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## Short-Chain Phosphatidylcholines as Superior Detergents in Solubilizing Membrane Proteins and Preserving Biological Activity<sup>†</sup>

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**ABSTRACT:** The solubilization of plasma and organelle membranes by diheptanoylphosphatidylcholine (DHPC) has been studied. This short-chain phosphatidylcholine is shown to act as a mild detergent, solubilizing effectively both kinds of membranes at DHPC concentrations of 10–20 mM (0.5–1%). The size of the resulting mixed protein–lipid–DHPC micelles ranges between 5 and 8 nm. The protein conformation and hence the enzymatic activity are well preserved over a rather large DHPC concentration range (up to 4–5 times the DHPC concentration required for solubilizing the membranes). Evidence is presented that short-chain phosphatidylcholines are superior to most detergents commonly used by biochemists. This is true not only regarding its excellent dispersing power on both phospholipid bilayers (Gabriel & Roberts, 1986) and biological membranes but also as to its capacity to preserve the native protein structure and hence enzymatic activity in the solubilized state. Due to its special properties DHPC lends itself very well not only to membrane solubilization but also to the purification of the solubilized membrane proteins and reconstitution of the proteins into simple lipid bilayers. Concerning the mechanism of membrane solubilization, evidence indicates that DHPC interacts primarily with the lipid bilayer of the membrane and not with the membrane proteins. DHPC solubilizes membranes by being distributed into the lipid bilayer and breaking it up. In the resulting small mixed micelles, the protein remains associated with its preferred intrinsic membrane lipids and is thus stabilized. The protein–intrinsic lipid complex is successfully shielded from unfavorable contacts with H<sub>2</sub>O by DHPC–intrinsic lipid interactions.

One problem encountered in the solubilization of membrane proteins by detergents is the preservation of the native protein structure and related to it the biological activity of the membrane proteins. A wide variety of detergents has been tested to this effect. No single detergent has yet emerged that can be generally and reliably applied to the solubilization and purification of membrane proteins (Helenius et al., 1979; Jones et al., 1986).

Nonionic industrial detergents such as Triton, Brij, and Tween permit the isolation of certain membrane proteins in their fully active form. Frequently, however, the detergent concentration required for the solubilization affects the

stability and activity of the solubilized membrane protein. Furthermore this class of detergents has disadvantages such as a low cmc,<sup>1</sup> which makes the removal of the detergent by dialysis cumbersome, the instability of the polyoxyethylene chains undergoing autoxidation in the presence of traces of heavy metal ions, the inhomogeneous chemical composition yielding mixed micelles of ill-defined composition, and for some of them, the presence of phenol groups absorbing in the UV range, which makes the application of spectrophotometric measurements difficult or impossible.

Bile salts and conjugated bile salts have been widely used for the solubilization of membrane proteins. They have the advantage of usually preserving the activity of solubilized membrane proteins. However, their bulky chemical structure, the distribution of polar groups that are located on one side of the apolar ring system, and the negative charge give rise to a complex micellar behavior strongly dependent on pH, ionic strength and composition as well as temperature. This restricts the choice of experimental conditions, and occasionally the negative charge of these detergents can induce protein denaturation (Helenius et al., 1979; Womack et al., 1983; Jones et al., 1986). Better results have been obtained with zwitterionic derivatives of bile salts such as CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate).

Satisfactory results have also been reported for well-defined, nonionic detergents such as octyl glucoside. These detergents effectively solubilize biological membranes at concentrations near their cmc, usually preserving the activity of membrane proteins (Womack et al., 1983; Jones & al., 1986). In some cases, however, even these detergents cause a loss in activity upon solubilization (MacNamee et al., 1986; Esmann, 1988).

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<sup>1</sup> Abbreviations: BBM, brush border membrane; BBMV, brush border membrane vesicles; CD, circular dichroism; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; cmc, critical micellar concentration; DFP, diisopropyl fluorophosphate; DHPC, 1,2-diheptanoyl-*sn*-phosphatidylcholine; DNase, deoxyribonuclease; DPPC, 1,2-dipalmitoyl-*sn*-phosphatidylcholine; DTT, 1,4-dithio-*rac*-threitol; EPC, egg phosphatidylcholine; FFEM, freeze-fracture electron microscopy; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid); INT, 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride; PBS, phosphate buffer saline (10 mM sodium phosphate buffer, pH 7.5, containing 0.15 M NaCl and 0.05% NaN<sub>3</sub>); PC, phosphatidylcholine; PMSF, phenylmethanesulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; TCA, trichloroacetic acid; Tris, tris(hydroxymethyl)aminomethane.

The purpose of this work is to investigate the ability of short-chain phosphatidylcholines such as 1,2-diheptanoyl-*sn*-phosphatidylcholine (DHPC) to solubilize biological membranes and to characterize the resulting solubilized membrane proteins. DHPC is structurally a phospholipid, but its short fatty acyl chains of seven carbon atoms endow it with detergent-like properties. It forms micelles (Tausk et al., 1974a) rather than bilayers when dispersed in water with a relatively high cmc of 1.4 mM and shows a broad size distribution depending on its concentration and on the NaCl concentration of the suspension (Tausk et al., 1974b). DHPC has the advantage that it is available in pure form, it has no net charge, it is stable over a wide pH range of 4–10, and it does not interfere with spectrophotometric measurements. Compared to other detergents, it is, however, rather expensive.

## MATERIALS AND METHODS

1,2-Diheptanoyl-*sn*-phosphatidylcholine (DHPC) and 1,2-dipalmitoyl-*sn*-phosphatidylcholine (DPPC) of purity greater than 99% were purchased from Avanti Polar Lipids (Alabaster, AL), PMSF and DNase were from Fluka (Buchs, Switzerland), CHAPS, sodium deoxycholate,  $\beta$ -D-octyl glucoside, emulphogene BC 720, DFP, Tris, and Hepes were from Sigma (St. Louis, MO), Sepharose-CL-4B and Dextran Blue were from Pharmacia (Dübendorf, Switzerland), Triton X-100 and glucose dehydrogenase were from *Bacillus megaterium* (solution ready for use, 5.2 units/mL), and NAD<sup>+</sup> from Merck (Darmstadt, Germany). 1-Palmitoyl-2-[9,10-<sup>3</sup>H]palmitoyl-3-*sn*-phosphatidylcholine (<sup>3</sup>H-DPPC, specific activity 85 Ci/mol) was prepared as described by Gupta et al. (1977). All phospholipids used were pure by TLC standard. DHPC was found to be pure by C, H, N microanalysis and by <sup>1</sup>H-NMR spectroscopy. All detergents were used as supplied. The purity of the detergents was given by the supplier as better than 98% except for emulphogen BC 720, for which no specification was available. All other chemicals were of analytical grade.

**Membrane Preparations.** Ghosts of human erythrocytes were prepared according to Beutler et al. (1976). Spectrin and ankyrin were released from erythrocyte ghosts as described by Bennett and Branton (1977) and Bennett and Stenbuck (1980). Brush border membrane vesicles (BBMV) were made by the Mg-EGTA procedure (Hauser et al., 1980) from rabbit small intestines stored at –80 °C prior to the preparation of BBMV. Suspensions of BBMV were supplemented with the proteinase inhibitors DFP (~0.6 mM) and iodoacetamide (4 mM) except for glucose uptake measurements where proteinase inhibitors were omitted. Mitochondria were prepared according to Gazzotti et al. (1979), and membranes from renal outer medulla according to Jørgensen (1988). Chromatophore membranes were prepared from *Rhodospirillum rubrum* G9<sup>+</sup> according to Snozzi and Bachofen (1979) with the following modifications: a 50 mM Tris buffer, pH 8.0, containing 50 mM NaCl and 2 mM MgCl<sub>2</sub> was used throughout the preparation and cell membranes were disrupted in a French press.

