In Vitro Evidence That Phospholipid Secretion into Bile May Be Coordinated Intracellularly by the Combined Actions of Bile Salts and the Specific Phosphatidylcholine Transfer Protein of Liver†

David E. Cohen,* Monika R. Leonard, and Martin C. Carey

Department of Medicine, Harvard Medical School and Harvard Digestive Diseases Center, Brigham and Women's Hospital, Boston, Massachusetts 02115

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ABSTRACT: Using model systems, we explored a potential function of hepatic phosphatidylcholine transfer protein to extract biliary-type phosphatidylcholines from intracellular membranes (e.g., smooth endoplasmic reticulum) and deliver them to canalicular plasma membranes where biliary secretion occurs. We measured transfer rates of parinaroyl phosphatidylcholine, a naturally fluorescent phospholipid, from small unilamellar vesicles composed of sn-1 palmitoyl, sn-2 parinaroyl phosphatidylcholine, and egg yolk phosphatidylcholine (molar ratio 75:25) wherein the fluorophore is self-quenched to small unilamellar vesicles composed of phosphatidylethanolamine, sphingomyelin, phosphatidylserine, phosphatidylinositol, and cholesterol (molar ratios 22:22:10:8:38) representing model microsomal and canalicular plasma membranes, respectively. Following addition of phosphatidylcholine transfer protein (purified from bovine liver), fluorescence intensity increased exponentially indicating net phosphatidylcholine transfer from donor to acceptor vesicles. Submicellar concentrations of a wide hydrophobicity range of common and uncommon taurine and glycine conjugated bile salts species (anionic steroid detergent-like molecules), sodium taurofusidate (a conjugated fungal bile salt analog), and sodium dodecyl sulfate and octylglucoside, anionic and nonionic straight chain detergents, respectively, markedly stimulated phosphatidylcholine transfer protein activity. This 40–115-fold effect was most pronounced for the common bile salts and correlated positively with bile salt hydrophobicity. Thermodynamic analysis of net transfer revealed that the rate-limiting step was extraction of phosphatidylcholine molecules from donor vesicles and that bile salts facilitated their capture by enhancing both phosphatidylcholine transfer protein binding as well as perturbing phospholipid packing in vesicle bilayers. These data, taken together with a kinetic analysis showing that bile salt-bound vesicles acted as both donors and noncompetitive inhibitors of phosphatidylcholine movement, suggest that protein-dependent phosphatidylcholine net transfer may function vectorially *in vivo* to deliver phosphatidylcholine molecules from endoplasmic reticulum to canalicular plasma membranes. Since we found a highly significant correlation between protein-mediated phosphatidylcholine transfer rates *in vitro* and reported phosphatidylcholine secretion rates *in vivo* as functions of secreted bile salt hydrophobicity [bile fistula hamsters [Gurantz, D., & Hofmann, A. F. (1984) *Am. J. Physiol. 247*, G736-G748] and prairie dogs [Cohen, D. E., Leighton, L. S., & Carey, M. C. (1992) *Am. J. Physiol. 263*, G386-G395]], our results are consistent with the hypothesis that the specific phosphatidylcholine transfer protein of liver may play a physiological role in bile formation.

Biliary phospholipids (>95% phosphatidylcholines, PCs[1]), which are crucial for efficient cholesterol solubilization in bile and dietary fat assimilation in the proximal small intestine, are highly enriched (>80%) in sn-1 palmitoyl PCs (Hay et al., 1993). Although bile salts, the common anionic detergent-like molecules of bile, promote hepatocellular secretion of biliary PCs together with cholesterol and solubilize these lipids in bile (Carey & Duane, 1994), the hepatocellular selection mechanisms responsible for class and molecular species specificities of biliary phospholipids remain unknown. In 1968, Wirtz and Zilversmit (Wirtz & Zilversmit, 1968) observed that rat liver cytosol stimulates phospholipid exchange between membranes, and Wirtz and colleagues (Teerlink et al., 1982) demonstrated subsequently that hepatocytes are enriched (~300 ng/mg cytosolic protein) in a transfer protein specific for PCs. Purified PC transfer protein (PC-TP) catalyzes both intermembrane exchange and net transfer of PCs, but not of other phospholipid classes (Wirtz, 1991). In particular, the protein displays high affinities for PCs with sn-1 palmitoyl chains (Wirtz, 1991). Although postulated previously that the protein might participate in intracellular PC synthesis (Khan & Helmkamp, 1990) and trafficking (Wirtz, 1991), we show here that the PC transfer activity of this protein is

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* Correspondence should be addressed to this author at the Department of Medicine, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115. Phone: (617) 732-5823; Fax: (617) 730-5807.

