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UDP-Glucuronosyltransferase: Phospholipid Dependence and Properties of the Reconstituted Apoenzyme†

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ABSTRACT: The effect of membrane lipid on the stability and catalytic activity of microsomal UDP-glucuronosyltransferase (EC 2.4.1.17) from rat liver was examined. Ninety-eight percent of membrane phospholipid was separated from total microsomal protein by gel filtration on a deoxycholate-equilibrated Sephadex G-50 column. Lipid removal reduced transferase activity, using *p*-nitrophenol as acceptor, to 0-6% that of a deoxycholate-treated control preparation. Incubation of the apoenzyme with liposomes, prepared from microsomal lipid, restored 30-44% of the original activity. Synthetic and naturally occurring lecithins as well as lysolecithin also reconstituted transferase function; however, dioleoyl- and bovine lecithins were more active than either dipalmitoyl- or plant lecithins. Bovine phosphatidylserine was without effect. *p*-Nitrophenol conjugation catalyzed by the reconstituted transferase exhibited similar responses to ionic strength, temperature, and pH as did the nondelipidated deoxycholate solubilized enzyme, although the reconstituted enzyme displayed a more restricted pH range (7.3-7.6 vs. 7.3-8.2) over which maximum activity was expressed. Ability to reactivate the apoenzyme decreased rapidly upon storage with a half-life of approximately 1 day. Storage in the presence of ethylene glycol markedly stabilized the apoenzyme; however, even in the presence of glycol, capacity of the apoenzyme to be reactivated decayed at a faster rate than did the activity of the fully reconstituted enzyme. Collectively, our results establish the phospholipid requirement of microsomal UDP-glucuronosyltransferase for maximal catalytic activity and implicate the involvement of lipid in the stabilization of the active enzyme.

Since the demonstration by Fleischer and Klouwen (1961) of the lipid dependence of mitochondrial electron transport, many other membrane-bound enzymes, such as glucose-6-phosphatase (Garland & Cori, 1972), cytochrome oxidase (Hinkle et al., 1972), β-hydroxybutyrate dehydrogenase (Nielsen & Fleischer, 1973), cytochrome b_5 reductase (Rogers & Strittmatter, 1973), lipopolysaccharide glycosyltransferases (Rothfield & Romeo, 1971), adenosine triphosphatase (Kimelberg & Papahadjopoulos, 1972; Hilden et al., 1974), and cytochrome P-450 (Lu et al., 1972), have been shown to require association with phospholipid for enzymatic function. A similar characterization of rat liver microsomal UDP-glucuronosyltransferase, *p*-nitrophenol as acceptor, has been hampered due to its inactivation upon membrane disruption. As a result, the bulk of current knowledge is based upon work utilizing particulate, microsomal membrane as a source of transferase activity. A number of investigations have shown that digestion of intact microsomes with phospholipase A or

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C activates *p*-nitrophenol conjugation (Vessey & Zakim, 1971; Winsnes, 1972; Berry et al., 1974). In addition, this treatment altered the specificity of the transferase for various nucleotide sugars in such a way that certain UDP sugars (e.g., UDP-glucose, UDP-mannose, and UDP-galactose), that had no effect on the activity of the native enzyme, now functioned as inhibitors (Zakim et al., 1973). On the basis of these results, Zakim et al. (1973) and Eletr et al. (1973) have concluded that phospholipid–protein interactions are not essential for glucuronidation but instead function in maintaining the appropriate conformation responsive to various allosteric regulators governing specificity.

On the other hand, separation of phospholipid hydrolysis products from microsomal protein partially inactivated *p*-nitrophenol glucuronidation, while subsequent lipid addition partially restored activity (Graham & Wood, 1969; Graham et al., 1974). However, because incomplete delipidation and inactivation were attained in these experiments, unequivocal assessment of the role of lipid in the catalytic process was difficult. Recently, Jansen & Arias (1975) reported that removal of more than 90% of phospholipid from rat liver microsomal membrane totally and irreversibly inactivated *p*-nitrophenol conjugation while Graham et al. (1977) using incompletely delipidated microsomes from guinea pig liver were able to regain activity by lipid supplementation.

