Biochimica et Biophysica Acta, 391 (1975) 28—38

BBA 67470

DELIPIDATION AND REACTIVATION OF UDPGLUCURONOSYLTRANSFERASE FROM RAT LIVER

PETER L.M. JANSEN and IRWIN M. ARIAS

Department of Medicine, Liver Research Center, Albert Einstein College of Medicine, Bronx, N.Y. 10461 (U.S.A.)

(Received November 8th, 1974)

Summary

UDPglucuronosyltransferase was solubilized by treating Wistar rat liver microsomes with deoxycholate. Chromatography of this preparation on Bio-Gel P-30 resulted in extraction of 92% of phospholipids and complete loss of enzyme activity. UDPglucuronosyltransferase was reactivated by dialysing this delipidated preparation in the presence of lecithin, a mixture of liver microsomal lipids or microsomal preparations from livers of UDPglucuronosyltransferase-deficient Gunn rats.

Virtually complete enzyme reactivation was obtained with regard to glucuronidation and glucosidation of bilirubin; however, the inactivation of UDPglucuronosyltransferase with *p*-nitrophenol as substrate was irreversible.

These findings demonstrate that UDPglucuronosyltransferase with bilirubin as substrate is a lipid-requiring enzyme.

Introduction

UDPglucuronosyltransferase (UDPglucuronate β-glucuronosyltransferase (acceptor unspecific EC 2.4.1.17) catalyzes glucuronosyl conjugation of several small-molecular compounds including hormones, products of endogenous metabolism, drugs and toxic compounds [1]. Many investigators have tried to determine whether glucuronidation of these compounds is mediated by a single enzyme or whether different UDPglucuronosyltransferases exist [2—6]. Attempts to separate and purify different UDPglucuronosyltransferases have been unsuccessful [7—9].

Gel filtration and ion-exchange chromatography in the presence of detergents permit purification of several membrane-bound enzymes which are mostly obtained in a delipidated form [10]. Before purification of UDPglucuronosyltransferase can be attempted, the influence of these procedures on enzyme activity must be investigated. The present study shows that delipidation com-

pletely and reversibly inactivates UDPglucuronosyltransferase. A method is described to restore enzyme activity to a delipidated preparation.

Materials and Methods

Animals

Adult male Wistar and homozygous UDPglucuronosyltransferase-deficient Gunn rats, weighing 200—250 g, were used, and were fed rat breeder chow and water ad libitum.

Chemicals

Bilirubin, uridine 5′-diphosphoglucuronic acid (ammonium salt), and uridine 5′-diphosphoglucose were obtained from Sigma, St. Louis, Mo.; *p*-nitrophenol was from Calbiochem, San Diego, Calif.; glycerol was from Fisher Scientific Company, Fair Lawn, N.J.; deoxycholic acid, sodium salt was obtained from Sigma, St. Louis, Mo.; and ethyl anthranilate was obtained from Eastman Kodak Company, Rochester, N.Y. For phosphorus quantitation, the Fiske and SubbaRow reducer from Sigma, St. Louis, Mo., was used. Trizma base, Sigma, St. Louis, Mo. was used for preparation of buffers. Glacial acetic acid, analytical grade, was from Mallinckrodt Chemical Works, St. Louis, Mo. All buffers were prepared with deionized distilled water. Bovine serum albumin was obtained from Sigma, St. Louis, Mo. Egg L-α-lecithin, chromatographically pure, was obtained from Grand Island Biological Company, New York, N.Y. Lysofree A grade, synthetic L-α-cephalin (β,γ-dipalmitoyl) and purified bovine brain phosphatidylserine, A grade, were obtained from Calbiochem, San Diego, Calif.

Bio-Gel P-30 (100—200 mesh) was obtained from Bio Rad, Richmond, Calif. For thin-layer chromatography, glass plates pre-coated with Silica Gel G, layer thickness 0.25 mm, size 20 cm × 20 cm (Analtech Inc.) distributed by Fisher Scientific Company, Pittsburgh, Pa. were used.