**Membrane Solubilization.** A freshly thawed suspension of the membrane preparation (30 mg of protein/mL) was diluted with either PBS (0.01 M sodium phosphate, pH 7.0–7.5, 0.15 M NaCl, 0.05% NaN<sub>3</sub>) or 0.01 M Tris buffer, pH 7.5, containing 0.15 M NaCl and 2 mM MgCl<sub>2</sub> to a final protein concentration of 3.2–6.4 mg/mL. Unless stated otherwise membranes were solubilized at 0 °C by adding dropwise a 200 mM stock solution of DHPC in the same buffer under vortexing to the desired final concentration of DHPC. The membrane suspension was vortexed another 30

s, and unless stated otherwise centrifuged at 100000g and 4 °C for 20 min. (Beckmann airfuge, rotor A-100). An operational criterion was used for defining solubilization: membrane proteins remaining in the supernatant after centrifugation at 100000g and 4 °C for 20 min were designated as solubilized. Chromatophore membranes suspended in 0.01 M Tris buffer, pH 7.5, containing 0.15 M NaCl and 2 mM MgCl<sub>2</sub> were solubilized at 0 °C in the dark under N<sub>2</sub>. Solubilized bacteriochlorophyll-containing proteins were expressed as percent based on absorbance measurements at 870 nm. Renal outer medulla membranes (10 mg of protein/mL) suspended in 0.1 mL of 0.025 M imidazole buffer, pH 7.2, containing 0.15 M KCl and 20 vol % glycerol were solubilized at room temperature by adding various detergents. In this case the amount of solubilized proteins was determined in the supernatant after centrifugation at 4 °C and 180000g for 30 min (Beckman L5-50, rotor 50 Ti).

**Measurement of Enzymatic Activities.** The activity of NADH dehydrogenase and succinate dehydrogenase of chromatophores was measured according to Morré (1971) with the following modifications: the reaction was performed at 30 °C in the dark, sucrose in the buffer was replaced by 2 mM DHPC, and the formation of the INT-formazan was followed spectrophotometrically at 490 nm as a function of time.

The F<sub>0</sub>F<sub>1</sub>-ATPase activity of chromatophores was determined at 30 °C in the dark. The suspension of chromatophores (30  $\mu$ L, ~5 mg of protein/mL) was diluted with 370  $\mu$ L of 50 mM Tris buffer, pH 7.5, containing 2 mM MgCl<sub>2</sub> (buffer A). The DHPC-solubilized chromatophores at the same protein concentration were diluted in buffer A containing 2 mM DHPC. The reaction was started by adding 100  $\mu$ L of 10 mM ATP in the same buffer. After incubation for 15 min at 30 °C, the reaction was stopped by adding 100  $\mu$ L of 10% TCA, the suspension was centrifuged at 12000g for 15 min, and the amount of inorganic phosphate present in the supernatant was determined according to Ohnishi and Gall (1978).

The activity of the reaction center of chromatophores from *R. rubrum* was determined by ESR spectroscopy based on the light-induced oxidation of the bacteriochlorophyll special pair of the reaction center. The ESR signal due to the oxidation of the special pair was measured under illumination with a 60-W lamp placed about 30 cm above the sample. ESR spectra were recorded at room temperature with a Varian X-band spectrometer (Model E-104A). The spectral baseline was recorded prior to illumination. For ESR measurements chromatophores suspended in 50 mM Tris buffer, pH 7.5 (6 mg of protein/mL), were injected into 100- $\mu$ L glass capillaries (internal diameter ~1 mm) and immediately inserted in the ESR spectrometer.

The sucrase activity of BBMV was determined as described by Banauch et al. (1975). The glucose liberated by the enzyme was determined using glucose dehydrogenase.

The Na<sup>+</sup>/K<sup>+</sup>-ATPase activity of renal outer medulla membrane was measured by a slightly modified method described by Jørgensen (1974). Native membranes or detergent-solubilized membranes (~50  $\mu$ L, 10 mg of protein/mL) were added to 1 mL of 39 mM imidazole buffer, pH 7.4, containing 0.13 mM EDTA and incubated at 20 °C for 30 min. The enzymatic reaction was started by adding 0.2 mL of a stock solution, yielding final concentrations of NaCl of 0.13 M, KCl of 20 mM, MgCl<sub>2</sub> of 3 mM, and ATP of 3 mM with or without 1 mM ouabain. After incubation at 37 °C for 10 min, the reaction was stopped by adding 0.1 mL of 10%

TCA and 5% SDS solution. After 0.2 mL of a 3.6% ammonium heptamolybdate solution in concentrated  $\text{H}_2\text{SO}_4$  and 0.1 mL of a 2% hydroquinone solution were added, the absorbance was measured at 820 nm. The  $\text{Na}^+/\text{K}^+$ -ATPase activity was calculated from the difference in inorganic phosphate produced in the absence and presence of ouabain.

Unless stated otherwise, solubilized enzymatic activities were quantitated as follows: the membrane was incubated with a certain amount of DHPC at 0 °C for 30 s under brief vortexing and the activity measured immediately (=100% value). The activity measured in the supernatant after centrifugation at 100000g and 4 °C for 20 min was expressed in percent of the activity measured immediately after solubilization.

The activity ratio  $F_A$  was obtained by dividing the 100% activity by the activity measured in intact membranes in the absence of DHPC. The  $F_A$  value is therefore identical to the ratio of activity measured in the presence and absence of DHPC.

**Reconstitution of the D-Glucose Carrier of Rabbit Small Intestinal BBM.** BBMV suspended in 0.3 mL of 0.01 M Hepes-Tris buffer, pH 7.0, 0.3 M D-mannitol, 5 mM EGTA at 28 mg of protein/mL was added to 1.2 mL of 2 mM Hepes-Tris buffer, pH 7.0, containing 0.1 M NaCl, 20% glycerol, 1 mM DTT, and 50 mM DHPC. The resulting dispersion was gently vortexed at room temperature for 2 min, immediately put on ice for 3 min, and centrifuged at 180000g for 30 min. The resulting clear solubilize was immediately added to a film of 30 mg of EPC dried on the glass wall of a 25-mL round-bottom flask under high vacuum for ~1 h. EPC was dispersed by gently shaking with two glass beads, and the dispersion was diluted with ~100 volumes of the same Hepes-Tris buffer except that DHPC was absent. Proteoliposomes formed upon dilution were spun down by centrifugation at 180000g for 90 min. The pellet of proteoliposomes was resuspended in the same buffer to 2 mg of protein/mL, and glucose uptake was immediately determined.

D-Glucose uptake by either BBMV or reconstituted proteoliposomes was measured at 25 °C as follows: membranes suspended in 30  $\mu\text{L}$  of 0.01 M Hepes-Tris buffer, pH 7.0, 0.3 M mannitol, 5 mM EGTA were diluted with 60  $\mu\text{L}$  of 0.01 M Hepes-Tris buffer, pH 7.0, containing 0.15 M KSCN or NaSCN and 0.6 mM radiolabeled [ $^{14}\text{C}$ ]-D-glucose to a final protein concentration of 0.67 mg/mL. After timed intervals D-glucose uptake was stopped by adding 2 mL of ice-cold stop solution of Hepes-Tris buffer, pH 7.0, containing 0.25 M NaCl. The membrane suspension was filtered through a Sartorius membrane filter of 0.65- $\mu\text{m}$  mean pore diameter. The filter was washed with 2 mL of stop solution and dissolved in scintillation liquid, and the radioactivity was counted in a Beckman LS 7500 liquid-scintillation counter.

**Purification of DHPC-Solubilized Membrane Proteins.** Sucrase-isomaltase solubilized with DHPC from rabbit small intestinal BBMV was purified by anion-exchange chromatography on DEAE-Sepharose (Pharmacia) according to the supplier's instructions followed by affinity gel filtration on Sephadex G-200 as described by Sigrist et al. (1975).

Bacteriochlorophyll-containing proteins solubilized with DHPC from chromatophores of *R. rubrum* were purified by anion-exchange chromatography on DEAE-Sepharose followed by density gradient centrifugation as described by Kessi (1994). All elution buffers and the density gradient buffer used in these purification procedures contained 2 mM DHPC.