This work seeks to clarify the effect of lipids on the catalytic activity and stability of *p*-nitrophenol conjugating UDP-glucuronosyltransferase. To this end a nonenzymatic method of delipidation was used to prepare the apoenzyme and purified phospholipids differing in head group as well as fatty acid composition were used to study the requirements for reconstitution. A comparison was also made among the activities of the deoxycholate solubilized, the reconstituted, and the native microsomal enzyme as a function of pH, ionic strength, and temperature.

Experimental Procedures

Dithiothreitol, *p*-nitrophenol (spectrophotometric grade), and the ammonium salt of UDP-α-D-glucuronic acid were obtained from Sigma Chemical Co., Lubrol WX was from Supelco Inc., purified phospholipids (which contained antioxidant) were from P-L Biochemical Co., nordihydroguaiaretic acid was from Aldrich Chemical Co., Sephadex G-50 (fine) was from Pharmacia Fine Chemicals, crystallized ovalbumin was from Worthington Biochemical Co., and sodium deoxycholate was from Schwarz/Mann; all other chemicals were analytical reagent grade. Organic solvents were redistilled once.

Preparation of Gradient-Purified Microsomes. Male Sprague-Dawley rats (150–175 g) were injected with sodium phenobarbital, 0.12 g/kg of body weight, according to Khandwala & Kasper (1973). Gradient-purified microsomal membrane was prepared from excised liver and stored frozen as previously reported (Gorski & Kasper, 1977).

Total protein was determined with the Lowry method (Lowry et al., 1951) using ovalbumin as standard.

Lipid Extraction and Liposome Preparation. A microsomal lipid fraction was obtained from noninduced animals as described by Folch et al. (1957), except that 20 μg/mL nordihydroguaiaretic acid was present during extractions. Lipid was stored at −20 °C with antioxidant for up to 4 weeks without a change in its ability to restore glucuronosyltransferase activity.

Liposomes were prepared from microsomal lipid as well as purified phospholipids (Bangham et al., 1965; Miyamoto & Stoeckenius, 1971; Rogers & Strittmatter, 1973) and stored in 20 mM Tris–acetate (pH 7.5) buffer, containing 0.2 mM EDTA at 4 °C in an atmosphere of nitrogen for periods of up to 1 week. The phosphorus content of liposome preparations was determined by the method of Bartlett (1959).

Solubilization and Delipidation. All operations were carried out at 0–4 °C within an 8–10-h period. Microsomal membrane was dispersed by sonication into 20 mM Tris–acetate, pH 7.5, containing 0.2 mM EDTA, at a protein concentration of 12 mg/mL. Solid sodium deoxycholate was added to a final concentration of 1% (w/v) and the suspension thoroughly mixed, without frothing, for 10 min before centrifuging at 183 600g_{max} for 40 min. Delipidation was accomplished by passing 1 mL of solubilized membrane over a 1.9 × 49 cm column of Sephadex G-50 (fine) equilibrated with 20 mM Tris–acetate, pH 7.5, containing 0.2 mM EDTA, 100 mM sodium chloride, 0.5 mM dithiothreitol, 0.2% (w/v) sodium deoxycholate, and 0.02% sodium azide. Effluent fractions containing delipidated microsomal protein were combined and used as a source of apoenzyme. Phosphorus and protein analyses confirmed and quantitated the extent of delipidation; an average of 1.3 μg of phosphorus/mg of protein was obtained for the void volume peak, in contrast to a value of 32.0 μg/mg for the initial detergent-solubilized membrane. Of the 1.3 μg of phosphorus/mg of protein, 50% was nonphospholipid phosphorus. Hence, essentially complete delipidation was achieved by this procedure.

The deoxycholate concentration of the delipidated protein sample was determined (Szalkowski & Mader, 1952) and found to range from 0.24 to 0.28% (w/v), on the basis of six separate experiments.

