipids. This characteristic of membrane-bound proteins has limited attempts to study their kinetic properties, however, because of the difficulties in preparing soluble forms of these enzymes. Moreover, work in this and other laboratories has shown that techniques which are useful for the separation of proteins from their attachment to membranes alter the properties of tightly-bound membrane enzymes[4–8]. It is becoming clear, therefore, that improved understanding of the properties and functions of membrane-bound enzymes requires the use of intact membranes despite the inherent limitations of working with a heterogeneous system. From the point of view of understanding complex physiological function, there may be disadvantages in working with purified forms of some enzymes.

Our interest in the possibility that ligand binding to tightly-bound membrane enzymes may alter their kinetic properties has been stimulated by two observations. The value for $K_{\text{UDPglucuronic acid}}$ for UDPglucuronyltransferase (EC 2.4.1.17) measured *in vitro*[9] is 2 orders of magnitude greater than reported values for the *in vivo* concentration of UDPglucuronic acid[10]. This suggests that rates of glucuronidation in liver microsomes are likely to be severely limited by the concentration of UDPglucuronic acid. On the other hand, it has been reported that UDP-*N*-acetylglucosamine, a physiologic intermediate, stimulates the rate of synthesis of several glucuronides[11,12]. Unfortunately, however, a mechanism for the observed effects of UDP-*N*-acetylglucosamine was not established. Moreover, UDPglucuronyltransferase used by previous investigators contained microsomal pyrophosphatase which hydrolyzes UDPglucuronic acid and UDP-*N*-acetylglucosamine. In the present studies, therefore, microsomes from guinea pig liver were used as the source of UDPglucuronyltransferase since these contain no pyrophosphatase. In addition to direct effects of UDP-*N*-acetylglucosamine on UDPglucuronyltransferase, we have found that the rates of glucuronidation of *p*-nitrophenol or *o*-aminobenzoate do not follow Michaelis–Menten kinetics at low concentrations of UDPglucuronic acid. The data suggest that deviations from Michaelis–Menten kinetics and activation by UDP-*N*-acetylglucosamine reflect ligand-induced modifications of the kinetic properties of UDPglucuronyltransferase.

MATERIALS AND METHODS

UDPglucuronic acid, UDP-*N*-acetylglucosamine, *p*-nitrophenol and *o*-aminobenzoate were purchased from Sigma Chemical Co.

Preparation of microsomes

Livers were obtained from retired male breeder guinea pigs and microsomes isolated and stored as described previously[9].

Enzyme assays

All assays were carried out at 37 °C in 50 mM P_i, pH 7.6. The concentration of

p-nitrophenol was 0.2 mM and *o*-aminobenzoate 0.2 mM. The concentrations of UDPglucuronic acid and UDP-*N*-acetylglucosamine are indicated in the figures and text. Initial rates of disappearance of *p*-nitrophenol were estimated as described previously[9] by removal of several serial aliquots from assay tubes during the course of each reaction. With *o*-aminobenzoate as substrate the rate of glucuronidation was also estimated serially during each assay by measurement of *o*-aminobenzoylglucuronic acid according to the method of Dutton and Storey[13], except that P_i buffer was substituted for glycine. Initial rates of reaction for control and UDP-*N*-acetylglucosamine stimulated preparations were directly proportional to the concentration of microsomal protein over a wide range of protein concentrations.

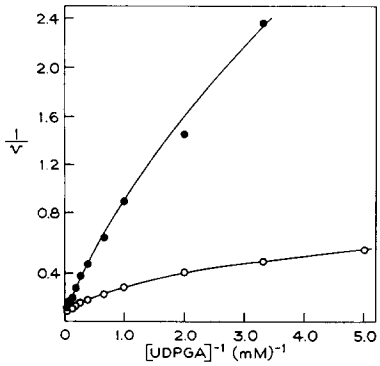
For investigation of possible non-specific irreversible binding of UDPglucuronic acid to microsomes, microsomes were treated with varying concentrations of UDP[^{14}C]glucuronic acid under exact conditions as for enzyme assays except for the omission of aglycones. After 5 min, microsomes were separated by centrifugation at 4 °C and recovery of UDP[^{14}C]glucuronic acid determined in the supernatants. Potential side pathways for the metabolism of UDPglucuronic acid were studied in a similar manner except that reactions were stopped by the addition of ethanol. UDP-[^{14}C]glucuronic acid was isolated by paper chromatography[11] and spots cut out and counted in a liquid scintillation counter. Destruction of aglycones and *p*-nitrophenylglucuronide in side reactions was investigated using standard colorimetric techniques for detection of these compounds[14]. Protein was measured by the biuret method[15].