**Gel Filtration on Sepharose CL-4B.**  $^3\text{H}$ -DPPC (~0.1  $\mu\text{Ci}$ ) dissolved in 0.5 mL of  $\text{CHCl}_3/\text{MeOH}$  (2:1 v/v) was taken to

dryness in a 25-mL round-bottom flask, and the resulting lipid film was dried under high vacuum for approximately 2 h. Solubilized membranes in 0.8–0.9 mL of PBS were added to the dry lipid film, and the lipid was dispersed by adding three glass beads and gently shaking for 5 min at room temperature. The radioactively labeled dispersion of mixed lipid-protein micelles (~1 mL) was applied to a Sepharose CL-4B column (50 cm  $\times$  1 cm) equilibrated with PBS containing 1.5 mM DHPC at 4 °C. The lipoprotein particles were eluted with the same buffer at a flow rate of 7.5 mL/h; 0.5-mL fractions of the eluate were collected and analyzed for lipid and protein.

The hydrodynamic radius  $r_H$  of lipoprotein particles is related to the elution volume  $V_e$  and the distribution parameter  $K_d$  by

$$r_H = a_0 + b_0 \text{erf}^{-1}(1 - K_d) \quad (1)$$

where  $K_d = (V_e - V_0)/(V_t - V_0)$  and  $V_0$  and  $V_t$  are the void and the total column volume, respectively (Ackers, 1967).  $V_0$  and  $V_t$  were determined by chromatographing Dextran Blue and [ $^3\text{H}$ ]-D-glucose, respectively. The constants  $a_0$  and  $b_0$  are characteristic of the gel filtration medium and were determined according to Schurtenberger and Hauser (1984).

**Electron Microscopy.** Freeze-fractured samples of mixed lipid-protein micelles were made, and electron micrographs were taken as described before (Hauser et al., 1983). Images on electron micrographs were measured using a calibrated magnifying glass of about tenfold magnification.

**Absorption Spectroscopy.** Visible light absorption spectra from 250–900 nm were recorded at room temperature with a Uvikon 860 spectrophotometer using quartz cells of 2-mm path length. Suspensions of intact or solubilized chromatophore membranes were diluted to an absorption at 870 nm of less than 0.8, and absorbance measurements were corrected for light scattering.

**Analytical Procedures.** Protein was determined according to Lowry et al. (1951) with bovine serum albumin as the standard. The radioactivity of samples containing  $^3\text{H}$ -DPPC was determined in a Beckman LS 7500 liquid-scintillation counter. SDS-PAGE analysis was performed according to the method of Laemmli (1970).

## RESULTS

### (I) Solubilization of Various Membranes by DHPC

Two different plasma membranes, rabbit small intestinal microvillus membrane (or brush border membrane) and human erythrocyte ghosts, and two organelle membranes, chromatophores from *R. rubrum* and rat liver mitochondria, were solubilized with DHPC. Solubilization was followed by determining changes in the sedimentability of membrane proteins in the presence of increasing concentrations of DHPC. The amount of solubilized protein as a function of the total DHPC concentration is shown in Figure 1A. Typically S-shaped curves were obtained, indicating that maximum solubilization of the membranes occurred at total DHPC concentrations of about 10–15 mM, corresponding to a detergent/protein wt ratio of 1–2. The position of the inflexion point of the S-shaped curves depended on the nature of the membrane, and the values of the DHPC concentrations at the inflexion points of the S-shaped curves are listed in Table 1. For each individual membrane the position of the inflexion point depended on the membrane protein concentration such

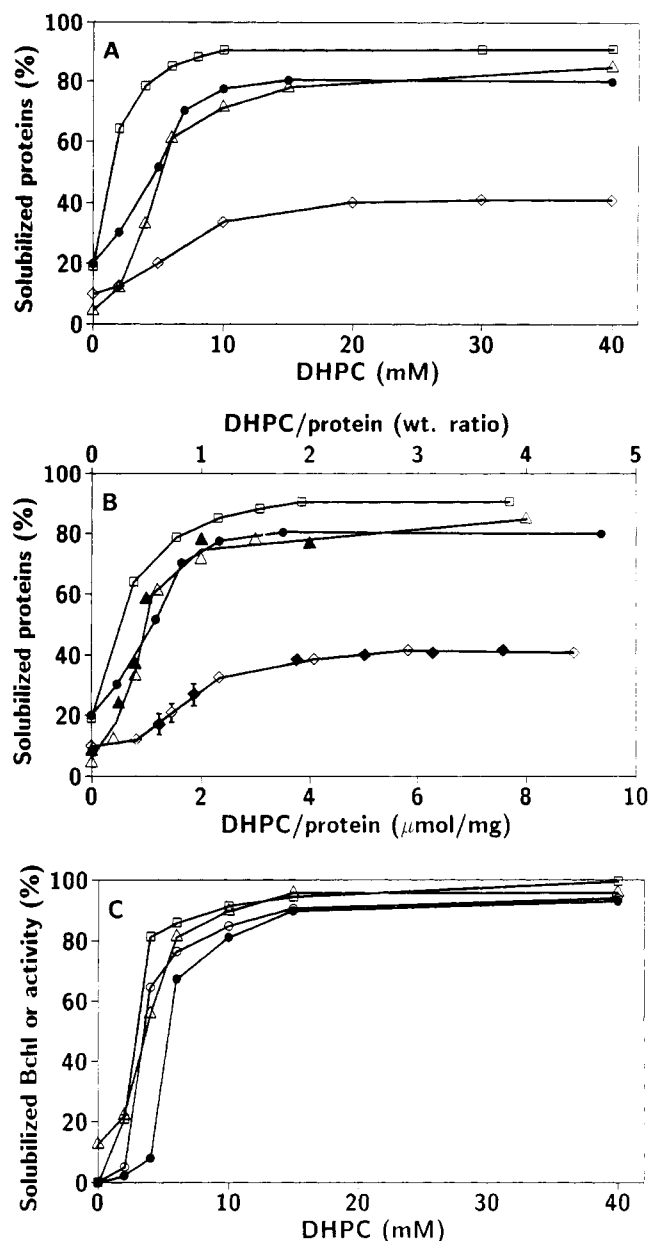


FIGURE 1: (A, top) Solubilization of plasma and organelle membranes by DHPC. To membranes suspended in PBS, a 200 mM stock solution of DHPC in the same buffer was added dropwise at 0 °C to the desired final DHPC concentration. The extent of solubilization (%) was determined as described in Materials and Methods and is shown as a function of the total DHPC concentration: ( $\square$ ) mitochondria, 2.6 mg of protein/mL; ( $\Delta$ ) chromatophores from *R. rubrum*, 5.0 mg of protein/mL; ( $\bullet$ ) BBMV, 4.3 mg of protein/mL; ( $\diamond$ ) erythrocyte ghosts, 4.5 mg of protein/mL. Final protein concentrations are given which were kept constant all along the solubilization curves. (B, middle) Normalization of the S-shaped solubilization curves shown in A. For this purpose the S-shaped curves are plotted as a function of the detergent/protein wt ratio (top abscissa) or as  $\mu\text{mol}$  of DHPC/mg of protein (bottom abscissa). In this plot the solubilization curves are independent of the protein concentration, as explicitly shown for chromatophores at 10 mg of protein/mL ( $\blacktriangle$ ) and 5.0 mg/mL ( $\triangle$ ) and erythrocyte ghosts at 4.5 mg/mL ( $\diamond$ ) and 1.6 mg/mL ( $\blacklozenge$ ). Otherwise the symbols have the same meaning as in A. (C, bottom) Solubilization of various proteins of chromatophores from *R. rubrum* by DHPC at 0 °C. The membranes were suspended in 0.01 M Tris-HCl buffer, pH 7.5, containing 0.15 M NaCl and 2 mM  $\text{MgCl}_2$  and were solubilized as described in Materials and Methods. The final protein concentration of the solubilized membrane varied from 4.8 to 5.2 mg/mL. ( $\square$ ) ATPase; ( $\circ$ ) succinate dehydrogenase; ( $\Delta$ ) NADH dehydrogenase; ( $\bullet$ ) BChl = bacteriochlorophyll-containing proteins.

that with increasing membrane concentrations the S-shaped curves shifted progressively to the right along the DHPC-axis

(data not shown). Upon normalization of these curves, i.e., plotting percent solubilization as a function of the DHPC/protein ratio, all the data points obtained for a particular membrane at different protein concentrations came to lie on a single S-shaped curve (Figure 1B). This result indicates that a particular membrane is solubilized at a constant detergent/protein ratio.