Single-Time-Point Assay. Delipidated microsomal protein (approximately 200 μg in 0.1 mL) was added to 0.2 mL of liposomes containing 3 μmol of phospholipid and the mixture vortexed intermittently for 10 min at room temperature. Approximately 150 μg of reconstituted enzyme was used per assay (Gorski & Kasper, 1977). Deoxycholate solubilized membrane was assayed by the same procedure after sixfold dilution with 20 mM Tris–acetate, pH 7.5, containing 0.10–0.12% (w/v) sodium deoxycholate, 120 mM sodium chloride, and 0.02% sodium azide. In this case, there was no supplementation with liposomes.

Kinetic Assay of Reconstituted Transferase. Delipidated microsomal protein (approximately 300 μg in 0.15 mL) was mixed with an equal volume of liposomes (0.5–0.85 μmol of phospholipids) in a cuvette and assayed as previously described (Gorski & Kasper, 1977). *p*-Nitrophenol displays a broad absorbance spectrum centered at 400 nm, and operationally a wavelength was chosen so as to obtain an initial absorbance between 1.5 and 2.0 OD. However, since the extinction coefficient for *p*-nitrophenol varied with changes in pH, temperature, and ionic strength, different assay conditions necessitated the use of wavelengths between 430 and 465 nm. Because 95% or more of the decrease in *p*-nitrophenol absorbance was routinely found to be due to conjugation, the reaction rate was calculated directly from this change with extinction coefficients determined for conditions (a) pH 6.0–9.0, (b) 0–1.0 M sodium chloride, and (c) 19–48 °C (refer to Gorski (1975) for coefficients).

Results

Effect of Deoxycholate on Enzymic Activity and Stability of Solubilized Transferases. Perturbation of the protein–lipid associations within the membrane brought about by the addition of low levels of deoxycholate resulted in a gradual stimulation of activity with a maximum twofold elevation being reached at a detergent to protein ratio (w/w) of 2.4 and a de-

TABLE I: Factors Affecting Stability of UDP-Glucuronosyltransferase Activity.[a]

	activity half-life (days)
deoxycholate-solubilized enzyme	
(a) no additions	1-2[b]
(b) + 35% ethylene glycol	9
(c) + antioxidant, 10 μg/mL	1-2[b]
(d) + 33% ethylene glycol, + antioxidant	13
(e) + 33% ethylene glycol, + N_2 atmosphere	13
Lubrol WX solubilized enzyme	
(a) no additions	13

[a] Deoxycholate-solubilized enzyme was prepared as described in the text. Lubrol solubilization was accomplished by mixing a microsomal suspension (10 mg of protein/mL) with an equal volume of 4% (v/v) detergent in 50 mM Tris-acetate buffer, pH 7.5, and centrifuging at 200 000g_{max} for 60 min at 4 °C to obtain the solubilized enzyme. Activity was measured as a function of time and the half-lives were determined from a least-squares fit of the data. [b] Calculated on the basis of two time points.

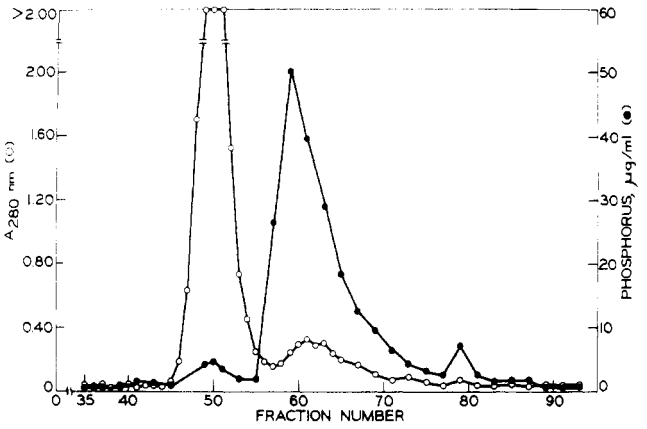

FIGURE 1: Delipidation of deoxycholate-solubilized microsomal membrane by gel filtration on Sephadex G-50.