RESULTS

Rate of synthesis of p-nitrophenylglucuronide and o-aminobenzoylglucuronide, as a function of the concentration of UDPglucuronic acid

In previous studies the kinetic parameters of UDPglucuronyltransferase, assayed with *p*-nitrophenol as aglycone, were estimated using a range of concentrations of UDPglucuronic acid of approx. 2.0–40.0 mM. As shown in a previous publication[5,9] double-reciprocal plots of $1/v$ *versus* 1/[UDPglucuronic acid] are linear over this range of concentrations. It was not appreciated previously, however, that below 2.5 mM UDPglucuronic acid plots of $1/v$ *versus* 1/[UDPglucuronic acid] are not linear but bend slightly downward (Fig. 1). Similar non-linearity in double reciprocal plots was observed when activity was assayed with *o*-aminobenzoate as aglycone (Fig. 2).

Since the source of UDPglucuronyltransferase in these experiments is the liver microsome, a complex and heterogeneous system, several alternate mechanisms might contribute to the production of apparent non-Michaelis–Menten kinetics. Most important is the consideration of artifacts in the assay procedure such as metabolism of substrates or products by alternate pathways. The data in Figs 1 and 2 indicate that the rates of glucuronidation of either aglycone are higher at low concentrations of UDPglucuronic acid than one would anticipate by extrapolation of double-reciprocal plots at high concentrations of UDPglucuronic acid. Thus, destruction of UDPglucuronic acid or aglycones in alternate metabolic pathways cannot explain these data. Nevertheless, attempts were made to demonstrate quantitatively significant participation of UDPglucuronic acid or aglycones in such side reactions, but in the absence of aglycone there was no metabolism of UDPglucuronic acid. Moreover, direct experimentation revealed that Michaelis conditions were attained in that the



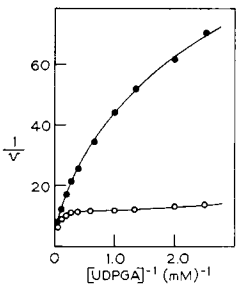


Fig. 1. Double-reciprocal plots of the rate of synthesis of *p*-nitrophenylglucuronide as a function of the concentration of UDPglucuronic acid (UDPGA). Initial rates of activity of UDPglucuronyltransferase with *p*-nitrophenol as aglycone were determined as in Materials and Methods at a *p*-nitrophenol concentration of 0.2 mM. The closed circles are control experiments and the open circles for assays carried out in the presence of 2.0 mM UDP-*N*-acetylglucosamine; *v* is expressed as nmoles per min per mg microsomal protein.

Fig. 2. Double-reciprocal plots of the rate of synthesis of *o*-aminobenzoylglucuronide as a function of the concentration of UDPglucuronic acid (UDPGA). Initial rates of activity of UDPglucuronyltransferase with *o*-aminobenzoate as aglycone were determined as in Materials and Methods at an *o*-aminobenzoate concentration of 0.2 mM. The closed circles are control experiments and the open circles for assays in the presence of 2.0 mM UDP-*N*-acetylglucosamine; *v* is expressed as the change in absorbance due to the formation of *o*-aminobenzoylglucuronide per min per mg microsomal protein.

effective concentration of free substrate was the same as the total concentration of substrate, and the concentration of substrate was in excess of the number of binding sites. At concentrations as low as 0.1 mM less than 1% of UDPglucuronic acid was bound to the microsomes. Additionally, glucuronide products at concentrations arising during assays in the forward direction were stable under the conditions of the assay, and there were no detectable blank reactions. These results indicate that the non-linearity of the data in Figs 1 and 2 is a real property of UDPglucuronyltransferases.

