BBA 66561

# REGULATION OF MICROSOMAL ENZYMES BY PHOSPHOLIPIDS

## IV. SPECIES DIFFERENCES IN THE PROPERTIES OF MICROSOMAL UDP-GLUCURONYLTRANSFERASE

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(Received November 10th, 1971)

SUMMARY

Modification of the activity of UDP-glucuronyltransferase (UDP-glucuronate glucuronyltransferase (acceptor unspecific), EC 2.4.1.17) by treatment of microsomes with phospholipase A (phosphatide acyl-hydrolase, EC 3.1.1.4) from both *Naja naja* and *Crotalus adamanteus* venoms and with *p*-chloromercuribenzoate has been studied in a variety of species: guinea pig, mouse, rat, beef, rabbit and human. Contrary to reports in the literature that –SH group reagents and phospholipase A inhibit the enzyme, activation of UDP-glucuronyltransferase by these agents was noted in all species examined. Thus, constraint on maximum potential activity is a general property of this enzyme in untreated microsomes from a variety of species. On the other hand, the extent of activation of glucuronyltransferase by treatment of microsomes with phospholipase A or *p*-chloromercuribenzoate varied with the species studied. In addition to variation in the extent of activation, species differences in the reactivities of the –SH groups associated with UDP-glucuronyltransferase were noted.

## INTRODUCTION

The activities and stabilities of some tightly-bound microsomal enzymes are altered by agents which affect primarily the structure and composition of microsomal phospholipids. Thus, extraction of microsomes with aqueous acetone reversibly inactivates stearyl-CoA desaturase[1] and NADH-cytochrome *c* reductase[2]; and treatment with phospholipases A (phosphatide acyl-hydrolase, EC 3.1.1.4) and C (phosphatidylcholine choline phosphohydrolase, EC 3.1.4.3) modifies the kinetic properties

Abbreviation: PCMB, *p*-chloromercuribenzoate.

and stabilities of acyl-CoA: L-glycerol-3-phosphate *O*-acyltransferase (EC 2.3.1.15)[3], glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9)[4,5], ATPase (ATP phosphohydrolase, EC 3.6.1.3)[6], and UDP-glucuronyltransferase (UDP-glucuronate glucuronyltransferase (acceptor unspecific), EC 2.4.1.17)[7–9]. A species difference in the effects of enzyme–phospholipid interactions is suggested by reports that phospholipases A and C activate UDP-glucuronyltransferase from beef[7] and rabbit[9] liver but inactivate the enzyme from guinea pig liver[8], when activity is assayed with *p*-nitrophenol as aglycone. Another indication of contrasting behavior of the enzyme from different species is that while in beef liver microsomes organic mercurials potentiate the activity of the enzyme[10,11], in mouse[12] and rabbit[9] microsomes they are reported to inhibit it. Considering the fact that prior treatment of beef liver microsomes with Triton X-100 or phospholipase A modifies the reactivity of the enzyme with –SH reagents[10], it is conceivable that the divergent effects of organomercurials on the enzyme from different species is also a reflection of differences in protein–phospholipid interactions.

In order to ascertain whether UDP-glucuronyltransferases from various species indeed respond differently to these treatments or whether the contrasting observations are due to differences in experimental conditions, it was desirable to compare the behavior of the enzyme from several species in the same laboratory under identical conditions. The results of such a comparative study are reported in the present paper. It is not yet clear how many different forms of UDP-glucuronyltransferase exist, nor what their substrate specificities are; but as used in this paper, UDP-glucuronyltransferase refers specifically to the form of this enzyme which catalyzes the synthesis of *p*-nitrophenylglucuronide.

