

The Phospholipid-Dependence of Uridine Diphosphate Glucuronyltransferase

REACTIVATION OF PHOSPHOLIPASE A-INACTIVATED ENZYME BY PHOSPHOLIPIDS AND DETERGENTS

BY D. ATTWOOD, A. B. GRAHAM AND G. C. WOOD

*Drug Metabolism Research Unit, Department of Pharmaceutical Chemistry,
Strathclyde University, Glasgow C.1, U.K.*

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Specific degradation of the phospholipid membrane of guinea-pig liver microsomal fraction with phospholipase A inactivated glucuronyltransferase. The inactivation was reversed by phosphatidylcholine and mixed microsomal phospholipid micelles at concentrations similar to those present in intact microsomal preparations. The other commonly occurring phospholipids did not reactivate phospholipase A-treated enzyme. Since the mixed microsomal phospholipids consisted mainly of phosphatidylcholine, it is concluded that the reactivation by phospholipids is phosphatidylcholine-specific. Reactivation was also achieved by low concentrations of the cationic detergents cetylpyridinium chloride and cetyltrimethylammonium bromide. Higher concentrations of these detergents inactivated the glucuronyltransferase activity of intact and phospholipase A-treated microsomal fractions. Anionic detergents were potent inactivators of the glucuronyltransferase activity of untreated and phospholipase A-treated microsomal fractions, whereas non-ionic detergents had little effect on the activity of either preparation. Measurements of the zeta-potentials of the micellar species used in this study showed that no obvious relationship existed between the zeta-potentials and the ability to reactivate glucuronyltransferase. However, high positive or negative zeta-potentials were correlated with the ability of the amphipathic compound to inactivate glucuronyltransferase.

Attempts to purify microsomal glucuronyltransferase (UDP-glucuronate glucuronyltransferase, EC 2.4.1.17) have frequently resulted in loss of enzymic activity and preparations of low stability (Pogell & Leloir, 1961; Isselbacher, Chabras & Quinn, 1962; Leventer, Buchanan, Ross & Tapley, 1965). We showed (Graham & Wood, 1969) that when guinea-pig liver microsomal fraction was treated with phospholipase A or phospholipase C, the activity of glucuronyltransferase (with *p*-nitrophenol as acceptor) was substantially decreased, to an extent that paralleled the degree of hydrolysis of microsomal phospholipids. Addition of mixed microsomal phospholipid micelles to phospholipase-inactivated preparations increased enzyme activity; when phospholipase A had been used the original activity was completely restored. It was concluded that the enzyme depends on phospholipid (and hence, presumably, on the integrity of the microsomal membrane) for full activity. Mowat & Arias (1970) have described a

scheme for purifying glucuronyltransferase which results in a product with substantially higher specific activity than those obtained by earlier workers. Their purest preparation still contained, however, membranous material. Rao, Rao & Breuer (1970) have also described a partially purified oestriol glucuronyltransferase from human liver cytosol fraction, which remained attached to membrane fragments. Failure of these two groups of workers to solubilize their enzymes is consistent with our view that attachment to an intact membrane is required for full activity.

Previous studies of phospholipid-dependent enzymes have established that specific phospholipids are often implicated in reactivation (see, e.g., Green & Tzagoloff, 1966). We have now therefore extended our initial observations by investigating the phospholipid specificity of the reactivation of glucuronyltransferase activity in phospholipase-treated microsomal preparations. In an attempt to obtain a better understanding of the reactivation

process we have extended our studies to include not only phospholipids but other amphipathic compounds, namely a number of cationic, anionic and non-ionic detergents. Some detergents have been reported to enhance markedly the activity of microsomal glucuronyltransferase (Leuders & Kuff, 1967; Van Roy & Heirwegh, 1968; Heirwegh & Meuwissen, 1968; Winsnes, 1969; Mulder, 1970; Henderson, 1970) and might reasonably be expected to affect the activity of phospholipase-treated microsomal preparations. To gain a further insight into reactivation we have also measured the zeta-potential of the micelles of a number of the reactivating agents.

MATERIALS AND METHODS

Microsomal preparations. Guinea-pig liver microsomal fractions and phospholipase A were prepared as described by Graham & Wood (1969). Except where otherwise stated microsomal preparations were used after being stored frozen for not more than 2 weeks. Microsomal fractions (12–15 mg of protein/ml) were incubated with phospholipase A (microsomal protein/phospholipase A protein ratio 20:1) at 19–21°C in the presence of 12.5 mM-tris-HCl buffer, pH 8.0, and 2.5 mM-CaCl₂; the reaction was terminated by adding excess of EDTA to a final concentration of 10 mM (Graham & Wood, 1969).