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[1] Abbreviations: PC, phosphatidylcholine; PC-TP, phosphatidylcholine transfer protein; TCDC, taurine conjugate of 3α,7α-dihydroxy-5β-cholanoate (chenodeoxycholate); GCDC, glycine conjugate of 3α,7α-dihydroxy-5β-cholanoate; TDC, taurine conjugate of 3α,12α-dihydroxy-5β-cholanoate (deoxycholate); GDC, glycine conjugate of 3α,12α-dihydroxy-5β-cholanoate; TC, taurine conjugate of 3α,7α,12α-trihydroxy-5β-cholanoate (cholate); GC, glycine conjugate of 3α,7α,12α-trihydroxy-5β-cholanoate; TUDC, taurine conjugate of 3α,7β-dihydroxy-5β-cholanoate (ursodeoxycholate); GUDC, glycine conjugate of 3α,7β-dihydroxy-5β-cholanoate; octyl glucoside, octyl β-D-glucopyranoside; taurofusidate, taurine conjugate of 3α,11α-dihydroxy-16β-acetoxyfusida-17(20)[16,21-*cis*],24-dien-21-oate; Tris-HCl, 2-(aminohydroxymethyl)-1,3-propanediol hydrochloride; Na_2EDTA, ethylenediaminetetraacetic acid, disodium salt.

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stimulated markedly *in vitro* by submicellar concentrations of the common bile salts. A strong positive correlation between PC-TP activity and biliary PC secretion rates *in vivo* implies that this transfer protein may have an important physiological function intracellularly in selecting and transporting specific PCs for biliary secretion.

EXPERIMENTAL PROCEDURES

A. Materials

Bile salts were taurine (T) and/or glycine (G) conjugates of 3α,7α-dihydroxy-5β-cholanoate (chenodeoxycholate, CDC), 3α,12α-dihydroxy-5β-cholanoate (deoxycholate, DC), 3α,7α,-12α-trihydroxy-5β-cholanoate (cholate, C), 3α,7β-dihydroxy-5β-cholanoate (ursodeoxycholate, UDC) (Sigma Chemical Co., St. Louis, MO, or Calbiochem, San Diego, CA), and 3α,7β,12α-trihydroxy-5β-cholanoate (ursocholate, UC) (gracious gift of Drs. G. Salen and A. Batta, Veterans Administration Medical Center, East Orange, NJ). Upon purification, each gave a single spot by thin layer chromatography following 200-μg sample application (greater than 98% pure). Highest purity (ULTROL grade) octyl β-D-glucopyranoside (octyl glucoside) was obtained from Calbiochem, reagent grade sodium dodecyl sulfate was from Sigma Chemical Co., and sodium taurofusidate was a gift from Dr. W. O. Godtfredsen (Leo Pharmaceuticals, Ballerup, Denmark). Grade I egg yolk PC and phosphatidylethanolamine were obtained from Lipid Products (South Nutfield, Surrey, U.K.). Egg yolk sphingomyelin, bovine liver phosphatidylinositol, bovine brain phosphatidylserine, and synthetic sn-1 palmitoyl, sn-2 *cis*-parinaroyl PC were purchased from Avanti Polar Lipids (Birmingham, AL). Each phospholipid was >99% pure by high-performance liquid chromatography (Cohen & Carey, 1991). Cholesterol was purchased from Nu-Chek-Prep (Elysian, MN) and found to be >99% pure by gas liquid chromatography. All other chemicals and solvents were ACS or reagent grade purity (Fisher Scientific Co., Medford, MA). ACS grade NaCl was roasted in a muffle furnace at 600 °C for 4 h to oxidize and remove organic impurities. Pyrex brand glassware was alkali alcohol washed for 24 h (EtOH–4 M KOH 1:1, v:v) followed by 24 h of acid washing (3 M HNO_3) and rinsed thoroughly with purified water prior to drying. Water was filtered, ion-exchanged, and glass distilled (Corning Glass Works, Corning, NY) and then further purified using a MilliQ filtration system (Millipore Corp., Bedford, MA).

