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REGULATION OF MICROSOMAL ENZYMES BY PHOSPHOLIPIDS

IX. PRODUCTION OF UNIQUELY MODIFIED FORMS OF MICROSOMAL UDP-GLUCURONYLTRANSFERASE BY TREATMENT WITH PHOSPHOLIPASE A AND DETERGENTS

DAVID ZAKIM and DONALD A. VESSEY

Departments of Medicine and Biochemistry and Biophysics, University of California Medical Center, San Francisco, Calif. 94143 and Division of Molecular Biology and Department of Medicine, Veterans Administration Hospital, San Francisco, Calif. 94121 (U.S.A)

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Summary

The kinetic parameters of microsomal UDPglucuronyltransferase (EC 2.4.1.17) were compared after treatment of microsomes with phospholipase A and Triton X-100. Treatments with phospholipase A and Triton had differential effects on activities at *V* (assayed in both forward and reverse directions), the affinity of the enzyme for UDPglucuronic acid and UDP, the specificity of binding of UDPsugars, and the sensitivity of the enzyme to treatment with UDP-*N*-acetylglucosamine. Comparisons of lysophosphatide, Triton, and cholate-activated forms indicated that different types of detergents activate by separate mechanisms. These data are taken as evidence that selective types of changes of the composition and structure of microsomal lipids lead to selective types of changes of the properties of UDPglucuronyltransferase. Activation of UDPglucuronyltransferase in response to different types of perturbations of the membrane lipids is thus a specific process.

It was found that constraint on the maximal potential activity of the reverse reaction catalyzed by UDPglucuronyltransferase is more extensive in untreated microsomes than constraint on the forward reaction. Also, the "activated forms" of UDPglucuronyltransferase have less activity than the untreated enzyme if assays are carried out under conditions presumed to exist in vivo. These results indicate the complexity of lipid-protein interactions as regulators of membrane-bound enzymes. They also indicate the functional significance of this type of regulation for the activity of UDPglucuronyltransferase.

Abbreviations: The subscripts UDPGA, PNP and PNPGA refer to UDP—glucuronic acid, *p*-nitrophenol and *p*-nitrophenylglucuronic acid, respectively.

Introduction

The lipid environment of some tightly bound membrane enzymes appears to be an integral part of their regulatory apparatus [1—9]. The basis for this conclusion is that perturbations of membrane lipids, induced by detergents or phospholipases, alter the properties of these enzymes. Temperature-induced phase transitions within the lipid portion of membranes have similar effects [10—14]. An important consideration, in view of these observations, is the manner in which the lipid environment influences the properties of membrane-bound enzymes.

Microsomal UDPglucuronyltransferase (EC 2.4.1.17)* is one of many enzymes that is activated in response to modification of its lipid environment [1,15]. Activity is increased after treatment of microsomes with detergents, phospholipase A, urea, chaotropes and sonic treatment [1,15—17]. It is uncertain whether UDPglucuronyltransferase is sensitive to specific modifications within its environment or whether activation reflects a non-specific response to a variety of different changes within the membrane. Resolution of this problem is important for delineating the regulatory mechanisms of this enzyme, and the role of lipid-enzyme interactions. A crucial unanswered question therefore is the relationship between different activated forms of UDPglucoronyltransferase, that is, do all agents that activate produce the same modified form of UDPglucuronyltransferase? We have begun to examine this question by comparing the kinetic properties of UDPglucuronyltransferase after treatment of microsomes with phospholipase A and detergents. The data presented below provide evidence that different types of activating agents produce forms of the enzyme with unique kinetic properties.

Materials and Methods

Microsomes from guinea pig livers were used as the source of UDPglucuronyltransferase. Microsomes were isolated as described previously [18] after homogenization in 0.25 M sucrose. UDPglucuronic acid (ammonium salt), *p*-nitrophenyl-β-D-glucuronide, and UDP-*N*-acetylglucosamine, were purchased from Sigma Chemical Co., and UDP from Pabst. Lysophosphatides were obtained from Applied Science Laboratories. Trace amounts of heavy metals were removed from the nucleotides by treatment with an ion-exchange resin [19].