Preparation of microsomes

All procedures were performed in a cold room at 4° C. Wistar rats and homozygous Gunn rats were killed by decapitation under light ether anesthesia. The livers were removed immediately, perfused with 50 ml ice-cold saline and cut into small pieces. A 15% (w/v) homogenate was prepared with a Teflon-glass homogenizer in 0.02 M Tris/acetate buffer, pH 8.1, containing 0.25 M sucrose and 0.2 mM disodium EDTA. The homogenate was centrifuged at 650 × *g* for 15 min in swinging buckets (IEC PR-J centrifuge). The supernatant was centrifuged twice at 9000 × *g* for 15 min (rotor, SS-34; Servall, superspeed, automatic refrigerated centrifuge, RC-2). The fatty layer on top of the 9000 × *g* supernatant was carefully removed by suction and a microsomal fraction was prepared from the remaining supernatant by centrifugation at 100 000 × *g* for 60 min (Beckman L2-65 B ultracentrifuge) in pre-weighed tubes. After centrifugation, the supernatant was removed, the tubes were reweighed and the pellets were resuspended with a few strokes of the Teflon-glass homogenizer in 4 ml 0.02 M Tris/acetate buffer, pH 8.1, containing glycerol (9 : 1, v/v), per gram of pellet. The suspensions were stored in a liquid nitrogen freezer

(−180° C). The microsomal fraction from Wistar and Gunn rat liver was stored up to 3 weeks. Storage did not alter UDPglucuronosyltransferase activity with bilirubin or *p*-nitrophenol as substrates.

Solubilization and delipidation

Microsomal suspensions from Wistar rat liver were thawed at 4° C and 1 ml 0.02 M Tris/acetate buffer, pH 8.1, containing glycerol (9 : 1, v/v) and 15 mg deoxycholate was added per 4 ml suspension. After standing for 20 min at 4° C, this suspension was centrifuged at 100 000 × *g* for 60 min. Centrifugation resulted in a clear supernatant, a loose cloudy fraction at the bottom of the tube and a small translucent pellet. Only the clear supernatant was processed further. Bovine serum albumin was added to the supernatant fraction (4 mg/ml). Two samples of 4 ml each were applied to two identical columns (Pharmacia K26/70, 2.6 cm × 70 cm, bed volume 370 ml) previously packed with Bio-Gel P-30 which was equilibrated with 0.02 M Tris/acetate, pH 8.1, containing glycerol (9 : 1, v/v), 4.8 mM sodium deoxycholate, 0.1 M NaCl and 0.1 mM disodium EDTA (modification of the procedure by Rogers and Strittmatter [11]). The same buffer was used for elution.

Enzyme reactivation

Immediately after elution from Bio-Gel, samples with the highest protein concentration were combined with a mixed lipid preparation, commercial phospholipids or Gunn rat liver microsomes. The protein-lipid or the protein-microsome mixtures were dialyzed against 4 l of 0.02 M Tris/acetate, pH 7.25, containing glycerol (97.4 : 2.6, v/v) and 5 mM $MgCl_2$. Dialysis was performed for 6 h at 18° C and for 16 h at 4° C.

After dialysis, the fractions were diluted (1 : 1, v/v) with 0.02 M Tris/acetate, pH 8.1, containing 0.25 M sucrose and 0.2 mM disodium EDTA. These solutions were centrifuged at 100 000 × *g* for 60 min. The resulting pellet was resuspended in Tris/sucrose/EDTA buffer with a few strokes of the Teflon-glass homogenizer. The enzyme assay was immediately performed.

Lipid preparations

Lipids were extracted from microsomal preparations from Wistar rat liver by the method of Folch et al. [12]. The organic solvent was evaporated from the lipid extract by a stream of nitrogen while keeping the solution at 37° C. The lipids were dispersed in 0.02 M Tris/acetate, pH 8.1, glycerol (9 : 1, v/v) by ultrasonication (Sonifier Cell Disruptor, Model W 140, Heat Systems and Ultradonics, Inc., Plainview, N.Y.). Phospholipids from commercial preparations were dissolved in the same way.