B. Methods

1. Small Unilamellar Vesicles. Small unilamellar (donor) vesicles for measurements of PC transfer (see below) were prepared by injection of sn-1 palmitoyl, sn-2 parinaroyl PC mixed with egg yolk PC (molar ratio 75:25) in ethanolic solution (4–8 μL, total PC concentration range, 0.7–1.8 mM) into buffer (150 mM NaCl, 20 mM Tris-HCl, 5 mM Na_2-EDTA, 3 mM NaN_3, pH 7.4) (Batzri & Korn, 1973; Somerharju et al., 1981). Small unilamellar (acceptor) vesicles were prepared by bath sonication (Special Ultrasonic Cleaner, Laboratory Supply Corp., Hicksville, NY) of phospholipid (5.5 mM) and cholesterol (3.4 mM) dispersed in buffer as above (DiCorleto & Zilvermit, 1977) and then diluted with buffer to achieve phospholipid concentrations of 0.014–1.1 mM. Acceptor vesicles generally contained phosphatidylethanolamine:sphingomyelin:phosphatidylserine:phosphatidylinositol:cholesterol (molar ratio 22:22:10:8:38); however, in some specific experiments (see below), acceptor vesicles were composed of egg yolk PC and cholesterol (molar ratio 62:38).

2. PC Transfer Protein (PC-TP). Bovine PC-TP was purified to homogeneity and stored at −20 °C in 50% glycerol (Westerman et al., 1983). Prior to transfer experiments, glycerol was removed by overnight dialysis against 1000 volumes of buffer containing 10 mM 2-mercaptoethanol at 4 °C. Protein concentration was determined using a reagent kit (Bio-rad Laboratories, Richmond, CA) based on the method of Bradford (Bradford, 1976). Following dialysis, PC-TP was stored at 4 °C and demonstrated no loss of activity when used within 36 h.

3. Fluorescence Measurements. PC transfer/exchange rates were measured using the intrinsic fluorescence of sn-1 palmitoyl, sn-2 parinaroyl PC which was self-quenched in donor vesicles when its membrane content exceeded 50 mol % (Somerharju et al., 1981). Fluorescence, which resulted from PC transfer, was detected as relative intensity using a Perkin-Elmer (Norwalk, CT) Model LS-50 fluorimeter fitted with a thermostated cuvette holder (temperature control to ±0.2 °C). The excitation wavelength was 324 nm and emission wavelength 420 nm (Somerharju et al., 1981). The final optical densities of all solutions were <0.1; hence, no corrections were made for inner filter effects (Berkhout et al., 1984).

C. Experimental Design

A 4-mL cuvette which contained submicellar concentrations of bile salts dissolved in 2.0 mL of buffer was mounted in the fluorimeter. Donor unilamellar vesicles were prepared in the cuvette and allowed to equilibrate for 180 s under continuous stirring. At time zero, 1.0 mL of acceptor small unilamellar vesicles was added. Following 200 s equilibration with continuous monitoring of fluorescence intensity, 0.05–0.25 mL of purified bovine PC-TP in buffer (approximately 25 μg/mL) was added to the cuvette using a Hamilton syringe pipette (Hamilton Corp., Reno, NV). In all experiments, the final concentration of ethanol was <0.3 vol %. Fluorescence intensities reflecting transfer of sn-1 palmitoyl, sn-2 parinaroyl PC from donor to acceptor vesicles were monitored as functions of time until values stabilized. Over the course of all experiments, vesicle fusion was excluded since no time-dependent changes in vesicle sizes were demonstrated by quasielastic light scattering spectroscopy (Cohen et al., 1990b). Apparent rates of PC exchange and net transfer between donors and acceptors were determined by fitting fluorescence intensities ($F(t)$) to the function $F(t) = A\exp(-kt) + B$ where k is the apparent first-order rate constant, A is the amplitude, and B is a constant (Zucker et al., 1992). Under conditions of PC exchange where both donor and acceptor vesicles were composed with PC, self-quenching of parinaroyl-PC in donor membranes was progressively depressed as the fluorophores removed by PC-TP from donors were replaced by natural PC molecules from acceptors (see Results and Discussion). Because the time dependent decreases in fluorescence quenching in donors artifactually increased experimentally determined PC exchange rates, true exchange rates were mathematically derived (Fullington et al., 1990) by correcting apparent exchange rates by a small factor (5–10%). Under conditions of net PC transfer to acceptor membranes devoid of PC, PC-TP extracted both parinaroyl and natural PCs from donor membranes but did not replace either molecule. The mole fraction of fluorescent PC in donor membranes therefore remained the same and the extent of self-quenching of the remaining fluorophore did not change with passage of time. Consequently, no correction was required for net transfer rates of PC.