The phospholipase A-treated form of UDPglucuronyltransferase was prepared by treating microsomes with purified phospholipase A [20]. The activity of the phospholipase A was 5600 units per mg protein, and the ratio of microsomal protein to phospholipase A protein was 200 to 1. Treatment with phospholipase A was at 25° C in 50 mM Tris, pH 8.0, for 30 s or 30 min. EDTA was added to a final concentration of 5.0 mM at the end of these times in order to inhibit the further action of phospholipase A [2]. EDTA has no effect on the

* Recent evidence indicates that there are several substrate-specific forms of UDPglucuronyltransferase, but the number of such enzymes is uncertain. As used in this paper the activity of UDP-glucuronyltransferase refers only to the enzyme catalyzing the glucuronidation of *p*-nitrophenol.

activity of UDPglucuronyltransferase using microsomes prepared as in ref. 18 if trace amounts of divalent metal ions are removed from substrates [19]. The conditions used in this paper reduce the concentrations of divalent metal to levels such that EDTA has no effect on the activity of the untreated enzyme or the phospholipase and detergent-treated forms.

In experiments using microsomes treated with detergent, Triton X-100 was added to assay tubes at a protein to detergent ratio of 5 : 1. This ratio yielded maximal activation. The amounts of detergent added, when agents other than Triton were used, are indicated in the legends to figures and tables.

Enzyme assays were carried out in 50 mM Tris, pH 7.6. The concentrations of UDPglucuronic acid, UDP-*N*-acetylglucosamine, other UDPsugars, UDP, *p*-nitrophenol and *p*-nitrophenylglucuronic acid are indicated in the legends of the figures and tables and in the text. Initial rates of activity were measured for each assay by removal of serial aliquots from the assay media, and determination of the rate of disappearance of *p*-nitrophenol (forward reaction) or the rate of appearance of *p*-nitrophenol (reverse reaction), using standard colorimetric techniques [18]. Saccharic acid-1,4-lactone was added to assays of the reverse reaction in order to inhibit β-glucuronidase [21]; 10 mM lactone gave complete inhibition of β-glucuronidase at concentrations of *p*-nitrophenylglucuronic acid as high as 20 mM. Activities are expressed as nmol of substrate metabolized per min per mg protein. Protein was determined by the biuret method [22].

Results

Effects of Triton X-100 and phospholipase A on K_{UDPGA} and K_{UDP} of UDPglucuronyltransferase

The kinetics of untreated UDPglucuronyltransferase fit the Michaelis-Menten model at concentrations of UDPglucuronic acid greater than 2.5 mM [19,23,24]. There is an abrupt discontinuity in double reciprocal plots, however, at lower concentrations, the plots bending concave downward. The cause of this kinetic anomaly is unproved, but seems to reflect negative cooperativity in the binding of UDPglucuronic acid [19,23,24]. The kinetic properties of the reverse reaction show a similar anomaly with regard to the relationship between activity and the concentration of UDP. The kinetic analyses of UDPglucuronyltransferase were carried out at substrate concentrations for which the enzyme adhered to the Michaelis-Menten equation. Under these conditions the enzyme conforms to a rapid-equilibrium, random order mechanism [25].

After brief treatment with phospholipase A (30 s) or Triton X-100, the kinetics of UDPglucuronyltransferase fit the Michaelis-Menten model over the entire range of concentrations of UDPglucuronic acid (Fig. 1) and UDP in forward and reverse directions, respectively. The pattern of end-product inhibition of UDPglucuronyltransferase after treatments with phospholipase A or Triton also conforms to a random order kinetic mechanism. It was possible, therefore, to determine K_{UDPGA} and K_{UDP} (enzyme-substrate and enzyme-product dissociation constants) from bisubstrate kinetic analysis of enzyme activities in forward and reverse directions, respectively [26]. Treatment with Triton or phospholipase A, under conditions which produced maximally acti-