The effect of UDP-N-acetylglucosamine on the rate of synthesis of p-nitrophenyl- and o-aminobenzoylglucuronides

Fixed concentrations of UDP-*N*-acetylglucosamine increased the rate of glucuronidation with either *p*-nitrophenol (Fig. 1) or *o*-aminobenzoate (Fig. 2) as aglycone. The extent of the rate enhancement was dependent on the concentration of UDPglucuronic acid (Figs 1 and 2), there being no enhancement at infinite concentrations of UDPglucuronic acid. Also, UDP-*N*-acetylglucosamine did not alter the affinity of UDPglucuronyltransferase for aglycones.

With only the data in Figs 1 and 2 it is not possible to exclude that UDPglucuronic acid and UDP-*N*-acetylglucosamine compete for binding at their respective sites, and that the rates of synthesis of glucuronides as a function of variable concentration of UDPglucuronic acid measured at fixed levels of UDP-*N*-acetylglucosamine reflect mixed effects. In order to investigate these possibilities the rate of synthesis of *p*-nitrophenyl- and *o*-aminobenzoylglucuronides were measured as a function of variable concentrations of UDP-*N*-acetylglucosamine. Double-reciprocal

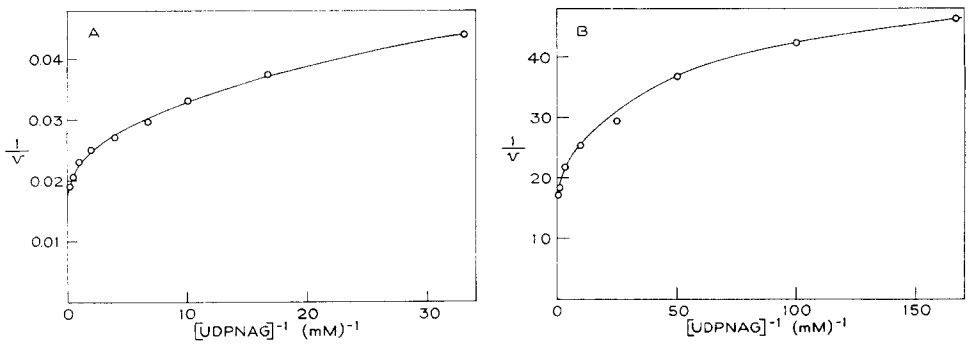


Fig. 3. Rate of UDPglucuronyltransferase catalyzed synthesis of glucuronides as a function of the concentration of UDP-*N*-acetylglucosamine (UDPNAG). Initial rates of activity were determined in (A) with 0.2 mM *p*-nitrophenol, and 2.0 mM UDPglucuronic acid, and in (B) with 0.2 mM *o*-aminobenzoate and 1.0 mM UDPglucuronic acid, and the indicated concentrations of UDP-*N*-acetylglucosamine as in Figs 1 and 2.

plots for the rate of synthesis of glucuronides as a function of the concentration of UDP-*N*-acetylglucosamine at fixed concentrations of substrates are shown in Fig. 3. The rates of glucuronidation of both *p*-nitrophenol and *o*-aminobenzoate continue to increase at concentrations of UDP-*N*-acetylglucosamine as high as 25 mM, or 12.5-fold greater than that used to obtain the data in Figs 1 and 2, indicating that UDP-*N*-acetylglucosamine does not bind at any UDPglucuronic acid sites important for the function of UDPglucuronyltransferase.

Unlike the data in Figs 1 and 2, those in Fig. 3 contain a blank reaction since rates of glucuronidation of *p*-nitrophenol and *o*-aminobenzoate are not zero in the absence of UDP-*N*-acetylglucosamine. In order to correct for this "blank" rate, the data were replotted after subtracting the rate in the absence of UDP-*N*-acetylglucosamine (Fig. 4). The replots in Fig. 4 reveal a linear relationship between the reciprocal of the concentration of UDP-*N*-acetylglucosamine and the reciprocal of the corrected velocity up to concentrations of 5.0 mM UDP-*N*-acetylglucosamine.