tergent concentration of 0.04% w/v. Deoxycholate concentrations above this level resulted in progressive inactivation until at 0.14% w/v complete loss of activity was noted. Inactivation, however, was partially or totally reversible depending on the conditions used to dilute out the detergent. For example, inactivation of UDP-glucuronosyltransferase resulting from solubilization in 1% w/v deoxycholate was completely reversed upon dilution with 20 mM Tris-acetate buffer, pH 7.5, containing 0.1 M EDTA to a final detergent concentration of 0.03% w/v. If, however, the same solubilized enzyme preparation was diluted sixfold with buffer containing 0.1% deoxycholate and then finally with detergent-free buffer to a concentration of 0.03%, only one-half of the expected activity was regained. Table I presents data on the stability of solubilized UDP-glucuronosyltransferase and the influence of several stabilizing agents. At a deoxycholate to protein ratio of 0.83 (w/w), 96% of UDP-glucuronosyltransferase activity was solubilized along with 89% of membrane protein and 90% of phospholipid. The half-life of deoxycholate solubilized enzyme is approximately 1-2 days at 4 °C (Table I). Addition of ethylene glycol to the level of 33% (v/v) markedly improved transferase stability, but inclusion of an antioxidant (nordihydroguaiaretic acid) by itself did not preserve activity. An interesting observation was that either antioxidant or a nitrogen atmosphere in conjunction with ethylene glycol conferred maximum stability ($t_{1/2}$ = 13 days). Since antioxidant was only effective in the presence of ethylene glycol, its effect, presumably the inhibition of lipid peroxidation (Minssen & Munkres, 1973; Tappel, 1973), seems secondary to that of the glycol.

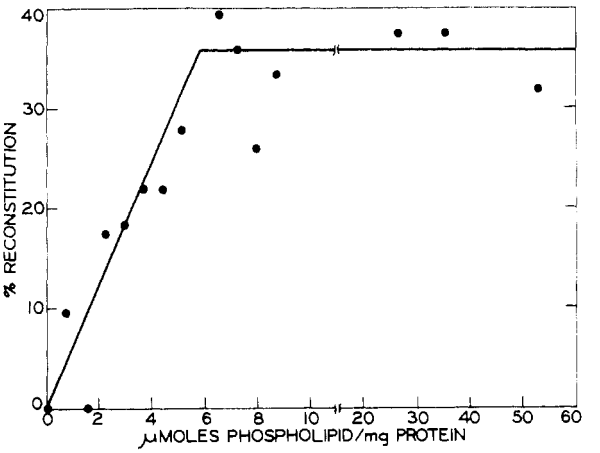

FIGURE 2: Reconstitution of apoenzyme with microsomal lipid. The extent of activity reconstituted is expressed as a percentage of the control rate, 22 nmol of *p*-nitrophenol conjugated/(min mg).

Delipidation of Solubilized Microsomal Protein. Mixed micelles of membrane phospholipid and deoxycholate were separated from detergent-protein complexes by gel filtration (Philippot, 1971; Helenius & Simons, 1971) on Sephadex G-50 (Figure 1). With this procedure, 4% of the total membrane phosphorus coeluted with protein at the column void volume, while the remaining 96% eluted well separated from the protein. Judged by its solubility in chloroform-methanol, approximately 50% of the phosphorus which coeluted with protein (Figure 1) was present as phospholipid phosphorus. Hence, the phosphorus to protein ratio (μg/mg) for the delipidated protein was 0.6, while that of the sample prior to column delipidation was 32. When 30% ethylene glycol was included in the column buffer to stabilize the transferase, no separation of phospholipid and protein was observed. Presumably this result is due to an increase in the critical micelle concentration of deoxycholate, as outlined by Tanford (1973). A similar outcome has been obtained when greater than 10% glycerol was included in (Jansen & Arias, 1975) or sodium chloride deleted from (Hinkle et al., 1972) the column buffer. Delipidation inactivated the enzyme, although analyses occasionally revealed a residual activity which ranged as high as 2.4 nmol/(min mg) or 65% of the control.