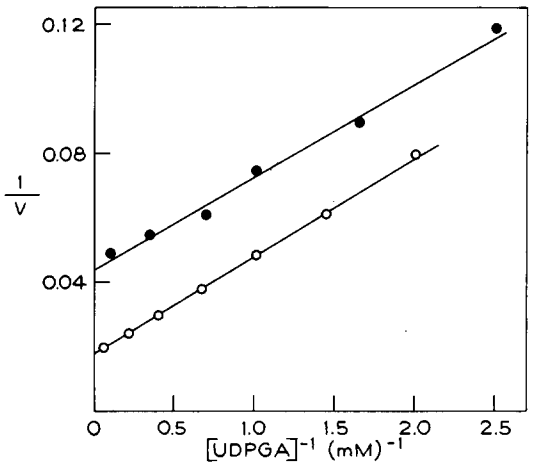

Fig. 1. The kinetics of glucuronyltransferase after treatment with purified phospholipase A from *Naja naja* venom (○) or Triton X-100 (●). Microsomes from guinea pig liver were treated for 30 s at 25°C with phospholipase A as in Materials and Methods. Initial rates of activity were measured at 37°C in 50 mM Tris, pH 7.6, 0.2 mM *p*-nitrophenol and the indicated amounts of UDPglucuronic acid. Units of activity were nmol *p*-nitrophenol metabolized per min per mg protein.

vated UDPglucuronyltransferase, both enhanced the affinity of the enzyme for UDPglucuronic acid and UDP in that K_{UDPGA} and K_{UDP} were lower after each of these treatments as compared with untreated enzyme (Table I). On the other hand, although both types of treatments decreased K_{UDPGA}, they appeared to do so by different mechanisms. Thus, K_{UDPGA} was smaller after treatment with Triton as compared with phospholipase A, but K_{UDP} was smaller after treatment with phospholipase A. Comparisons of ΔG° of binding for UDPglucuronic acid and UDP, calculated from the kinetic parameters in Table I, indicate the significance of these differences.

Treatment of UDPglucuronyltransferase with Triton decreases ΔG° for the binding of UDP and UDPglucuronic acid to enzyme to nearly an identical extent, about −1800 cal per mol (Table II). This enhanced binding of UDP-

TABLE I

EFFECT OF TREATMENT WITH PHOSPHOLIPASE A AND TRITON X-100 ON THE AFFINITY OF UDP-GLUCURONYLTRANSFERASE FOR UDP-GLUCURONIC ACID AND UDP

Initial rates of activity of UDP-glucuronyltransferase were measured in the forward or reverse direction as indicated in Materials and Methods. The preparation of the phospholipase A-treated and Triton-treated forms of the enzyme is described in Materials and Methods. Treatment with phospholipase A was stopped by addition of EDTA (final concentration of 5 mM) after 30 s. Phospholipase A-treated microsomes were kept at 0°C prior to assay. The kinetic constants K_{UDP} *and* K_{UDPGA} were determined graphically from double reciprocal plots of reaction rates as a function of variable concentrations of UDP or UDP-glucuronic acid at several different fixed concentrations of *p*-nitrophenol or *p*-nitrophenylglucuronic acid. The *K* terms are for the binding of substrate prior to aglycone or aglycone glucuronide; units are mM.

Treatment	K_{UDPGA}	K_{UDP}
None	14.0	2.0
Phospholipase A	1.0	0.03
Triton	0.6	0.11

TABLE II

EFFECT OF TREATMENT WITH PHOSPHOLIPASE A AND TRITON X-100 ON THE CALCULATED VALUES OF ΔG° FOR THE BINDING OF UDP-GLUCURONIC ACID AND UDP TO UDP-GLUCURONYLTRANSFERASE

The values of ΔG° were calculated from the values of K_{UDPGA} and K_{UDP} in Table I according to the equation $\Delta G^\circ = RT\ln(K)^{-1}$. Units are cal per mol.

