Submicellar bile salts stimulate phosphatidylcholine transfer activity of sterol carrier protein 2

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Abstract **To explore a potential role for sterol carrier protein 2 (SCP2, also known as non-specific lipid transfer protein) in hepatocellular phospholipid trafficking, we examined the influence of submicellar bile salt concentrations on phosphatidylcholine (PC) transfer activity of SCP2. We measured rate constants for first-order transfer of *sn*-1 palmitoyl, *sn*-2 parinaroyl PC, a naturally fluorescent self-quenching phospholipid between model membranes. Purified bovine liver SCP2 promoted transfer of PC from donor to acceptor small unilamellar vesicles. Taurine- and glycine-conjugated bile salts (anionic steroid detergent-like molecules), at concentrations well below their critical micellar concentrations, stimulated PC transfer activity of SCP2 80- to 140-fold. Rate constants increased in proportion to bile salt concentration, temperature, and bile salt–membrane binding affinity. Sodium taurofusidate, a conjugated fungal bile salt analog, also activated PC transfer whereas no effect was observed with the anionic and nonionic straight chain detergents sodium dodecyl sulfate and octylglucoside, respectively. Thermodynamic and kinetic analyses of PC transfer support a mechanism in which bile salts stimulate SCP2 activity by partitioning into donor vesicles and enhancing membrane association of SCP2. These results imply that under physiological conditions, SCP2 may contribute to hepatocellular selection and transport of biliary PCs.—**Leonard, A. N., and D. E. Cohen. **Submicellar bile salts stimulate phosphatidylcholine transfer activity of sterol carrier protein 2. *J. Lipid Res.* 1998.** 39: **1981–1988.**

Supplementary key words phospholipid • membrane • detergent • fluorescence

Biliary secretion of phospholipids in response to transhepatic transport of bile salts is essential for cholesterol elimination as well as solubilization in the biliary tree and small intestine (1). Recent studies have suggested that bile salts promote biliary phospholipid secretion by inducing vesiculation of the outer leaflet of the canalicular plasma membrane (cLPM) (2, 3). Because this process is responsible for the daily secretion of ~11 g of phospholipid into bile, a high capacity intracellular mechanism must exist to resupply the cLPM with phospholipids.

The 1968 observation by Wirtz and Zilversmit (4) that liver cytosol promotes intermembrane exchange of phospholipids led to purification and characterization of two abundant lipid transfer proteins. Phosphatidylcholine transfer protein (PC-TP) is a 25 kDa protein that catalyzes intermembrane transfer of PCs exclusively (5). Because biliary phospholipids are composed (>95%) of PCs (6), we previously explored whether PC-TP might participate in hepatocellular selection and transport biliary PCs (7). Using model systems, we demonstrated that submicellar bile salt concentrations, such as might be present in liver cytosol, markedly stimulated activity of PC-TP by partitioning into PC bilayers and facilitating removal of PCs. This suggests a physiological role for PC-TP in bile formation.

Sterol carrier protein 2 (SCP2, also called nonspecific lipid-transfer protein) is a 13 kDa protein that, in vitro, catalyzes intermembrane movement of PCs and a variety of other phospholipid classes as well as sterols (8–10). In steroidogenic tissues where it is expressed, SCP2 is localized principally in peroxisomes (11). Peroxisomal SCP2 in hepatocytes is believed to participate in metabolism of cholesterol, bile acids, and steroid hormones and possibly in β-oxidation of fatty acids (11). In liver, an appreciable proportion of SCP2 that is also present in cytosol (12, 13) appears to participate in trafficking of newly synthesized cholesterol into bile (14) as well as hypersecretion of biliary cholesterol during gallstone formation (15, 16). Here we explore a potential role for SCP2 hepatocellular trafficking of biliary phospholipids by examining in model systems the influence of submicellar bile salt concentrations on intermembrane PC transport.

Abbreviations: PC, phosphatidylcholine; SCP2, sterol carrier protein 2; PC-TP, phosphatidylcholine transfer protein; cLPM, canalicular plasma membrane; GCDC, glycochenodeoxycholate; TCDC, taurochenodeoxycholate; GC, glycocholate; GUDC, glycoursodeoxycholate; TUC, tauroursocholate; TF, taurofusidate; PnA-PC, *sn*-1 palmitoyl, *sn*-2 *cis*-parinaroyl PC.