Enzyme assays. Glucuronyltransferase activity was measured essentially according to Hollmann & Touster (1962) with *p*-nitrophenol (spectrophotometric grade; BDH Chemicals Ltd., Poole, Dorset, U.K.) as acceptor. There was no significant difference between the activities of fresh microsomal preparations and those of the same preparations after they had been frozen and thawed, with or without intervening storage. Microsomal marker-enzyme activities (glucose 6-phosphatase and nucleoside diphosphatase) were measured by methods given elsewhere (Graham & Wood, 1969).

Protein concentration. This was determined by a biuret method (Layne, 1957) with bovine serum albumin as standard.

Detergents. Triton X-100, sodium deoxycholate and cetyltrimethylammonium bromide were purchased from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K.; digitonin and sodium lauryl sulphate were from BDH Chemicals Ltd.; Lubrol W was from I.C.I. Ltd., Wilmslow, Ches., U.K.; cetylpyridinium chloride was from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K., and Cetomacrogol 1000 was from Macarthys Ltd., Romford, Essex, U.K.

Phospholipids. Except for phosphatidylcholine, individual phospholipids (Folch fractions, bovine brain) and cardiolipin were obtained from Koch-Light Laboratories. Phosphatidylcholine was purified from a commercial grade egg-yolk preparation [Sigma (London) Chemical Co. Ltd.] by the method of Attwood (1965). The material eluted from the silicic acid-Celite column was recrystallized three times from ethyl methyl ketone, redissolved in a minimum quantity of that solvent, flushed with nitrogen and stored at 0–4°C. Mixed microsomal phospholipids

were prepared from guinea-pig liver microsomal fraction as described by Graham & Wood (1969).

Individual phospholipids were examined by t.l.c. either by using silica gel G (Merck) as a stationary phase and chloroform-methanol-water (14:6:1, by vol.) or by the two-dimensional system of Morrison (1968). Purified phosphatidylcholine and cardiolipin gave single spots, and phosphatidylethanolamine was shown also to contain a small amount of phosphatidylcholine by using the one-dimensional system. Analyses by the two-dimensional method showed that the phosphatidylinositol and the phosphatidylserine contained traces of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine, and phosphatidylcholine and phosphatidylethanolamine respectively.

Morrison's (1968) method was also used to analyse the mixed microsomal phospholipids. After the spots had been detected and removed from the plates, total phosphorus contents were determined after digestion with H₂SO₄ and H₂O₂ (Morrison, 1964). The mixed microsomal phospholipids consisted of: phosphatidylcholine, 62%; phosphatidylethanolamine, 25%; phosphatidylinositol, 15%.

Phospholipid dispersions. For enzyme-reativation experiments, dispersions were prepared by dissolving the required amount of phospholipid in a small volume of ether, to which was then added a calculated volume of 20 mM-tris-1 mM-EDTA, pH 8.0; the ether was removed with a stream of nitrogen and the resulting aqueous suspension was sonicated for seven 1 min periods alternating with similar periods of cooling on ice. An MSE ultrasonicator, set at 28 KHz and adjusted to maximum efficiency, was used. The micellar dispersions were clarified by centrifuging at 88000g_{av.} for 30 min. The phosphorus content of the dispersions was measured by the Chen, Toribara & Warner (1956) method. To test phospholipid dispersions for reactivation, samples were added to phospholipase-treated microsomal fractions under conditions detailed below. To ensure that measured zeta-potentials should be those pertaining in the reactivation medium, physicochemical measurements were made on phospholipids dispersed in a solution at pH 8.0 containing 28.9 mM-KCl, 17.2 mM-tris, 9.37 mM-EDTA and 0.94 mM-CaCl₂. The concentrations of the dispersions were estimated from measurements of their refractive indices by using a Hilger-Rayleigh interference refractometer.

Detergent solutions. For enzyme-reativation experiments and physicochemical studies these were prepared by using the same buffer systems employed for the phospholipid dispersions.