Activation energies (E_a) were determined by fitting k values to the Arrhenius equation: $\ln(k) = -E_a/RT + C$, where T

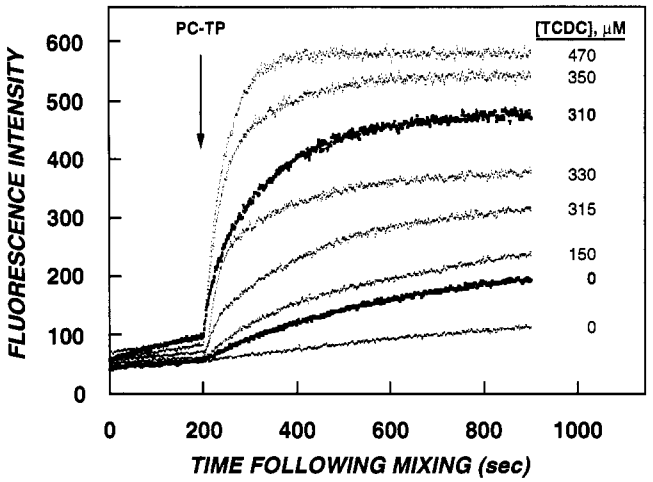

FIGURE 1: Fluorescent assay of submicellar bile salt activation of PC transfer protein (PC-TP) *in vitro*. Fluorescence intensities in arbitrary units are plotted as functions of time following mixing of donor unilamellar vesicles (5.5 nmol of sn-1 palmitoyl, sn-2 parinaroyl PC:egg PC, molar ratio 75:25) with a 100-fold excess of acceptor unilamellar vesicles in the presence of submicellar concentrations (inscribed μM values) of the taurine conjugate of 3α,7α-dihydroxy-5β-cholanoate (taurochenodeoxycholate, TCDC). Other conditions were 150 mM NaCl, 20 mM Tris-HCl, 5 mM Na_2EDTA, 3 mM NaN_3, pH 7.4, 37 °C with continuous stirring. The arrow indicates 200 s when PC-TP (65 nM final concentration) was added. The lightly stippled lines indicate net PC *transfer* to acceptor vesicles devoid of PC (550 nmol of phosphatidylethanolamine:sphingomyelin: phosphatidylserine:phosphatidylinositol:cholesterol, molar ratio 22: 22:10:8:38). Dark stippling denotes PC *exchange* with acceptor vesicles containing PC as the sole phospholipid (550 nmol of egg PC:cholesterol, molar ratio 62:38). (See text for details.)

is temperature (K), R is the gas constant, and C is a constant. Changes in free energy of activation ($\Delta G^{\ddagger}$), enthalpy ($\Delta H^{\ddagger}$), and entropy ($\Delta S^{\ddagger}$) were calculated from experimentally determined values of E_a (Zucker et al., 1992). To determine maximum fluorescence intensities on the hypothetical basis that all fluorescent PC molecules were transferred, a single population of vesicles was prepared by mixing donor and acceptor membrane lipids (Berkhout et al., 1984): Phospholipids and cholesterol were coprecipitated from chloroform (Carey & Small, 1978), redispersed in buffer, and then sonicated under an atmosphere of argon, in the dark at 4 °C, a procedure that prevented oxidation of parinaroyl PC.

