Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 6147–6151, August 1988
Medical Sciences

Functional reconstitution of the canalicular bile salt transport system of rat liver

(taurocholate/proteoliposomes/membrane carrier/glycoproteins)

STEPHAN RUETZ*†, GABRIEL HUGENTOBLER*, AND PETER J. MEIER*‡

*Division of Clinical Pharmacology, Department of Medicine, University Hospital, Zurich, Switzerland; and †Laboratorium für Biochemie, Eidgenössische Technische Hochschule, Zurich, Switzerland

Communicated by Rudi Schmid, May 9, 1988 (received for review February 25, 1988)

ABSTRACT **Recent studies have suggested that the canalicular bile salt transport system of rat liver corresponds to a 100-kDa membrane glycoprotein. In the present study we attempted to functionally reconstitute the 100-kDa protein into artificial proteoliposomes. *Canalicular* membrane proteins were solubilized with octyl glucoside in the presence of asolectin phospholipids. The extracts were treated with preimmune serum or the 100-kDa protein selectively immunoprecipitated with a polyclonal antiserum. Proteins remaining in the supernatant were then incorporated into proteoliposomes by gel-filtration chromatography. Canalicular proteoliposomes containing the 100-kDa protein exhibited transstimulatable taurocholate uptake that could be inhibited by 4,4′-diisothiocyanato-2,2′-stilbenedisulfonic acid (DIDS). In contrast, no DIDS-sensitive transstimulatable taurocholate uptake was found in 100-kDa protein-free canalicular proteoliposomes. However, when the immunoprecipitated 100-kDa protein was dissociated from the antibodies and exclusively incorporated into liposomes, reconstitution of DIDS-sensitive transstimulatable and electrogenic taurocholate anion transport was again positive. Although incorporation of solubilized *basolateral* membrane proteins into liposomes also resulted in a prompt reconstitution of Na^+ gradient-driven taurocholate uptake, the anti-100-kDa antibodies had no effects on the reconstituted transport activity of basolateral proteins. Thus, the findings establish that the previously characterized canalicular-specific 100-kDa protein is directly involved in the transcanalicular secretion of bile salts.**

To maintain ongoing vectorial secretion of bile salts from blood into bile, hepatocytes localize distinct transport systems at their basolateral (sinusoidal and lateral) and canalicular surface domains (1, 2). The involved transport systems have recently been identified and partially characterized by using photoaffinity-labeling techniques and kinetic transport studies in intact hepatocytes as well as isolated plasma membrane vesicles (3–6). Thus, basolateral uptake of bile salts is mediated by one or two polypeptides with molecular masses between 48 and 54 kDa (3, 7, 8). In contrast, a distinct polypeptide with a molecular mass of 100 kDa appears to be involved in the canalicular secretion of bile salts. The latter conclusion was primarily supported by the following observations: (*i*) photoaffinity-labeling studies in highly purified canalicular membrane vesicles as well as in tiny liver snips, in which the structural and functional polarity of individual hepatocytes is maintained, resulted in the exclusive incorporation of the photoreactive taurocholate analogue (7,7-azo-3α,12α-dihydroxy-5β-[3β-^{3}H]cholan-24-oyl)-2-aminoethanesulfonate into a canalicular 100-kDa glycoprotein (9, 10) and (*ii*) antibodies against the purified 100-kDa bile salt binding protein inhibited taurocholate uptake into as well as taurocholate efflux from isolated canalicular vesicles (10). However, although these studies provided suggestive evidence, they did not definitely establish that the characterized 100-kDa bile salt binding protein can directly mediate transcanalicular movement of bile salts. To prove this assumption we developed a reconstitution procedure that permitted the definite demonstration of the transport function of the 100-kDa bile salt binding polypeptide in artificial proteoliposomes (PLP). Part of this work has been presented in abstract form (11).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (SUT:SDT Süddeutsches Tierzuchtinstitut, Tuttlingen, F.R.G.) weighing 200–250 g were used throughout this study. The animals had free access to water, were fed ad libitum (Nafag 690 diet, Gossau, Switzerland), and housed at a constant temperature and humidity with alternating 12-hr light (6:30 a.m. to 6:30 p.m.) and dark cycles. Fed animals were regularly killed by decapitation between 7:30 and 8:30 a.m.

Isolation of Canalicular and Basolateral Liver Plasma Membrane Subfractions. Highly purified canalicular and basolateral membrane vesicles were isolated from rat liver as described (12). The membrane subfractions were resuspended either in 300 mM mannitol/100 mM KCl/20 mM Hepes/Tris, adjusted to pH 7.4 (MBK_{100} buffer; Figs. 2, 3, and 5), or in 200 mM mannitol/50 mM tetramethylammonium, 100 mM K^+/150 mM gluconate/20 mM Hepes/Tris, adjusted to pH 7.4 ($MBTK_{100}$ buffer; Fig. 4). The membrane suspensions were stored frozen in liquid nitrogen (protein concentration >5 mg/ml) for up to 2 weeks. Immediately before use the samples were quick-thawed by immersion into a 37°C water bath.