As evident from Figure 1A and B, even in the absence of DHPC some protein remained in the supernatant after centrifugation at 100000g for 20 min. The amount of protein liberated from membranes varied between 5 and 20% (Figure 1A and B); in the case of BBM, which is known to be prone to degradation by intrinsic proteinases (Thurnhofer & Hauser, 1990), this amount was between 10 and 30%. Freeze-thaw cycles and storage of the membrane at temperatures  $> 0$  °C increased this amount. Freeze-thaw cycles had a particularly destabilizing effect on mitochondria, and for this reason only freshly prepared mitochondria were used. The plateau values observed at  $[\text{DHPC}] > 10\text{--}15$  mM differ significantly for the two types of membranes (Figures 1A and B): in the case of organelle membranes, 85–95% of the membrane proteins were solubilized while the yield was much less for plasma membranes, 65–80% for BBM and only 40–45% for erythrocyte ghosts (Table 1). The yield of solubilized erythrocyte ghosts increased approximately linearly with ionic strength; for instance a plateau value of 80% was reached with 20 mM DHPC in 10 mM sodium phosphate buffer, pH 7.5, containing 0.8 M NaCl (Table 1). Treatment of erythrocyte ghosts with increasing concentrations of NaCl alone, i.e., in the absence of DHPC, did not significantly enhance the release of protein compared to the blank. High ionic strength was shown to cause dissociation of cytoskeletal proteins from erythrocyte membranes (Bennett & Branton, 1977). The NaCl experiments then suggested to us that the low yield of solubilized ghosts is due to the presence of the intact cytoskeleton. This interpretation is supported by SDS-PAGE. Erythrocyte ghosts solubilized with 20 mM DHPC under standard conditions (cf. Materials and Methods) yielded band 3 as the major protein on SDS-PAGE and very little spectrin. If, however, ghosts were solubilized with 20 mM DHPC in the presence of 0.8 M NaCl, the pattern of the SDS-PAGE of solubilized ghosts closely resembled that of original ghosts. In this case  $\alpha$ - and  $\beta$ -spectrin were major protein bands (data not shown). The SDS-PAGE pattern of BBM, chromatophores, and mitochondrial membranes solubilized with 20 mM DHPC under standard conditions were practically indistinguishable from those obtained with the original membranes (data not shown). Furthermore, removal of cytoskeletal proteins according to Bennet and Branton (1977) and Bennett and Stenbuck (1980) resulted in inside-out ghosts which could be solubilized with DHPC under standard conditions, yielding about 80% solubilized proteins (data not shown).

## (II) Solubilization of Chromatophores by DHPC and Recovery of Enzymatic Activities

The solubilization of various proteins of the chromatophore membrane essential for photosynthesis is depicted in Figure 1C. The S-shaped curves provide evidence for the differential solubilization of proteins. For instance, at 4 mM DHPC about 80% of the ATPase activity but less than 10% of bacteriochlorophyll-containing proteins were solubilized. More than 80% of all the enzymatic activities were solubilized at 10 mM DHPC. The inflexion points of the curves shown in Figure 1C were at total DHPC concentrations of 2.6, 3.0, 3.7, and

Table 1: Solubilization of Plasma and Organelle Membranes by DHPC

plasma membrane	organelle membrane	lipid/protein wt ratio in the intact membrane	detergent/protein wt ratio at the inflexion point of the S-shaped curve <sup>a</sup>	detergent/protein wt ratio at maximum solubilization <sup>b</sup>	amount of protein (%) solubilized at 40 mM DHPC <sup>c</sup>
erythrocyte ghosts		0.8/1 <sup>d</sup>	0.75 ± 0.05	1.6	43 ± 3 (80 ± 5)
brush border membrane		0.5/1 <sup>e</sup>	0.45 ± 0.05	1.6	73 ± 7
	chromatophores	0.5/1 <sup>f</sup>	0.45 ± 0.05	1.6	85 ± 5
	mitochondria	0.3/1 <sup>g</sup>	0.26 ± 0.05	1.6	93 ± 3

<sup>a</sup> This is the approximate detergent/protein wt ratio taken at the inflexion point of the normalized S-shaped curves such as those in Figure 1. <sup>b</sup> This is the detergent/protein wt ratio taken at the beginning of the plateau region of the normalized S-shaped curve (cf. Figure 1). <sup>c</sup> Solubilization was carried out with 40 mM DHPC as described under Materials and Methods. Erythrocyte ghosts were solubilized to 80% (value in parentheses) after removal of the cytoskeleton according to Bennett and Branton (1977) and Bennett and Stenbuck (1980). <sup>d</sup> Steck, 1974. <sup>e</sup> Hauser et al., 1980. <sup>f</sup> Snozzi & Bachofen, 1979. <sup>g</sup> Tzagoloff, 1982.

5.0 mM for ATPase, succinate dehydrogenase, NADH dehydrogenase, and bacteriochlorophyll-containing proteins, respectively. The enzymatic activities observed at 40 mM DHPC were slightly increased as compared to those measured at 10 mM DHPC.

The  $F_A$  values representing the ratios of the activities measured in the presence and absence of DHPC were determined as a function of the total DHPC concentration (Table 2).  $F_A$  values greater than 1 were obtained for ATPase and NADH dehydrogenase while these values were smaller than 1 for succinate dehydrogenase, indicating that solubilization with DHPC activates the first two enzymes but partially inhibits succinate dehydrogenase. These values were independent of the DHPC concentration, and activation or inhibition occurred at sublytic DHPC concentration (Table 2).

The activity of the reaction center of chromatophores was well preserved after solubilization in 40 mM DHPC. The solubilized activity determined by ESR spectroscopy was  $105 \pm 5\%$  ( $n = 3$ ), and the  $F_A$  value was 1.05 (Table 2). The light absorption spectrum of chromatophores solubilized in 40 mM DHPC was identical to that of intact chromatophores except for a reversible slight blue shift of the main bacteriochlorophyll peak from 873 to 868 nm (data not shown). The  $F_A$  value of the bacteriochlorophyll-containing proteins in 40 mM DHPC was 0.92 (Table 2), indicating that these proteins are still well preserved in 40 mM DHPC. Another piece of evidence for the stability of the bacteriochlorophyll-containing proteins in the presence of DHPC was the preservation of their absorption spectrum for several days when solubilized in 10 mM DHPC and stored at 0 °C in the dark.

### (III) Solubilization of BBMV with DHPC and Reconstitution of the D-Glucose Cotransporter into a Simple, Artificial Membrane System

BBMV were solubilized in 40 mM DHPC as described in Materials and Methods. The D-glucose carrier was reconstituted from mixed lipid-protein micelles resulting from the solubilization of BBMV in 40 mM DHPC as described in Materials and Methods. The proteoliposomes reconstituted in this way were used to measure D-glucose uptake in the presence of an inwardly directed 0.1 M NaSCN gradient. The reconstituted proteoliposomes behaved qualitatively similar to BBMV: as shown in Figure 2 a small but statistically significant overshoot of D-glucose accumulation was measured, which was about 20% of that measured with intact BBMV. Furthermore, the equilibrium value of D-glucose uptake measured after 3 h of incubation with glucose was similar to that found for intact BBMV. These results indicated that the Na<sup>+</sup>-dependent D-glucose cotransporter is at least partially retained in a functional state when BBMV are solubilized in

Table 2:  $F_A$  of Various Chromatophore Proteins<sup>a</sup>

protein	DHPC (mM)					
	2	4	6	10	15	40
ATPase	1.64	1.64	1.50	1.53	1.64	1.70
NADH dehydrogenase	1.75	2.11	1.94	1.99	1.98	2.10
succinate dehydrogenase	0.60	0.60	0.64	0.66	0.69	0.70
reaction center	nd <sup>b</sup>	nd	nd	nd	nd	1.05
bacteriochlorophyll-containing proteins	1.08	1.04	1.03	0.94	0.91	0.92

<sup>a</sup>  $F_A$  values were determined as described under Materials and Methods.

<sup>b</sup> nd = not determined.