Enzyme assay

The conjugation of bilirubin with glucuronic acid was determined exactly as described previously [13]. Incubation mixtures (volume: 1.2 ml) contained 0.2 ml enzyme preparation and 83 μM bilirubin, 3 mM UDPglucuronate and 3.3 mM $MgCl_2$. For determination of bilirubin glucosidation, UDPglucuronate was replaced by 3 mM UDPglucose. The mixtures were incubated at 37° C in a shaking water bath for 20 min. Diazo reaction and measurement of the azopig-

ments were performed as described by Fevery er al. [14]. Nature of the azopigments was confirmed by thin-layer chromatography [14].

The assay for *p*-nitrophenol glucuronidation was according to Henderson and Kersten [15]. Incubation mixtures (volume: 0.6 ml) contained 0.1 ml enzyme preparation and 1.66 mM *p*-nitrophenol, 3 mM UDPglucuronate and 3.3 mM $MgCl_2$. The mixtures were incubated for 20 min at 37° C.

Other determinations

Protein was determined according to Lowry et al. [16]. The phosphorus assay of Bartlett [17] was used. Multiplication of the phosphorus concentration by the conversion factor 25 gave the phospholipid concentration.

Phospholipid analysis was performed by thin-layer chromatography with chloroform/methanol/water (65 : 25 : 4, v/v/v) as solvent system. After development, the plates were sprayed with ninhydrin (0.25%, w/v) in 90% butanol, heated for 5 min at 90° C, sprayed again with 80% H_2SO_4 and heated for 10 min at 120° C prior to examination.

Results

As shown in Table I, total activity (activity in pellet + supernatant) with regard to glucuronidation of *p*-nitrophenol and bilirubin, increased as a result

TABLE I

ACTIVATION AND SOLUBILIZATION OF UDPGLUCURONOSYLTRANSFERASE

Samples (2 ml) from a microsomal suspension from Wistar rat liver (0.25 g microsomes/ml) were mixed with 0.5 ml of a deoxycholate solution of varying concentrations. Samples and deoxycholate contained 0.02 M Tris/acetate buffer pH, 8.1, containing glycerol (9:1, v/v). After standing for 20 min at 4° C, the samples were centrifuged at 100 000 × *g* for 60 min. Enzyme activities were measured in the clear supernatant and in the pellet. The pellet was resuspended in a final volume of 2.5 ml. Total activity represents activity in pellet + supernatant. Activities were calculated per ml enzyme suspension. The activity of the resuspended pellet was called 1.00. Percentage deoxycholate relates to the amount of detergent per gram microsomal pellet.

Substrates	Percentage Deoxycholate (w/w)	Total activity	Activity in supernatant
1. Bilirubin			
+ UDPglucuronate	0	1.00	0
	0.5	3.18	0.12
	1.0	3.36	1.51
	1.5	2.76	1.85
	2.0	1.78	1.26
2. Bilirubin			
+ UDPglucose	0	1.00	0
	0.5	0.78	0
	1.0	1.03	0.31
	1.5	0.37	0.20
	2.0	0.33	0.33
3. *p*-Nitrophenol			
+ UDPglucuronate	0	1.00	0
	0.5	6.55	1.63
	1.0	9.63	3.37
	1.5	7.50	4.88
	2.0	5.66	2.81