## MATERIALS AND METHODS

Microsomes from beef, mouse, guinea pig, rat, rabbit and human liver were prepared and stored as previously described except that after resuspension for washing, the microsomes were centrifuged at 12 000 × *g* in a Spinco No. 30 rotor for 10 min[7]. The resulting pellet was discarded and the supernatant was centrifuged at 30 000 rev./min for 60 min in a No. 30 rotor to obtain the final microsomal pellet. Human liver was obtained *post-mortem* from a cadaveric kidney transplant donor and was packed in ice until the microsomes were isolated approx. 8 h after death. The purification of phospholipase A from *Naja naja* venom and assays of UDP-glucuronyltransferase were as previously described[7]. Crude phospholipase C from *Clostridium welchii* was obtained from Sigma Chemical Co.

The activity of UDP-glucuronyltransferase was assayed with *p*-nitrophenol as aglycone at a *p*-nitrophenol concentration of 0.6 mM and 4.0 mM UDP-glucuronic acid. As discussed in a previous paper[7] β-glucuronidase (β-D-glucuronide glucuronohydrolase, EC 3.2.1.31) and UDP-glucuronic acid pyrophosphatase, if present, do not affect the measurements of initial rates of UDP-glucuronyltransferase under these assay conditions. For assays of phospholipase A treated microsomes, assay tubes contained EDTA at a final concentration of 5.0 mM (ref. 5). Enzyme activities are expressed as nmoles of *p*-nitrophenol metabolized per min per mg microsomal protein, and represent initial rates. Protein was measured by the biuret method[13].

## RESULTS

### *Effect of treatment with phospholipase A and C on the activity of UDP-glucuronyltransferase*

Treatment of microsomes with phospholipase A from *Naja naja* venom led to activation of hepatic UDP-glucuronyltransferase in all species studied (Table I).

TABLE I

COMPARISON OF THE EFFECT OF TREATMENT WITH PHOSPHOLIPASE A ON THE ACTIVITY OF UDP-GLUCURONYLTRANSFERASE FROM GUINEA PIG, MOUSE, BEEF, HUMAN AND RABBIT LIVER MICROSOMES

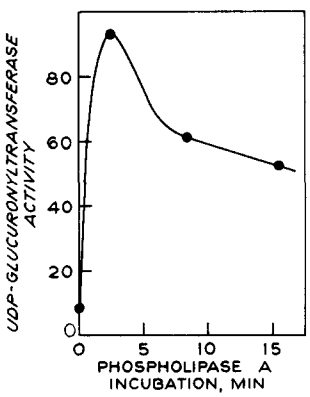
Microsomes were treated with a partially purified preparation of phospholipase A from *Naja naja* venom at a microsomal to phospholipase A protein ratio of 100/1 at 23° in the presence of 50 mM Tris, pH 8.0. Serial samples were removed and UDP- glucuronyltransferase assayed at 37° as in MATERIALS AND METHODS. For each species studied, the value noted below is the maximum activity attained, and was reached within 2–2.5 min of incubation.

| *Source of microsomes* | *Treatment*: | |
|---|---|---|
| | *None* | *Phospholipase A* |
| Guinea pig | 8.14 | 93.0 |
| Mouse | 0.68 | 8.45 |
| Rat | 1.43 | 15.5 |
| Beef | 0.90 | 3.30 |
| Human | 1.98 | 8.00 |
| Rabbit | 3.97 | 57.7 |

This was true also for the guinea pig enzyme, which has been reported to be inactivated by phospholipase A from *Crotalus adamanteus*[8]. Although the data in Table I are for microsomes treated with *Naja naja* venom, a quantitatively identical activation was observed when guinea pig liver microsomes were treated with a partially purified preparation of phospholipase A from *C. adamanteus*. In order to exclude that the method of preparation of microsomes, *i.e.* homogenization in 0.25 M sucrose as we use versus 0.15 M KCl as used by Graham and Wood[8] was without effect on the subsequent action of phospholipase A on UDP-glucuronyltransferase, microsomes from guinea pig liver were prepared according to the method of Graham and Wood[8]. It was found that treatment of these microsomes with phospholipase A from either venom led to activation of UDP-glucuronyltransferase.

