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THE PHOSPHOLIPID DEPENDENCE OF UDP-GLUCURONYLTRANSFERASE: CONFORMATION/REACTIVITY STUDIES WITH PURIFIED ENZYME

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Highly-active purified UDP-glucuronyltransferase from guinea-pig liver microsomal membranes is associated with phospholipids. Removal of these phospholipids inactivated the transferase and caused profound changes in the enzyme's circular dichroism spectrum indicating that its secondary structure was drastically altered. Treatment of the delipidated fraction with phosphatidylcholine restored the enzyme to a much more helical, high reactivity conformation. These results show clearly that an intact phospholipid environment is required to maintain the transferase in a reactive conformation.

UDP-glucuronyltransferase (EC 2.4.1.17) is an integral enzyme of liver microsomal membranes whose activity is regulated by the intact phospholipid structure of the membrane (for reviews see refs. 1 and 2). By studying crude enzyme fractions, it would appear that the enzyme requires phospholipids for expression of full activity (see ref. 3). Recently, we purified guinea-pig transferase and showed that the enzyme protein, which catalysed glucuronidation of p-nitrophenol with high specific activity, was associated with considerable amounts of microsomal phospholipids (170-550 mol/mol), and phosphatidylcholine was the major species present (4). The enzyme was almost completely inactivated by delipidation and efficient reconstitution of high activity was observed only with fluid phosphatidylcholines (4). Apparent requirements for phospholipid of the rat liver transferases conjugating testosterone, oestrone and bilirubin have been reported (5,6).

The mechanism by which the enzyme interacts with membrane phospholipids to maintain activity is unknown. Many workers have suggested that phospholipids confer

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on such membrane enzymes a reactive conformation and that this is drastically changed when the enzymes are inactivated by delipidation or phospholipid modification (see e.g. ref. 7). In this paper we report data supporting this idea. The conformation and reactivity of purified guinea-pig transferase have been investigated before and after delipidation and after reconstitution with phosphatidylcholine.

Materials and Methods

The purifications of UDP-glucuronyltransferase and phosphatidylcholine (egg-yolk), delipidation and reconstitution of the enzyme, and the determinations of the transferase's activity (with 0.6mM-p-nitrophenol and 4mM-UDP-glucuronate), protein and phospholipid contents were described previously (4).

Enzyme conformational changes were studied by circular dichroism (CD) spectroscopy. CD spectra (320-205nm) were recorded in duplicate at 27°C using a Cary 60 spectropolarimeter fitted with a Cary 6003 CD attachment and 1 cm pathlength strain-free cylindrical quartz cells. The instrument was calibrated with D-camphor-10-sulphonic acid (8). Enzyme solutions used 5mM-Tris/0.005% Lubrol/5% glycerol buffer, pH 7.4 as solvent which had $A_{200} = 0.7$ (determined with a Varian 210 spectrophotometer). Mean residue ellipticities, $[\theta']$, were calculated assuming a mean residue weight of 115. α-Helix contents were estimated from the mean residue ellipticities at 224.5nm (9).

Results and Discussion

Highly-active purified UDP-glucuronyltransferase displayed negative ellipticity bands below 240nm (Fig. 1). The enzyme fraction used in the experiment of Fig. 1a had a specific activity of 4,700nmol/min per mg of protein and a phospholipid content of 570 mol/mol. Increasing extents of phospholipid removal from this preparation caused progressive decrease in the enzyme's activity (Fig. 2) and this was accompanied by progressive decrease in the amplitude of the ellipticity bands below 240nm (Fig. 1a). These spectral changes show that a substantial modification of the enzyme's secondary structure had occurred and that this involves a marked reduction in helicity. When 98% or more of the phospholipid was removed these ellipticity bands virtually disappeared and the almost completely delipidated enzyme fractions had extremely low specific activities.

When 98% delipidated enzyme (130nmol/min per mg of protein) was treated with phosphatidylcholine (375 mol/mol) an enzyme fraction with an activity (3,500nmol/