Zeta-potentials (ζ) of the micellar dispersions. These were calculated from electrophoretic mobilities (u) determined at 25±0.5°C by the dye-tracer method (Hoyer, Mysels & Stigter, 1954) as described by Attwood (1969). The micelles were 'tagged' by shaking the dispersions with the dye Orange O.T. for 3–4 days at room temperature and dye concentrations were estimated from the E_{492} . The conductivity of the solutions at 25±0.005°C was determined with a conductivity bridge (L.K.B. Instruments Ltd., South Croydon, Surrey, U.K.). Electrophoretic mobility was essentially independent of solution concentration and the values quoted are the means of values obtained at a series of concentrations. For the cationic

detergent micelles ζ was calculated from u by means of eqn. (1) (Stigter & Mysels, 1955):

$$\Phi = \frac{u'}{X_1^*} - \frac{X_3^* + Y_3^* + q_3^* Z_3^*}{X_1^*} \left(\frac{u'}{X_1^*} \right)^3 - \frac{q_4^* Z_4^*}{X_1^*} \left(\frac{u'}{X_1^*} \right)^4 \quad (1)$$

which takes into account deformation of the double layer due to electrophoretic motion, in which $\Phi = e\zeta/kT$, $u' = (6\pi\eta e/DkT)u$ and where e is electronic charge, η is the viscosity of the medium, D is the dielectric constant of the medium, k is the Boltzmann constant and T is the temperature ($^{\circ}\text{K}$). The factors X^* , Y^* and Z^* depend on the micellar radius and double-layer thickness, and q^* is related to ionic mobility. The subscripts refer to coefficients in the power series relating mobility and zeta-potential, from which eqn. (1) is derived. Owing to the very large micelles present in the phospholipid dispersions their zeta-potentials were calculated by using the Helmholtz-Smoluchowski relationship (eqn. 2):

$$\zeta = 4\pi\eta u/D \quad (2)$$

RESULTS AND DISCUSSION

In our earlier work (Graham & Wood, 1969) it was found that complete reactivation of glucuronyltransferase by mixed microsomal phospholipids occurred only after the enzyme had been inactivated by preincubating microsomal fractions with phospholipase A; only partial reactivation was observed when the enzyme had been inactivated by phospholipase C. We therefore used phospholipase A in the present work.

Reactivation by phospholipids. In a series of experiments microsomal fractions were treated with phospholipase A for 20 min, when the reaction was stopped by adding EDTA. This resulted, as in our earlier work (Graham & Wood, 1969), in a substantial loss of glucuronyltransferase activity, which in the various experiments ranged from 61–87% of the initial activity (the activity of untreated microsomal fraction). After addition of EDTA, portions of the mixture (0.3 ml, 4.4–4.5 mg of microsomal protein) were added to phospholipid dispersions (0.5 ml) of different concentrations. After 5 min, portions (0.2 ml) of these mixtures were assayed for glucuronyltransferase activity. As previously observed (Graham & Wood, 1969), at higher concentrations, the mixed microsomal phospholipids almost completely restored the activity of the microsomal glucuronyltransferase (Fig. 1), and these higher concentrations are approximately equivalent to those originally present in untreated microsomal preparations. Phosphatidylcholine was a more efficient reactivator giving, at the highest concentrations, maximum activity some 30% higher than that of the original microsomal fraction. It gave about 100% re-

activation at a concentration equal to that initially present in microsomal fraction. In a parallel series

of experiments carried out in an identical manner, except that phospholipase A was omitted from the preincubation medium, phosphatidylcholine and the mixed microsomal phospholipids slightly decreased the glucuronyltransferase activity of the microsomal fractions (Fig. 2). Phosphatidylinositol, phosphatidylserine and cardiolipin, however, caused the loss of about 80% of the enzyme activity, and phosphatidylethanolamine had an intermediate effect. It is perhaps not surprising that those phospholipids that inactivated the glucuronyltransferase activity of intact microsomal preparations did not reactivate the enzyme in phospholipase treated fractions (Fig. 1). Of the phospholipids present in significant amounts in microsomal fractions, only phosphatidylcholine produced significant reactivation and it is reasonable to suggest that the reactivation by mixed microsomal phospholipids is primarily due to their phosphatidylcholine content. The partial reactivation by phosphatidylethanolamine may also be due to presence of