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EXPERIMENTAL PROCEDURES

Materials

Reagents. Taurine (T) and glycine (G) conjugates of the bile salt 3α,7α-dihydroxy-5β-cholanoate (chenodeoxycholate CDC) as well as the glycine conjugates of 3α,7β-dihydroxy-5β-cholanoate (glycoursodeoxycholate, GUDC) and 3α,7α,12α-trihydroxy-5β-cholanoate (glycocholate, GC) were obtained from Sigma Chemical Company (St. Louis, MO). The taurine conjugate of 3α, 7β, 12α-trihydroxy-5β-cholanoate (tauroursocholate, TUC) was a generous gift from Drs. G. Salen and A. Batta (Veterans Administration Medical Center, East Orange, NJ). After purification (17), each bile salt was >98% pure as judged by thin-layer chromatography. ULTROL® grade (highest purity) octyl-β-d-glucopyranoside (octylglucoside) was purchased from Calbiochem, reagent grade sodium dodecyl sulfate was from Sigma Chemical Co., and sodium taurofusidate (taurine conjugate of 3α,11α-dihydroxy-16β-acetoxyfusida-[17, 20 (16-21 *cis*) 24]-diene-21-oate) 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.). Bovine brain phosphatidylserine, bovine liver phosphatidylinositol, egg yolk sphingomyelin, and synthetic sn-1 palmitoyl, *sn*-2 *cis*-parinaroyl PC (PnA-PC) were purchased from Avanti Polar Lipids (Birmingham, AL). Phospholipids were found to be >99% pure by high-performance liquid chromatography, and cholesterol from Nu-Chek Prep (Elysian, MN) was >99% pure by gas–liquid chromatography. ACS grade NaCl was roasted in a muffle furnace at 600°C for 4 h to oxidize and remove organic impurities. All other chemicals were either ACS or reagent grade purity (Fisher Chemical Co., Medford, MA). Pyrex brand glassware was alkali alcohol and then acid washed as previously described (7). Water used in experiments was purified by ion-exchanging followed by Milli-Q filtration (Millipore Corp., Bedford, MA).

Model membranes. Small unilamellar donor vesicles were prepared by injection (18, 19) of 730 μm *sn*-1 palmitoyl, *sn*-2 parinaroyl PC (PnA-PC) combined with egg yolk PC (molar ratio 75:25) in ethanolic solution (4–20 μl) into buffer (150 mm NaCl, 20 mm Tris-HCl, 5 mm Na_2EDTA, 3 mm NaN_3, pH 7.4) to achieve final PC concentrations of 0.9–4.6 μm. Small unilamellar acceptor vesicles were prepared by bath sonication (Special Ultrasonic Cleaner, Laboratory Supply Corp., Hicksville, NY) of cholesterol (340 μm) and phospholipid (550 μm) dispersed in buffer. Unless otherwise stated, acceptor vesicles were composed with phosphatidylethanolamine:sphingomyelin:phosphatidylserine:phosphatidylinositol:cholesterol 22:22:10:8:38 (molar ratios). In preliminary experiments, quasielastic light scattering spectroscopy (20) demonstrated that sizes of donor and acceptor vesicles did not differ appreciably (mean hydrodynamic radii ~150–200 Å).

Sterol Carrier Protein 2 (SCP2). Bovine SCP2 was purified through the octyl agarose step described by Crain and Zilversmit (8). Purified SCP2 yielded a single band by 14% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and its identity was confirmed by Western blot analysis using anti-SCP2 antisera (a generous gift from Dr. Terence J. Scallen, Albuquerque, NM). Purified protein was stored in 50% glycerol at –20°C. Prior to experiments, glycerol was removed by overnight dialysis at 4°C against 1000 volumes of buffer containing 2-mercaptoethanol (10 mm). After dialysis, protein concentration was determined using a reagent kit (Bio-Rad Laboratories, Richmond, CA) based upon the method of Bradford (21). Preliminary experiments demonstrated no loss of PC transfer activity when SCP2 was kept at 4°C for use within 24 h of dialysis.

Methods

Rate constants and activation mechanisms of PC transfer from donor to acceptor vesicles were determined essentially as previously described (7). Briefly, a stirred cuvette containing submicellar concentrations of detergent dissolved in buffer was mounted in an Aminco-Bowman Model 2 Luminescence Spectrometer (SLM-Aminco, Urbana, IL). Donor unilamellar vesicles, prepared by ethanol injection directly into the cuvette, were allowed to equilibrate for 180 s. Acceptor small unilamellar vesicles were then admixed. After a 200-s equilibration period, purified SCP2 (0–360 nm) was added using a Hamilton microsyringe (Hamilton Corp., Reno, NV). Measurements of PC transfer exploited the intrinsic fluorescence of PnA-PC (excitation wavelength of 324 nm: emissions wavelength of 420 nm) which was self-quenched in donor vesicles due to its high membrane concentration of 75 mol% (19). Increases in fluorescence intensity indicated removal of fluorescent PC from donor vesicles. In all experiments, the total volume was 3.2 ml, and unless otherwise stated, the ratio of acceptor to donor vesicle phospholipid was 100:1. In preliminary experiments, appreciable transfer of fluorescent PC from donors to SCP2 in the absence of acceptor vesicles was not detected, indicating that SCP2 promoted PC transfer from donor to acceptor membranes. It was also observed that, when the cholesterol content of acceptor vesicles was reduced below ~38 mol%, bile salt molecules partitioned into acceptor membranes and induced phase transitions of the lipid bilayers (22) which precluded measurements of inter-vesicle fluorescent PC transfer. The final ethanol concentration of ≤0.7 vol% did not promote intermembrane transfer of PC. Because final optical densities of all solutions were <0.1, no corrections were required for inner filter effects (23).