Reconstitution of Apotransferase. Restoration of glucuronidation activity was achieved upon the addition of membrane derived liposomes to apotransferase (Figure 2). Several points with regard to this process should be stressed. First, the reconstitution process demonstrated saturation with respect to the amount of lipid added. Maximal conjugation was obtained at a ratio of approximately 6 μmol of phospholipid per mg of protein, which is close to fourfold higher than that of the native membrane. Second, for a large number of experiments, restoration of transferase activity was in the range of 30–44%, with an average of 36%. Third, detergent was necessary for optimal restoration of activity, since its removal before addition of liposomes resulted in extensive protein aggregation and poor recoveries. Efforts to improve reconstitution efficiency by altering detergent concentration were unsuccessful.

Liposomes prepared from certain classes of phospholipids were able to substitute for microsomal lipid, as shown in Figure

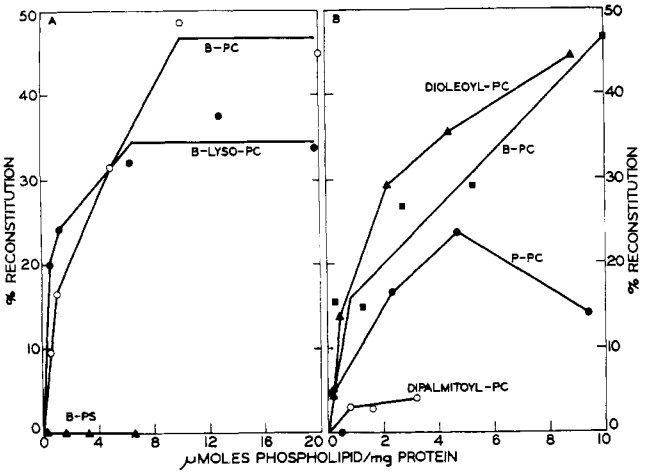

FIGURE 3: Reconstitution of apoenzyme with purified phospholipids. Abbreviations used are: B, bovine; Lyso-PC, lysophosphatidylcholine; PS, phosphatidylserine; P, plant (soybean); and PC, phosphatidylcholine.

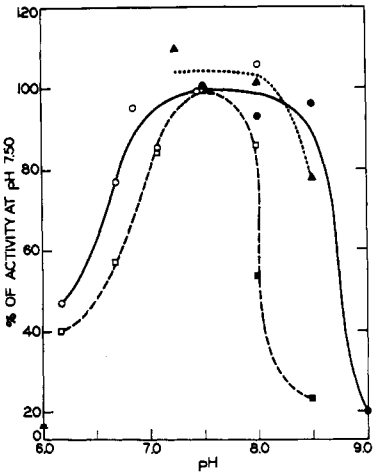

FIGURE 4: pH–activity profile for reconstituted, DOC-solubilized, and native UDP-glucuronosyltransferase. Reaction rates were determined at 24 °C with the kinetic assay described in Experimental Procedures. Open symbols refer to the 70 mM Tris–35 mM malate buffer system in the range pH 6.2–8.0, and the closed symbols refer to 140 mM Tris in the range pH 7.2–9.0. Final pH adjustment of these buffers employed glacial acetic acid or sodium hydroxide. The three preparations and their specific activities at pH 7.50 were: sonicated membrane, 48 nmol/(min mg) (▲); DOC-solubilized membrane, 42 (○, ●); and reconstituted enzyme, 12 (□, ■). Reconstitution was carried out with bovine lecithin at a phospholipid/protein ratio of 2.4–2.8 μmol/mg.