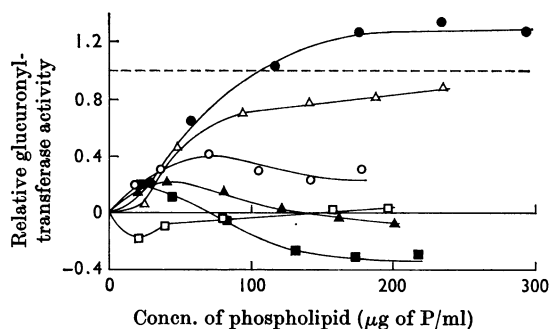


Fig. 1. Effect of phospholipid micelles on the activity of glucuronyltransferase in microsomal fractions pretreated with phospholipase A. Microsomal fractions were preincubated with phospholipase A as described in the Materials and Methods section and the text. Treated microsomal fractions were added to phospholipid dispersions giving the indicated concentrations. Relative enzyme activity is given by the relationship $(a - a_0)/(a' - a_0)$, where a is the activity of treated microsomal fraction after phospholipid-reativation, a_0 is the activity of treated fraction and a' is the activity of untreated fraction. ●, Phosphatidylcholine; ○, phosphatidylethanolamine; ■, phosphatidylinositol; □, phosphatidylserine; ▲, cardiolipin; △, mixed microsomal phospholipids.

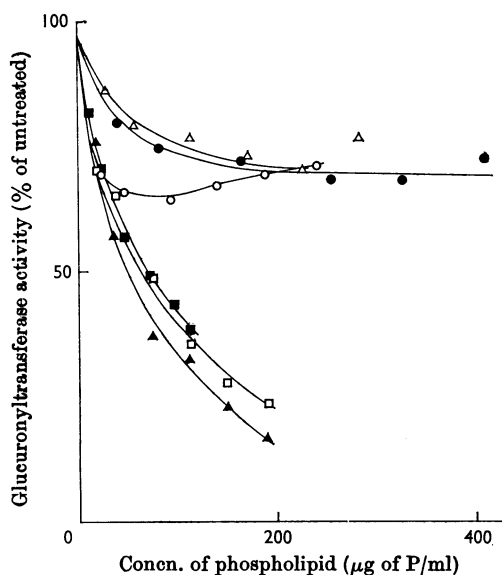


Fig. 2. Effect of phospholipid micelles on the activity of microsomal glucuronyltransferase. Microsomal fractions were preincubated for 20 min as described in the Materials and Methods section, but without addition of phospholipase A. Preincubated microsomal fractions (0.3 ml, 3.7–4.2 mg of protein) were added to phospholipid dispersions (0.5 ml), giving the indicated concentrations. After 5 min, samples of these mixtures (0.2 ml) were assayed for glucuronyltransferase activity. Activity is expressed as a percentage of that of preincubated microsomal fraction without addition of phospholipid. ●, Phosphatidylcholine; ○, phosphatidylethanolamine; ■, phosphatidylinositol; □, phosphatidylserine; ▲, cardiolipin; △, mixed microsomal phospholipids.

phosphatidylcholine of which the Folch fraction used in these experiments contained a small but significant amount.

Microsomal glucuronyltransferase is thus similar to several other membrane-dependent enzymes, which also appear to be phosphatidylcholine-dependent. Bound phosphatidylcholine has been shown to be essential for the full activities of mitochondrial D- β -hydroxybutyrate dehydrogenase from ox heart (Sekuzu, Jurtshuk & Green, 1963) and rat liver (Gotterer, 1967) and mitochondrial GTP-dependent acyl-CoA synthetase (Galzigna, Sartorelli, Rossi & Gibson, 1969). Microsomal NADH-cytochrome *c* reductase is reactivated by (γ -oleoyl- β -butyryl) phosphatidylcholine (Jones & Wakil, 1967) and phosphatidylcholine is specifically required for the reduction of cytochrome *P*-450 and in fatty acid, hydrocarbon and drug hydroxylation catalysed by a soluble microsomal enzyme system (Strobel, Lu, Heidma & Coon, 1970).