Solubilization of Plasma Membrane Proteins. Membrane solubilization was routinely performed in the presence of soybean phospholipids (asolectin; Associated Concentrates, Woodside, NY) and octyl glucoside (*n*-octyl β-D-glucopyranoside; Bachem, Burgdorf, Switzerland) at final concentrations of 3.3 mg/ml and 100 mM, respectively (detergent/phospholipid ratio, 9:1). A phospholipid stock solution was first prepared in diethyl ether (25 mg/ml) and filtered once through a nitrocellulose filter (pore size, 0.45 μm; Millex-HA, Millipore, Molsheim, France). From this stock solution an aliquot of 161 μl (4.0 mg of asolectin phospholipids) was evaporated under vacuum in a glass tube for 1 hr at room temperature. The dried phospholipids were redissolved in 0.6 ml of appropriate membrane resuspension buffer (i.e.,

Abbreviations: PLP, proteoliposome(s); PLP + 100, PLP containing only the 100-kDa bile salt binding polypeptide; cPLP, canalicular PLP; cPLP + 100, cPLP containing all canalicular membrane proteins; cPLP − 100, cPLP immunodepleted of the 100-kDa bile salt binding polypeptide; DIDS, 4,4′-diisothiocyanato-2,2′-stilbenedisulfonic acid.

‡To whom reprint requests should be addressed at: Division of Clinical Pharmacology, Department of Medicine, University Hospital, CH-8091 Zurich, Switzerland.

MBK_{100} or $MBTK_{100}$, respectively) that contained in addition 200 mM octyl glucoside. After addition of a 0.6-ml aliquot of isolated plasma membrane subfractions (4 mg of protein) the whole suspension (total volume, 1.2 ml) was gently stirred on ice for 15 min and finally centrifuged at 105,000 × *g* for 30 min. The clear supernatant (= membrane solubilization extract) represented the starting material for all subsequent immunoprecipitation experiments.

Immunoprecipitation of the Canalicular Bile Salt Binding Polypeptide. The production and characterization of the monospecific antiserum against the canalicular 100-kDa bile salt binding polypeptide has been described (10). Protein A-Sepharose was prepared as a 30% stock solution in MBK_{100} or $MBTK_{100}$ buffer. One-milliliter stock aliquots were incubated overnight at 4°C with either 0.5 ml of preimmune or 0.5 ml of anti-100-kDa serum. The immunoglobulin-coated protein A-Sepharose beads were then subjected to four washing steps with the corresponding membrane resuspension buffer containing either 0.2% bovine serum albumin (steps 1 and 2) or 100 mM octyl glucoside and 3.3 mg of asolectin phospholipids per ml (steps 3 and 4). Subsequently, 1.2 ml of membrane solubilization extract was added. The mixture was then incubated with slight shaking for 60 min at 4°C. After careful sedimentation of the beads (13) two different membrane solubilization extracts were obtained, one containing all solubilized membrane proteins including the 100-kDa antigen (protein A-Sepharose beads incubated with preimmune serum) and the other specifically devoid of the 100-kDa bile salt binding protein (protein A-Sepharose beads incubated with the anti-100-kDa serum) (see Fig. 1).

In addition, a third extract containing only the solubilized 100-kDa protein was prepared by dissociating the antigen from the pelleted antibody-coated protein A-Sepharose beads (see Fig. 1). For this purpose the protein A-Sepharose-antibody/antigen complexes were washed twice with membrane resuspension buffer containing 100 mM octyl glucoside and 3.3 mg of asolectin phospholipids per ml and subsequently incubated at 4°C in 1.2 ml of dissociation buffer (300 mM mannitol/100 mM KCl/20 mM Hepes/HCl, pH 3.0/100 mM octyl glucoside/3.3 mg of asolectin phospholipids per ml). After 5 min the pH of the incubation mixture was titrated back to 7.4 with concentrated Tris. The efficiency of the antigen/antibody dissociation procedure was checked by $NaDodSO_4$/PAGE of the final supernatant.