Treatment	Substrate	Calculated ΔG° of binding	Difference in ΔG° of binding: UDPglucuronic acid — UDP
None	UDPglucuronic acid	—2602	
			1052
	UDP	—3654	
Triton	UDPglucuronic acid	—4469	
			885
	UDP	—5354	
Phospholipase A	UDPglucuronic acid	—4062	
			2058
	UDP	—6118	

glucuronic acid to the Triton-treated form of UDP glucuronyltransferase appears to occur because of an increase in the affinity of the enzyme for the UDP moiety of the substrate. In contrast, treatment with phospholipase A enhances the affinity of UDPglucuronyltransferase for UDP to a greater extent than it does for UDPglucuronic acid (Table II). Treatment with phospholipase A thus appears to decrease the affinity of UDPglucuronyltransferase for the glucuronic acid moiety. The validity of these conclusions was examined in another way since they predict that treatment with phospholipase A and Triton should have

TABLE III

EFFECT OF UDP ON THE RATE OF SYNTHESIS OF *p*-NITROPHENYLGLUCURONIC ACID CATALYZED BY UNTREATED, PHOSPHOLIPASE A-TREATED, AND TRITON-TREATED FORMS OF UDP-GLUCURONYLTRANSFERASE

Initial rates of activity of UDP-glucuronyltransferase were determined as in Materials and Methods in 50 mM Tris, pH 7.6, and 0.2 mM *p*-nitrophenol. When added, the concentration of UDP was 1.0 mM. The concentration of UDPglucuronic acid was 1.0 mM. UDPglucuronyltransferase was treated at 23°C with phospholipase A for 30 s as in Materials and Methods, and EDTA was added at a final concentration of 5.0 mM in order to inhibit the further action of phospholipase A. For the preparation of the Triton-treated form of the enzyme Triton X-100 was added to assays at a Triton : protein ratio of 1 : 5, which gave optimum activation. Units are nmol of *p*-nitrophenol metabolized per min per mg.

Treatment	Additions to assay	Activity (units)
None	None	4.57
	UDP	1.50
Phospholipase A	None	22.6
	UDP	1.52
Triton	None	22.3
	UDP	5.5

differential effects on the sensitivity of UDPglucuronyltransferase to end-product inhibition.

The extent of end-product inhibition of glucuronidation by UDP depends on the relative affinities of UDPglucuronyltransferase for UDPglucuronic acid and UDP. If the difference between ΔG° of binding of UDPglucuronic acid and UDP is 2000 cal per mol for phospholipase A-treated enzyme, but 1000 cal per mol for untreated enzyme, treatment with phospholipase A will potentiate end-product inhibition by UDP as compared with untreated enzyme. Treatment of UDPglucuronyltransferase with Triton, on the other hand, will have no effect on the extent of end-product inhibition by UDP. The data in Table III indicate that the predictions based on the derived thermodynamic constants are correct. Inhibition by UDP is much greater after treatment of UDPglucuronyltransferase with phospholipase A as compared with the untreated enzyme, but treatment with Triton does not alter the extent of inhibition by UDP.

In addition to altering the affinity of UDPglucuronyltransferase for UDPglucuronic acid (Table I), treatment with phospholipase A modifies the specificity of binding of sugar nucleotides [27]. Thus, only UDPglucuronic acid, of all nucleotide sugars tested, binds to the active site of the untreated form of UDPglucuronyltransferase, but all nucleotide sugars tested appear to bind to the active site of the phospholipase A-treated form of UDPglucuronyltransferase [27]. In contrast to this effect of treatment with phospholipase A, the Triton-treated form of UDPglucuronyltransferase retained specificity for UDPglucuronic acid in that all UDPsugars tested were without effect on the activity of the Triton-treated enzyme (data not shown). These data provide clear evidence that the properties of the UDPglucuronic acid binding site of UDPglucuronyltransferase are different after treatment with phospholipase A as compared with treatment by Triton.

Effect of treatment with Triton X-100 and phospholipase A on the activities at V of UDPglucuronyltransferase

UDPglucuronyltransferase catalyzes a reversible reaction [1,25]. Several reports suggest that detergents have a differential activating effect on rates of the forward and reverse reactions [1,28,29]. The effects of treatment with phospholipase A and Triton on activities at V of forward and reverse reactions hence were determined. The results of these experiments (Table IV) are of interest for several reasons. Treatment with phospholipase A increases activity at V of the forward reaction about 4-fold, but increases activity at V of the reverse reaction 10-fold. Treatment with Triton has no effect on the activity at V of the forward reaction, although it does increase the maximum rate of the reverse reaction (Table IV). There was no constant relationship between activities at V of forward and reverse reactions for any form of UDPglucuronyltransferase. Phospholipase A and Triton hence have differential effects on maximal activities of the forward and reverse reactions catalyzed by UDPglucuronyltransferase. Moreover, these treatments have unique effects on the relative rates of the forward and reverse reactions.