In each experiment, fluorescence intensity was monitored until equilibrium was achieved (i.e., until values of fluorescence intensity leveled off). Apparent rate constants and relative amounts of PC transfer from donor to acceptor vesicles were determined by curve-fitting fluorescence intensity curves (F(t)) to the function $F(t) = A - B\exp(-kt)$, where A is the equilibrium fluorescence intensity, B is the amplitude of the fluorescence increase, and k is the apparent first-order rate constant. Because SCP2 promotes intermembrane transfer of other phospholipid classes in addition to PC (5), it was assumed that time-dependent accumulation of acceptor phospholipid in donor vesicles artifactually increased actual rate constants of PC transfer by reducing fluorescence self-quenching of parinaroyl PC. Actual rate constants were therefore calculated for each experiment from apparent values by applying a correction factor as described by Fullington, Schoemaker, and Nichols (24). We found that apparent rate constants of PC exceeded actual values by 10–15%. Vesicle fusion as a means of PC transfer from donor to acceptor vesicles was excluded by quasielastic light scattering spectroscopy (20), which demonstrated the absence of time-dependent changes in vesicle sizes.

In order to determine the fraction of PC molecules in donor membranes that was transferred to acceptor vesicles, we measured the maximum fluorescence that could be achieved in the hypothetical case that all donor PC was transferred to acceptors (7). This was simulated by coprecipitation of donor and acceptor membrane lipids from chloroform, followed by resuspension in buffer and sonication under argon gas. Maximum fluorescence intensity was then measured using the same spectrometer settings as were used to determine rate constants for PC transfer. The fraction of PC molecules transferred by expressing the amplitude fluorescence increase, B, as a percentage of the maximum fluorescence intensity.

Thermodynamic parameters associated with activation of PC transfer were determined as previously described (7) by linear

least-squares fitting of k values to the Arrhenius equation, $\ln(k) = -E_a/RT + C$, where E_a represents activation energy, R is the universal gas constant, T is absolute temperature (°K), and C is a constant. Changes in free energy of activation ($\Delta G^{\ddagger}$), enthalpy ($\Delta H^{\ddagger}$) and entropy ($T\Delta S^{\ddagger}$) were calculated from experimentally determined values of E_a (25).

RESULTS

To explore whether bile salts stimulated PC transfer activity of SCP2, rate constants for transfer of fluorescent parinaroyl-PC from donor to acceptor vesicles were determined in the presence SCP2 and/or submicellar concentrations of bile salts (**Fig. 1**). Prior to addition of SCP2 at 200 seconds, slow increases in fluorescence were consistent with TCDC-induced PC transfer from pure PC donor vesicles to acceptor vesicles that lacked PC (phosphatidylethanolamine:sphingomyelin:phosphatidylserine:phosphatidylinositol:cholesterol 22:22:10:8:38) (7). After addition of SCP2, each increment in TCDC concentration resulted in a rapid first-order increase in fluorescence intensity, consistent with stimulation of transfer protein activity. A time of 1200 sec in Fig. 1 represents the point at which PC transfer for the most rapid reaction (for 550 μm TCDC) reached equilibrium. In the presence of lower bile salt concentrations, slightly longer time periods elapsed before fluorescence intensities leveled off. In contrast, SCP2 alone required ~10 h to reach equilibrium (inset to Fig. 1). Rate constants after addition of SCP2 rose linearly as functions of TCDC concentration to 5.6×10^{-3} sec^{-1} (at 550 μm TCDC), a 140-fold increase compared with SCP2 in the absence of bile salt. Because preliminary experiments demonstrated that rate constants for PC transfer stimulated by bile salts alone were <5% of those observed after addition of transfer protein, rate constants for bile salt-stimulated SCP2 activity were not corrected for the small contribution from bile salts acting alone. Over the range of TCDC concentrations shown in Fig. 1, a linear increase was observed in percentage of PC in donors that was transferred to acceptor vesicles from 25% to 40%.