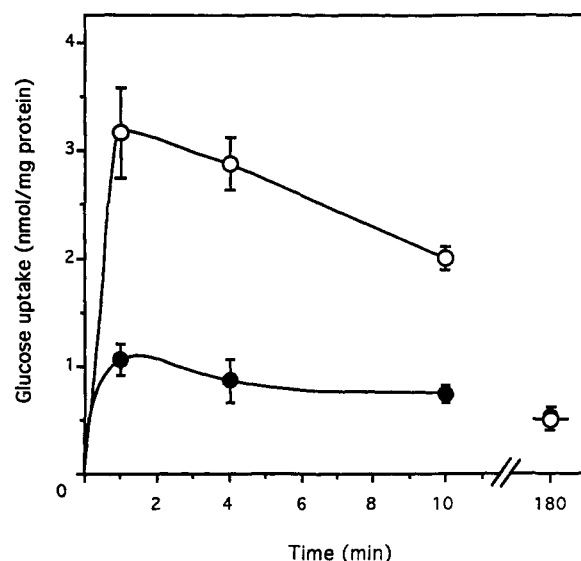


FIGURE 2: Kinetics of D-glucose uptake by rabbit small intestinal BBMV (O) and proteoliposomes reconstituted from BBM proteins solubilized in 40 mM DHPC (●). D-glucose uptake was measured in the presence of an initial 0.1 M NaSCN gradient (0.1 M outside, 0.0 M inside). Results are represented as the mean ± standard deviation of three independent measurements.

excess DHPC and the D-glucose cotransporter is reconstituted into EPC bilayers.

### (IV) Solubilization of the Na<sup>+</sup>/K<sup>+</sup>-ATPase from the Outer Renal Medulla Membrane. Comparison of the Effect of Different Detergents

The Na<sup>+</sup>/K<sup>+</sup>-ATPase was solubilized from the renal medulla membrane using different detergents. In order to compare the effect of different detergents, the amount of protein and Na<sup>+</sup>/K<sup>+</sup>-ATPase activity solubilized (both as percent) are shown as a function of the detergent/protein wt ratio (Figure 3). With regard to the amount of solubilized protein, the different detergents behaved similarly: at detergent/protein wt ratios greater than 2–3, about 70–80%

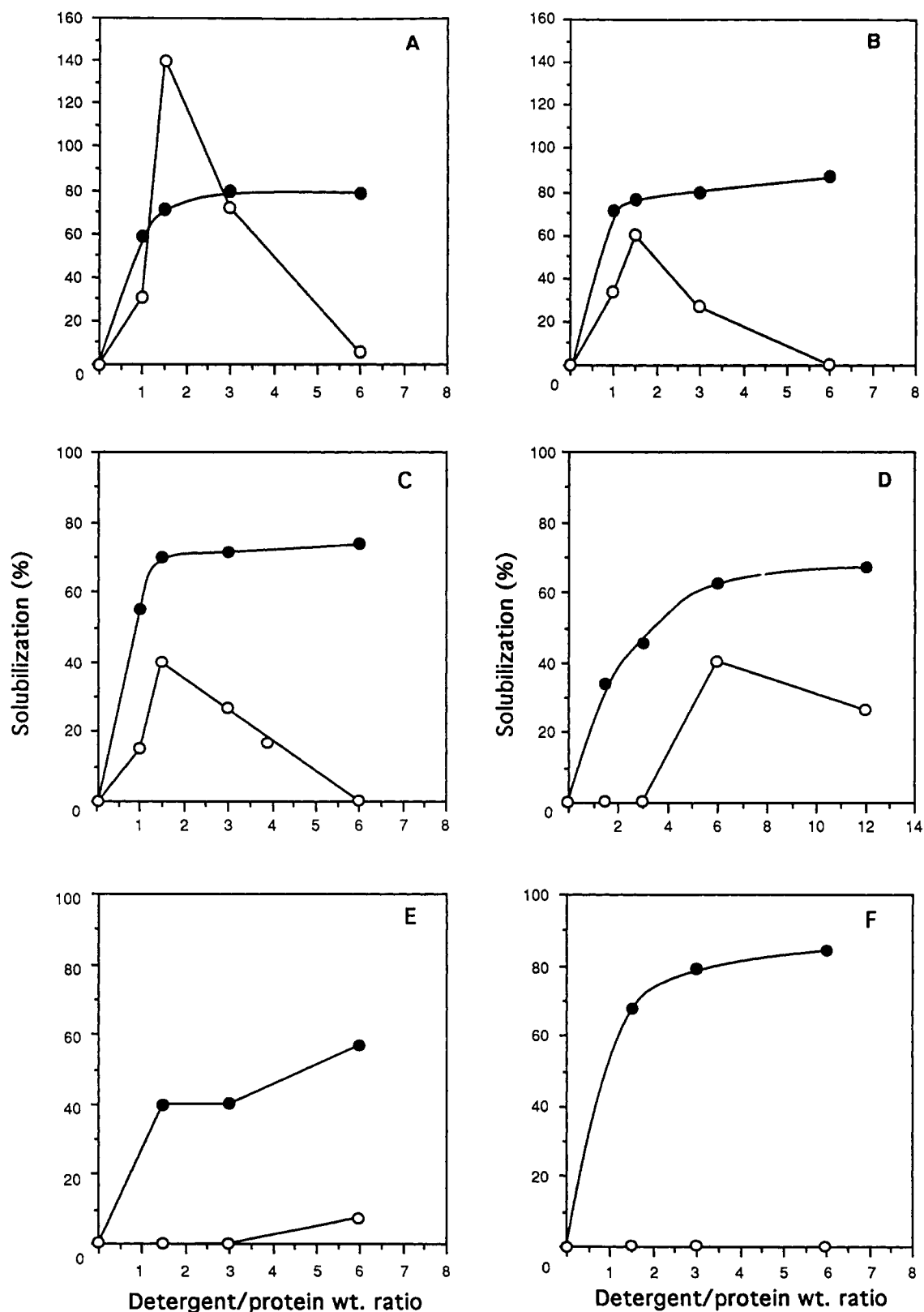


FIGURE 3: Solubilization of the  $\text{Na}^+/\text{K}^+$ -ATPase from the renal outer medulla membrane by various detergents. Membranes (10 mg of protein/mL) suspended in 0.1 mL of 0.025 M imidazole buffer, pH 7.2, containing 0.15 M KCl and 20 vol % glycerol were solubilized by adding various detergents. Protein (●) and  $\text{Na}^+/\text{K}^+$ -ATPase activity (○) were measured in the supernatant obtained by centrifugation at 180000g for 30 min at 4 °C: (A) DHPC ( $M_r = 500$ ); (B) Triton X-100 ( $M_r = 628$ ); (C)  $\text{Na}^+$  deoxycholate ( $M_r = 415$ ); (D) CHAPS ( $M_r = 615$ ); (E)  $\beta$ -D-octyl glucoside ( $M_r = 292.5$ ); (F) emulphogene BC 720 ( $M_r = 616$ ). 100% values refer to the activity measured in the original membrane suspension prior to the addition of detergent.

of the membrane proteins appeared to be solubilized except for octyl glucoside and CHAPS, which solubilized only about 40% and 50% of the membrane proteins, respectively (Figure 3E and D, respectively). Regarding the solubilized  $\text{Na}^+/\text{K}^+$ -ATPase activity the detergents differed greatly: DHPC behaved exceptionally because it was the only detergent that stimulated  $\text{Na}^+/\text{K}^+$ -ATPase. The activity exhibited a well-defined maximum of ~140% at a DHPC/protein wt. ratio

of 1.5. For all other detergents, the activity was either low or not detectable. The activity of  $\text{Na}^+/\text{K}^+$ -ATPase in the supernatant was measured by the method of Kessler and Kessler (1988). The activity was measured in the supernatant obtained by centrifugation at 180000g for 30 min at 4 °C. The activity was measured in the supernatant obtained by centrifugation at 180000g for 30 min at 4 °C. The activity was measured in the supernatant obtained by centrifugation at 180000g for 30 min at 4 °C.

of 1.5. Above this ratio the activity decreased progressively up to a DHPC/protein wt. ratio of  $\sim 6$  at which the enzyme had lost its activity (Figure 3A). Triton X-100 and  $\text{Na}^+$  deoxycholate behaved qualitatively similarly to DHPC exhibiting maxima in activity at detergent/protein wt. ratios of 1.5, but with activities of only 60% and 40%, respectively, which is significantly lower than the activity measured in the presence of DHPC (Figures 3B and C). Octyl glucoside and emulphogene BC 720 solubilized the membrane, but both detergents inactivated  $\text{Na}^+/\text{K}^+$ -ATPase (Figures 3E and F).