Values of K_{eq} of different forms of UDPglucuronyltransferase were determined from the kinetic constants in order to validate the internal consistency of the kinetic data. The equilibirum constant of an enzyme with a rapid equi-

TABLE IV

THE EFFECT OF PHOSPHOLIPASE A OR TRITON X-100 ON THE ACTIVITY AT V FOR UDP-GLUCURONYLTRANSFERASE, ASSAYED IN THE FORWARD AND REVERSE DIRECTION

Initial rates of activity of UDP-glucuronyltransferase were measured as in Materials and Methods. Activities were determined as a function of the concentration of UDP-glucuronic acid (forward reaction) or UDP (reverse reaction) at several fixed concentrations of *p*-nitrophenol (forward reaction) and *p*-nitrophenylglucuronic acid (reverse reaction). The data were plotted in double reciprocal form in order to obtain activities at infinite concentrations of UDP-glucuronic acid and UDP as a function of the concentration of the second substrate. Intercepts on the $1/v$ axis were replotted versus 1/[*p*-nitrophenol] or 1/[*p*-nitrophenolglucuronic acid] to obtain the activities at V of forward and reverse reactions, respectively. All assays were at 37°C in 50 mM Tris, pH 7.6. Phospholipase A-treated microsomes were treated with purified phospholipase A (*Naja naja*) for 30 s at 23°C, in 50 mM Tris, pH 8.0, as in Materials and Methods. EDTA (5 mM) was added to inhibit further activity of phospholipase A. The ratio of phospholipase A protein to microsomal protein was 1/50. In experiments with Triton-treated microsomes redistilled Triton X-100 was added to assays at a Triton to protein ratio of 1/5. These treatments yielded maximal activations in each case. Units of activity are nmol of *p*-nitrophenol metabolized per min per mg protein.

Treatment	Activity at V	
	Forward reaction	Reverse reaction
None	47.5	8.5
Phospholipase A	177	89
Triton	40	29.3

librium, random order mechanism can be calculated from kinetic constants according to the equation [30]

$$K_{eq} = \frac{V_f}{V_r} \frac{K_{UDP} K'_{PNPGA}}{K_{UDPGA} K'_{PNP}}$$

The K terms are then enzyme-substrate dissociation constants when the substrate is the first to bind (Table I), and the K' terms refer to binding of the second substrate, when the enzyme is saturated with the first substrate [25]. Values of K_{eq} are approximately equal when calculated from the kinetic data

TABLE V

K_{eq} OF UDP-GLUCURONYLTRANSFERASE CALCULATED FROM KINETIC CONSTANTS OF UNTREATED PHOSPHOLIPASE A, AND TRITON-ACTIVATED FORMS OF THE ENZYME

K_{eq} was calculated according to the Haldane relationship for a random order kinetic mechanism. Kinetic parameters were determined from bisubstrate analysis, in the forward and reverse reactions as in Tables I and IV. Phospholipase A-treated microsomes were treated for 30 s at 25°C with purified phospholipase A (*Naja naja*) which gives maximal activation. Further action of phospholipase A was inhibited by addition of EDTA. For Triton treatment, Triton X-100 was added to assay tubes in a ratio of Triton to microsomal protein of 1 to 5.

Source of enzyme	Treatment	Calculated K_{eq}
Guinea pig	None	10.4
	Phospholipase A	6.7
	Triton	10.3
Wistar Rat	None	7.2
Gunn Rat	None	8.2

of untreated, phospholipase A, or Triton-modified forms of UDPglucuronyltransferase (Table V). Also shown in this table are calculated values of K_{eq} for UDPglucuronyltransferase from two strains of rats. Of note is that the ratio of activities at V of the forward and reverse reactions catalyzed by the rat enzyme is 35.