It is apparent from the data in Table I that although phospholipase A-induced activation is a general property in all species tested, the extent of activation of UDP-glucuronyltransferase is variable in microsomes from different animals. The fact that phospholipase A activates UDP-glucuronyltransferase 12–16-fold in rat, rabbit, guinea pig, and mouse liver but only 3–4-fold in beef and human liver microsomes indicates that the amount of constraint on the activity of UDP-glucuronyltransferase imposed by the phospholipids of the untreated microsome is variable in different species. Additional species differences in the way in which phospholipid–protein interactions modulate the activity of UDP-glucuronyltransferase became apparent when the time course of phospholipase A effects on the activity of UDP-glucuronyltransferase was examined. The data presented in Table I are the maximal activities

reached during treatment of microsomes with phospholipase A in any given experiment. As illustrated for guinea pig liver microsomes in Fig. 1, however, phospholipase A has a biphasic effect on the activity of UDP-glucuronyltransferase: there is an initial rapid activation followed by a slower secondary decline of activity. This was a typical response for all species except the rat, in which activity plateaued after the rapid initial activation. Despite the apparent similarities in the secondary decline phase for guinea pig and beef liver, for example (Fig. 1 and ref. 7), the nature of the secondary decline in activity was not the same in these species. Phospholipase A treatment of beef liver microsomes for longer periods than those needed to obtain maximum activation produced an unstable form of UDP-glucuronyltransferase, the activity of which fell on further incubation after EDTA was added to stop the action of phospholipase A[7]. In contrast, extensive treatment of guinea pig liver microsomes did not produce an unstable form of UDP-glucuronyltransferase, but inactivated the enzyme directly during the secondary decline phase, as evidenced by the prevention of phospholipase A-induced inactivation by 5.0 mM EDTA (Fig. 2).



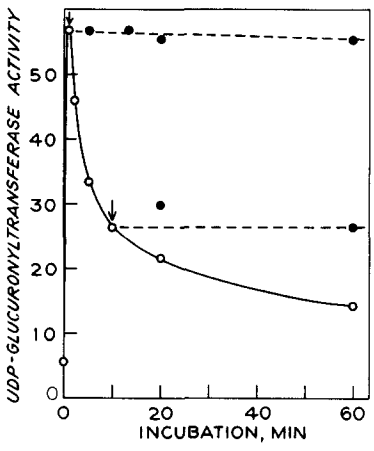




Fig. 1. Effect of treatment with partially purified phospholipase A from *Naja naja* venom on the activity of UDP-glucuronyltransferase from guinea pig liver microsomes. Microsomes were treated with phospholipase A at a microsomal to phospholipase A protein ratio of 100/1 in 50 mM Tris, pH 8.0, at 23°. At the indicated times aliquots were removed and assayed at 37° as in MATERIALS AND METHODS.

Fig. 2. Effect of treatment with phospholipase A on the stability of guinea pig liver UDP-glucuronyltransferase. Guinea pig liver microsomes were treated with phospholipase A at a microsomal protein to phospholipase A ratio of 100/1, as in Fig. 1. At the times indicated by the arrows, portions of the mixture of microsomes and phospholipase A were added to separate tubes containing EDTA so that the final EDTA concentration was 5.0 mM and the incubation continued at 23°. UDP-glucuronyltransferase was assayed at 37° as in MATERIALS AND METHODS.

Although the extent of activation of guinea pig liver UDP-glucuronyltransferase by phospholipase A clearly depends on the time and frequency of sampling, the prior report that phospholipase A inactivates rather than activates the enzyme[8] remains unexplained since in guinea pig liver the secondary decline in the activity of UDP-glucuronyltransferase does not extend below the initial level of activity even after a long period of incubation (Figs. 1 and 2). Identical results were obtained with phos-

pholipase A from *Naja naja* or *C. adamanteus* venoms. Also in contrast to previous reports it was found that phospholipids, in the form of Asolectin micelles, did not alter the activity of UDP-glucuronyltransferase if added after treatment with phospholipase A.