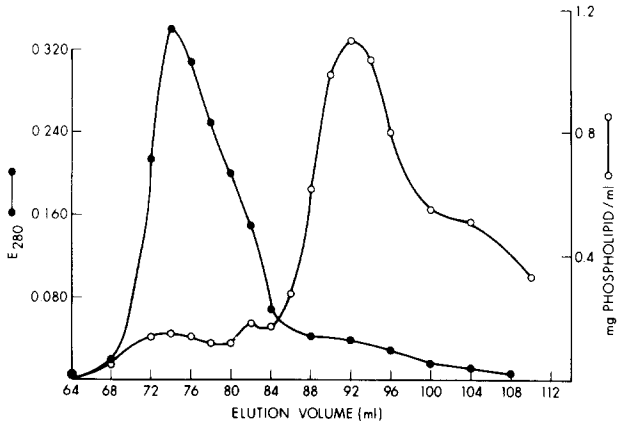

Fig. 1. Separation of microsomal proteins and phospholipids following chromatography on Bio-Gel P-30. Wistar rat liver microsomes were treated with deoxycholate (15 mg deoxycholate/gram microsomal pellet) and chromatography of the 100 000 × *g* supernatant was performed. The chromatography column contained Bio-Gel P-30. As equilibration and elution buffer, 0.02 M Tris/acetate, pH 8.1, containing glycerol (9 : 1, v/v), 4.8 mM sodium deoxycholate, 0.1 M NaCl and 0.1 mM disodium EDTA, was used. Before application to the column, the sample was mixed with bovine serum albumin (4 mg/ml). The protein peak was eluted with the void volume.

of deoxycholate treatment. Maximal activation occurred at 10 mg deoxycholate per g microsomal pellet. No activation was found for glucosidation of bilirubin. For maximal solubilization, a somewhat higher amount of deoxycholate, 15 mg per gram microsomal pellet, was required. Higher deoxycholate concentrations were inhibitory for glucuronidation of *p*-nitrophenol and bilirubin. Supernatant glucosidation was not significantly different at 10, 15 an 20 mg deoxycholate per gram microsomal pellet.

The data in Table I are ratios of activity per ml enzyme suspension. Calculation of ratios as activity per mg microsomal protein, yielded similar results. UDPglucuronosyltransferase and glucosyltransferase activity were solubilized together with the bulk of the microsomal proteins. Selective solubilization was not obtained. Solubilization in this text denotes non-sedimentability after centrifugation at 100 000 × *g*. The 100 000 × *g* supernatant probably consists of highly dispersed membrane fractions which are kept in solution by deoxycholate.

Bovine serum albumin was added to the solubilized fraction (microsomal protein/albumin at approx. 2 : 1, w/w) and this solution was applied to a Bio-Gel P-30 column. Elution with a deoxycholate-containing buffer resulted in separation of the microsomal proteins and phospholipids (Fig. 1). The elution buffer contained 10% glycerol. At 20% glycerol, no separation was obtained. In the absence of glycerol or albumin, enzyme activity was irreversibly destroyed. Chromatography on Bio-Gel P-30 resulted in 92% extraction of phospholipids. The delipidated preparation was extracted according to Folch et al. [12]. Analysis of the extract by thin-layer chromatography revealed only one clearly circumscribed spot with an identical R_F and color as glycerol. The 8% of phospholipid remaining after extraction was presumably in multiple, very faint spots which could not be further identified within the sensitivity of the chromatography system.

TABLE II

REACTIVATION OF BILIRUBIN GLUCURONIDATION WITH MIXED MICROSOMAL LIPID

Delipidated microsomal protein was dialyzed together with mixed microsomal lipids as described in Materials and Methods. After dialysis, an aliquot was removed for enzyme assay. The remainder was diluted 1:1 and centrifuged at 100 000 × *g* for 60 min. Enzyme activity was measured in supernatant and resuspended pellet.

	nmol/mg protein	mg protein/ml	Phospholipid/ protein ratio
Delipidated protein	0	3.78	0.06
Reactivated preparation			
After dialysis	2.98	3.01	0.77
After centrifugation			
Supernatant	0	1.81	0.33
Pellet	4.00	4.55	1.14