Production of Canalicular PLP (cPLP). After immunoprecipitation the three solubilization extracts of canalicular membranes were supplemented with Triton X-100 to a final concentration of 100 μM. Furthermore, where the final cPLP had to be preloaded with taurocholate (e.g., transstimulation experiments; Figs. 2 and 3), 100 μM of the conjugated bile salt was also added. The detergents octyl glucoside and Triton X-100 were then removed from the mixed micellar solutions by gel filtration over Sephadex G-50 superfine columns (1.5 × 30 cm; Pharmacia, Uppsala, Sweden) (14–16). The columns were preequilibrated and eluted at a flow rate of 0.33 ml/min either with MBK_{100} plus 100 μM taurocholate (Figs. 2 and 3) or with $MBTK_{100}$ without taurocholate (Fig. 4). Samples of 2.5 ml were collected. The fractions containing unilamellar vesicles (turbidity measurements at 700 nm) were pooled and centrifuged at 290,000 × *g* for 90 min. The recovered cPLP were finally resuspended in 0.4 ml of appropriate transport medium (see below) and kept on ice for taurocholate uptake studies.

Production of Basolateral PLP. For reconstitution of Na^+-dependent basolateral taurocholate transport the same liposomal preparation procedure as outlined above was used; the only modification was that the Sephadex G-50 columns were preequilibrated and eluted with taurocholate-free MBK_{100} buffer.

Taurocholate Transport in PLP. [^{3}H]Taurocholate (6.6 Ci/mmol; 1 Ci = 37 GBq; DuPont–New England Nuclear) uptake into the various PLP preparations was determined by a rapid filtration assay as described (2). PLP prepared from 4 mg of plasma membrane protein were routinely resuspended and preincubated for 10 min at 25°C in 0.4 ml of appropriate resuspension buffers (buffer preloading of PLP). All vesicles were treated with valinomycin (3.0 μg/mg of phospholipids) to collapse transmembrane potential differences (i.e., in Figs. 2, 3, and 5) or to create an intravesicular positive K^+ diffusion potential (i.e., in Fig. 4). Aliquots of 10 μl of vesicle suspensions were incubated at 37°C in 190 μl of the respective transport media that were supplemented with 1 μM [^{3}H]taurocholate and, where indicated, also with 0.5 mM 4,4'-diisothiocyanato-2,2'-stilbenedisulfonic acid (DIDS). The exact compositions of the PLP resuspension buffers (intravesicular buffers) as well as of the various transport media (extravesicular buffers) are detailed in the corresponding figure legends. After the indicated time peri-

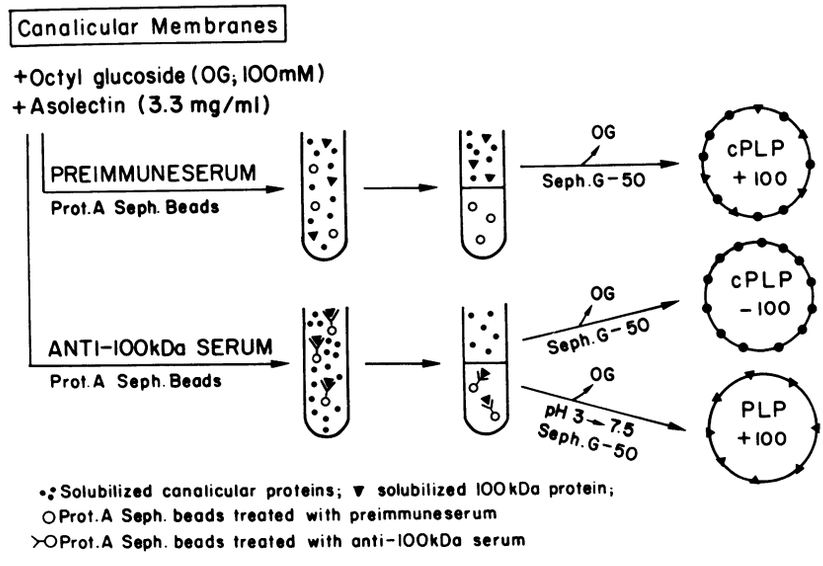

FIG. 1. Schematic outline of preparation of cPLP for reconstitution experiments. cPLP + 100, cPLP containing all canalicular membrane proteins; cPLP − 100, cPLP immunodepleted of the 100-kDa bile salt binding protein; PLP + 100, PLP containing only the 100-kDa bile salt binding polypeptide.