Since reports of the qualitative differences in the response of UDP-glucuronyltransferase from different species to phospholipase A treatment could not be confirmed, similar studies were carried out with phospholipase C. Guinea pig microsomes were selected, since the enzyme from this animal was reported to be inactivated by phospholipase C[8]. In a previous paper[7] we have documented reversible activation of the enzyme from beef liver microsomes by phospholipase C. As shown in Fig. 3, UDP-glucuronyltransferase from guinea pig liver microsomes behaved similarly except that after attainment of the maximal activity no secondary decline occurred, in contrast to beef liver microsomes which show a biphasic curve just as on treatment with phospholipase A[7]. Comparison of Figs. 1 and 3 also reveals that activation of the guinea pig liver enzyme by phospholipase C is less extensive than by phospholipase A.

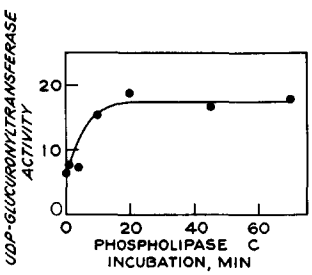



Fig. 3. Effect of treatment with phospholipase C from *C. welchii* on the activity of UDP-glucuronyltransferase from guinea pig liver microsomes. Microsomes were incubated at a microsomal to phospholipase C protein ratio of 16/1 in 50 mM Tris, pH 8.0, and 2.5 mM $CaCl_2$ at 23°. At the indicated times aliquots were removed and assayed at 37° as in MATERIALS AND METHODS.

*Effect of treatment with p-chloromercuribenzoate on the activity of UDP-glucuronyl transferase*

Treatment of microsomes with *p*-chloromercuribenzoate (PCMB) increased the activity of UDP-glucuronyltransferase in all species tested. Again, however, there was a variable extent of activation after PCMB which, except for the rat, paralleled the activation produced by phospholipase A (Table II).

It has been shown for beef liver microsomes that at least three types of –SH groups influence the activity of UDP-glucuronyltransferase[10,11]. Two of these (Types 2 and 3) react with mercurials and in so doing produce characteristic changes in activity. A third (Type 1) appears to be unreactive with, or inaccessible to, organic mercurials in untreated microsomes but reacts with *N*-ethylmaleimide. Mercaptide formation with Type 2 –SH occurs at relatively low concentrations of mercurial (0.5 mM PCMB) and leads to activation whereas the titration of Type 3 –SH requires high concentrations of mercurial (>0.5 mM PCMB) and results in deactivation of the previously activated enzyme. Titration of beef liver microsomes with PCMB thus gives a biphasic plot for activity *versus* the concentration of PCMB[10]. The titration of UDP-glucuronyltransferase from mouse, rat, rabbit and human liver microsomes

TABLE II

THE EFFECT OF PCMB ON THE ACTIVITY OF UDP-GLUCURONYLTRANSFERASE FROM GUINEA PIG, MOUSE, RAT, HUMAN AND RABBIT LIVER MICROSOMES

Microsomes at a concentration of 10.0 mg microsomal protein per ml were incubated in 50 mM Tris, pH 7.6 with PCMB as in MATERIALS AND METHODS. The final concentrations of PCMB required for maximum stimulation of activity (activities noted below) were 1.0 mM for guinea pig, rat and rabbit and 0.5 mM for human, mouse, and beef liver microsomes. Activity was measured as in MATERIALS AND METHODS.