Effects of exhaustive treatment with phospholipase A on the properties of UDPglucuronyltransferase

The activity of UDPglucuronyltransferase is dependent on the duration of treatment with phospholipase A [1,15,31]. When liver microsomes from guinea pig are the source of UDPglucuronyltransferase, activity increases to a maximum after 30 s of treatment with phospholipase A, and then declines [31]. This decline of activity from the level of maximal activation is prevented by inhibiting phospholipase A, and hence does not reflect instability of a phospholipase A-modified form [31]. It is likely therefore that the values of specific kinetic parameters of UDPglucuronyltransferase depend on the extent of treatment with phospholipase A. The data in Table VI confirm this idea. The lower activity of UDPglucuronyltransferase after treatment with phospholipase A for 30 min as compared with 30 s is related, at least in part, to differences between the kinetic parameters of these two forms of the enzyme (compare Tables I and VI). Exhaustive treatment with phospholipase A (30 min) altered the affinity of UDPglucuronyltransferase for UDP and UDPglucuronic acid, as compared with a brief treatment. Of interest was that the extent of UDP and UDPglucose-induced inhibition of rates of glucuronidation were the same for preparations of UDPglucuronyltransferase treated with phospholipase A for 30 s or 30 min.

A rather remarkable effect of exhaustive treatment with phospholipase A (30 min), as compared with brief treatment (30 s), was a modification of the nature of the relationship between the concentration of UDPglucuronic acid and rates of glucuronidation. Double reciprocal plots of rates of glucuronidation as a function of the concentrations of UDPglucuronic acid are non-linear after exhaustive treatment with phospholipase A (Fig. 2). These plots bend sharply at concentrations of UDPglucuronic acid less than 2.5 mM. This is not seen after treatment with phospholipase for 30 s (Fig. 1), although it is a prop-

TABLE VI

PROPERTIES OF UDP-GLUCURONYLTRANSFERASE AFTER EXHAUSTIVE DIGESTION WITH PHOSPHOLIPASE A

Microsomes were treated with phospholipase A, as in Materials and Methods, for 30 min. EDTA (5 mM) was added to inhibit the further action of phospholipase A. Microsomes were kept subsequently at 0°C until assay. Initial rates of activity of UDP-glucuronyltransferase were measured in forward and reverse directions as in Materials and Methods. Kinetic constants were determined by graphical methods as in Tables I and IV. V_f and V_r are activities at V of the forward and reverse reactions, respectively.

Kinetic constant	Units
K_{UDPGA}	5.0 mM
K_{UDP}	0.18 mM
V_f	110 nmol
V_r	40 nmol

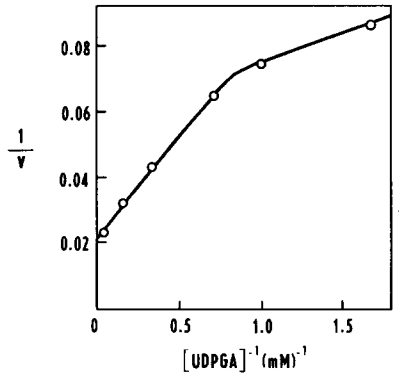

Fig. 2. Double reciprocal plots for the rate of glucuronidation of *p*-nitrophenol as a function of the concentration of UDPglucuronic acid. Microsomes were treated with phospholipase A as in Materials and Methods for 30 min at 25°C. EDTA was added to a final concentration of 5.0 mM at the end of this time and the activity of UDPglucuronyltransferase determined at 37°C in 0.4 mM *p*-nitrophenol and the indicated concentrations of UDPglucuronic acid. Activity is expressed as nmol *p*-nitrophenol metabolized per min per mg protein.

erty of untreated UDPglucuronyltransferase [19,23,24]. The values in Table VI were obtained at concentrations of UDPglucuronic acid greater than 2.5 mM.