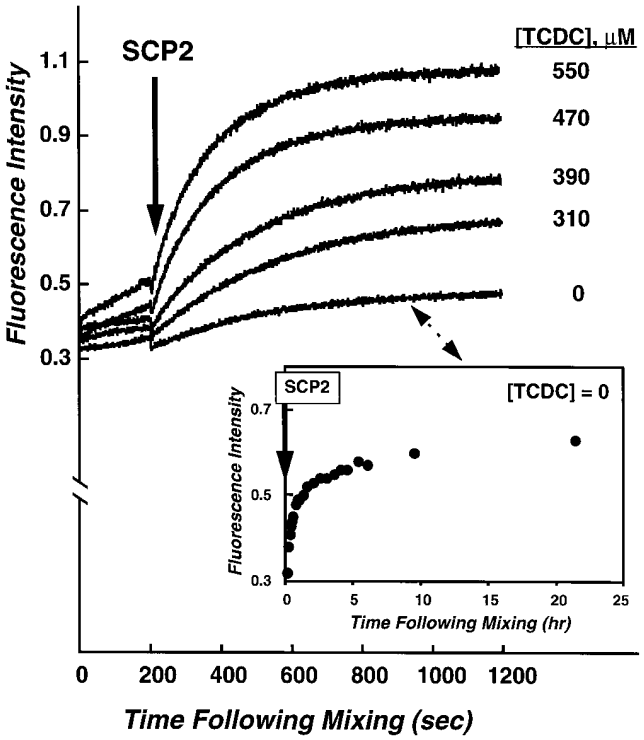

Fig. 1. Fluorescence assay demonstrating activation of SCP2-mediated PC transfer by submicellar TCDC concentrations. Fluorescence intensities in arbitrary units are plotted as functions of time after mixing of TCDC with donor vesicles composed of pure PC (PnA-PC: egg yolk, PC molar ratio 75:25; total PC concentration 1.7 μm) and acceptor vesicles lacking PC (phosphatidylethanolamine: sphingomyelin: phosphatidylserine: phosphatidylinositol: cholesterol, molar ratio 22:22:10:8:38; total phospholipid concentration 172 μm). Addition of SCP2 to a final concentration of 360 nm is indicated by the arrow. The inset shows SCP2-mediated PC transfer in the absence of bile salts when monitored until the fluorescence intensity reached equilibrium. Other conditions were 150 mm NaCl, 20 mm Tris-HCl, 5 mm NaN_3, pH 7.4, and 37°C.

As observed for TCDC, other conjugated common and uncommon bile salts as well as the bile salt analog taurofusidate stimulated PC transfer activity of SCP2 in direct proportion to their concentrations. **Figure 2** demonstrates the influence on SCP2 activity of submicellar concentrations of these steroid detergents as functions of their membrane binding affinities, which were previously quantified as binding enthalpies using immobilized artificial membrane (IAM) HPLC (26). When plotted in this format, transfer activities of SCP2 normalized per mole of added bile salt or taurofusidate were positively correlated ($R^2 = 0.73$) with corresponding IAM binding enthalpies. A linear correlation ($R^2 = 0.62$) was also observed between PC transfer activities of SCP2 and bile salt hydrophobic indices determined by reversed phase HPLC (27)

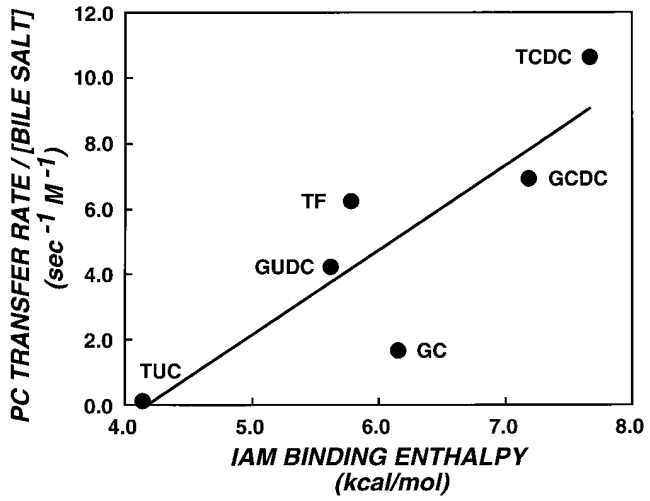

Fig. 2. Correlation between rate constants for PC transfer and bile salt–membrane binding as quantified by immobilized artificial membrane (IAM) HPLC. Rate constants for PC transfer are normalized to concentrations of bile salt (GCDC 470 μm, TCDC 470 μm, GUDC 470 μm, GC 2,000 μm, TUDC 12,600 μm) or fusidate (TF 780 μm) and plotted as functions of the magnitude of binding enthalpy for each bile salt or fusidate (26). Other conditions were as described in Fig. 1.

(data not shown). Activity of SCP2 was not stimulated by any submicellar concentration of the anionic or non-ionic straight chain detergents, sodium dodecyl sulfate or octylglucoside, respectively.