The delipidated protein was enzymatically inactive (Table II). Dialysis of the delipidated preparation with mixed lipid, extracted from Wistar rat microsomes reactivated bilirubin glucuronidation. The reactivated preparation was concentrated by centrifugation at 100 000 × *g* (Table II). Dialysis of the delipidated preparation with microsomes from UDPglucuronosyltransferase-deficient Gunn rat liver also reactivated the bilirubin glucuronidation (Table III). This was due to reactivation of the delipidated Wistar rat preparation and not to activation of the Gunn rat preparation. When Gunn rat microsomes were mixed with the deoxycholate-containing elution buffer and dialyzed without added Wistar rat microsomal protein, no activity was obtained. In addition, lipids extracted from Gunn rat microsomes equally reactivated the delipidated preparation. Reactivation only occurred when delipidated protein and lipid preparations or Gunn rat liver microsomes were dialyzed together. Simple addition of lipid or Gunn rat microsomes to non-dialyzed delipidated protein or to the protein after dialysis did not result in reactivation. Delipidated protein, dialyzed in the absence of lipid, was insoluble and precipitated in the dialysis bags.

TABLE III

REACTIVATION OF BILIRUBIN GLUCURONIDATION BY MICROSOMAL MEMBRANES FROM GUNN RAT LIVER

Delipidated microsomal protein from Wistar rat liver was dialyzed with a microsomal preparation from Gunn rat liver and processed as described in the legend of Table II. The value between brackets represents enzyme activity per mg Wistar rat protein. A correction was made for the presence of an estimated amount of Gunn rat liver microsomal protein.

	nmol/mg protein	mg protein/ml	Phospholipid/ protein ratio
Delipidated protein	0	3.78	0.06
Gunn rat microsomes	0	15.40	0.59
Reactivated preparation			
After dialysis	1.04 (2.49)	8.80	0.51
After centrifugation			
Supernatant	0	4.05	0.18
Pellet	0.85	20.00	0.44

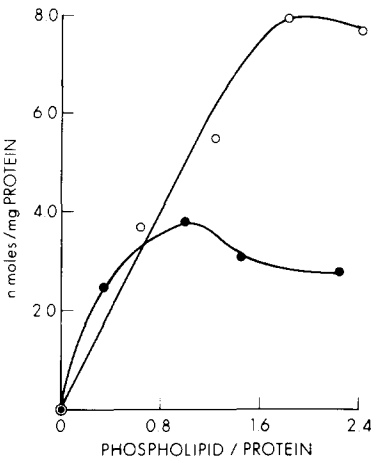

Fig. 2. Bilirubin glucuronidation with preparations reactivated by different amounts of mixed lipid or Gunn rat microsomal membranes. Delipidated Wistar rat microsomal protein was mixed with different amounts of mixed lipid or Gunn rat microsomal membranes and dialyzed. After dialysis the preparations were centrifuged at 100 000 × *g* for 1 h and bilirubun glucuronidation was measured in the pellet. ○———○, reactivated with Gunn rat microsomes; ●———●, reactivated with mixed microsomal lipids. The ordinate represents enzyme activity per mg Wistar rat protein and the abscissa represents the phospholipid: Wistar rat protein ratio. The data were corrected for the presence of Gunn rat liver microsomal protein.

The optimal phospholipid/delipidated protein ratio was determined by adding varying amounts of mixed lipid or Gunn rat microsomes to a constant amount of delipidated protein (Fig. 2). The optimal ratio was approx. 1 when mixed lipids were used for reactivation, and approx. 2 when Gunn rat liver microsomes were used.

Commercial preparations of lecithin, phosphatidylserine and phosphatidylethanolamine were tested as reactivators of the delipidated preparation (Table IV). No reactivation was found with phosphatidylethanolamine. Lecithin was the most potent reactivating phospholipid preparation. Activity was found with phosphatidylserine. A lecithin/protein ratio of about 1 appeared to be optimal for both glucuronidation and glucosidation of bilirubin (Fig. 3).