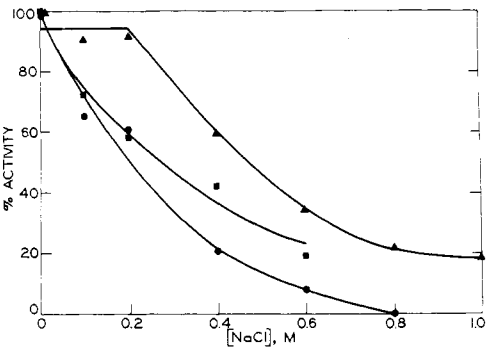

FIGURE 5: The effect of sodium chloride concentration on glucuronidation. Reaction rates for the three preparations were determined as in Figure 4, except that the buffer used in the assays was 56 mM Tris-acetate, pH 7.50. Activities for the three preparations examined are expressed as a percentage of that determined in the absence of sodium chloride and these absolute values were: (▲) sonicated membrane, 51 nmol/(min mg); (●) DOC-solubilized membrane, 33; and (■) reconstituted enzyme, 9. The latter had been reconstituted with bovine lecithin at a phospholipid/protein ratio of 2.3 μmol/mg.

3A. Bovine phosphatidylcholine and lysophosphatidylcholine, neutral phospholipids at pH 7.5, were active in this system whereas anionic phosphatidylserine was inactive at all concentrations tested. Reconstitution of apotransferase with purified lecithin reached a plateau in a manner similar to that obtained with microsomal lipid (Figure 2); however, a higher phospholipid/protein ratio was necessary to achieve maximum activity with bovine lecithin. By way of comparison, egg lecithin has been shown to substitute fully for microsomal lipid in restoring delipidated bilirubin transferase function (Jansen & Arias, 1975).

Variation of the fatty acid composition of phosphatidylcholine also had a striking effect on reconstitution (Figure 3B). For example, dioleoyllecithin was far more effective in restoring transferase activity than was either dipalmitoyl- or plant lecithin. The results with soybean lecithin may be due to the fact that more than half of the total fatty acids of soybean lecithin are multiply unsaturated C_{18} fatty acids (Galliard, 1973).

Properties of Reconstituted Apotransferase. A detailed comparison was made between reconstituted UDP-glucuronosyltransferase and the native enzyme before and after deoxycholate solubilization. Initial reaction rates were obtained as a function of pH (Figure 4), ionic strength (Figure 5), and temperature (Figure 6). It should be stressed that in these experiments changes in enzymic activity measured under different assay conditions may be due directly to effects on the protein–phospholipid complex or indirectly through enzyme bound or free detergent. Reconstituted transferase exhibited a rather narrow pH profile with activity rapidly falling off below pH 7.3 and above pH 7.6 (Figure 4). The DOC-solubilized enzyme, however, displayed a broad maximum in the range of pH 7.2–8.2. Both the reactivated and the DOC-solubilized enzyme showed a marked loss of activity with increasing ionic strength (Figure 5). The most notable feature was in the concentration range of 0–0.2 M sodium chloride where the sonicated particulate enzyme was fully active, whereas the solubilized and the reconstituted forms had lost approximately 40% of their activity at the higher salt concentration. Furthermore, in 1 M salt the insoluble transferase retained 19% of the original activity, whereas the solubilized enzyme was totally inactive in 0.8 M sodium chloride.

The influence of temperature on initial rates of conjugation indicated interesting similarities and differences among the preparations (Figure 6). The temperature dependence displayed by the reconstituted and the DOC-solubilized enzyme was essentially the same, with a broad maximum which ranged from 24 to 34 °C for the former and from 25 to 37 °C for the latter. Although not shown, higher deoxycholate levels in the assay significantly lowered the temperature optimum for the solubilized enzyme. On the other hand, within the temperature range studied, the sonicated, particulate transferase had not yet attained a maximal rate. Digitonin treatment of microsomal membrane has also been reported to lower the optimum temperature of the enzyme relative to that of untreated membrane (Norling & Hanninen, 1974).

The above experiments establish that the conditions of pH, ionic strength, and temperature required for sensitive assay of the DOC-solubilized transferase remain unchanged after delipidation and reconstitution.