To gain insights into the molecular mechanism(s) by which bile salts stimulate PC transfer activity of SCP2, thermodynamic parameters associated with activation of PC transfer from donor to acceptor vesicles were determined from variations in rate constants as functions of temperature. **Figure 3A** shows Arrhenius plots for transfer of PC in the presence of SCP2 and/or TCDC. At each temperature, rate constants for SCP2 and TCDC combined were found to be approximately 70- and 25-fold greater than observed for SCP2 and TCDC, respectively. Figure 3B presents $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $T\Delta S^{\ddagger}$ values for activation of PC transfer. In order to facilitate direct comparisons (see Discussion) of PC transfer by SCP2 and PC-TP under identical experimental conditions (7), we calculated $\Delta G^{\ddagger}$ values using rate constants for PC transfer at 31°C. Similar values for $\Delta G^{\ddagger}$ were associated with PC transfer due to either SCP2 (24.0 kcal/mol) or TCDC (23.2 kcal/mol) acting alone. Under these conditions PC transfer was associated with high values of $T\Delta S^{\ddagger}$ (22.1 kcal/mol for SCP2; 16.5 kcal/mol for TCDC) and low values for $\Delta H^{\ddagger}$ (1.9 kcal/mol for SCP2; 6.7 kcal/mol for TCDC). When SCP2 was used in combination with TCDC, the $\Delta G^{\ddagger}$ value decreased by 2.5 kcal/mol, reflecting stimulation of transfer activity by TCDC. In the presence of TCDC, the magnitude of $T\Delta S^{\ddagger}$ for activation of PC transfer by SCP2 decreased 8.8 kcal/mol compared with SCP2 alone, whereas $\Delta H^{\ddagger}$ increased 6.3 kcal/mol.

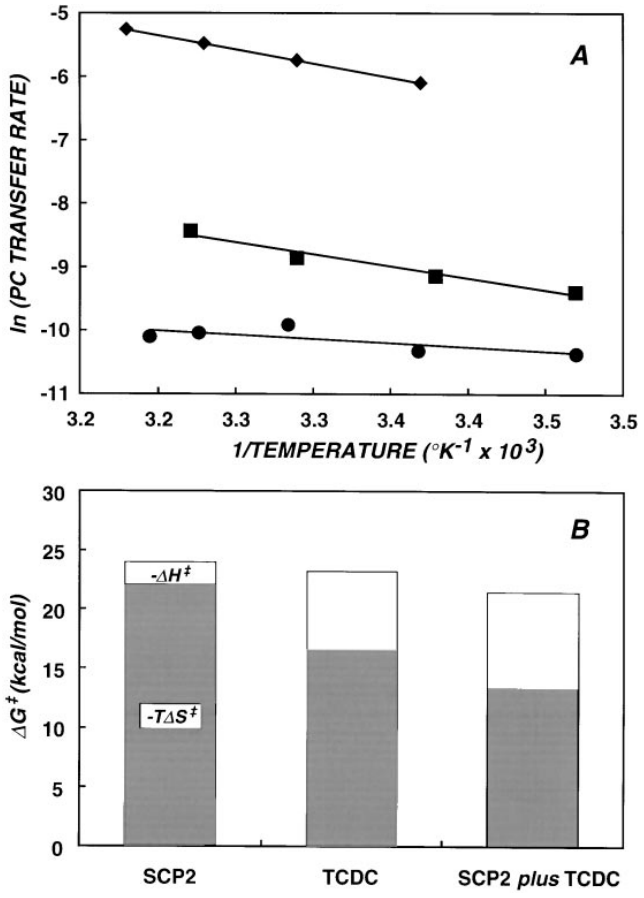

Fig. 3. (A) Arrhenius plots for activated PC transfer. Rate constants for PC transfer were determined as functions of temperature (15–41°C) in the presence of either SCP2 (360 nm) (●), TCDC (470 μm) (■) or both (◆). Membrane compositions and concentrations of donor and acceptor vesicles were as described in the Fig. 1. Standard deviations of triplicate measurements lie within the symbol sizes of the data points. (B) Barplots of thermodynamic activation parameters associated with PC transfer. The height of each bar represents the free energy of activation ($\Delta G^{\ddagger}$) at 31°C. The components of $\Delta G^{\ddagger}$, enthalpy ($\Delta H^{\ddagger}$), and entropy ($T\Delta S^{\ddagger}$) are depicted within the bars as open and shaded areas, respectively. Energy values were calculated from Arrhenius plots in panel (A) as described in the text.