RESULTS AND DISCUSSION

We tested whether micromolar bile salt concentrations could activate PC transfer protein (PC-TP) by modeling the native hepatocyte membrane systems of smooth endoplasmic reticulum (putative site of PC selection) and bile canalicular plasma membranes (site of biliary PC secretion) (Carey & LaMont, 1992). All bile salt concentrations were submicellar and spanned a range typical of hepatic cytosol (Carey & LaMont, 1992). Net transfer of PC from vesicles enriched in parinaroyl PC to PC-deficient vesicles composed of the principal lipid components of canalicular plasma membranes (Evans et al., 1976) was compared with PC exchange rates between PC-rich vesicles (Figure 1). Prior to addition of PC-TP, micromolar concentrations of taurochenodeoxycholate (TCDC) (Carey & LaMont, 1992) induced very slow PC movement. In agreement with other observations (Wirtz, 1991), purified bovine PC-TP alone (no bile salts present) promoted PC exchange (bold curves) at a rate (0.2×10^{-2} s^{-1}) that was 1 order of magnitude faster than net trasfer (light curves). All submicellar bile salt concentrations activated both PC-TP-mediated net transfer and exchange in proportion to increments in concentration. Maximal exchange (0.7×10^{-2} s^{-1}) occurred at 310 μM TCDC, whereas maximal net transfer which was 3-fold faster (2.2×10^{-2} s^{-1}) occurred at 470 μM TCDC. Higher bile salt concentrations yielded no further increases in maximal transfer or exchange rates (data not shown). Therefore, while PC-TP alone promoted PC exchange, the addition of bile salt in micromolar concentrations unmasked rapid net PC transfer.

Experimental measurements at later time points than those displayed in Figure 1 demonstrated that an identical equilibrium fluorescence intensity was reached for each bile salt concentration. Therefore, bile salts enhanced rates but not quantities of PC transferred or exchanged. Furthermore, the equilibrium fluorescence intensity was 60% of the maximum value achieved with vesicles composed of mixed donor and acceptor lipids. This indicates that only three-fifths of donor PC molecules were available for both transfer and exchange. For PC exchange, this value is in good agreement with the observation of others (Berkhout et al., 1984; Johnson et al., 1975) that indicate only PC molecules in the outer monolayer of donor membranes are available for exchange. In contrast, prior measurements of parinaroyl-PC self-quenching in acceptor membranes have suggested that a minimum of 10% donor PC is available for net transfer (Berkhout et al., 1984), whereas electron spin resonance spectral shifts of a spin-labeled PC incorporated into acceptor vesicles imply that the transfer is on the order of 20% (Wirtz et al., 1980). The difference between our observation (60%) and the latter estimate may be explained by the higher temperature employed in our work (37 °C compared with 20 °C) and differences in lipid compositions of donor and acceptor membranes which could promote greater PC transfer. Under the present conditions, removal of a significant fraction of PC molecules from outer monolayers by net transfer may induce structural changes in donor vesicles. One possibility is that net extraction of PC by PC-TP facilitates translocation ("flip–flop") of PC from inner to outer membrane leaflets that is not observed under conditions of PC exchange (Berkhout et al., 1984; Johnson et al., 1975).

Figure 2A shows Arrhenius plots for PC transfer activated by PC-TP, TCDC, and PC-TP *plus* TCDC. Because data sets for TCDC and PC-TP intersect near 31 °C, the similar $\Delta G^{\ddagger}$ values (23.1 and 23.2 kcal/mol, respectively) at this temperature facilitated comparisons of thermodynamic constants for PC transfer as shown in Figure 2B. A high $\Delta G^{\ddagger}$ value (23.2 kcal/mol) for TCDC-induced PC transfer without PC-TP was associated with a small $\Delta H^{\ddagger}$ (6.7 kcal/mol) and a large decrease in $T\Delta S^{\ddagger}$ (–16.5 kcal/mol). The small enthalpy term reflects facile desorption of PCs from membrane vesicles presumably perturbed by bile salts, whereas the high entropic contribution reflects the very low water solubility of desorbed PC monomers ($\sim 10^{-12}$ M) (Nichols, 1986). With PC-TP but no bile salt (Figure 2B), the $\Delta G^{\ddagger}$ value (23.1 kcal/mol) was essentially identical to that with TCDC alone and resulted from a relatively large positive $\Delta H^{\ddagger}$ term (17.7 kcal/mol) which represented the energy required to form a tight complex between PC and PC-TP (Devaux et al., 1977). The much smaller decrease in $T\Delta S^{\ddagger}$ (–5.4 kcal/mol) reflected the higher water solubility of the PC plus PC-TP complex compared with PC alone. The $\Delta G^{\ddagger}$ value for stimulated PC transfer with TCDC plus PC-TP was reduced $\sim$3 kcal/mol compared with PC-TP alone (Figure 2B) as a result of a substantially lower $\Delta H^{\ddagger}$ term (14.4 kcal/mol), but constant $T\Delta S^{\ddagger}$ value (–5.9 kcal/mol). These thermodynamic quantities