TABLE IV

REACTIVATION OF BILIRUBIN GLUCURONIDATION BY DIFFERENT PHOSPHOLIPID PREPARATIONS

Delipidated microsomal protein was dialyzed with different phospholipid preparations. Bilirubin glucuronidation was assayed immediately after dialysis.

	nmol/mg protein	Phospholipid/protein ratio
Mixed lipid	6.15	1.29
Lecithin	6.24	2.97
Phosphatidylserine	1.29	2.34
Phosphatidylethanolamine	0	2.95

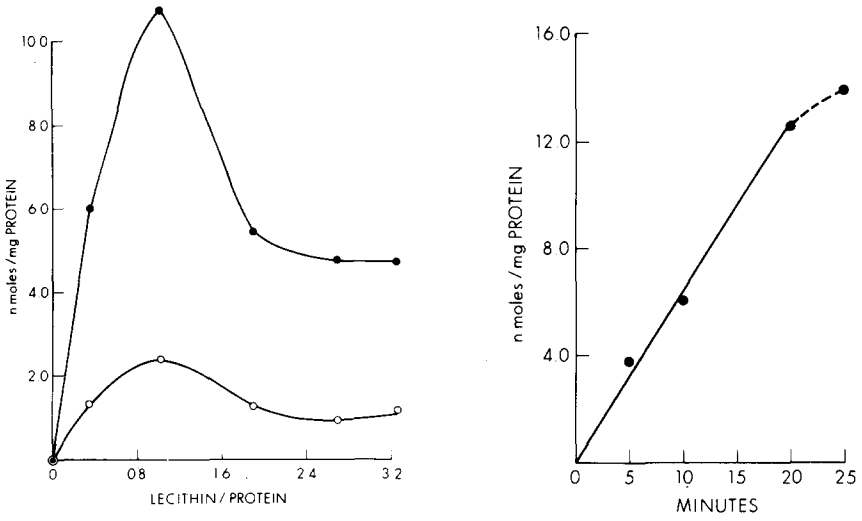

Fig. 3. Glucuronidation and glucosidation of bilirubin by preparations reactivated with different amounts of lecithin. Delipidated microsomal protein was mixed with different amounts of lecithin and dialyzed. After dialysis, the preparations were centrifuged at 100 000 × *g* for 1 h. Glucuronidation and glucosidation of bilirubin were measured in the resuspended pellet. ●——●, bilirubin glucuronidation; ○——○, bilirubin glucosidation.

Fig. 4. Bilirubin glucuronidation as function of incubation time. Delipidated protein was mixed with lecithin in the optimal lecithin/protein ratio. After dialysis and centrifugation (100 000 × *g*, for 1 h), bilirubin glucuronidation was measured in the resuspended pellet.

Marked differences were found when enzyme activity in the reactivated preparations was measured with different substrates. The preparation was completely inactive with *p*-nitrophenol as substrate for glucuronidation (Table V). Relatively high activity was found for glucosyl conjugation of bilirubin. The activity of the lecithin-reactivated preparation amounted to 181% as compared with bilirubin glucosidation activity in normal microsomes. Reactivation with

TABLE V

CONJUGATION OF BILIRUBIN AND *p*-NITROPHENOL WITH NORMAL MICROSOMES AND REACTIVATED PREPARATIONS

Glucuronidation of bilirubin and *p*-nitrophenol and glucosidation of bilirubin were determined in normal microsomes and in reactivated preparations

	Bilirubin +UDPglucuronate	Bilirubin +UDPglucose	*p*-Nitrophenol +UDPglucuronate	Phospholipid/protein ratio
Microsomes	12.00	1.63	474	0.78
Delipidized protein	0	0	0	0.06
Reactivated with mixed lipid	4.71	1.27	0	1.19
Percentage reactivation	39	78		
Reactivated with lecithin	10.79	2.43	0	1.02
Percentage reactivation	90	181		

TABLE VI

STABILITY OF THE DELIPIDATED PREPARATION BEFORE AND AFTER REACTIVATION

A fraction of the delipidated preparation was incubated immediately after elution from Bio-Gel at 37° C. After incubation for 24 and 48 min, samples of this fraction were mixed with lecithin and dialyzed. Another fraction of the delipidated preparation was first mixed with lecithin, reactivated by dialysis and then incubated at 37° C.