Stability of Apoenzyme. In the course of these studies, irreversible inactivation of the apoenzyme was noted and at-

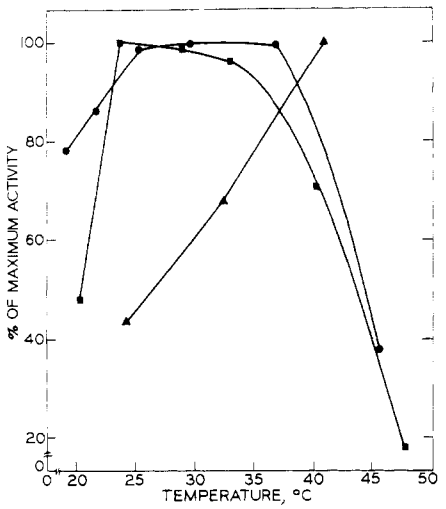

FIGURE 6: The effect of temperature on glucuronosyltransferase activity. Reaction rates for the three preparations were measured as in Figure 4, except that the buffer used in the assays was 70 mM Tris–35 mM malate, pH 7.50. Activity is expressed as a percentage of that at the optimum temperature within the range examined. Maximum activities of the different preparations were: (▲) sonicated membrane, 90 nmol/(min mg); (●) DOC-solubilized membrane, 26; and (■) reconstituted enzyme, 8. Reconstitution was carried out with bovine lecithin at a phospholipid/protein ratio of 2.3 μmol/mg.

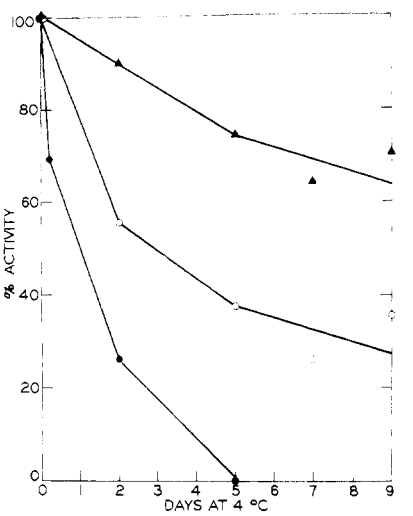

FIGURE 7: Stability of apotransferase. Deoxycholate-solubilized membrane was delipidated as in Figure 2 and stored under the following conditions: (▲) nitrogen atmosphere at 0.87 mg/mL protein and 2.7 μmol/mL microsomal phospholipid in 16 mM Tris-acetate (pH 7.5) containing 0.10% (w/v) sodium deoxycholate, 20% (v/v) ethylene glycol, 0.02% sodium azide; (○) 1.45 mg/mL protein in 13 mM Tris-acetate (pH 7.5) containing 0.17% (w/v) sodium deoxycholate, 33% (v/v) ethylene glycol, and 0.02% sodium azide; and (●) 2.17 mg/mL protein in 20 mM Tris-acetate (pH 7.5) containing 0.26% (w/v) sodium deoxycholate and 0.02% sodium azide. Reaction rates were determined directly (▲) or after reconstitution with fresh liposomes (○, ●). Reconstitution with microsomal liposomes was carried out at phospholipid/protein ratio in the range of 1.9–3.1 μmol/mg.

tempts were made to determine the participating factors so that such a loss might be prevented. Stability of the delipidated enzyme was monitored over a period of days by measuring regeneration of activity upon supplementation with fresh liposomes (Figure 7). The effects of ethylene glycol and of ethylene glycol plus liposomes in an inert atmosphere were examined. Both regimens slowed irreversible inactivation of the apoenzyme; however, the latter combination with liposomes was most successful. Two points should be noted. First, both the activity of the transferase after solubilization with deoxycholate and the reconstitutability of the apoenzyme stored in the absence of any stabilizing agents displayed similar rates of decay at 4 °C (compare Table I and Figure 7). Second, retention of transferase activity after solubilization and/or delipidation is greatly enhanced by inclusion of ethylene glycol and is further increased by the presence of phospholipid.

