Rapid Paper

Effect on Uridine Diphosphate Glucuronyltransferase Activity of Depleting and Restoring Phospholipids to Guinea-Pig Liver Microsomal Preparations

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Phospholipid depletion substantially inhibited the maximum demonstrable activities of the forward (glucuronidation) and reverse reactions of UDP-glucuronyltransferase towards *p*-nitrophenol in guinea-pig liver microsomal preparations. Dispersions of liver phospholipids restored activity, whereas non-phospholipid amphipaths failed to do so effectively. These results suggest that the system is probably phospholipid-dependent rather than conformationally constrained by phospholipids.

Effects of phospholipids on the activity of liver UDP-glucuronyltransferase (EC 2.4.1.17) are controversial. Reports from one laboratory (e.g. Vessey & Zakim, 1971, 1972; Zakim *et al.*, 1973; Zakim & Vessey, 1974, 1975) suggest that the system is conformationally phospholipid-constrained and that such constraint is essential for its physiological regulation. However, other reports appear to be consistent with phospholipid-dependence of the transferase activity (Graham & Wood, 1969; Attwood *et al.*, 1971; Hänninen & Puukka, 1971; Rao *et al.*, 1972; Winsnes, 1972; Berry *et al.*, 1974; Jansen & Arias, 1975).

Recently we have shown that glucuronidation of *o*-aminophenol and both glucuronidation and the reverse reaction with *p*-nitrophenol appear to be structurally latent in guinea-pig liver microsomal preparations (Berry *et al.*, 1975). Substantial phospholipid depletion of other structurally latent phospholipid-dependent enzymes (Hallinan, 1974; Coleman & Bramley, 1975; Cater *et al.*, 1975) simultaneously caused two superimposed and contrary effects: increased activity due to removal of latency, and decreased activity due to diminished availability of essential phospholipids, though 'full demonstrable activity', assayed with latency removed, was always inhibited by delipidation. We now report the effect of phospholipid depletion, by phospholipase C at 5°C, on *p*-nitrophenol glucuronidation and its reverse reaction in paired samples of fresh guinea-pig liver microsomal preparations. Microsomal vesicles were either untreated and presumably intact before delipidation or had been previously treated to remove glucuronyltransferase latency as far as possible.

Materials and Methods

Preparation of guinea-pig liver microsomal fractions in 0.25M-sucrose, the removal of glucuronyltransferase latency by ultrasonication, the preparation and ultrasonic dispersion of acetone-precipitated guinea-pig liver phospholipids, the assay of *p*-nitrophenol glucuronidation and of its reverse reaction and protein determinations were as described by Berry *et al.* (1975). Phospholipid depletion of microsomal preparations by using C-type phospholipases at 5°C was as described by Berry *et al.* (1974). Delipidation was always monitored by following hydrolysis of radioactive phosphatidylcholine, by using a glass-fibre-disc precipitation method, and in some experiments hydrolysis of total phospholipids was also measured by chemically determining phosphorus in extracted washed phospholipids (Cater *et al.*, 1975).

Results and Discussion

Fig. 1 shows the effect of increasing phospholipid depletion on both *p*-nitrophenol glucuronidation and its reverse reaction in paired samples from the same freshly prepared microsomal fraction. Before treatment with phospholipase C, the microsomal vesicles were either untreated (and their glucuronyltransferase thus highly latent) or else had been ultrasonicated for 4 min under conditions that give an estimate of maximally expressed transferase activity (Berry *et al.*, 1975). Extensive phospholipid depletion greatly inhibited this maximally expressed transferase activity in ultrasonicated microsomal preparations (40–85% inhibition, mean 70%, in 12

Fig. 1. Effect on UDP-glucuronyltransferase of increasing phospholipid depletion of previously untreated or disrupted microsomal preparations

Microsomal pellets, prepared about 30 min previously and kept throughout at 0°C, were resuspended in 0.25M-sucrose. Part was ultrasonicated to remove latency and was then kept for 180 min at 5°C, before assay of the forward and reverse UDP-glucuronyltransferase reactions towards *p*-nitrophenol, measured at 37°C (nmol/min per mg of microsomal protein). Sonicated and non-sonicated microsomal preparations (10 mg of protein/ml) were also treated at 5°C with 0.7 mg of *Clostridium perfringens* phospholipase C/ml, samples being removed after 4–193 min to measure UDP-glucuronyltransferase activities, and to assess phosphatidylcholine hydrolysis radiochemically. In five similar experiments 89–98% phosphatidylcholine hydrolysis was accompanied by 70–82% (mean 76%) hydrolysis of total microsomal phosphatides. ■, *p*-Nitrophenol glucuronidation after ultrasonication; □, reverse reaction after ultrasonication; ●, *p*-nitrophenol glucuronidation in untreated microsomal preparations; ○, reverse reaction in untreated microsomal preparations.