| *Source of microsomes* | *Treatment:* | |
|---|---|---|
| | *None* | *PCMB* |
| Guinea pig | 5.70 | 50.0 |
| Mouse | 0.68 | 10.60 |
| Rat | 0.95 | 2.40 |
| Beef | 1.30 | 4.30 |
| Human | 1.98 | 6.80 |

with PCMB does not yield biphasic plots but a plateau is reached instead as shown for human and rat microsomes in Fig. 4. These data suggest that the Type 3 –SH group present in beef microsomes is either absent or unreactive in the other species tested. Since, however, the Type 3 –SH group of beef liver UDP-glucuronyltransferase has greater reactivity with mersalyl than with PCMB[10], rat liver microsomes were also treated with 3.0 mM mersalyl, which resulted in deactivation of the enzyme (Fig. 4). This figure also shows activation of rat UDP-glucuronyltransferase by 0.5 mM mersalyl. A similar result, *i.e.* a biphasic effect of mersalyl on the activity of UDP-glucuronyltransferase was obtained with human, mouse and guinea pig liver microsomes (data not shown). Thus, the Type 3 –SH group appears to be present in these species but its properties are different from those in the beef enzyme[10] in that it fails to react with PCMB.

PCMB-induced activation of beef liver UDP-glucuronyltransferase is reversed by subsequent treatment of microsomes with dithiothreitol[10], as is the PCMB-induced secondary decline of activity at high concentrations of mercurial. However, after pre-treatment of microsomes with phospholipase A, PCMB leads to an irreversible

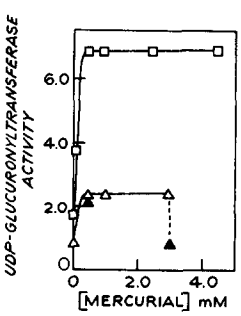



Fig. 4. Effect of PCMB and mersalyl on the activity of UDP-glucuronyltransferase. Microsomes from rat (△) and human liver (□) at a final protein concentration of 10 mg/ml were treated for 2 min at 23° with the indicated concentrations of PCMB (open symbols) or mersalyl (closed symbols) in 50 mM Tris, pH 7.6. Aliquots were removed and UDP-glucuronyltransferase activity measured as in MATERIALS AND METHODS.

inactivation of UDP-glucuronyltransferase. This also seems to be true of rat, rabbit and guinea pig liver microsomes (Fig. 5). The inactivation observed in these data was unaffected by dithiothreitol. Although it cannot be established with certainty, it is likely that inactivation of UDP-glucuronyltransferase by PCMB in microsomes pretreated with phospholipase A results from reaction of the Type 3 –SH group. Thus, treatment with phospholipase A seems to produce a form of UDP-glucuronyltransferase in which the reactivity of the Type 3 –SH groups become identical in guinea pig, rat and beef liver microsomes. If the deactivation noted in Fig. 5 is due to Type 3 –SH, it would appear that prior phospholipase treatment exposes Type 3 –SH in these species, so that the behavior of the enzyme in microsomes so treated becomes indistinguishable from that of the beef liver enzyme in untreated microsomes.

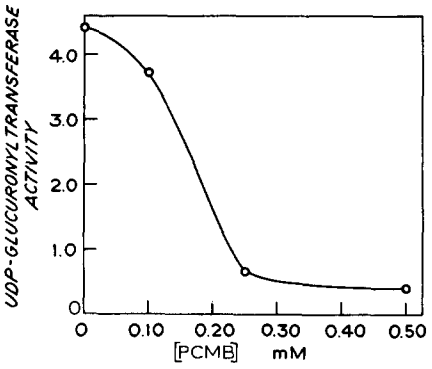



Fig. 5. Effect of PCMB on the activity of the phospholipase A-treated form of UDP-glucuronyltransferase. Rat liver microsomes were treated with phospholipase A as in Fig. 1 at a microsomal to phospholipase A protein ratio of 25:1. At the end of 5 min of treatment at 23° EDTA was added to a final concentration of 5.0 mM. Aliquots were then treated with the indicated concentrations of PCMB for 2 min at pH 7.6, 23°. UDP-glucuronyltransferase activity was measured at 23°, as in MATERIALS AND METHODS but at a *p*-nitrophenol concentration of 0.45 mM.