Discussion

Two main conclusions are supported by the data presented in this paper. They are that rat liver microsomal UDP-glucuronosyltransferase, with *p*-nitrophenol as substrate, requires lipid for catalytic function and maximum stability, and that a preference for specific phospholipids is expressed. Titration of the inactive apoenzyme with either microsomal lipid, purified lecithins, or lysolecithin restored *p*-nitrophenol conjugation up to 30–47% that of control. Although regeneration of activity upon addition of lipid to apoenzyme appeared to follow a typical saturation curve, phosphatidylserine and dipalmitoyllecithin were largely inactive at all concentrations tested.

Previous reconstitution studies with the rat liver enzyme (Wistar strain) were unsuccessful in restoring *p*-nitrophenol conjugation; however, essentially complete reactivation of bilirubin glucuronidation was achieved with either microsomal lipid or egg lecithin (Jansen & Arias, 1975). Similar studies utilizing partially delipidated microsomal membrane from guinea pig liver did succeed in restoring *p*-nitrophenol conjugating activity upon supplementation with a mixture of lecithin and lysolecithin (Graham et al., 1977). Related to these findings is the observation that isoelectric focusing of affinity column purified UDP-glucuronosyltransferase (Gorski & Kasper, 1977) resolved three discrete electrophoretic forms of the enzyme, each having identical polypeptide compositions. The implication is that the lipid composition of the active phospholipid-protein complex may be altered to bring about a change in physicochemical properties of the transferase and perhaps a change in enzymic behavior. It is plausible that specific glucuronidating activities are dependent upon the association of a particular type of phospholipid with the polypeptide chain to either generate the appropriate substrate binding site or to confer the proper conformation required for activity. Consequently, in the case of lipid modulated acceptor specificity, the recovery of a specific enzymic character in lipid reconstitution studies would totally depend upon the nature of the phospholipid head group as well as the acyl side chains. For example, failure of dipalmitoyllecithin to restore a significant level of activity may be related to the fact that below 41 °C such bilayers display a low permeability (de Gier et al., 1968) and possess a solid-phase hydrophobic core (Philips et al., 1969). In this connection, Eletr et al. (1973) have presented data based on paramagnetic resonance measurements utilizing spin-labeled fatty acids to suggest a close relationship between transferase activity and membrane fluidity. Also, temperature-activity studies produced a discontinuous Arrhenius plot in which the break occurred at the thermal transition temperature for microsomal lipid (Eletr et al., 1973) further suggesting a dependence of enzymic activity on the physical state of membrane lipids. If lipid does function in the determination of acceptor specificity, failure of Gunn rats to glucuronidate bilirubin may be associated with a defect in lipid metabolism which could prevent the formation of an active phospholipid-protein complex capable of recognizing bilirubin. One report that detracts from this possibility deals with the observation that microsomal lipid from Gunn rats can restore bilirubin conjugation when added to lipid free liver microsomes

from Wistar rats (Jansen & Arias, 1975). Although *p*-nitrophenol conjugating activity is associated with a lipid–protein complex containing a polypeptide chain of 59 000 molecular weight (Gorski & Kasper, 1977), a protein of 52 600 molecular weight did copurify with the complex. This was also noted with the rabbit liver microsomal enzyme.[1] The suggestion has been made that the smaller protein may function either alone or in conjunction with the 59 000 molecular weight protein in the conjugation of acceptors other than *p*-nitrophenol (Gorski & Kasper, 1977). Experiments dealing with the kinetics of UDP-glucuronosyltransferase have demonstrated a sequential mechanism for *p*-nitrophenol conjugation which requires the existence of two distinct binding sites, one for UDP-glucuronic acid and another for *p*-nitrophenol (Vessey & Zakim, 1972). Furthermore, the acceptor substrate site has been shown to be hydrophobic in nature, since the degree of competitive inhibition of short chain aliphatic alcohols is directly proportional to their hydrophobicity (Alanen & Hanninen, 1966). The lipophilic binding site could be formed by a folding of the polypeptide chain or could be located within the domain generated by the association of phospholipid with specific amino acid side chains. In the latter case, the acceptor combining site could be either at the juncture at which the protein enters the phospholipid bilayer or actually embedded within the lipid matrix.

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