#### (V) Particle-Size Analyses of Solubilized Membranes

**Gel Filtration on Sepharose CL-4B.** Gel filtration on Sepharose CL-4B provided direct evidence for the solubilization of biological membranes in the presence of DHPC. This method was also used to optimize the DHPC concentration. Solubilization of chromatophores in DHPC resulted in mixed micelles of at least two different sizes (Figure 4A). With progressively higher DHPC concentrations, the second peak, which was eluted at the larger elution volume (corresponding to smaller micelles), grew at the expense of the first one (corresponding to larger micelles). DHPC concentrations in excess of 40 mM had an increasing tendency of denaturing bacteriochlorophyll-containing proteins, as evident from changes in the near-infrared spectrum. The absorbance at 870 nm decreased, and a new peak appeared at 775 nm, indicating the partial denaturation of the bacteriochlorophyll-containing proteins (Ghosh et al., 1988). The DHPC concentration was optimized such that both the size of the resulting micelles and the protein denaturation were minimal. Chromatophores like all other membranes exhibited maximum solubilization at 40 mM DHPC (cf. Figure 1). Since the activity of the solubilized chromatophore proteins and the absorption spectra of the bacteriochlorophyll-containing proteins were well preserved at 40 mM DHPC (Table 2), this concentration was chosen for the particle-size analysis of solubilized membrane proteins. Chromatophore proteins solubilized with 40 mM DHPC were eluted as two peaks corresponding to mean Stokes or hydrodynamic radii of 4.0 and 6.8 nm, respectively (Table 3; Figure 4A and C). The gel filtration patterns of erythrocyte ghosts, BBMV, and mitochondrial membranes solubilized with 40 mM DHPC are shown in Figure 4B, D, and E, respectively. In all cases solubilized proteins were eluted at elution volumes  $V_e$  significantly larger than the column void volume  $V_0$ , indicating that the resulting mixed micelles are significantly smaller than  $\sim 30$  nm. The protein elution pattern of solubilized erythrocyte ghosts consisted of two broad peaks corresponding to Stokes radii of 5.5 and 8.3 nm (Table 3). Solubilized BBMV and mitochondrial membrane proteins were eluted as broad, asymmetric peaks at elution volumes  $V_e$  corresponding to Stokes radii of  $r_H = 6.3$  nm and  $r_H = 5.8$  nm, respectively. The lipid elution profiles based on the incorporation of  $^3\text{H}$ -DPPC in the mixed micelles are included in Figure 4. With all four membranes most of the lipids were eluted as a single, rather symmetric peak. Converting the elution volumes of the lipid peaks to Stokes radii, values ranging from about 3 to 5 nm were obtained (Table 3). These values are comparable to the Stokes radius measured for pure DHPC micelles which were eluted as a single, symmetric peak corresponding to a Stokes radius of  $4.4 \pm 0.4$  nm (data not shown). Assuming that these DHPC micelles are spherical and using the partial specific volume of Tausk et al. (1974b), the average aggregation number of these DHPC micelles was calculated to be about 60. The Stokes radius of pure DHPC micelles together with the protein and lipid elution patterns of DHPC-solubilized

membranes (Figure 4B–E) indicate that the lipid peaks in Figure 4 consist of both lipids associated with solubilized proteins as well as lipid–DHPC micelles.

**Freeze-Fracture Electron Microscopy (FFEM).** A representative electron micrograph of a freeze-fractured preparation of mixed lipid–protein micelles obtained by solubilization of BBMV with 40 mM DHPC is shown in Figure 5. The electron micrograph shows mainly small spherical particles with hydrodynamic radii ranging from  $\sim 4$  to 8 nm. The sizes of several hundred particles were measured, and the results are presented as a bar histogram (Figure 6C). The same particle-size analysis was carried out with solubilized erythrocyte ghosts, chromatophores, and mitochondria (Figure 6A, B, and D, respectively). The particle size distribution of solubilized erythrocyte ghosts is multimodal, consisting of three peaks corresponding to hydrodynamic radii of 4.3, 6.0, and 8.0 nm (Figure 6A; Table 3). This is in good agreement with the particle size distribution derived from gel filtration on Sepharose CL-4B (cf. Figure 4B). A multimodal particle size distribution was also obtained with solubilized chromatophores. Peaks in the bar histogram were at hydrodynamic radii of 3.0, 5.5, and 7.5 nm, corresponding to lipid–DHPC micelles and two different sizes of mixed lipid–protein micelles, respectively (Figure 6B). Again the particle size distribution is consistent with that derived from gel filtration (cf. Table 3). Broad, unresolved bar histograms were obtained with freeze-fractured preparations of solubilized BBMV and mitochondria (Figure 6C and D). The bar histograms range from Stokes radii of  $\sim 2.5$  to  $\sim 12$  nm and  $\sim 3.0$  to  $\sim 10$  nm, respectively, exhibiting maxima in the size distribution at 5.5 and 5.0 nm, respectively (Figure 6C and D; Table 3). Inspection of Table 3 shows that for both BBM and mitochondria, the maxima as well as the spread of the bar histograms are in satisfactory agreement with the average hydrodynamic radii and the peak widths of the protein peaks, respectively, derived from gel filtration on Sepharose CL-4B.

#### (VI) Purification of Sucrase-Isomaltase from BBMV and Its Reconstitution into a Simple, Artificial Membrane System

BBMV were solubilized in 40 mM DHPC as described in Materials and Methods. The sucrase activity in the resulting lipid–protein micelles was  $92 \pm 2\%$  ( $n = 3$ ) of the original activity measured in BBMV in the absence of DHPC. Sucrase-isomaltase solubilized with DHPC was purified as described in Materials and Methods. The enzyme complex eluted from Sephadex G-200 was pure by SDS-10% PAGE (Figure 7A), and the total activity recovered was 22–30%, corresponding to 1.5–2 times the activity recovered from Triton X-100-solubilized BBMV under comparable conditions (Sigrist et al., 1975). The solubilized and purified sucrase-isomaltase was incorporated into EPC bilayers as described by Brunner et al. (1978), and the resulting proteoliposomes behaved very similarly to those described in this reference (data not shown).

#### (VII) Purification of the Bacteriochlorophyll-Containing Proteins from Chromatophores of *R. rubrum*

Chromatophores suspended in Tris-HCl buffer were solubilized in 15 mM DHPC. Bacteriochlorophyll-containing proteins were purified as described in Materials and Methods. The yield of the purified bacteriochlorophyll-containing proteins was about 50%, and the reaction center activity of the recovered bacteriochlorophyll-containing proteins was about 80% of the activity measured in the intact membrane. The SDS-PAGE pattern (Figure 7B) shows that a complex consisting of reaction center and light-harvesting peptides was