Incubation at 37° C	—Lecithin	+Lecithin	Ratio
0	8.22	8.22	1
24	4.69	7.15	0.66
48	0.36	4.59	0.08

respect to glucuronidation of bilirubin amounted to 90% when the optimal lecithin/protein ratio was used.

The stability of the enzyme protein in the absence and presence of lecithin was tested. Immediately after elution from the Bio-Gel column, delipidated protein was incubated at 37° C for 24 and 48 min and subsequently mixed with lipid and dialyzed. The activity of this preparation was compared with a preparation incubated under the same circumstances after reactivation. The former preparation was considerably more labile than the latter (Table VI). Upon reactivation, the reaction rate remained constant for at least 20 min (Fig. 4).

Discussion

UDPglucuronosyltransferase activity is predominantly localized in the microsomal fraction of liver homogenates [1]. Many microsomal enzymes require phospholipids for normal function. For example, lecithin is required for reduction of cytochrome P450 [18]; a lecithin/lysolecithin mixture reactivates delipidated NADH-cytochrome b_5 reductase [19]; and phosphatidylethanolamine is required for glucose-6-phosphatase activity [20]. Graham et al. [21] showed that removal of phospholipids from guinea pig liver microsomes by treatment with phospholipase A and washing with serum albumin, partially inactivates UDPglucuronosyltransferase. Activity was restored on addition of a phospholipid mixture. Our results show that delipidation inactivates UDPglucuronosyltransferase with respect to both bilirubin and *p*-nitrophenol glucuronidation activities. Delipidation also inactivated bilirubin glucosidation.

Deoxycholate is an efficient detergent for the dissolution of membranes [22,23]. Dissociated membranes can be functionally and morphologically reconstituted by dialysis of membrane proteins, lipids and deoxycholate against a $MgCl_2$-containing buffer [22]. Meissner and Fleischer [23] showed that the functional reconstitution of sarcoplasmic reticulum vesicles is closely related to removal of deoxycholate by dialysis. Better reconstitution was obtained when dialysis was performed at room temperature rather than at low temperature. In the present study, addition of lipids or Gunn rat microsomes to delipidated microsomal protein, before or after dialysis, did not reactivate UDPglucuronosyltransferase. Dialysis of delipidated protein, phospholipids or Gunn rat microsomes and deoxycholate under optimal conditions for reconstitution of biologi-

cal membranes [22,23], restored bilirubin glucuronidation and glucosidation activities. The molecular explanation for this reactivation is unknown. Perhaps UDPglucuronosyltransferase is a protein-lecithin complex, which is reconstituted by the employed procedure.

UDPglucuronosyltransferase activity increases in vitro when microsomes are treated with detergents and phospholipases [4,24—30]. Vessey and coworkers [3,24] suggest that digestion of phospholipids per se activates the enzyme, phospholipids in untreated microsomes may constrain enzyme activity, and hydrolysis of phospholipids may cause conformational changes producing an activated but unstable form of UDPglucuronosyltransferase. Our results also suggest that phospholipid may affect conformation of the enzyme protein. In a delipidated form, the enzyme protein was thermolabile and irreversibly inactivated at 37°C whereas the enzyme was more stable in the presence of lecithin. The present studies, however, demonstrate that a specific amount of lecithin is required for UDPglucuronosyltransferase activity. The explanation of the activating effect of phospholipase treatment [3,24] may be that one of the hydrolytic products can replace lecithin.

Delipidation irreversibly inactivated *p*-nitrophenol glucuronidation which was unaffected by procedures suited for reactivation of bilirubin glucuronidation. Other data suggest that bilirubin and *p*-nitrophenol glucuronidation may be mediated by different enzymes [1,6,31—34]. For example, homozygous Gunn rats are deficient in bilirubin glucuronidation but liver homogenates catalyze glucuronidation of *p*-nitrophenol and other compounds [6,31—33].