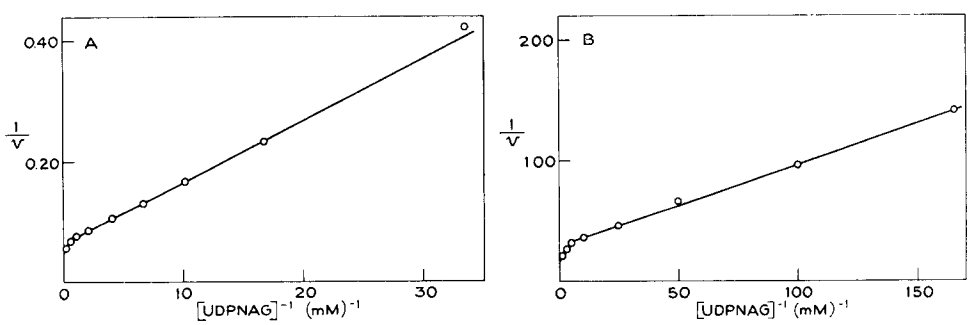
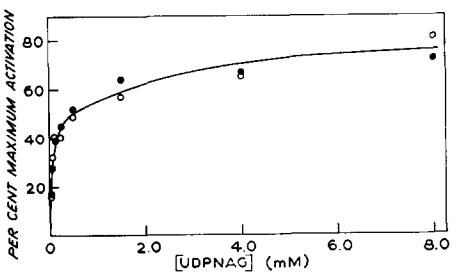


Fig. 4. Rate of glucuronidation of *p*-nitrophenol (A) and *o*-aminobenzoate (B) as a function of variable concentrations of UDP-*N*-acetylglucosamine (UDPNAG). The rates of synthesis of glucuronides in the absence of UDP-*N*-acetylglucosamine were subtracted from the rate obtained in the presence of the indicated concentrations of UDP-*N*-acetylglucosamine as in Fig. 3. The data are plotted in double-reciprocal form; for assays with *p*-nitrophenol *v* is expressed as nmoles *p*-nitrophenol conjugated per min per mg protein and for assays with *o*-aminobenzoate as the absorbance change per min per mg due to the formation of *o*-aminobenzoylglucuronide.

In order to investigate the possibility that UDPglucuronic acid could bind at the UDP-*N*-acetylglucosamine site, contributing thereby to the lack of stimulation at V, the rates of synthesis of glucuronides as a function of the concentration of UDP-*N*-acetylglucosamine were studied at two different fixed levels of UDPglucuronic acid. The rate of glucuronidation at each concentration of UDP-*N*-acetylglucosamine was normalized by plotting the data as the percent of the maximum activation determined by extrapolation of double-reciprocal plots to infinite concentrations of UDP-*N*-acetylglucosamine. With *p*-nitrophenol as glucuronyl acceptor activation by UDP-*N*-acetylglucosamine is independent of the concentration of UDPglucuronic acid (Fig. 5). Thus, at levels of 5 mM, UDPglucuronic acid does not bind at the UDP-*N*-acetylglucosamine site. Also, the binding of UDPglucuronic acid at the active site does not alter the binding of UDP-*N*-acetylglucosamine. In contrast, prior binding of UDPglucuronic acid alters the subsequent binding of UDP-*N*-acetylglucosamine for



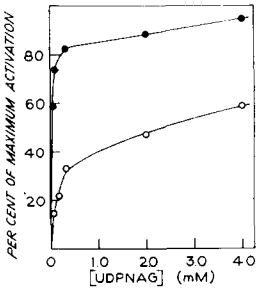


Fig. 5. Activation of UDPglucuronyltransferase by UDP-*N*-acetylglucosamine (UDPNAG) at variable concentrations of UDPglucuronic acid. Initial rates of activity were determined as in Fig. 1 with 0.2 mM *p*-nitrophenol and plotted in double reciprocal form in order to obtain v at infinite UDP-*N*-acetylglucosamine. The plots were normalized by calculation of the percent of maximum activation for each concentration of UDP-*N*-acetylglucosamine at 0.6 mM (○) or 10.0 M (●) UDPglucuronic acid.