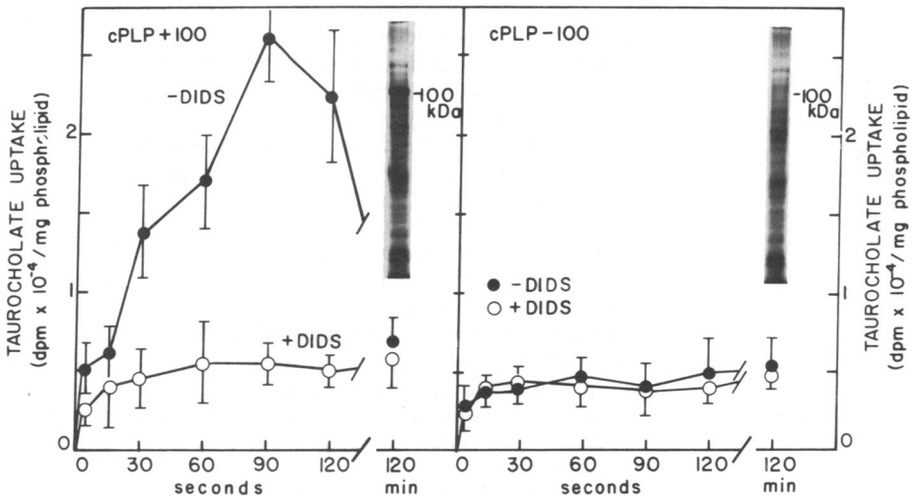

FIG. 2. DIDS-sensitive transstimulatable taurocholate uptake into cPLP in the presence (cPLP + 100) and absence (cPLP − 100) of the 100-kDa bile salt binding polypeptide. Taurocholate (100 μM)-preloaded cPLP were resuspended and preincubated (10 min, 25°C) in 300 mM mannitol/20 mM KCl/20 mM Hepes/Tris, pH 7.4, and treated with valinomycin (3.0 μg/mg of phospholipid). Ten-microliter cPLP suspensions were incubated in 190 μl of transport medium that was of similar composition as the cPLP resuspension buffer. Tracer taurocholate (1 μM) uptake was determined in the presence (○) or absence (●) of 0.5 mM DIDS. Data represent the means ± SD of triplicate determinations in three separate cPLP preparations. In parallel, protein compositions of the two types of cPLP were analyzed by $NaDodSO_4$/PAGE (*Insets*).

ods, tracer taurocholate uptakes were terminated by addition of 2 ml of ice-cold stop solution consisting either of 300 mM mannitol/20 mM KCl/20 mM Hepes/Tris, pH 7.4 (Figs. 2, 3, and 5), or of 50 mM mannitol/50 mM tetramethylammonium/100 mM K^+/150 mM gluconate/20 mM Hepes/Tris, pH 7.4 (Fig. 4). After two additional washing steps, the vesicle-associated radioactivity retained on the filters was determined by liquid scintillation counting as described (10).

Other Methods. Protein concentrations were determined according to Lowry *et al.* (17) using bovine serum albumin as standard. Total phospholipids were estimated by the method of Chen *et al.* (18). $NaDodSO_4$/PAGE was performed as described (10), and the separated polypeptide bands were stained with Coomassie brilliant blue R (19) or by the silver stain technique (20).

RESULTS

The various types of cPLP produced are schematically illustrated in Fig. 1. The average diameter of all isolated PLP amounted to 137 ± 66 nM (means ± SD; n = 3), as determined by light-scattering analysis. Furthermore, electron micrographs of freeze-fracture replicas revealed the exclusive presence of unilamellar vesicles with numerous incorporated protein particles (data not shown).

Taurocholate Uptake into cPLP. Previous studies with native canalicular membrane vesicles demonstrated the following characteristics of transcanalicular [^{3}H]taurocholate transport: (*i*) marked stimulation by the simultaneous presence of unlabeled taurocholate on the trans side of the canalicular membrane (transstimulation or countertransport) (2, 10, 21, 22); (*ii*) sensitivity to the anion transport inhibitor DIDS (10, 22); and (*iii*) stimulation of taurocholate uptake into or efflux from canalicular vesicles by intravesicular positive or negative K^+ diffusion potentials, respectively (2, 10, 21–23). Consequently, the same transport characteristics were also used to test the functional integrity of the canalicular bile salt transport system in the PLP. Thereby, uptake rather than efflux studies were exclusively performed since they did not require the inclusion of radioactive taurocholate during the cPLP preparation procedure. As demonstrated in Fig. 2, preloading of cPLP + 100 with unlabeled taurocholate (100 μM) transiently stimulated [^{3}H]taurocholate (1 μM) uptake ≈5-fold above equilibrium values at 2 hr ("overshoot"). This transstimulatable taurocholate uptake was completely blocked by 0.5 mM DIDS. In contrast, no DIDS-sensitive transstimulatable taurocholate uptake was observed in cPLP − 100, in which the 100-kDa bile salt binding polypeptide had been selectively removed by immunoprecipitation (Fig. 2). However, if the immunoprecipitated 100-kDa protein was dissociated from the antibodies and selectively incorporated into the liposomes (PLP + 100), transstimulation of [^{3}H]taurocholate uptake was again positive and could also be inhibited by DIDS (Fig. 3). Furthermore, in taurocholate-free PLP + 100, an inside positive K^+ diffusion potential (K^+ out > in, 100 mM; plus valinomycin) markedly stimulated [^{3}H]taurocholate uptake as compared to voltage-clamped conditions (K^+ out = in, 100 mM; plus valinomycin) (Fig. 4). Thus, the most characteristic properties of taurocholate anion transport in intact canalicular vesicles, such as transstimulation, inhibition by DIDS, and potential sensitivity, could be fully reconstituted by incorporation of the 100-kDa bile salt binding polypeptide (10) into PLP.