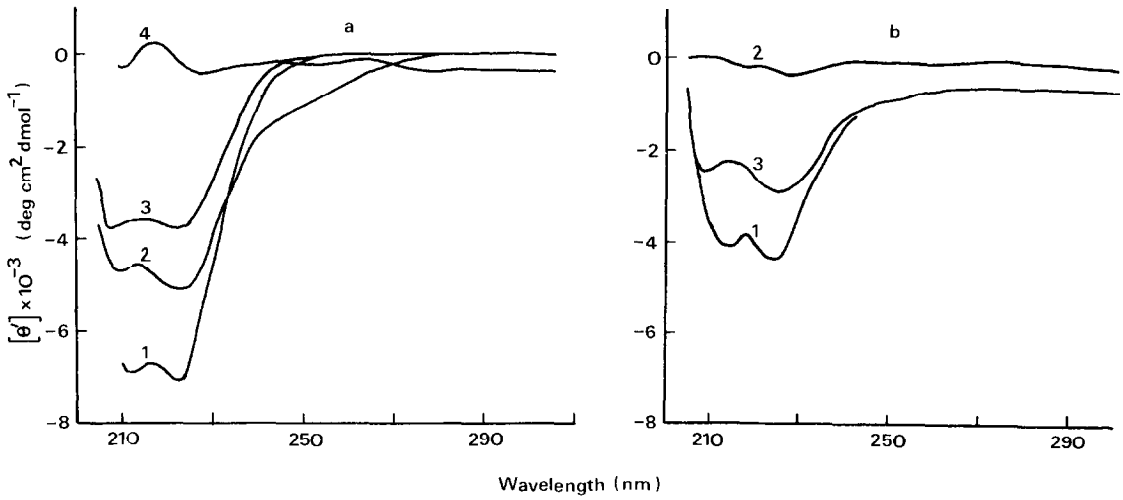

Fig. 1 CD spectra of purified UDP-glucuronyltransferase

(a) Spectra were recorded before delipidation (1) and after delipidation to various extents; 66% (2); 73% (3); 99% (4).

(b) Spectra were recorded before delipidation (1), after 98% of the lipid was removed (2), and after delipidation and treatment with 375 mol of phosphatidylcholine/mol (3). See also text.

min per mg of protein) close to that of the purified enzyme before delipidation (4,200 nmol/min per mg of protein, 170mol of phospholipid/mol) was recovered. This enzyme fraction showed distinct negative ellipticity bands below 240nm (Fig. 1b) and it is

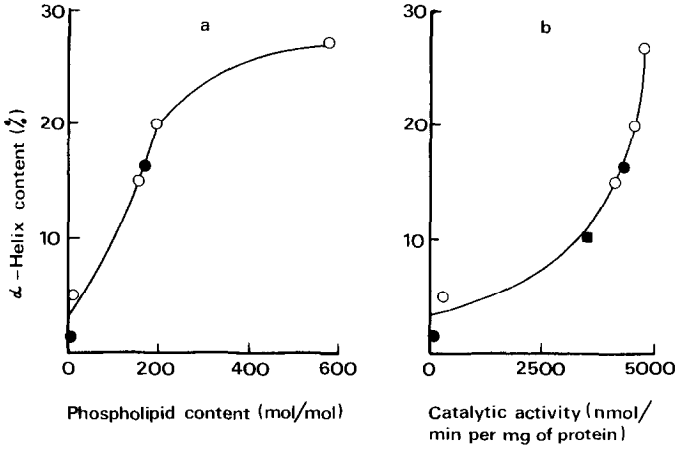

Fig. 2 Relationships between the α-helix content of purified UDP-glucuronyltransferase and (a) its phospholipid content and (b) its catalytic activity

α-Helix contents were calculated from the spectra of Fig. 1. Data from the experiment of Fig. 1a (○); data from that of Fig. 1b (●). Data for the phosphatidylcholine-reactivated enzyme fraction of Fig. 1b (■). See also text.

concluded that phosphatidylcholine restored the inactive enzyme to a high-activity conformation resembling more closely that of the active purified transferase.

Although the proportions of the various ordered polypeptide structures in a protein can not be calculated with precision from its CD spectrum when the protein contains less than 50% α-helix, our results demonstrate that the transferase adopts a conformation of much lower helicity when it is inactivated by delipidation. When 99% of the phospholipid was removed from the enzyme preparation of Fig. 1a its α-helix content (estimated according to ref. 9) was decreased from 27% to about 5%. When 98% was removed from the preparation of Fig. 1b the α-helix content decreased from about 16% to less than 2%; reactivation of this fraction with phosphatidylcholine increased the helix content to a value about 10%. The transferase exhibited very low catalytic activities at helicities less than 10% (Fig. 2b) and it seems that a critical content of phospholipid is required to maintain the enzyme in a reactive conformation. From Fig. 2a this was estimated to be 100-200 mol/mol and it is interesting to note that when lipid-depleted enzyme was titrated with egg-yolk and microsomal phosphatidylcholines complete reactivation was approached at concentrations greater than 120 mol/mol (4,10).

Although very little direct evidence is available, data for other phospholipid-dependent integral enzymes suggest that their membrane environments also might be involved in supporting reactive conformations. It has been estimated (by CD spectroscopy) that purified sarcoplasmic reticulum Ca^{2+}-ATPase has an α-helix content near 35% (11) and its inactivation by delipidation has been correlated with decreased helicity (see also ref. 12). Rapid rates of microsomal glucuronidation and high activities of impure transferase fractions require phospholipids (3). The findings of this study with purified enzyme affords the first insight into the mechanism of this form of membrane modulation; they show that UDP-glucuronyltransferase's essential membrane phospholipid environment provides the forces required to maintain the enzyme in a reactive conformation.

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