Fig. 6. Activation of UDPglucuronyltransferase by UDP-*N*-acetylglucosamine (UDPNAG) at variable concentrations of UDPglucuronic acid. Initial rates of activity were determined as in Fig. 2 with 0.2 mM *o*-aminobenzoate and either 0.5 mM (○) or 10 mM (●) UDPglucuronic acid. Data were plotted in double reciprocal form in order to estimate v at infinite concentrations of UDP-*N*-acetylglucosamine. The plots were normalized as in Fig. 5.

assays conducted with *o*-aminobenzoate as aglycone (Fig. 6). Increasing concentrations of UDPglucuronic acid decrease the amount of UDP-*N*-acetylglucosamine needed to produce a half maximum rate enhancement for the synthesis of *o*-aminobenzoylglucuronide.

Reversibility of the effects of UDP-N-acetylglucosamine

It appears from the data in Figs 1 and 2 that UDP-*N*-acetylglucosamine could be a regulator of the activity of UDPglucuronyltransferase *in vivo*. For a regulatory mechanism to be useful physiologically, however, it should be reversible. The data in Table I indicate that the effect of UDP-*N*-acetylglucosamine can be reversed by dilution of the mixture of microsomes and UDP-*N*-acetylglucosamine. The effect of this compound is reversed also by washing of microsomes treated previously with 2 or 4 mM UDP-*N*-acetylglucosamine.

TABLE I

REVERSIBILITY OF UDP-*N*-ACETYLGLUCOSAMINE STIMULATION OF UDP-GLUCURONYLTRANSFERASE

UDPglucuronyltransferase was assayed in the presence or absence of the indicated concentrations of UDP-*N*-acetylglucosamine as in Materials and Methods with 0.2 mM *p*-nitrophenol and 1.0 mM UDPglucuronic acid. To test for reversibility microsomes were treated with 1.0 mM UDP-*N*-acetylglucosamine and diluted subsequently in the assay portion of the experiment to a final concentration of 0.05 mM UDP-*N*-acetylglucosamine. Activities are nmoles *p*-nitrophenol glucuronidated per min per mg microsomal protein.

Assay conditions	*Activity*
No additions	2.82
1 mM UDP-*N*-acetylglucosamine	6.66
0.05 mM UDP-*N*-acetylglucosamine	4.20
Treatment of microsomes with 1.0 mM UDP-*N*-acetylglucosamine with subsequent dilution to 0.05 mM in the assay	4.53

DISCUSSION

Interpretation of the data demonstrating that UDP-*N*-acetylglucosamine activates UDPglucuronyltransferase is relatively simple. The significance of the nonlinearity of double-reciprocal plots must be interpreted cautiously, however, because of the heterogeneity of the microsomal fraction. Two generalized models must be analyzed as to their usefulness in providing an explanation for the anomalous kinetics. These are that *p*-nitrophenol and *o*-aminobenzoate are each conjugated by at least two isoenzymes, and that the rates of glucuronidation of these aglycones are limited by the accessibility of UDPglucuronic acid to the enzyme.

Are there isoenzymes for the glucuronidation of p-nitrophenol and o-aminobenzoate?