Taurocholate Uptake into Basolateral PLP. Basolateral uptake of taurocholate is a Na^+-dependent cotransport process that can also be inhibited by DIDS (1–3, 5, 8, 24–27). To evaluate the suitability of the adopted procedure for the eventual additional reconstitution of the basolateral bile salt transport system(s), we next determined Na^+ gradient-driven taurocholate uptake into basolateral PLP. Indeed, as demonstrated in Fig. 5, incorporation of octyl glucoside-solubilized basolateral proteins into asolectin liposomes resulted in a transient, DIDS-sensitive accumulation of taurocholate within the vesicles (overshoot). Furthermore, and most important, the anti-100-kDa serum exerted no effects on

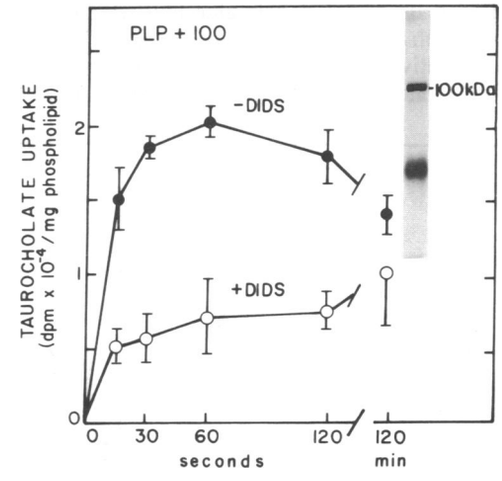

FIG. 3. DIDS-sensitive transstimulatable taurocholate uptake into PLP + 100. [^{3}H]Taurocholate uptake studies were performed as outlined in the legend to Fig. 2. Data represent the means ± SD of nine determinations in three separate PLP preparations. (*Inset*) The low molecular mass protein band on the demonstrated $NaDodSO_4$/polyacrylamide gel represents IgG.

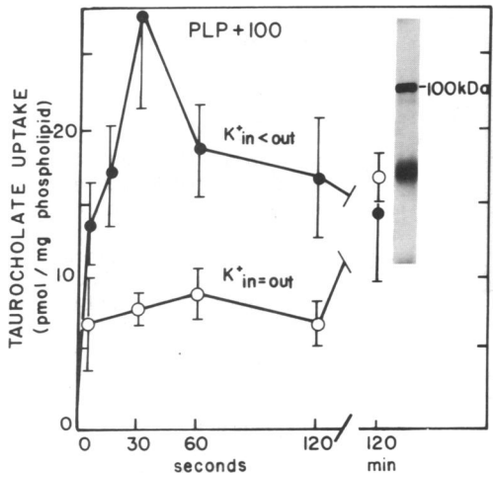

FIG. 4. Electrogenic taurocholate uptake into PLP + 100. PLP + 100 subfractions were resuspended and preincubated (10 min, 25°C) either in K^+-free buffer (●) (250 mM mannitol/50 mM tetramethylammonium/50 mM gluconate/20 mM Hepes/Tris, pH 7.4) or in a K^+-containing medium (○) (50 mM mannitol/50 mM tetramethylammonium/100 mM K^+/150 mM gluconate/20 mM Hepes/Tris, pH 7.4). After treatment with valinomycin, aliquots (10 μl) of K^+-free (●) and K^+-preloaded (○) vesicles were incubated in transport medium (190 μl), the composition of which was identical to the K^+-containing resuspension buffer. Hence, [^{3}H]taurocholate (1 μM) uptakes were determined in the presence of an intravesicular-positive K^+ diffusion potential (K^+ in < out; 100 mM) or under voltage-clamped conditions (K^+ in = out; 100 mM), respectively. Data represent the means ± SD of nine determinations in three separate PLP preparations. A representative $NaDodSO_4$/polyacrylamide gel of the PLP + 100 subfraction used is also shown. (*Inset*) The low molecular mass protein band corresponds to IgG.

the reconstituted Na^+ gradient-driven basolateral uptake of taurocholate (Fig. 5*B*) as compared to the preimmune serum (Fig. 5*A*). Thus, the developed reconstitution procedure should be very useful for further characterization of the basolateral bile salt transport system(s) as well.