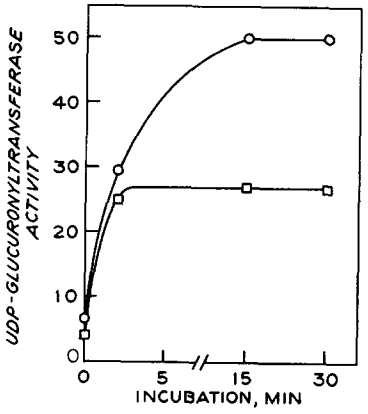



Fig. 6. Influence of duration of treatment on PCMB induced activation of UDP-glucuronyltransferase. Microsomes from guinea pig liver (○) and rabbit liver (□) were treated with 1.0 mM PCMB as in Fig. 3. At the indicated times aliquots were removed and assayed as in MATERIALS AND METHODS for UDP-glucuronyltransferase activity.

A further difference between species was found in studies of the time required for attainment of maximum activation by PCMB. As reported previously[10], PCMB treatment of beef liver microsomes at 23° gives an almost instantaneous maximum activation. In contrast, somewhat more than 2 min of incubation with PCMB at 23° are required for full activation of UDP-glucuronyltransferase from rabbit liver microsomes and an even longer period is needed for full activation of the enzyme from guinea pig liver microsomes (Fig. 6). Thus, there are species differences in the reactivity of the Type 2 –SH group, as well as those ascribed to Type 3 –SH.

## DISCUSSION

A general property of hepatic microsomal UDP-glucuronyltransferase from many different species is that the maximum potential activity is constrained by the

phospholipid environment of the native microsome, but several lines of evidence suggest that there are important species differences in the nature of the enzyme–phospholipid interactions in the microsomal membrane. For example, the extent of constraint on maximum activity, as evidenced by phospholipase A-induced activation of UDP-glucuronyltransferase is variable from one species to another. Also, the role of membrane phospholipids for the stability of UDP-glucuronyltransferase differs in different species; and in guinea pig liver microsomes the secondary decline of activity (Figs. 1 and 2), is not due to phospholipase A-induced instability, but results from the direct action of phospholipase A on the microsomal membrane.

In addition to quantitative differences in the effects of phospholipases on the activity of UDP-glucuronyltransferase in a number of species, differences have also been noted in the reactivities of the –SH groups associated with the function of this enzyme, though it should be stressed that constraint on activity could be relieved in all species by reaction of the Type 2 –SH group with organic mercurials. Since the properties of the –SH groups of beef liver UDP-glucuronyltransferase can be altered by prior treatment of microsomes with phospholipase A and Triton X-100 (ref. 10), species variability in the reactivities of the Type 2 and 3 –SH groups may be related to differences in how UDP-glucuronyltransferase interacts with its lipid environment. This conclusion is supported by the apparent effect of phospholipase A on the reactivity of the Type 3 –SH group; thus, prior treatment with phospholipase A seems to produce a form of UDP-glucuronyltransferase in which different species have Type 3 –SH groups with similar properties.

At the present time it is not possible to decide whether the variable constraint on activity in native microsomes and differences in the reactivities of the Type 2 and 3 –SH groups result from alterations in the primary structure of the enzyme which affect protein–phospholipid interactions, from species differences in the composition of the microsomal phospholipids or both. Nevertheless, it is clear that there is species variability in the possible ways in which the catalytic properties of UDP-glucuronyltransferase can be altered by protein–phospholipid interactions. The potential for variability in the regulation of this enzyme could be related to the widely differing patterns of development of UDP-glucuronyltransferase activities in fetal animals, and also to the problem of apparent multiplicity of this enzyme.

ACKNOWLEDGEMENTS

The authors thank Dr. Thomas P. Singer for his continued interest and support, and for many helpful discussions during the course of this work, and Dr. Samuel Koontz for his help in obtaining the sample of human liver.

These investigations were supported in part by grants from the U.S. Public Health Service (HE 10027) and the National Science Foundation (GB 20814) to Dr. Thomas P. Singer.

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