Reactivation by cationic detergents. In a series of

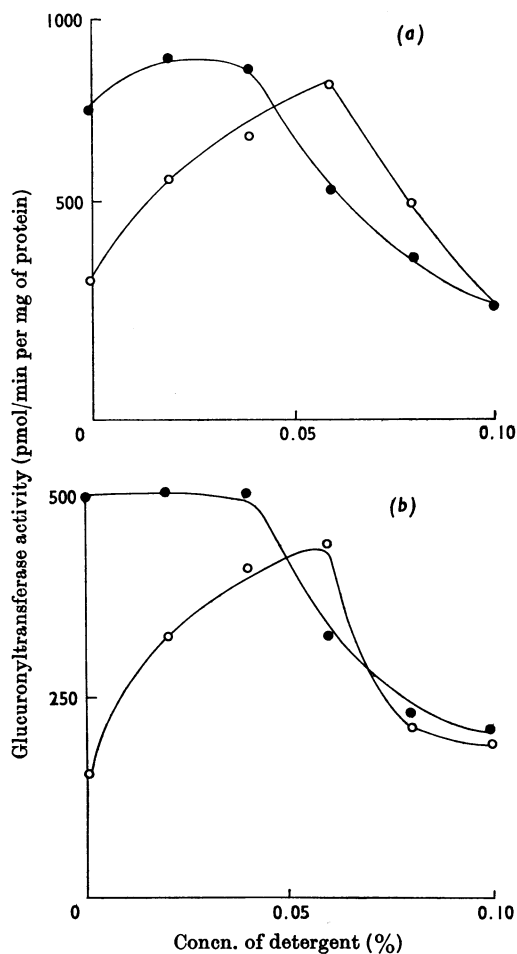


Fig. 3. Effect of cationic detergents on the glucuronyltransferase activity of untreated and phospholipase A-treated microsomal fractions. Microsomal fractions were preincubated in the presence and absence of phospholipase A as described in the Materials and Methods section and the text. Treated and untreated preparations were added to detergent solutions to give the indicated concentrations. (a) Cetylpyridinium chloride; (b) cetyltrimethylammonium bromide. ●, Untreated fraction; ○, treated fraction.

experiments microsomal fractions were treated with phospholipase A for 10 min. This resulted in loss of 55–68% of the initial activity (500–800 pmol/min per mg of protein). After the reaction had been stopped with EDTA, samples of the mixture (0.3 ml, 3.6 mg of microsomal protein) were added to detergent solutions (0.5 ml) of different concentrations. After 5 min, samples (0.2 ml) of the mixtures were assayed for glucuronyltransferase activity. In control experiments phospholipase was

omitted. Cetylpyridinium chloride and cetyltrimethylammonium bromide both reactivated the glucuronyltransferase activity of phospholipase-treated microsomal fractions at low concentrations (Figs. 3a and 3b), the dependence of the effect on concentration being similar to that observed with phosphatidylcholine. At detergent concentrations greater than 0.06%, however, cetylpyridinium chloride and cetyltrimethylammonium bromide both inhibited the reactivated enzyme, increasing detergent concentration causing increasing inhibition. This biphasic variation of enzyme activity with detergent concentration is reflected in the effect of the detergents on the glucuronyltransferase activity of intact microsomal preparations. Thus, up to a concentration of 0.06%, these detergents had little significant effect; at higher concentrations, however, they progressively inactivated the enzyme in a way similar to phosphatidylinositol, phosphatidylserine and cardiolipin.

To establish that the increase in the enzyme activity of phospholipase-treated microsomal preparations observed with lower detergent concentrations is a true reactivation, microsomal fractions were treated with phospholipase A in the usual way. At different times the reaction was stopped by adding portions of the reaction mixture (0.3 ml, 4.1 mg of microsomal protein) to 0.5 ml of detergent solution in 20 mM-tris-15 mM-EDTA, pH 8.0, bringing the final concentration of detergent to 0.06%. In control experiments the tris-EDTA buffer was used instead of detergent. After 5 min, samples (0.2 ml) of these mixtures were assayed for glucuronyltransferase activity. The results (Figs. 4a and 4b) show that at all extents of inactivation by phospholipase full reactivation was achieved by 0.06% cetylpyridinium chloride or cetyltrimethylammonium bromide. These results closely parallel those of a similar experiment in which reactivation by mixed microsomal phospholipids was studied (Graham & Wood, 1969), and it is concluded that they represent a genuine form of reactivation.

Effect of anionic detergents. The effects of sodium deoxycholate and sodium lauryl sulphate on the glucuronyltransferase activity of intact and phospholipase-treated microsomal fractions were investigated by experiments similar to those carried out with the cationic detergents. Between 0.02 and 0.1% concentration both anionic detergents effectively inhibited enzyme activity in both fresh and treated microsomal preparations. At the same time they clarified the microsomal suspensions, indicating 'solubilization' of the membrane. While this work was in progress similar results were reported by Mowat & Arias (1970). Leuders & Kuff (1967) reported, however, that comparable concentrations (0.02-0.04%) of deoxycholate increased the activity of rat liver microsomal glucuronyltransferase