DISCUSSION

The present study demonstrates successful reconstitution of the canalicular and basolateral taurocholate transport systems into artificial PLP. In *canalicular* PLP (Fig. 1), the reconstitution of native canalicular taurocholate transport properties, such as transstimulation, inhibition by DIDS, and potential sensitivity (2, 10, 21–23), required the presence of the 100-kDa bile salt binding polypeptide (10) within the liposomal membrane (Figs. 2–4). These findings prove that the previously characterized canalicular-specific 100-kDa glycoprotein (10) can directly mediate transmembrane movement of bile salts and, therefore, most probably represents the canalicular bile salt transport system or a component of it. Furthermore, the lack of any inhibitory effects of the anti-100-kDa serum on Na^+-dependent taurocholate uptake into *basolateral* PLP (Fig. 5) provides further evidence that the canalicular and basolateral bile salt transport systems represent different membrane proteins in hepatocytes (9, 10). Interestingly, similar to native basolateral plasma membrane vesicles (2, 24–26), the basolateral PLP also exhibited transient intravesicular accumulation of [^{3}H]taurocholate in the presence of an out-to-in Na^+ gradient (Fig. 5). This preservation of the Na^+ gradient-dependent overshoot phenomenon generally indicates that the followed procedure may be especially well suited for reconstitution of membrane transport functions.

The adopted reconstitution method was modeled after those previously described for other purposes (14–16). In principle, it consists of forming PLP directly from protein-containing, phospholipid-supplemented, and detergent-rich mixed micelles (14) rather than starting with mixtures of phospholipid and protein in separate micellar solutions (8, 28). By this means possible harmful delipidation and/or aggregation of hydrophobic proteins can be prevented. Thereby, octyl glucoside rather than Triton X-100 should be used as detergent, since octyl glucoside can be more efficiently removed from mixed micellar complexes due to its high critical micellar concentration (25 mM) (14, 15). In addition, our studies confirm that at a detergent/lipid ratio above 5:1 the rapid chromatographic removal of octyl glucoside from protein-containing mixed micelles results in the predominant formation of large unilamellar vesicles with diameters ranging between 100 and 200 nm (14). Thus, better preservation of the lipid environment and/or formation of more uniform and larger PLP may partially explain the better reconstitution of Na^+ gradient-dependent taurocholate uptake into basolateral PLP (Fig. 5) compared to Triton X-100-solubilized sinusoidal plasma membrane proteins incorporated into liposomes prepared by sonication and freeze-thaw procedures (8).

Furthermore, the developed reconstitution procedure provided the unique possibility of quantitatively immunoprecipitating the 100-kDa bile salt binding polypeptide from the initial mixed micellar solutions (Fig. 1). As a consequence, all PLP could be formed under identical conditions, thus permitting the direct correlation of typical canalicular taurocholate transport properties with the presence of incorpo-

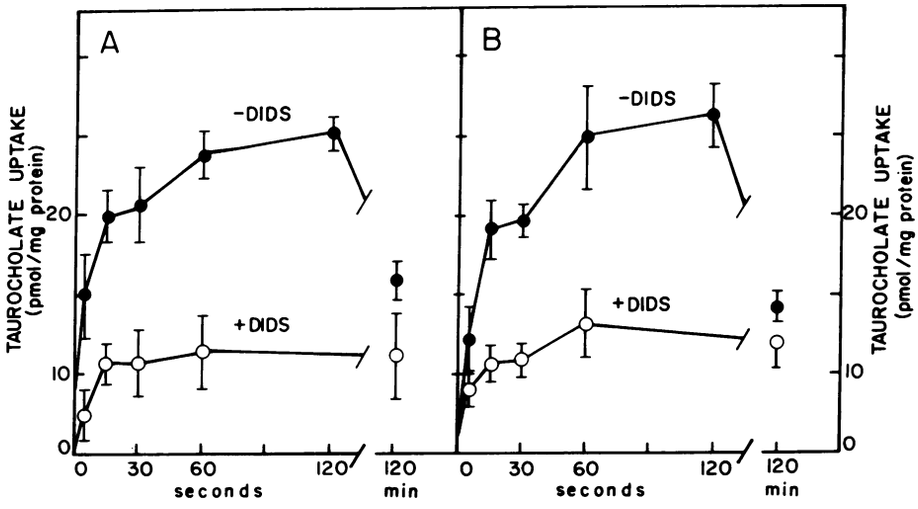

FIG. 5. Reconstitution of Na^+ gradient-driven taurocholate uptake in basolateral PLP. Solubilized basolateral membrane proteins were exposed to protein A-Sepharose treated with either preimmune serum (*A*) or anti-100-kDa serum (*B*). From the corresponding supernatants, basolateral PLP were prepared, resuspended, and preincubated (10 min, 25°C) in 300 mM mannitol/20 mM KCl/20 mM Hepes/Tris, pH 7.4. After treatment with valinomycin, [^{3}H]-taurocholate (1 μM) uptakes were determined by incubation of 10 μl of PLP suspensions in 190 μl of transport medium (100 mM mannitol/20 mM KCl/100 mM NaCl/20 mM Hepes/Tris, pH 7.4) containing either no DIDS (●) or 0.5 mM DIDS (○). Data represent the means ± SD of nine determinations in three separate PLP preparations.