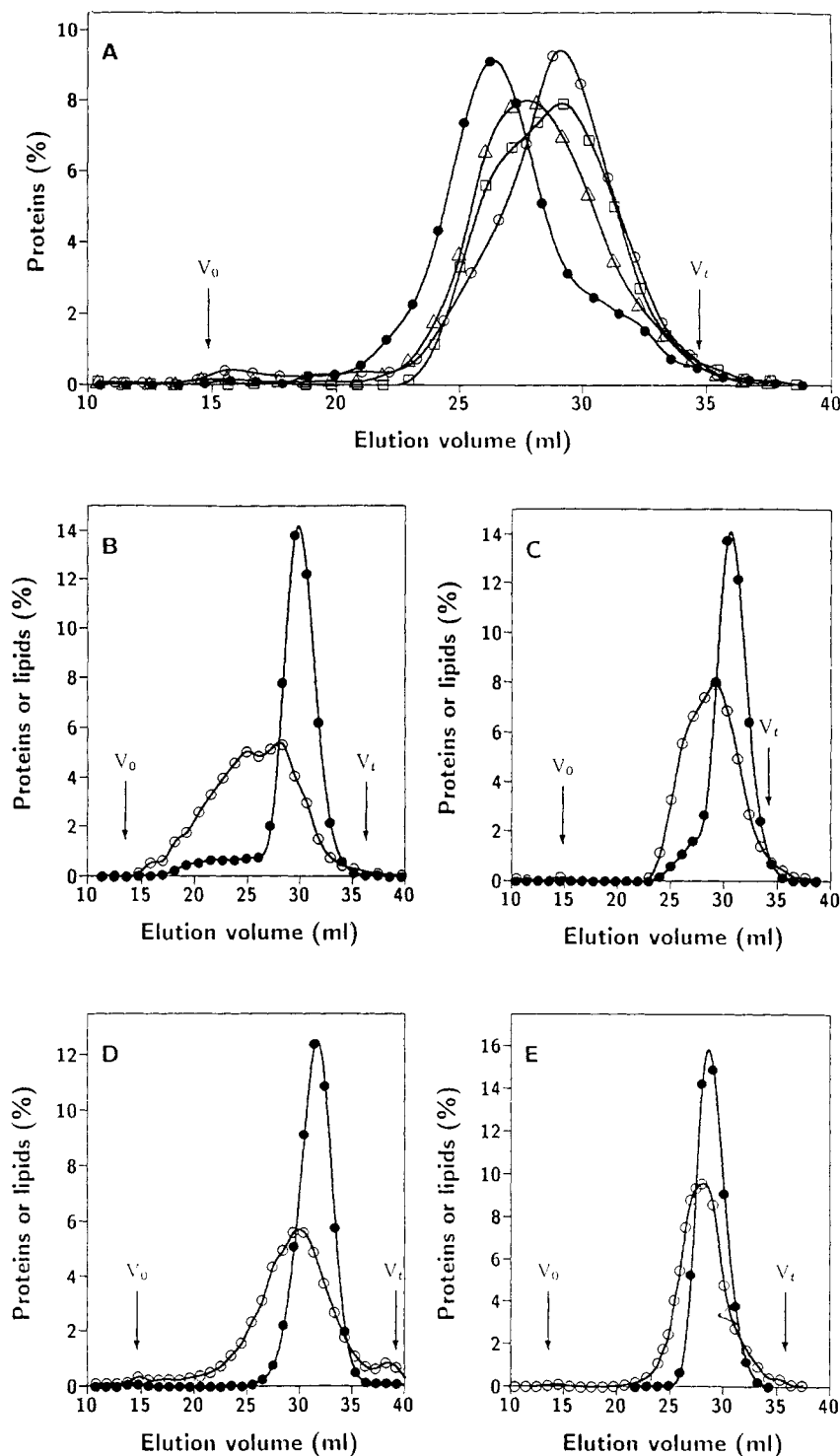


FIGURE 4: Gel filtration patterns on Sepharose CL-4B of solubilized chromatophores from *R. rubrum*. Chromatophores suspended in PBS buffer to a final protein concentration of 3–4 mg/mL were solubilized by DHPC at room temperature. Gel filtration experiments were performed in duplicate, and reproducible gel filtration patterns were obtained. (A) Protein elution patterns of chromatophores solubilized with total DHPC concentrations of 10 mM (●), 20 mM (Δ), 40 mM (□), and 80 mM (○). (B–E) The solubilized plasma and organelle membranes were labeled with  $^3\text{H}$ -DPPC as described in Materials and Methods and chromatographed on Sepharose CL-4B. The column eluate was analyzed for protein (○) and  $^3\text{H}$ -DPPC radioactivity (●): (B) erythrocyte ghosts ( $95 \pm 2\%$ ,  $92 \pm 2\%$ ); (C) chromatophores from *R. rubrum* ( $86 \pm 5\%$ ,  $93 \pm 2\%$ ); (D) rabbit intestinal BBMV ( $96 \pm 5\%$ ,  $82 \pm 2\%$ ); (E) rat liver mitochondria ( $96 \pm 5\%$ ,  $81 \pm 3\%$ ). The numbers in parentheses are the mean  $\pm$  standard deviations of the protein and lipid recovery, respectively. The column (50 cm  $\times$  1 cm) was equilibrated and run at 4  $^{\circ}\text{C}$  with PBS containing 1.5 mM DHPC.  $V_0$  and  $V_t$  marked by arrows are the void and total column volumes, respectively.

purified. The main bands observed on SDS-PAGE were the L, M, and H subunits of the reaction center and the light-harvesting peptides (LH). It was shown before for preparation of pure reaction center that the subunit H splits into two bands on SDS-PAGE (H and H' in Figure 7B). This result agrees with a kinetic analysis reported by van Grondelle (1976)

providing evidence for two types of reaction center. Near-infrared CD and absorption spectra indicated that the three-dimensional structure of the reaction center–light-harvesting complex is well preserved in the purified bacteriochlorophyll-containing proteins (data not shown). A minor impurity of ATPase was detected (labeled C); on the basis of activity

Table 3: Stokes or Hydrodynamic Radii  $r_H$  of Solubilized Mixed Lipid Protein Micelles Compared to the Size of the Intact Membrane Particles<sup>a</sup>

membrane	size of the intact membrane (nm)	Stokes or hydrodynamic radii $r_H$ (nm)			
		gel filtration on Sepharose CL-4B		freeze-fracture electron microscopy	
		phospholipid	protein	phospholipid	protein
chromatophores	45 <sup>b</sup>	2.9	4.0; 6.8	3.0	5.5; 7.5
erythrocyte ghosts	6000–7000 <sup>c</sup>	4.3	5.5; 8.3	4.3	6.0; 8.0
brush border membrane vesicles	80 (range 40–140) <sup>b</sup>	4.9	6.3, range 2–13	nd	5.5 (range 2.5–12)
mitochondria	oval particles 1000–2000 long 500–1000 wide <sup>d</sup>	5.3	5.8, range 3–9	nd	5.0 (range 3–10)

<sup>a</sup> Plasma and organelle membranes were suspended in PBS to a final protein concentration of 3.5–5.2 mg/mL. The membranes were solubilized to mixed lipid–protein micelles by adding DHPC to a final concentration of 40 mM at room temperature. <sup>b</sup> Perevucnik et al., 1985. <sup>c</sup> Dodge et al., 1963.

<sup>d</sup> Tzagoloff, 1982.



FIGURE 5: Electron micrograph of a freeze-fractured sample of BBMV solubilized in the presence of 40 mM DHPC. Samples were prepared as described by Hauser et al. (1983). The bar represents 100 nm.

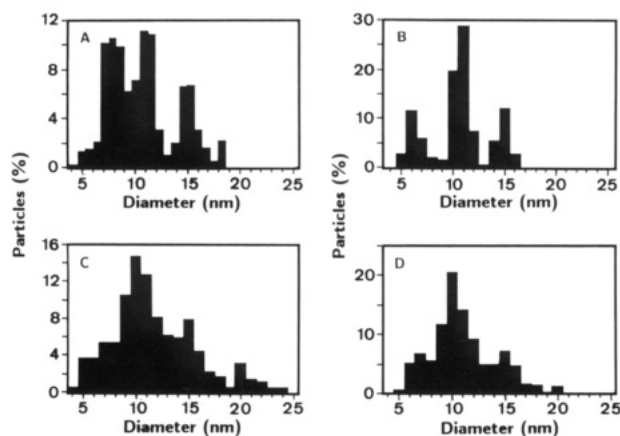


FIGURE 6: Bar histograms derived from electron micrographs of freeze-fractured samples of various plasma and organelle membranes solubilized in 40 mM DHPC at room temperature: (A) erythrocyte ghost; (B) chromatophores from *R. rubrum*; (C) rabbit intestinal BBMV; (D) rat liver mitochondria suspended in PBS buffer to 3–4 mg of protein/mL were solubilized in the presence of 40 mM DHPC. The number of particles measured on the electron micrographs were  $n = 240$ ,  $n = 268$ ,  $n = 353$ , and  $n = 345$  for DHPC-solubilized erythrocyte ghosts, chromatophores, BBMV, and mitochondria, respectively.

measurements, the impurity was less than 2% of the original ATPase activity.