Kinetic analysis of PC transfer was based upon effects on rate constants of variations in donor and acceptor vesicle concentrations. **Figure 4A** (left side) demonstrates rate constants as functions of varying donor concentrations at a constant acceptor concentration. A 10-fold increase in donor PC concentration was associated with a 2-fold decrease in rate constant. Because this effect was apparently inconsistent with either a collision-based or a monomeric PC transfer mechanism, we next investigated whether vesicle charge and/or bile salt–membrane partitioning influenced rate constants for PC transfer. This was accomplished by varying acceptor vesicle composition. Vesicles were prepared that contained identical phospholipid:cholesterol molar ratios (62:38), but were either composed with a mixture of PC and phosphatidylserine (PC:phosphatidylserine:cholesterol 52:10:38) or with PC as the sole phospholipid (PC:cholesterol 62:38). The former vesicle composition was negatively charged (due to the presence of phosphatidylserine), whereas the latter was uncharged because PC molecules are zwitterionic. In addition, the high molar content of PC increased the membrane partition coefficient of bile salts for both types of vesicles (28, 29). Irrespective of acceptor vesicle composition, identical results were observed when donor vesicle concentrations were varied at constant acceptor vesicle concentration of 86 μm (data not shown).

On the right side of Fig. 4A, rate constants for transfer of PC are shown as functions of acceptor phospholipid concentrations at a constant donor vesicle concentration. For acceptor vesicles composed without PC (phosphatidylethanolamine–sphingomyelin–phosphatidylserine–phosphatidylinositol–cholesterol 22:22:10:8:38), a 10-fold increase in acceptor concentration resulted in a 2-fold decline in rate constant. In contrast, when PC was substituted as the sole phospholipid (PC:cholesterol 62:38), a 1.8-fold increase in rate constants was observed for the same 10-fold increase in acceptor vesicle concentrations. With a mixture of PC and phosphatidylserine (PC:phosphatidylserine:cholesterol 52:10:38), the same variation in acceptor phospholipid concentration did not influence rate constants (mean rate constant ± SD = $5.5 \times 10^{-3} \pm 5.3 \times 10^{-4}$ sec^{-1}, data not displayed).

To explore whether rate constants of PC transfer depended upon total SCP2 concentration, Fig. 4B shows the effects of variations in SCP2 concentration on rate constants. At constant donor vesicle PC concentrations of 1.8

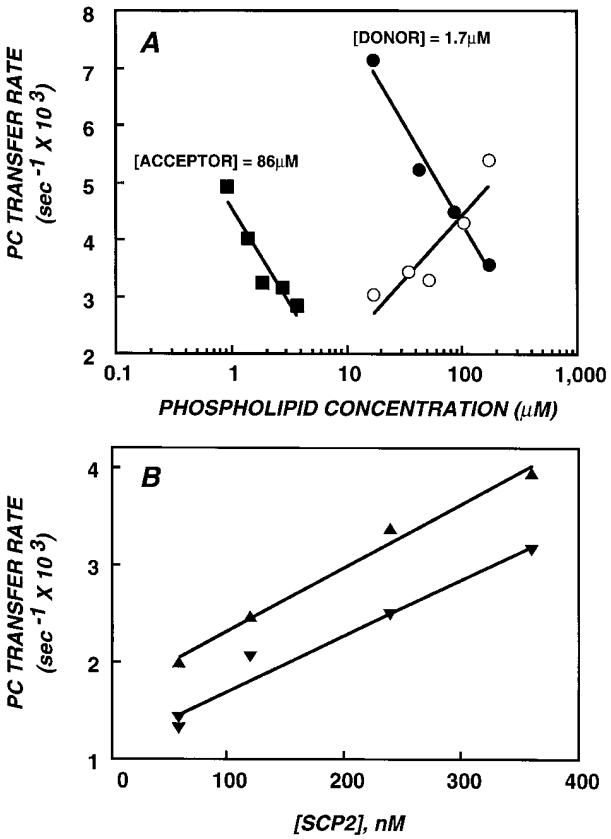

Fig. 4. (A) Influence of donor and acceptor membrane lipid concentrations and phospholipid compositions on rate constants for PC transfer at constant concentrations of TCDC (470 μm) and SCP2 (360 nm). Rate constants are plotted on the left as a function of donor PC concentration at constant acceptor phospholipid concentration (86 μm, ■) or on the right as functions of acceptor phospholipid concentration at constant donor PC concentration (1.7 μm). Opposite effects on rate constants for PC transfer were observed when acceptor vesicles were composed with PC (PC: cholesterol 62:38, ●) or without PC (phosphatidylethanolamine: sphingomyelin: phosphatidylserine: phosphatidylinositol: cholesterol, molar ratio 22:22:10:8:38, ○). For clarity, data points are not displayed for acceptor vesicles composed of PC and phosphatidylserine (PC: phosphatidylserine: cholesterol 52:10:38) for which there was no influence on PC transfer rates. Irrespective of acceptor vesicle phospholipid composition, rate constants for PC transfer decreased as functions of increasing donor PC concentration. (B) Influence of SCP2 concentrations on PC transfer. Rate constants are plotted as functions of SCP2 concentrations for two concentrations of donor PC, 1.8 μm (▲) and 4.6 μm (▼) in the presence of acceptor vesicles composed without PC (phosphatidylethanolamine: sphingomyelin: phosphatidylserine: phosphatidylinositol: cholesterol 22:22:10:8:38; total phospholipid concentration 172 μm).

or 4.6 μm, rate constants increased linearly as functions of SCP2 concentration. However, at each SCP2 concentration, rate constants of PC with 1.8 μm donor phospholipid were greater than with 4.6 μm donors.