The simplest approach to the analysis of the data in terms of an isoenzyme model is to consider only the case for *p*-nitrophenol, and that it is metabolized by two enzymes which follow typical Michaelis–Menten kinetics. Thus, there would seem to be a high K_m form of the enzyme and a low K_m form (Fig. 1). Since the double-reciprocal plot in Fig. 1 for assays of untreated microsomes (closed circles) is linear at concentrations of UDPglucuronic acid greater than 2.5 mM, all forms of UDPglucuronyltransferase except one, the postulated high K_m form, would be inhibited completely by concentrations of UDPglucuronic acid greater than 2.5 mM. By varying the concentration of UDPglucuronic acid it should be possible therefore to measure activities which are the sum for high and low K_m forms of the enzyme (at low concentrations of UDPglucuronic acid) or to measure only that due to the high K_m form of the enzyme (at high concentrations of UDPglucuronic acid). The data are not compatible with this explanation, however, since at concentrations of UDPglucuronic acid as high as 5.0 mM plots of $1/v$ *versus* 1/[UDP-*N*-acetylglucosamine] are nonlinear. It could be argued that there are two high K_m forms of UDPglucuronyltransferase, and that these are differentiated only in the presence of UDP-*N*-acetylglucosamine; but the extent of activation as a function of the concentration of UDP-*N*-acetylglucosamine is identical at 0.5 mM and 5.0 mM UDPglucuronic acid.

It is equally difficult to reconcile the data with the concept of isoenzymes, if

it is assumed that the low K_m form of UDPglucuronyltransferase is not inhibited by substrate. Extrapolation of the activity of the low K_m form of the enzyme to infinite concentrations of UDPglucuronic acid indicates that UDP-*N*-acetylglucosamine increases the activity at V of this form of the enzyme. Since total activity at V is unchanged by UDP-*N*-acetylglucosamine, however, this compound would have to inhibit the activity of the high K_m form of the enzyme so that the activation of the low K_m form is balanced exactly by the inhibition of the high K_m form. Similar exact balancing of activation and inhibition would have to occur to explain the effects of UDP-*N*-acetylglucosamine on the glucuronidation of *o*-aminobenzoate. It is clear, therefore, that a large number of additional qualifying assumptions would have to be made in order to explain the data on the basis of an isoenzyme model. Moreover, no isoenzyme model could clarify the nature of the UDP-*N*-acetylglucosamine effects on the activity of UDPglucuronyltransferase.

Are rates of glucuronidation limited by the access of substrate to the active sites?

Limitation of the rates of synthesis of glucuronides by diffusion of UDPglucuronic acid through the matrix of the membrane cannot explain the data since the bend in the plots in Figs 1 and 2 is downward rather than upward. Goldman *et al.*[16] have shown for diffusion limited reactions of membrane-bound enzymes that rates at low concentrations of substrate are smaller than anticipated by extrapolation of rates obtained at high concentrations of substrate. Similarly, the data cannot be accounted for on the basis of simple carrier mediated transport. Combinations of passive diffusion through the matrix *plus* carrier mediated transport could produce double reciprocal plots as for control assays in Figs 1 and 2; but as for the isoenzyme model, a large number of qualifying assumptions would have to be made. Furthermore, as indicated below a diffusion limited model would still not obviate the need for explaining the actions of UDP-*N*-acetylglucosamine on the basis of non-Michaelis–Menten binding to some type of receptor site.

A plausible model for anomalous kinetics of UDPglucuronyltransferase and activation by UDP-N-acetylglucosamine

The simplest model compatible with all the data and one which requires no additional qualifying assumptions, is that UDPglucuronyltransferase is a multisubunit enzyme, and the binding of UDPglucuronic acid to the first open subunit makes it progressively more difficult for the binding of subsequent molecules of UDPglucuronic acid, that is, there is so-called "negative homotropic cooperativity"[3,17,18] in the binding of UDPglucuronic acid. Replots of the data in Figs 1 and 2 in the form log $v/(V-v)$ *versus* log [UDPglucuronic acid] are compatible with this notion in that they have limiting slopes (Hill coefficients) of 1 at high concentrations of UDPglucuronic acid (greater than 2.5 mM with *p*-nitrophenol as aglycone), but slopes of less than 1 for concentrations of UDPglucuronic acid less than 2.5 mM (ref. 19). The non-linearity of plots of $1/v$ *versus* 1/[UDP-*N*-acetylglucosamine] also can be explained on the basis of homotropic negative cooperativity.

We have previously published a complete kinetic analysis of UDPglucuronyltransferase with *p*-nitrophenol as aglycone[9]. These studies were restricted to UDPglucuronic acid concentrations in excess of 2 mM, a range of concentration over which the reciprocal plots were linear. These data represent, therefore, normal Michaelis–

Menten kinetics for the binding of substrate to the *n*th subunit of the enzyme.