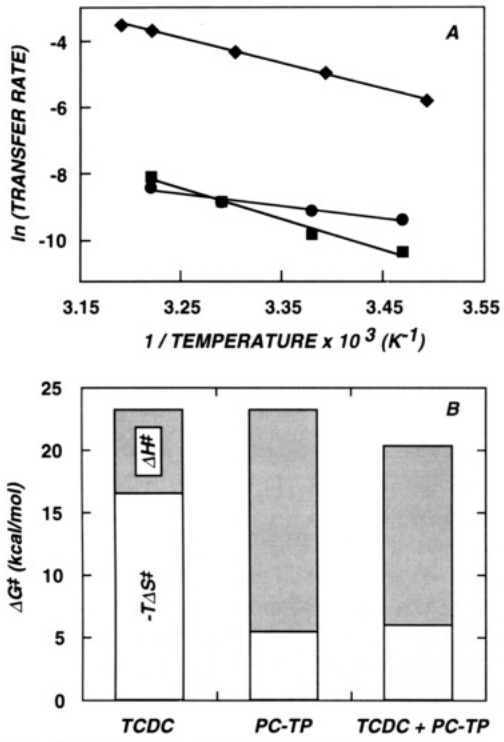

FIGURE 2: (A) Arrhenius plots for facilitated PC transfer. First-order net transfer rates of PC were determined in the presence of taurochenodeoxycholate (TCDC, 470 μM) (●), purified bovine PC-TP (65 nM) (■), or both (◆) as functions of temperature (13–41 °C). Standard deviations lie within the symbol sizes of the data points. (B) Thermodynamic activation parameters for PC transfer from donor to acceptor membranes calculated from Arrhenius plots in Figure 2A at 31 °C (see text for details of calculations). The height of each column represents free energy change for activation ($\Delta G^{\ddagger}$) which equals the sum of the changes in enthalpy ($\Delta H^{\ddagger}$, values represented by shaded area) and entropy ($-T\Delta S^{\ddagger}$, values represented by open areas). (See text for details.)

imply that bile salts facilitate PC-TP capture of PC molecules from donor vesicles by loosening PC packing without exposing the phospholipid to the intervesicular water.

To determine the influence of bile salts on kinetic properties of PC-TP transfer, we varied both absolute and relative concentrations of donor and acceptor vesicles in one set of experiments (Figure 3). Increases in acceptor vesicle concentration increased PC transfer rates (Figure 3A) suggesting a potential collision-based PC transfer mechanism. However, this possibility was excluded because increases in donor membrane concentrations inhibited PC transfer noncompetitively (Figure 3A). Our observations may be explained by membrane binding of PC-TP that occurs secondary to partitioning of anionic bile salts into PC membranes (Cohen et al., 1990a), thereby decreasing the fraction of PC-TP available in the intervesicular water for transferring PC molecules. Increases in donor vesicle concentration, by providing more vesicle binding sites for PC-TP, should decrease the free (aqueous) fraction and hence decrease transfer rates. Increases in acceptor vesicle concentration, which were routinely added in great excess compared with donors (13- to 281-fold), should have the opposite effect by competing with donors for bile salts and hence increasing transfer rates. Figure 3B supports this hypothesis since it demonstrates that PC transfer rates increase linearly in proportion to total PC-TP concentration. Further, by doubling the donor vesicle concentration, the slope of the line decreased by a factor of 2, which reflects a proportional decrease in the free fraction of PC-TP. These findings are also consistent with the observation that membrane incorporation of 2–20 mol % of negatively charged phospholipids (e.g., phosphatidic acid) decreases PC transfer rates by increasing PC-TP binding to PC vesicles (Berkhout et al., 1984; van den Besselaar et al., 1975). Therefore, depending upon experimental conditions, PC vesicles can serve both as donors and as noncompetitive inhibitors of PC-TP activity.