DISCUSSION

The capability of SCP2 to catalyze intermembrane transfer of a broad spectrum of membrane lipids in vitro (11), taken together with recent in vivo observations suggesting its role in biliary cholesterol trafficking (14–16), led us to investigate a possible role for SCP2 in hepatocellular trafficking of biliary PCs. Our current observations demonstrate that submicellar bile salt concentrations that fall close to those estimated for liver cytosol (30) markedly stimulate of PC transfer activity of SCP2.

SCP2 activity varied in direct proportion to both concentration and membrane binding affinity of bile salt molecular species. Whereas similar effects were observed with PC-TP (7), only steroid detergents (bile salts and a fusidate) enhanced PC transfer activity of SCP2: octylglucoside and sodium dodecyl sulfate, which stimulated PC-TP activity 40- to 50-fold, did not activate SCP2. In addition, increasing rate constants by SCP2 were associated with increases in the proportion of PC molecules transferred from donor to acceptor membranes, whereas with PC-TP, bile salts enhanced only the rate and not amount of PC transfer. These observations suggest that micromolar bile salt concentrations stimulate PC transfer activity of SCP2 by a mechanism distinct from that observed for PC-TP, whereby detergent-mediated loosening of PC molecular packing in donor membranes facilitated extraction of PCs (7). We now present a mechanistic interpretation of our thermodynamic and kinetic data which suggests that bile salts stimulate SCP2-mediated PC transfer by binding to donor vesicle membranes and promoting association of SCP2.

Activation of PC transfer with either SCP2 or bile salts alone was associated with high entropy ($T\Delta S^{\ddagger}$) and low enthalpy ($\Delta H^{\ddagger}$) (Fig. 3B). With bile salts, this finding is likely explained by movement of highly insoluble monomeric PCs from the membrane into the aqueous phase (7, 31). SCP2 has been shown to bind PCs to form water-soluble complexes that mediate intermembrane transfer of PCs while protecting them from the polar water environment (32–34). However, there is an apparent requirement for the PC to dissociate from the membrane prior to binding SCP2 (35). Exposure of PCs to the aqueous phase during this initial phase of the binding process presumably explains the large $T\Delta S^{\ddagger}$ values we observed. By contrast, a high value for $\Delta H^{\ddagger}$ and low $T\Delta S^{\ddagger}$ associated with PC transfer by PC-TP in the absence of bile salt (7) was attributable to tight binding of PC by PC-TP which shielded the phospholipid from the aqueous phase.

A substantial decrease in the $T\Delta S^{\ddagger}$ term in the presence of both bile salts and SCP2 is consistent with an activation mechanism whereby anionic (i.e., negatively charged) bile salts partition into donor vesicles and promote membrane association of positively charged SCP2 (pI ~ 9.6, (11)). This would be consistent with observations that SCP2 is stimulated by membrane incorporation of acidic (negatively charged) phospholipids (36–38). Bile salts bind to membranes with their steroid nuclei parallel to the membrane–water interface (39, 40) and condense PCs in proportion to their hydrophobicities (39). This condensing effect is due to interactions between fatty acyl chains of phospholipids and hydrocarbon backbones of bile salt molecules (39). We postulate that condensation of PCs

around bile salts facilitates removal of PCs by the protein from the membrane in an environment that partially shields PC molecules from the aqueous phase, thereby reducing $T\Delta S^{\ddagger}$ associated with PC movement. Such an interpretation is consistent with the observed stimulation of SCP2 activity by both bile salts and structurally related taurofusidate in proportion to their membrane-binding affinities (Fig. 2), but not by charged or uncharged straight-chain detergents. In addition to enhancing PC transfer rates, our data indicate that increasing total bile salt concentrations enhanced the amount of PC transferred to acceptor membranes by up to 60%. This was presumably due to perturbations induced by bile salts in the molecular packing of the donor membrane.

Irrespective of acceptor membrane composition, we found that increasing donor PC concentrations decreased rate constants for SCP2-mediated PC transfer (Fig. 4A). As observed with PC-TP (7), PC vesicles in the presence of bile salts apparently function as both donors of PC and inhibitors of transfer protein activity. In this manner, increasing donor vesicle concentrations create additional regions of PC condensation, which facilitate PC transfer. However, there is also increased nonspecific binding of SCP2 to donor vesicles, which inhibits transfer. The net result is a decrease in rate constants for PC transfer that likely results from a decrease in the free fraction of SCP2 engaged in shuttling PCs from donor to acceptor membranes. This finding is in keeping with the observation of Gadella and Wirtz (36) that SCP2-mediated PC transfer rates from donor to acceptor vesicles were decreased by binding of SCP2 in solution to an admixed population of vesicle/micelle aggregates composed of pure negatively charged phospholipids. Figure 4B supports this assertion by demonstrating a linear dependence of rate constants on total concentration of SCP2, where lower donor PC concentrations yielded higher rate constants at each SCP2 concentration.