rated 100-kDa protein (Figs. 2–4). The data cannot be explained by unspecific effects of incorporated proteins on liposomal permeability since (*i*) transient intravesicular accumulation (overshoot) of tracer taurocholate was observed under all DIDS-free incubation conditions (Figs. 2–5) and (*ii*) DIDS-sensitive transstimulatable taurocholate uptake was completely lost in cPLP − 100 despite the presence of numerous other canalicular proteins within the liposomal membrane (Fig. 2). Parenthetically, protein-free liposomes exhibited only minimal taurocholate uptake activity within a 2-min period (data not shown). Finally, and most important, the immunoprecipitation-induced annihilation of transstimulatable taurocholate uptake activity (Fig. 2) could almost completely be restored by subsequent antibody dissociation and selective incorporation of the 100-kDa polypeptide into the PLP (Fig. 3). Hence, if normalized to the amount of 100-kDa protein that accounts for ≈5–8% of total canalicular membrane proteins (10), the selective reconstitution of immunopurified 100-kDa protein (PLP + 100) was associated with a 10- to 20-fold increase in specific transport activity compared to the reconstitution of the whole canalicular membrane extract (cPLP + 100). However, since the degree of physical purification was considerably higher (compare gels in Figs. 2 and 3), it is likely that formation of PLP + 100 nevertheless resulted in some inactivation of transport activity. This may have been caused by either (*i*) incomplete incorporation of the immunopurified 100-kDa protein into the liposomes, (*ii*) altered conformation and orientation of the 100-kDa protein within the lipid bilayer, and/or (*iii*) the continuous presence of residual amounts of inhibitory IgG antibodies (Figs. 3 and 4). As it may be, it is the qualitative similarity between native and reconstituted canalicular taurocholate transport properties rather than the quantitative increase in specific transport activity that essentially proves the bile salt transport function of the 100-kDa polypeptide.

The reconstituted canalicular taurocholate transport properties further support the concept that canalicular secretion of bile salts is mediated by potential-sensitive facilitated diffusion (2, 10, 21–23, 29). However, the studies permit no definite conclusions yet with respect to the type of transport system or the gating mechanism(s) involved, since both simple "carriers" as well as complex "channels" can demonstrate the phenomenon of transstimulation or countertransport (30). Furthermore, it remains to be investigated whether the reconstituted 100-kDa bile salt transporting polypeptide is identical with one of the recently characterized 100- to 110-kDa canalicular-specific glycoproteins of unknown function (31–40). One of these proteins has recently been cloned, and its primary amino acid sequence was determined (40). The reported data indicate that the so-called gp110 glycoprotein contains only one membrane-spanning domain, which makes it a rather unlikely candidate for a membrane transport protein, unless it is organized *in vivo* as a multimeric membrane protein complex. Although further studies are required to ultimately elucidate its exact molecular properties, the present study establishes that the canalicular bile salt transport system corresponds to a 100-kDa membrane polypeptide. Furthermore, the developed reconstitution procedure should be of considerable help in identification, isolation, and functional characterization of other membrane transport systems as well.

We thank Prof. Dr. K. Winterhalter from the Swiss Federal Institute of Technology, Zurich, for his continuous support and many useful suggestions. We also thank Prof. Dr. H. G. Weder from the Pharmaceutical Institute, Zurich, for routine light-scattering analysis of the proteoliposomes. The studies were supported by Swiss National Science Foundation Grant 3.983.0.84.