The influence of bile salt detergency on PC-TP transfer activity was studied with glycine and taurine conjugated bile salt species representing a wide range of hydrophobicities (Heuman, 1989) as well as with other detergents of varied molecular structure and charge (Table 1). Submicellar concentrations of all detergents stimulated PC-TP transfer activity by a 40–115-fold range. Whereas maximum PC transfer rates increased in proportion to bile salt hydrophobic index (Heuman, 1989), more hydrophobic bile salts maximized PC transfer rates at lower submicellar concentrations than hydrophilic bile salts. Under the same experimental conditions, submicellar concentrations of sodium taurofusidate (Carey & Small, 1973), sodium dodecyl sulfate, and octyl glucoside stimulated PC-TP activity, but their maximum rates ($(0.9–1.2) \times 10^{-2}$ s^{-1}) were appreciably smaller than those observed with the common bile salts ($(1.6–2.5) \times 10^{-2}$ s^{-1}). These findings suggest that the common bile salts of humans (Carey, 1985) most efficiently stimulate hepatic PC-TP transfer activity compared with the various nonphysiological detergents tested.

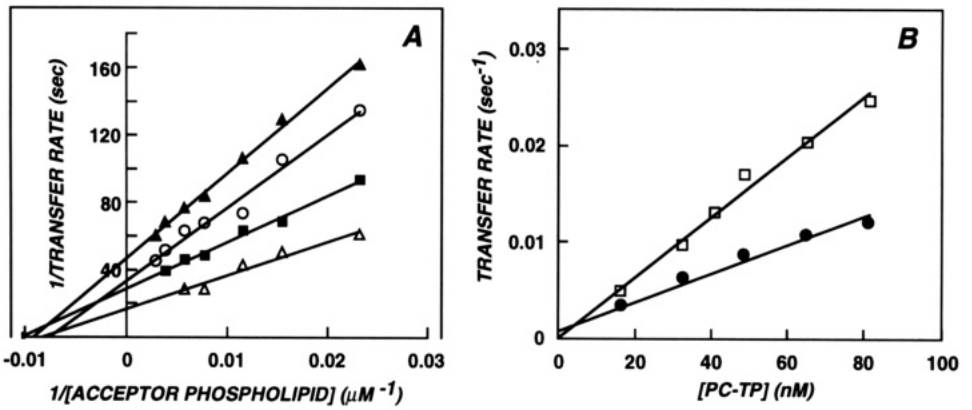

FIGURE 3: (A) Lineweaver–Burk plots demonstrating noncompetitive inhibition of PC-TP (65 nM) net transfer of PC in the presence of TCDC (470 μM which maximally stimulated PC-TP activity (Figure 1)). Total phospholipid concentrations of acceptor membranes were 43–347 μM and PC concentrations of donor membranes were 0.9 μM (△), 1.8 μM (■), 2.2 μM (○), and 3.4 μM (▲). (B) Linear dependence of PC transfer rates upon total concentration of PC-TP at constant concentrations of TCDC (470 μM) and acceptor membrane phospholipid (86 μM). Donor PC concentrations were 0.9 μM (□) and 1.8 μM (●). Sizes of symbols represent ± 1 SD.

Table 1: Influence of Bile Salt Hydrophobicity and Detergent Structure on Phosphatidylcholine Transfer Protein Activity[a]

detergent[b]	maximum phosphatidylcholine transfer rate[c] ($\times 10^2$ s^{-1} ± SD)	detergent concn[d] (μM)	bile salt hydrophobic index[e]
taurodeoxycholate (TDC)	2.5 ± 0.2	470	0.59
glycochenodeoxycholate (GCDC)	2.3 ± 0.2	470	0.51
taurochenodeoxycholate (TCDC)	2.2 ± 0.2	470	0.46
glycodeoxycholate (GDC)	2.2 ± 0.1	470	0.65
tauroursodeoxycholate (TUDC)	2.1 ± 0.3	1,600	–0.47
taurocholate (TC)	1.9 ± 0.4	2,300	0.00
glycocholate (GC)	1.6 ± 0.2	2,000	0.07
taurofusidate	1.2 ± 0.2	780	
sodium dodecyl sulfate	1.2 ● 0.1	240	
octyl glucoside	0.9 ± 0.2	6,300	
tauroursocholate (TUC)	0.9 ± 0.1	12,600	–0.94

[a] Phosphatidylcholine transfer activity fron donor to acceptor membranes in the presence of detergent was determined according to the experimental conditions described in the text and the legend to Figure 1. [b] Bile salts are listed with abbreviations used in the text. Nonbile salt detergents are listed using italics (systematic nomenclature given in footnote 1 and text). [c] Phosphatidylcholine transfer activity is expressed as maximum phosphatidylcholine transfer rates determined with increasing detergent concentrations (see text). [d] Minimum detergent concentrations at which maximum phosphatidylcholine transfer rates were observed. [e] Values are based on reversed-phase HPLC retention times of Heuman (1989).