Comparison of the effects of lysophosphatides and other detergents on the properties of UDPglucuronyltransferase

The phospholipase A used in our work is purified to homogeneity, and hence contains no proteolytic activites [20]. The effects of phospholipase A on the properties of UDPglucuronyltransferase result therefore from the production of lysophosphatides and fatty acids, or the removal of normal amounts of choline-containing phosphatides from the membrane. Graham and Wood [32] have shown recently that lysophosphatides activated UDPglucuronyltransferase. If this is the mechanism of phospholipase A-induced activation of UDP-glucuronyltransferase, addition of lysophosphatides would be expected to pro-

TABLE VII

EFFECT OF LYSOLECITHIN AND CHOLATE ON THE ACTIVITY OF UDP-GLUCURONYLTRANSFERASE

Microsomes were treated at 0°C with lysolecithin (1-oleoyl) or cholate. The ratio of protein to detergent was 3 to 1. Initial rates of activity were determined as in Materials and Methods using 1 mM UDP-glucuronic acid and 0.4 mM mM *p*-nitrophenol. When added, the concentration of UDP-glucose was 5 mM. Activities are nmol *p*-nitrophenol metabolized per min per mg protein.

Treatment	Additions to assay	Activity (units)
None	None	5.23
	UDPglucose	5.00
Lysolecithin	None	53.1
	UDPglucose	27.4
Cholate	None	31.4
	UDPglucose	30.5

TABLE VIII

EFFECT OF PHOSPHOLIPASE A AND TRITON X-100 ON THE ACTIVITIES OF UDP-GLUCURONYLTRANSFERASE ASSAYED IN THE PRESENCE OF UDP AND UDP-*N*-ACETYLGLUCOSAMINE

Initial rates of activity were determined as in Materials and Methods at 37°C in 50 mM Tris, pH 7.6, 0.2 mM *p*-nitrophenol, 1.0 mM UDP-glucuronic acid, 1.0 mM UDP, and 2.5 mM UDP-*N*-acetylglucosamine. Microsomes were treated with phospholipase A for 30 s as in Materials and Methods and then added directly to the assay tubes. No EDTA was added. Triton treatment was as in Table I. Activities are nmol *p*-nitrophenol metabolized per min per mg protein.

Treatment	Activity
None	7.8
Phospholipase A	1.05
Triton	2.5

duce a form of UDPglucuronyltransferase that is similar to the phospholipase A-treated form, but different from the Triton-treated form. Complete, bisubstrate kinetic analyses were not carried out in the investigation of this aspect of the problem. The parameter of function used for distinguishing between different kinetic forms of the enzyme was the specificity of binding at the UDP-glucuronic acid site as determined by measuring the effect of UDPglucose on rates of glucuronidation. Activation by treatment with lysolecithin (1-oleoyl) produces a form of UDPglucuronyltransferase that is inhibited by UDPglucose (Table VII). Treatment with lysolecithin thus produces a form of UDPglucuronyltransferase that is similar to the phospholipase A-treated form, but distinct from the Triton X-100-activated form. This result indicates that different amphiphilic compounds alone may have unique effects on the properties of UDP-glucuronyltransferase. Of interest in this regard is that only the lysophosphatide, of the amphiphiles tested, appeared to alter the specificity of binding at the UDPglucuronic acid site.

Are phospholipase- and detergent-treated forms of UDPglucuronyltransferase activated forms?

Treatments with phospholipase A and detergents increase the activity of UDPglucuronyltransferase, but they also alter the response of the enzyme to UDP-*N*-acetylglucosamine, UDP, and other nucleotide sugars [27]. These last effects raise the question of whether the so-called "activated forms" of UDP-glucuronyltransferase may have less activity than the untreated enzyme under some assay conditions. This is the case, as shown by the data in Table VIII. In the presence of UDP-*N*-acetylglucosamine and UDP, both of which are physiological metabolites, the untreated form of UDPglucuronyltransferase is the most active form of the enzyme.

Discussion

Selective changes of the composition of the microsomal membrane lead to selective and unique types of alterations of the function of UDPglucuronyltransferase. Especially interesting is that treatments with different amphiphiles also produce unique forms of UDPglucuronyltransferase. These last data sug-

gest, in fact, that the dependence of the kinetic parameters of UDPglucuronyltransferase on the extent of treatment with phospholipase A is due to the production of various types of phosphatides during the course of lipase activity.