experiments). By contrast, on starting with the low, highly latent, activity of untreated microsomal preparations, delipidation increasingly stimulated the transferase until activity for both forward and reverse reactions approximated to the inhibited activities seen in the delipidated ultrasonicated particles (Fig. 1). Similar results were also obtained after removal of latency differently: thus in 11 experiments the maximum activity increase, when previously untreated microsomal vesicles were extensively delipidated, averaged only 26% (range 8–55%) of that produced from the same microsomal preparations by removing glucuronyltransferase latency by 'spontaneous activation' (Lueders & Kuff, 1967), or with dilute alkali (Kuriyama, 1972), or by exposure to optimum concentrations of Triton X-100, cetyltrimethylammonium bromide or oleic acid or to 2 mM-UDP-*N*-acetylglucosamine and 4 mM-Mg²⁺. This behaviour seems consistent with delipidation eliciting two opposing effects on glucuronyltransferase: inhibition from decreased availability of essential phospholipids, and a simultaneous stimulation from removal of latency. Significantly, treatment of an ultrasonicated microsomal preparation with pure *Bacillus cereus* phospholipase C at 5°C also inhibited *p*-nitrophenol glucuronidation by 86%, when glucuronidation was also assayed at 5°C, and bi-substrate kinetic analysis of *p*-nitrophenol glucuronidation and of its reverse reaction indicates that extensive delipidation lowers both apparent *V* parameters and also increases apparent *K* values for both co-substrates (C. S. Berry & T. Hallinan, unpublished work). Finally, glucuronidation of *o*-

Table 1. Reactivation of delipidated UDP-glucuronyltransferase with ultrasonicated guinea-pig liver phospholipids

Ultrasonicated but lipid-replete microsomal preparations and microsomal preparations delipidated for 90–180 min at 5°C by using *Cl. perfringens* phospholipase C (reaction then stopped with 4 mM-EDTA) were supplemented with guinea-pig liver phospholipid dispersions (2.3–7.7 mg/mg of microsomal protein). After 10 min at 0°C, glucuronyltransferase activities were assayed. Phosphatidylcholine hydrolysis is reported in each experiment. Assessment by *t* tests shows that delipidation of ultrasonicated microsomal preparations significantly inhibited the reverse transferase reaction ($P < 0.001$), and that phospholipids significantly restored it ($P < 0.01$).

UDP-glucuronyltransferase activity (nmol/min per mg of microsomal protein)					
Expt. no.	Ultrasonicated	Ultrasonicated+ delipidated	Ultrasonicated+ phospholipids	Ultrasonicated+ delipidated+ phospholipids	Phosphatidylcholine hydrolysis (%)
(A) <i>p</i> -Nitrophenol glucuronidation					
3	26.5	7.4	29.5	26.2	94.8
4	24.0	5.1	21.5	17.0	97.4
(B) Reverse reaction					
1	23.0	6.7	30.4	27.5	94.2
2	19.0	4.8	25.3	20.9	93.7
3	17.3	5.3	22.1	20.3	94.8
4	22.3	4.3	25.7	18.5	97.4
5	—	7.4	—	27.0	95.0
Mean ± S.D. (Expts. 1–4)	20.4 ± 2.7	5.3 ± 1.0	25.9 ± 3.4	21.8 ± 3.9	95.0 ± 1.6

aminophenol is even more markedly inhibited by extensive phospholipid depletion (C. S. Berry & T. Hallinan, unpublished work).

Ultrasonically dispersed guinea-pig liver phospholipids can fully reactivate glucuronyltransferase in extensively phospholipid-depleted microsomal preparations (Table 1). This is in qualitative agreement with the 40–44% reactivation reported by Graham & Wood (1969), but is contrary to another finding, quoted in support of conformational constraint by phospholipids, that phospholipids either inhibited or were without effect on *p*-nitrophenol glucuronidation in phospholipase C-treated guinea-pig liver microsomal preparations (Vessey & Zakim, 1971). Further, we find that a range of concentrations (% w/v) of digitonin (0.31–1.25), of Triton X-100 (0.05–0.20), of oleic acid (0.01–0.10) or of cetyltrimethylammonium bromide (0.02–0.10) all failed to reactivate the transferase effectively after extensive phospholipid depletion, though these surfactants all markedly stimulated the enzyme on addition to untreated lipid-replete guinea-pig liver microsomal preparations (cf. Attwood *et al.*, 1971). This result reinforces the concept of specific reactivation by phospholipids and also suggests that little residual latency persists after phospholipase C treatment.

In conclusion, the substantial inhibition of full demonstrable activity on phospholipid depletion and its effective reactivation by phospholipids fails to support conformational phospholipid constraint of this glucuronyltransferase activity, and rather suggests that phospholipids are required to support activity. However, phospholipase C may decrease transferase activity in two ways: by phospholipid depletion and by the effect of its diglyceride products, since moderate inhibition of *p*-nitrophenol glucuronidation by glycerol dioleate can be shown under suitable conditions in phospholipid-replete

microsomal preparations (C. S. Berry, M. Caldecourt & T. Hallinan, unpublished work).

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