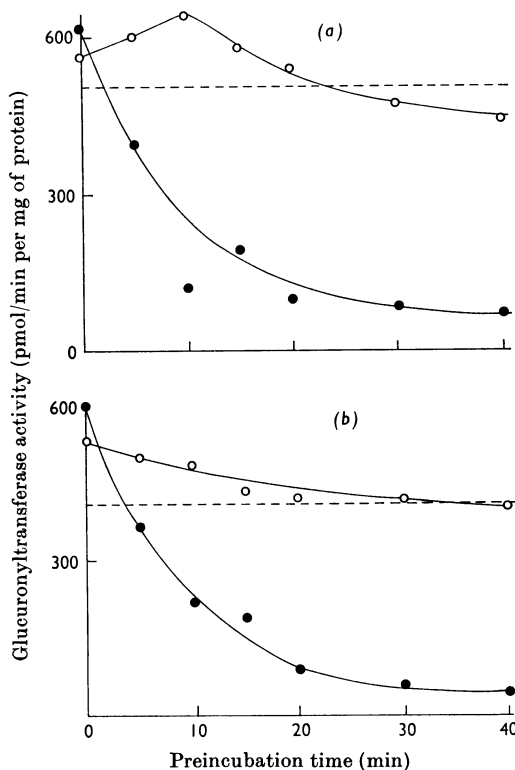


Fig. 4. Reactivation of glucuronyltransferase activity by cationic detergents after pretreatment of microsomal fractions with phospholipase A. Microsomal fractions were preincubated with phospholipase A for the times indicated and added to the detergents as described in the Materials and Methods section and the text. Initial activity (----) represents that of untreated microsomal fraction. (a) Cetylpyridinium chloride; (b) cetyltrimethylammonium bromide. ●, No detergent; ○, +0.06% detergent.

sixfold. Inhibition and clarification of their microsomal suspensions were observed only at much higher concentrations (0.2-0.5%). The results of Leuders & Kuff (1967) were supported by the observations of Van Roy & Heirwegh (1968). The reason for this apparent species difference is not known but it is possible that stimulation of the rat enzyme is due to inhibition of UDP-glucuronate pyrophosphatase (Pogell & Leloir, 1961; Adlard & Lathe, 1970) and/or β -glucuronidase, which are present in rat liver microsomal preparations but are usually present at only low activity in those from guinea-pig liver (Pogell & Leloir, 1961). Alternatively, as discussed below in relation to the effects of non-ionic detergents, the difference might be due to minor differences in procedures for preparing microsomal fractions.

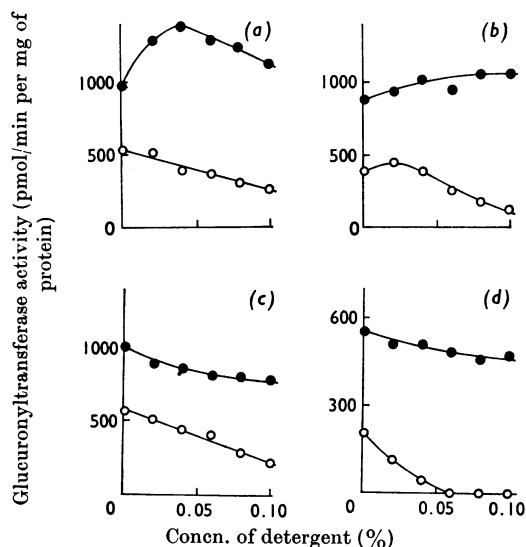


Fig. 5. Effect of non-ionic detergents on the glucuronyltransferase activity of untreated and phospholipase A-treated microsomal fraction. Microsomal fractions were preincubated for 10 min in the presence and absence of phospholipase A as described in the Materials and Methods section. Samples (0.3 ml, 3.3–3.6 mg of protein) of treated and untreated preparations were added to detergent solutions giving the concentrations indicated. After 5 min, samples (0.2 ml) were assayed for glucuronyltransferase activity. (a) Lubrol W; (b) digitonin; (c) Triton X-100; (d) Cetomacrogol 1000. ●, Untreated fraction; ○, phospholipase A-treated fraction.

Effect of non-ionic detergents. This was investigated by the same experimental technique as was used to study the effects of the other detergents. Lubrol W, Triton X-100, digitonin and Cetomacrogol 1000 all failed to reactivate the activity of phospholipase-treated microsomal fractions (Fig. 5); rather they caused further loss of activity, this effect being most pronounced with Cetomacrogol 1000. They had little effect on the activity of untreated microsomal fractions although Lubrol W produced a significant increase of activity at low concentrations. At concentrations about 0.1% they clarified the microsomal suspensions indicating 'solubilization' of the membrane. To confirm this, in experiments similar to those shown in Fig. 5, detergent-microsomal fraction (untreated) mixtures were centrifuged at $34000g_{av}$ for 1 h. With 0.1% Lubrol W, 81% of the microsomal protein and 72% of the glucuronyltransferase activity were found in the supernatant fraction, whereas with 0.1% Triton X-100 the values were 60% and 43% respectively.