In contrast to donor vesicles, increasing concentrations of acceptor vesicles composed of pure PC enhanced rate constants for PC transfer (Fig. 4A). This was likely due to the effects of cholesterol on acceptor membranes: at 38 mol% cholesterol, condensing effects of bile salts on PC bilayers are eliminated (39). However, the same vesicle cholesterol content does not abolish bile salt–membrane partitioning (28). Under these conditions, incorporation of bile salts into acceptors would have failed to promote non-specific binding of SCP2, but the 50- to 100-fold excess of acceptors could have competed with donor PC for available bile salts. A reduction in bile salt binding to donor vesicles would have, in turn, led to a decline in non-specific association of SCP2 with donors. Therefore, these data are consistent with the argument that the observed increases in rates constants for PC transfer were due to increases in free concentration of SCP2.

The opposite effect was observed using acceptors composed without PC: rate constants decreased as functions of increasing acceptor vesicle concentrations (Fig. 4A). This effect is best explained by the observation by Gadella and Wirtz (36) that introduction of negatively charged phospholipids into acceptor membranes decreases PC transfer activity of SCP2 by promoting non-specific membrane binding of SCP2. In this connection, acceptor vesicles that lacked PC in Fig. 4A did contain phosphatidylserine and phosphatidylinositol, and were therefore net negatively charged. Moreover, the presence of phosphatidylserine, phosphatidylethanolamine, and sphingomyelin has been shown to render membranes relatively resistant to partitioning of bile salts compared with those composed principally of PCs (28, 29). Consequently, these acceptor vesicles could not have competed effectively with donor membranes for bile salts in order to reduce the proportion of SCP2 bound non-specifically to donor membranes. Instead, the negatively charged acceptor vesicles presumably enhanced non-specific binding of SCP2 and thereby decreased the free fraction of SCP2 available to transfer PC.

When acceptors were composed with PC and phosphatidylserine, variations in their concentrations did not alter rate constants for PC transfer appreciably. Because their high membrane concentration of PC (52 mol%) provides substantial membrane binding capacity for bile salts (22), these acceptor vesicles could have competed with donor membranes for available bile salts and thereby decrease non-specific binding of SCP2 to donor membranes. However, negative charges imparted by phosphatidylserine would have been expected to enhance non-specific SCP2 binding to acceptor membranes. As a result, no net change in rate constants was observed apparently because decreased binding of SCP2 to donor membranes was offset by increased binding to acceptor membranes.

To assess the potential contribution of SCP2 to hepatocellular trafficking of biliary PCs, we compared its bile salt-stimulated PC transfer activity with that of PC-TP under identical experimental conditions in vitro (7). When normalized per mole of lipid transfer protein and per mole of bile salt, PC transfer activities of SCP2 were 12- to 35-fold lower than those observed for PC-TP upon stimulation by the same bile salt molecular species (data from **Table 1** in ref. 7). Based on measurements in rat liver of cytosolic concentrations of PC-TP (~300 ng/mg protein) (41) and SCP2 (~700 ng/mg protein) (13, 42), hepatic cytosol contains a 4.5-fold molar excess of SCP2 compared with PC-TP. However, immunolocalization studies (12, 43) demonstrating concentration of SCP2 in liver peroxisomes led Wirtz (11) to conclude that quantitative determinations of cytosolic SCP2 concentrations were elevated artifactually due to leaking of SCP2 from peroxisomes during tissue homogenization. Assuming instead equimolar concentrations of SCP2 and PC-TP, our in vitro data based upon rate constants for PC transfer suggest that SCP2 could contribute 3–8% of bile salt-stimulated hepatocellular transport of PCs. Based on an average biliary phospholipid secretion rate of 11 g/day (44), SCP2 could account for the daily hepatocellular transport of 300–900 mg of PC to the cLPM. If amounts of PC transfer are also influenced by bile salts in vivo as was observed in vitro (Fig. 1), this contribution could range up to 2-fold higher. Because our current experimental design was limited to

characterizing bile salt-stimulated PC transfer activity of SCP2, further studies are necessary to ascertain whether bile salt-stimulated transfer of other phospholipid classes could account for hepatocellular transport of the small but significant proportion of phosphatidylethanolamines (up to 5%) and traces of sphingomyelins that are secreted together with PCs into bile (1).

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