The variability of modified forms of UDPglucuronyltransferase that are produced by treatment with phospholipases and amphiphiles is somewhat surprising. This type of behavior may be characteristic, however, of tightly bound membrane enzymes. The kinetic properties of most of these have not been studied in sufficient detail as yet, but recent data indicate that the activity and stability of β-hydroxybutyrate dehydrogenase depend on the type of lipid added to the purified apoenzyme [33]. Several different forms of the holoenzyme are produced in this manner. It is possible, therefore, to produce multiple kinetic forms of at least two tightly bound membrane enzymes by selective modifications of their lipid environments.

The property of the lipid phase that accounts for the apparent variability of lipid-induced modifications of UDPglucuronyltransferase, or other enzymes of this type, is unknown. It is not certain, for example, whether these enzymes respond to a change in a property of the bulk phase of the membrane lipids, or whether changes of enzyme function are due only to modifications of their immediate environments. Work from several different laboratories suggests that the extent of molecular motion (fluidity) within the lipid portion of the membrane is important for regulating the activities of membrane-bound proteins [10—13,34,35]. Correlations betwen changes in the physical properties of the membrane lipids and enzyme function provide a basis for understanding how modifications of the membrane lipids can alter the function of membrane-bound enzymes. On the other hand, properties of the lipid-phase other than fluidity also may be significant in this regard. This problem can be approached by correlating changes of enzyme function with changes in the physical and chemical properties of the membrane lipids under different experimental conditions. An important aspect of the data for UDPglucuronyltransferase therefore is to indicate that correlations between membrane structure and enzyme function cannot be based on assays of activity under arbitrary conditions. Treatment with Triton and phospholipase A appear, for example, to have identical effects on activity in assays carried out as in Table II, but the kinetic parameters of the two modified forms are different. Resolution of the manner in which membrane lipids influence the function of membrane-bound enzymes depends on complete characterizations of the kinetic properties after treatment with different perturbing agents. Correlation between membrane structure and enzyme function may be misleading in the absence of complete characterization of the latter.

In addition to illustrating the complexity of enzyme-lipid interactions, the data contain new observations on the importance of the lipids of the microsomal membrane for determining the functional status of UDPglucuronyltransferase. The lipids of untreated microsomes regulate the properties of the UDP-glucuronic acid binding region in such a way as to limit end-product inhibition by UDP, and to maintain specificity for substrate binding. The lipids of the untreated microsomes also are required for normal sensitivity to UDP-*N*-acetylglucosamine (Table VIII and ref. 27). Constraint on the maximal potential

activity of UDPglucuronyltransferase, by limiting affinity for UDPglucuronic acid and by limiting activity at V, appears to be an essential aspect of efficient function because the unconstrained forms of UDPglucuronyltransferase are too susceptible to inhibitions by nucleotides and nucleotide sugars, and unresponsive to UDP-*N*-acetylglucosamine. Another interesting feature of lipid-enzyme interactions is that constraint on the activity of the reverse reaction of UDP-glucuronyltransferase is more extensive than on the forward reaction. The lipids of untreated microsomes hence also limit the rate of UDP-dependent hydrolysis of *p*-nitrophenol glucuronic acid. Modification of UDPglucuronyltransferase by a variety of treatments which alter the structure and composition of the lipid phase of the membrane produce forms of the enzyme which are catalytically inefficient, as compared with untreated enzyme, under conditions likely to exist in vivo.

These data and arguments also are important for resolving the question of how the maximal potential activity of UDPglucuronyltransferase is limited in untreated microsomes. One explanation is that some molecules of the enzyme have no access to substrates in untreated microsomes [25]. Activation is considered to reflect the opening up of compartments of previously inactive enzyme. The data presented above cannot be reconciled with this concept of activation unless several additional assumptions are made. We feel, therefore, that these data reinforce prior conclusions that the lipid environment influences the conformation and hence the kinetic properties of UDPglucuronyltransferase.

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