In agreement with our results, Mowat & Arias

(1970) found that treatment of guinea-pig liver microsomal fraction with 0.1% Triton X-100 resulted in partial loss and slight solubilization of glucuronyltransferase activity (their criterion of solubilization being the presence of activity in the supernatant solution after centrifugation at $100000g$ for 45 min). Leuders & Kuff (1967), on the other hand, observed that Triton X-100 (0.02–0.04%) increased the activity of glucuronyltransferase in fresh microsomal fractions from rat and guinea-pig liver tenfold, and similar results have been obtained by other workers using a variety of non-ionic detergents and microsomal preparations (Heirwegh & Meuwissen, 1968; Winsnes, 1969; Mulder, 1970; Henderson, 1970). Leuders & Kuff (1967) noted, however, that with guinea-pig preparations the stimulating effect of Triton X-100 was markedly dependent on the length of time the microsomal suspensions had been stored and on the ratio of microsomal protein to detergent. It seems likely that the apparent discrepancy between our results and those of Mowat & Arias (1970) and the results of earlier workers is due to differences in these parameters or possibly to minor differences in fractionating techniques.

Zeta-potential of micelles. To interpret the diverse effects that amphipathic compounds appear to have on intact and phospholipase-treated microsomal fractions the measurement of other parameters in addition to enzyme activity is required. Since the micelles presumably approach and react with the microsomal vesicles so as to effect the observed changes in enzyme activity, measurements were made of micellar zeta-potential, a measure of surface charge. The surface charge of microsomal vesicles, if they behave like most biomembranes, is expected to be less than $-20mV$ (Bangham, 1963). Thus positively charged micelles would be expected to be able to approach the phospholipase-treated microsomal membrane more readily than uncharged or negatively charged micelles and would therefore be expected to have a greater effect on enzyme activity if, in fact, approach is the limiting factor.

Values of the electrophoretic mobilities and the derived zeta-potentials of a number of the compounds used in this study are presented in Table 1 together with a qualitative summary of the effects of these compounds on glucuronyltransferase activity. For a number of compounds values of zeta-potential are quoted from the literature; although these values were obtained with dispersions in media whose ionic composition is different from that used in the present work they are not likely to be sufficiently different from the true values to affect the limited conclusions that may be drawn. It is noteworthy that the electrophoretic mobility determined for sonicated phosphatidyl-

Table 1. *Amphipathic compounds, their zeta-potentials and effects on microsomal suspensions and glucuronyltransferase activity*

Effect of glucuronyltransferase activity: +, increase; -, decrease; 0, no change. Clarification: +, observed; -, no visible effect. ND, not determined; data from literature not available.

Amphipathic compound	Zeta-potential (mV)	Electrophoretic mobility ($\mu\text{m}\cdot\text{s}^{-1}\cdot\text{V}^{-1}\cdot\text{cm}^{-1}$)	Glucuronyltransferase activity		Clarification of untreated microsomal fraction ('solubilization')
			Effect on phospholipase-treated microsomes	Effect on untreated microsomal fraction	
Phosphatidylcholine	+ 0.4	+0.03	++	0	-
Mixed microsomal phospholipids	-38.9	-3.03	++	0	-
Phosphatidylethanolamine	-40.9	-3.18	+	-	-
Cardiolipin	ND	ND	±	---	-
Phosphatidylinositol	-42.0	-3.27	-	---	-
Phosphatidylserine	-60*	-3.4*	±	---	-
Cetylpyridinium chloride	+23.8	+1.37	++ (low concn.) -- (high concn.)	0 (low concn.) -- (high concn.)	-
Cetyltrimethylammonium bromide	+27.0	+1.42	++ (low concn.) -- (high concn.)	0 (low concn.) -- (high concn.)	-
Lubrol W	ND (probably near zero)		-	+	++
Triton X-100			-	-	++
Cetomacrogol 1000			---	0	+
Digitonin			-	0	+
Deoxycholate	ND	ND	---	---	++
Lauryl sulphate	-75†	-3.6†	---	---	++

* Data from Bangham & Papahadjopoulos (1966) and Bangham (1968) for dispersions in 0.154M-KCl, pH 8.0.