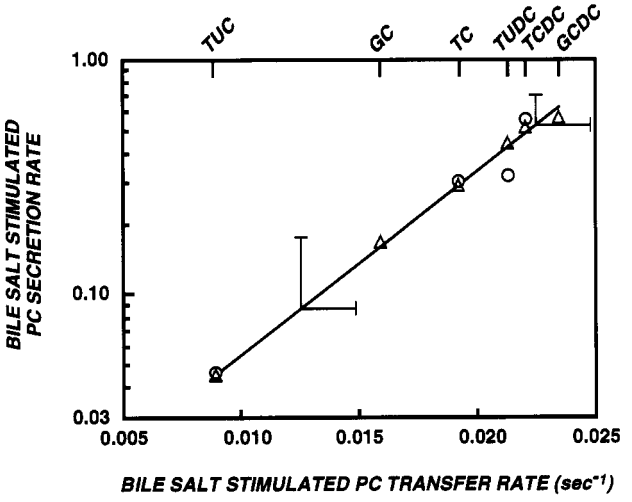

FIGURE 4: Linear least-squares fitted positive correlation between maximum PC-TP-mediated net transfer rates of PC *in vitro* (bottom abscissa, data from Table 1) as stimulated by individual bile salt molecular species (listed on the top abscissa, see abbreviations listed in footnote 1) versus logarithm of *in vivo* biliary PC secretion rates promoted by the same bile salt species in bile fistula hamsters (Gurantz & Hofmann, 1984) (△) and prairie dogs (Cohen et al., 1992) (○). Biliary PC secretion rates in the experimental animals normalized for both bile salt secretion rate and body weight (kg^{-1}) were measured following interruption of the enterohepatic circulation, drainage of the endogenous bile salt pool, and stimulation of biliary PC secretion by either intraduodenal (hamster) or intravenous (prairie dog) bile salt infusions. Error bars indicate +1 SD.

Among a variety of physiological factors that control biliary lipid secretion rates in experimental animals (Carey & LaMont, 1992), bile salt hydrophobicity appears to be a major determinant of biliary PC secretion rates (Cohen et al., 1992; Gurantz & Hofmann, 1984). Figure 4 displays a tight positive correlation between the maximum PC-TP transfer rates *in vitro* from the present work and bile salt stimulated PC secretion rates *in vivo* using bile fistula hamsters (Gurantz & Hofmann, 1984) and prairie dogs (Cohen et al., 1992). This highly significant correlation suggests a possible physiological role of PC-TP in selection and transport of PCs during bile formation.

It has long been appreciated that no precursor PC pool exists within hepatocytes (Curstedt & Sjövall, 1974), although the source for bile ultimately resides in microsomal membrane PC which constitutes the majority of intracellular membranes. While it has been proposed that biliary PC in the rat may be derived from preexisting PCs in high density lipoproteins (Portal et al., 1993), PC newly synthesized by the Kennedy pathway (Chanussot et al., 1990), and to a lesser extent, methylation of phosphatidylethanolamine (Robins & Brunengraber, 1985), recent evidence (Patton et al., 1994a,b) suggests that acyl remodeling of preexisting PCs as well as hepatic acylglycerides provides most of the microsomal substrates required for synthesis of bile specific PCs. Furthermore, it appears that transcytosolic vesicle movement from smooth endoplasmic reticulum to the bile canaliculus cannot account quantitatively for the flow of membrane PC into bile (Crawford & Gollan, 1991). Our results, taken in context with recent evidence of a PC translocator ("flippase") in canalicular plasma membranes (Berr et al., 1993), imply that bile salt regulation of PC-TP activity could provide a potential intracellular selection mechanism for delivery of highly specific PCs to the canalicular plasma membranes for secretion into bile. Future physical–chemical, physiological, and molecular biological experiments should help considerably in elucidating the specific functions of PC-TP suggested herein and perhaps of other hepatocellular proteins in the pre-canalicular aspects of bile formation.

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