The mechanism of action of allosteric effectors is not necessarily based on co-operative interactions between effectors and substrates[2]. There are, however, several reasons for concluding that UDP-*N*-acetylglucosamine-induced activation of UDP-glucuronyltransferase represents such cooperative interaction. Thus, UDP-*N*-acetylglucosamine appears to facilitate the binding of very low concentrations of UDPglucuronic acid to UDPglucuronyltransferase. Further, as would be expected for an effector which decreases cooperative interactions in the binding of substrate, there is less homotropic cooperativity for the binding of the effector than for the substrate. Finally, for assays with *o*-aminobenzoate as aglycone, high concentrations of UDPglucuronic acid appear to facilitate the binding of UDP-*N*-acetylglucosamine to the enzyme.

As mentioned above, one of the interesting aspects of the function of UDP-glucuronyltransferase is the discrepancy between the $K_{\text{UDPglucuronic acid}}$ measured *in vitro*[9] and the concentrations of UDPglucuronic acid *in vivo*[10]. High rates of glucuronidation may be achieved even at the concentrations of UDPglucuronic acid *in vivo*, however, because of the large increase in the affinity of UDPglucuronyltransferase for glucuronyl donor produced by prior binding of UDP-*N*-acetylglucosamine. More recent data show that this effect of UDP-*N*-acetylglucosamine depends on the presence of endogenous divalent metal ions, and that it is potentiated by addition of such ions to assay media (Zakim, D., Vessey, D. A. and Goldenberg, J., unpublished).

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REFERENCES

1 Atkinson, D. E. (1966) *Annu. Rev. Biochem.* 35, 85–124
2 Stadtman, E. R. (1966) *Adv. Enzymol.* 28, 41–154
3 Koshland, D. E. (1970) in *The Enzymes* (Boyer, P. D., ed.) Vol. 1, pp. 342–396, Academic Press, New York
4 Zakim, D. (1970) *J. Biol. Chem.* 245, 4953–4961
5 Vessey, D. A. and Zakim, D. (1971) *J. Biol. Chem.* 246, 4649–4656
6 Vessey, D. A. and Zakim, D. (1972) *Biochim. Biophys. Acta* 268, 61–69
7 Raison, J. K., Lyons, J. M. and Thomson, W. W. (1971) *Arch. Biochem. Biophys.* 142, 83–90
8 Seiler, D. and Hasselbach, W. (1971) *Eur. J. Biochem.* 21, 385–387
9 Vessey, D. A. and Zakim, D. (1972) *J. Biol. Chem.* 247, 3023–3028
10 Keppler, D. O. R., Rudiger, J. F. M., Bischoff, E. and Decker, K. F. A. (1970) *Eur. J. Biochem.* 17, 246–253
11 Pogell, B. M. and LeLoir, L. F. (1961) *J. Biol. Chem.* 236, 293–298
12 Wisnes, A. (1969) *Biochim. Biophys. Acta* 191, 279–291
13 Dutton, G. J. and Storey, I. D. E. (1962) in *Methods in Enzymology* (Colowick, S. P. and Kaplan, N. O. eds), Vol. 5, pp. 159–164, Academic Press, New York
14 Dutton, G. J. (1966) in *Glucuronic Acid, Free and Combined* (Dutton, G. J. ed.), pp. 185–299, Academic Press, New York
15 Gornall, A. G., Bardawill, C. S. and David, M. M. (1949) *J. Biol. Chem.* 177, 751–766
16 Goldman, R., Kedem, O., and Katchalski, E. (1968) *Biochemistry* 7, 4518–4532
17 Levitzki, A. and Koshland, Jr., D. E. (1969) *Proc. Natl. Acad. Sci. U.S.* 62, 1121–1128
18 Teipel, J. and Koshland, Jr, D. E. (1969) *Biochemistry* 8, 4656–4663
19 Zakim, D., Goldenberg, J., and Vessey, D. A. (1973) *Biochim. Biophys. Acta* 309, 67–74.