† Data of Stigter & Mysels (1955) for solutions in 50mM-NaCl.

ethanolamine is more highly negative than the value ($-1.8\mu\text{m}\cdot\text{s}^{-1}\cdot\text{V}^{-1}\cdot\text{cm}^{-1}$ at pH 8.0) reported (Bangham, 1968) for samples not dispersed by this method.

In general, effects on enzyme activity are not restricted to, or even most marked with, compounds whose micelles have positive surface charge. Micellar surface charge, therefore, does not appear to have a dominant effect in the sense discussed above. No relationship can be discerned between zeta-potential and ability of the various compounds to reactivate phospholipase-treated microsomal preparations. It does appear, however, that those compounds whose micelles have relatively high zeta-potential (positive or negative) are the most effective in inactivating the enzyme activity of intact microsomal preparations; the mixed microsomal phospholipids do not, however, conform to this relationship. The electrophoretic mobility of the mixed microsomal phospholipids is more highly negative than might at first sight be expected for micelles consisting of 62% phosphatidylcholine, 25% phosphatidylethanolamine and 15% phosphatidylinositol with mobilities of +0.03, -3.18 and $-3.27\mu\text{m}\cdot\text{s}^{-1}\cdot\text{V}^{-1}\cdot\text{cm}^{-1}$ respectively. Our

results are, however, consistent with the findings of Bangham (1968) that an increase in the mobility of phosphatidylcholine micelles from 0 to $1.2\mu\text{m}\cdot\text{s}^{-1}\cdot\text{V}^{-1}\cdot\text{cm}^{-1}$ occurs on adding only 10% of phosphatidylserine, and addition of a further 10% of this compound causes a further increase to $-2.2\mu\text{m}\cdot\text{s}^{-1}\cdot\text{V}^{-1}\cdot\text{cm}^{-1}$ which approaches the mobility of phosphatidylserine itself ($-3.4\mu\text{m}\cdot\text{s}^{-1}\cdot\text{V}^{-1}\cdot\text{cm}^{-1}$). Although micellar charge may well have some effect on the ability of amphipathic compounds to reactivate glucuronyltransferase, it is evident that other factors must be considerably more important. If we are correct in supposing that the reactivating effect of mixed microsomal phospholipids is due to their phosphatidylcholine content the one common feature of all the reactivators is the presence of a quaternary nitrogen atom. It is suggested that this grouping may be required for enzyme reactivation.

Winsnes (1969) and Mulder (1970) found that treatment of post-mitochondrial fractions of rat and mouse livers with detergents, or preincubation of these preparations at 37°C, greatly increased their glucuronyltransferase activities. They suggested that such treatments expose active sites on the

enzyme not normally accessible to substrates. Since treatment with detergents in our experiments failed to stimulate glucuronyltransferase in microsomal preparations whose activities were comparable with, or lower than, those of the above untreated post-mitochondrial fractions, we conclude that the proposal of Winsnes (1969) and Mulder (1970) does not apply in our system. Rather our results support the view that full expression of enzyme activity depends on the phospholipid part of the microsomal membrane.

Our results also indicate that low concentrations of the cationic detergents cetylpyridinium chloride and cetyltrimethylammonium bromide can replace phosphatidylcholine as a reactivator of glucuronyltransferase. In previous work only rarely has such replacement of lipid by detergent been observed. For example, according to Tobar (1964), Tween 80 can, under certain conditions, restore full enzyme activity to lipid-depleted malate dehydrogenase from *Mycobacterium avium*, and the same detergent could almost totally replace phosphatidylcholine in protecting $\text{Na}^+ + \text{K}^+$ -stimulated adenosine triphosphatase and mitochondrial adenosine triphosphatase against inhibition by oligomycin (Palatini & Bruni, 1970). Martonosi, Donley & Halpin (1968) reported that the adenosine triphosphatase activity of a phospholipase C-treated microsomal preparation is reactivated by non-ionic and anionic detergents in addition to phospholipids. The reactivations of glucuronyltransferase by phosphatidylcholine and the cationic detergents show striking differences in their concentration-dependencies and it is possible that the two types of compound reactivate by different mechanisms, which nevertheless produce the same end result. This emphasizes the need for measuring parameters other than enzyme activity alone in studying this type of interaction.

Since it is likely that investigation of the interaction of enzymes with membrane components would be greatly facilitated if a truly soluble form of the enzyme could be prepared, it is noteworthy that the non-ionic detergents seemed the most effective in dispersing microsomal material with only minimal loss of enzyme activity. Their use may therefore provide a first step in further attempts to purify glucuronyltransferase.

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