References

1 Dutton, G.J. (1966) in Glucuronic Acid, Free and Combined (Dutton, G.J., ed.), Academic Press, New York
2 Zakim, D., Goldenberg, J. and Vessey, D.A. (1973) Biochim. Biophys. Acta 309, 67—74
3 Vessey, D.A., Goldenberg, J. and Zakim, D. (1973) Biochim. Biophys. Acta 309, 75—82
4 Mulder, G.J. (1971) Biochem. J. 125, 9—15
5 Mulder, G.J. (1972) Biochim. Biophys. Acta 289, 284—292
6 Jansen, P.L.M. and Henderson, P.Th. (1972) Biochem. Pharmacol. 21, 2457—2462
7 Mowat, A.P. and Arias, I.M. (1970) Biochim. Biophys. Acta 212, 65—78
8 Halac, E. and Reff, A. (1967) Biochim. Biophys. Acta 139, 328—343
9 Isselbacher, K.J., Chrabas, M.F. and Quinn, R.C. (1962) J. Biol. Chem. 237, 3033—3036
10 Lu, A.Y.H. and Levin, W. (1974) Biochim. Biophys. Acta 344, 205—240
11 Rogers, M.J. and Strittmatter, P. (1973) J. Biol. Chem. 248, 800—806
12 Folch, J., Lees, M. and Sloane-Stanley, G.A. (1957) J. Biol. Chem. 226, 497—509
13 Jansen, P.L.M. (1974) Biochim. Biophys. Acta 338, 170—182
14 Fevery, J., Van Hees, G.P., Leroy, P., Compernolle, F. and Heirwegh, K.P.M. (1971) Biochem. J. 125, 803—810
15 Henderson, P.Th. and Kersten, K.J. (1970) Biochem. Pharmacol. 19, 2343—2351
16 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
17 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466—468
18 Strobel, H.W., Lu, A.Y.H., Heidema, J. and Coon, M.J. (1970) J. Biol. Chem. 245, 4851—4854
19 Jones, P.D. and Wakil, S.L. (1967) J. Biol. Chem. 242, 5267—5273
20 Duttera, S.M., Byrne, W.L. and Ganoza, M.C. (1968) J. Biol. Chem. 243, 2216—2228
21 Graham, A.B., Pechey, D.T., Wood, G.C. and Woodcock, B.G. (1974) Biochem. Soc. Trans., in the press
22 Razin, S. (1972) Biochim. Biophys. Acta 265, 241—296
23 Meissner, G. and Fleischer, S. (1974) J. Biol. Chem. 249, 302—309
24 Vessey, D.A. and Zakim, D. (1971) J. Biol. Chem. 246, 4649—4656
25 Graham, A.B. and Wood, G.C. (1973) Biochim. Biophys. Acta 311, 45—50
26 Graham, A.B., Woodcock, B.G. and Wood, G.C. (1974) Biochem. J. 137, 567—574

27 Winsnes, A. (1969) Biochim. Biophys. Acta 191, 279—291
28 Winsnes, A. (1972) Biochim. Biophys. Acta 284, 394—405
29 Lueders, K.K. and Kuff, E.L. (1967) Arch. Biochem. Biophys. 120, 198—203
30 Hänninen, O. and Puukka, R. (1971) Chem. Biol. Interactions 3, 282—284
31 van Leusden, H.A.I.M., Bakkeren, J.A.J.M., Zilliken, F. and Stolte, L.A.M. (1962) Biochem. Biophys. Res. Commun. 7, 67—69
32 Drucker, W.D. (1968) Proc. Soc. Exp. Biol. Med. 129, 308—311
33 Arias, I.M. (1961) Biochem. Biophys. Res. Commun. 6, 81—84
34 Bock, K.W., Fröhling, W., Remmer, H. and Rexer, B. (1973) Biochim. Biophys. Acta 327, 46—56