1. Coleman, R. (1987) *Biochem. J.* **244,** 229–261.
2. Meier, P. J., Meier-Abt, S., Barrett, C. & Boyer, J. L. (1984) *J. Biol. Chem.* **259,** 10614–10622.
3. Berk, P. D., Potter, B. J. & Stremmel, W. (1987) *Hepatology (Baltimore)* **7,** 165–176.
4. Kramer, W., Bickel, U., Buscher, H. P., Gerok, W. & Kurz, G. (1982) *Eur. J. Biochem.* **129,** 13–24.
5. von Dippe, P. & Levy, D. (1983) *J. Biol. Chem.* **258,** 8896–8901.
6. Ziegler, K., Frimmer, M. & Fasold, H. (1984) *Biochim. Biophys. Acta* **769,** 117–129.
7. Wieland, T., Nassal, M., Kramer, W., Fricker, G., Bickel, U. & Kurz, G. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5232–5236.
8. von Dippe, P., Ananthanarayanan, M., Drain, P. & Levy, D. (1986) *Biochim. Biophys. Acta* **862,** 352–360.
9. Fricker, G., Schneider, S., Gerok, W. & Kurz, G. (1987) *Biol. Chem. Hoppe-Seyler* **368,** 1143–1150.
10. Ruetz, S., Fricker, G., Hugentobler, G., Winterhalter, K., Kurz, G. & Meier, P. J. (1987) *J. Biol. Chem.* **262,** 11324–11330.
11. Ruetz, S., Hugentobler, G. & Meier, P. J. (1987) *Hepatology (Baltimore)* **7,** 1105 (abstr.).
12. Meier, P. J., Sztul, E. S., Reuben, A. & Boyer, J. L. (1984) *J. Cell Biol.* **98,** 991–1000.
13. Hauri, H. P., Sterchi, E. E., Bienz, D., Fransen, J. A. M. & Marxer, A. (1985) *J. Cell Biol.* **101,** 838–851.
14. Mimms, L. T., Zampighi, G., Nozaki, Y., Tanford, C. & Reynolds, J. A. (1981) *Biochemistry* **20,** 833–840.
15. Green, P. R. & Bell, R. M. (1984) *J. Biol. Chem.* **259,** 14688–14694.
16. Fischl, A. S., Homann, M. J., Poole, M. A. & Carman, G. M. (1986) *J. Biol. Chem.* **261,** 3178–3183.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
18. Chen, P. S., Torigara, T. Y. & Warner, H. (1956) *Anal. Chem.* **28,** 1756–1758.
19. Steck, G., Leuthard, P. & Bürk, R. P. (1980) *Anal. Biochem.* **107,** 21–24.
20. Merril, C. R., Dunaum, M. L. & Goldman, D. (1981) *Anal. Biochem.* **110,** 201–207.
21. Inoue, M., Kinne, R., Tran, T. & Arias, I. M. (1984) *J. Clin. Invest.* **73,** 659–663.
22. Meier, P. J., Meier-Abt, A. S. & Boyer, J. L. (1987) *Biochem. J.* **242,** 465–469.
23. Griffiths, J. C., Sies, H., Meier, P. J. & Akerboom, P. M. (1987) *FEBS Lett.* **213,** 34–38.
24. Duffy, M. C., Blitzer, B. L. & Boyer, J. L. (1983) *J. Clin. Invest.* **72,** 1470–1481.
25. Inoue, M., Kinne, R., Tran, T. & Arias, I. M. (1982) *Hepatology (Baltimore)* **2,** 572–579.
26. Suchy, F. J., Bucuvalas, J. C., Goodrich, A. L., Moyer, M. S. & Blitzer, B. L. (1986) *Am. J. Physiol.* **251,** G665–G673.
27. Zimmerli, B., Valantinas, J. & Meier, P. J. (1987) *Hepatology (Baltimore)* **7,** 1036 (abstr.).
28. Hokin, L. E. (1981) *J. Membr. Biol.* **60,** 77–93.
29. Weinman, S. A., Scaramuzza, D. M. & Boyer, J. L. (1987) *Hepatology (Baltimore)* **7,** 1105 (abstr.).
30. Macey, R. I. (1986) in *Physiology of Membrane Disorders*, eds. Andreoli, T. E., Hoffman, J. F., Fanestil, D. D. & Schultz, S. G. (Plenum, New York), pp. 111–131.
31. Kreisel, W., Volk, A. B., Büchsel, R. & Reutter, W. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1828–1831.
32. Cook, J., Hou, E., Hou, Y., Cairo, A. & Doyle, D. (1983) *J. Cell Biol.* **97,** 1823–1833.
33. Hixson, D. C., Allison, J. P., Chesner, J. E., Leger, M. J., Ridge, L. L. & Walborg, E. F. (1983) *Cancer Res.* **43,** 3874–3884.
34. Hixson, D. C., McEntire, K. D. & Obrink, B. (1985) *Cancer Res.* **45,** 3742–3749.
35. Bartles, J. R., Braiterman, L. T. & Hubbard, A. L. (1985) *J. Biol. Chem.* **260,** 12792–12802.
36. Becker, A., Neumeier, R., Park, C. S., Gossrau, R. & Reutter, W. (1985) *Eur. J. Cell Biol.* **39,** 417–423.
37. Sadoul, J. L., Peyron, J. F., Balloti, R., Debaut, A., Fehlmann, M. & Van Obberghen, E. (1985) *Biochem. J.* **227,** 887–892.
38. Odin, P., Tingström, A. & Obrink, B. (1986) *Biochem. J.* **236,** 559–568.
39. Petell, J. K., Diamond, M., Hong, W., Bujanover, Y., Amarri, S., Pittschieler, K. & Doyle, D. (1987) *J. Biol. Chem.* **262,** 14753–14759.
40. Hong, W. & Doyle, D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7962–7966.