## DISCUSSION

**Efficiency of DHPC in Membrane Solubilization.** DHPC is a short-chain phospholipid that can be readily synthesized

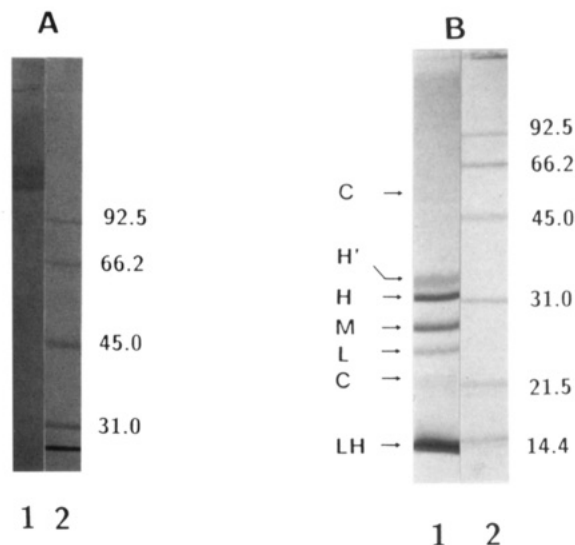


FIGURE 7: SDS-PAGE patterns. PAGE was performed according to the method of Laemmli (1970), and the gels were stained with Coomassie brilliant blue. (A) Lane 1: 10% PAGE of the purified sucrase-isomaltase. lane 2: Molecular weight standards. (B) Lane 1: 12.5% PAGE of the purified bacteriochlorophyll-containing proteins. L, M, H, and H' designate the subunits of the reaction center, and LH designates the subunits of the light-harvesting complex. C = contamination arising from the ATPase. The diffuse bands in the molecular weight range >92 kDa probably represent aggregates of the subunits of the reaction center and light harvesting complex. Lane 2: Molecular weight standards. The molecular weights of the standard proteins are given in kDa.

in large quantities. It is commercially available as a pure compound though rather expensive compared to other detergents. As a short-chain diacyl phospholipid it has detergent-like properties. The dispersing effect of short-chain phosphatidylcholines on long-chain phospholipid bilayers was first reported by Gabriel and Roberts (1986). As shown here DHPC acts as an effective solubilizing agent for biological membranes. Organelle membranes lacking a cytoskeleton are solubilized to 80–95% in the presence of a total DHPC concentration of 10–20 mM (0.5–1.0%), corresponding to DHPC/protein wt ratios of 1–2 (cf. Table 1). Plasma membranes which have a cytoskeleton are solubilized to a similar extent only after removal of the cytoskeletal proteins or disruption of the interactions between integral membrane proteins and the cytoskeleton. For instance, the effective solubilization of erythrocyte ghosts with yields of up to 80% requires the removal or disruption of the cytoskeleton (Table 1).

The comparison of the particle-size analysis of the solubilized mixed protein–phospholipid–DHPC micelles with the original size of the membrane vesicles (cf. Table 3) provides clear-cut

evidence for the effective solubilization of both organelle and plasma membranes. Solubilization yields mixed micelles of Stokes radii of 5–8 nm (Table 3), which is a small fraction of the size of the original membrane vesicles. The dimensions of the protein–lipid–detergent mixed micelles, particularly the smallest ones, are probably determined by the size of the integral membrane proteins. They are in good agreement with published data (Møller et al., 1986; Esmann, 1984). The similarity in dimension of these mixed micelles may be explained by the similarity in dimension of integral membrane proteins, the size of which is related to the thickness of the lipid bilayer.

The detergent/protein wt ratio at the inflexion point of the solubilization curve is a characteristic value for each membrane. As demonstrated in Table 1, this value is closely related to the lipid/protein wt ratio of the original membrane. Such a correlation implies that all membranes investigated are solubilized at an identical DHPC/lipid ratio. This finding is taken as evidence that DHPC primarily interacts with the lipid bilayer and not with the membrane proteins (see discussion below).

**Preservation of Protein Structures and Activities.** Important features of the solubilization of biological membranes by DHPC are (i) the preservation of the native conformation and hence the activity of the solubilized proteins. In all cases studied here the activity of the solubilized membrane proteins is retained upon solubilization. (ii) The three-dimensional structure and activity of proteins are retained not only at 10–15 mM DHPC concentrations at which maximum solubilization usually occurs but also over a large range of DHPC concentrations, e.g. up to 40 mM. The preservation of the native protein structure over a large concentration range of the detergent is characteristic of DHPC. For instance,  $\beta$ -D-octyl glucoside, which ranks among the mildest detergents, was shown to induce massive denaturation of bacteriochlorophyll-containing proteins of *R. rubrum* at concentrations twice that required for the solubilization of chromatophores (Ghosh & Bachofen, 1989). The concentration range of DHPC is also significantly larger than that of Triton X-100 and lauryldimethylamine *N*-oxide routinely used for the solubilization of chromatophores. For instance, chromatophores at ~17 mg of protein/mL are solubilized by 22 mM (0.5%) lauryldimethylamine *N*-oxide, yet the solubilized proteins become unstable above this detergent concentration and undergo massive denaturation in excess of ~26 mM (0.6%) (Kessi, 1994). In contrast, DHPC concentrations >40 mM are required before denaturation and loss in enzymatic activity occur. (iii) Not only are the three-dimensional structure and hence the activity of most proteins retained in excess DHPC (e.g. up to 40 mM) but the proteins also appear to be stable in DHPC. Near-infrared spectroscopy indicates that the three-dimensional structure of the bacteriochlorophyll-containing proteins solubilized from chromatophores of *R. rubrum* is maintained in 10 mM DHPC at 0 °C in the dark for several days. In this respect DHPC is also superior to lauryldimethylamine *N*-oxide.

In some cases studied here the solubilized proteins retain their biological activities in the presence of 10–40 mM DHPC as compared to the activity measured in the intact membrane, i.e., in the absence of DHPC; in others the activity is even enhanced, as observed for the  $F_0F_1$ -ATPase, NADH-dehydrogenase (Table 2), and  $Na^+/K^+$ -ATPase (Figure 3A). With succinate dehydrogenase and the D-glucose transporter (Table 2; Figure 2), the activity appears to be reduced in the solubilized state. However, except for the  $Na^+/K^+$ -ATPase the enzyme

activities are retained over a large DHPC concentration range, e.g., up to 4–5 times the concentration required to solubilize these proteins. This result suggests that the direct interaction of DHPC with integral membrane proteins is weak.

The  $Na^+/K^+$ -ATPase from the renal medulla membrane appears to be more sensitive to denaturation by increasing concentrations of DHPC than  $F_0F_1$ -ATPase, NADH-dehydrogenase, succinate dehydrogenase, and bacteriochlorophyll-containing proteins of the chromatophore membrane (Figure 3A). Still a comparison of Figure 3A with Figure 3B–E clearly shows the superiority of DHPC in solubilizing and preserving the activity of this enzyme over all other detergents tested. Triton X-100 and bile salts which were equally efficient as DHPC in solubilizing membrane proteins from the renal medulla membrane were much less effective regarding the recovery of the  $Na^+/K^+$ -ATPase activity. The loss in activity observed in the presence of excess DHPC (Figure 3A) is probably due to the loss of the quaternary structure of the enzyme under these conditions (Esmann, 1988).

The activity of the D-glucose carrier is also sensitive to the presence of detergents. All previous attempts carried out in this and other laboratories to solubilize BBMV in various detergents and to reassemble the D-glucose carrier into phospholipid bilayers gave unsatisfactory results (Semenza et al., 1984). One problem encountered in the reconstitution of integral BBM proteins is the activation of intrinsic proteinases in the presence of detergents (Gains & Hauser, 1981). Poirée et al. (1986) reported the successful reconstitution of the  $Na^+$ -D-glucose cotransporter from a Triton X-100 solubilized kidney BBM. These authors demonstrated the accumulation of D-glucose in proteoliposomes in the presence of  $Na^+$ , but no overshoot was observed under any of the conditions tested. In our hands the use of DHPC to solubilize small intestinal BBM proteins and to reconstitute the solubilized proteins into phospholipid bilayers leads to a statistically significant accumulation of D-glucose over the equilibrium value.

The observation that enzymatic activities are retained over a large DHPC concentration range, e.g., up to 4–5 times the concentration required to solubilize the membrane proteins, is taken as evidence that DHPC interacts weakly with membrane proteins. This is consistent with the finding that the DHPC concentration needed for solubilizing biological membranes is dependent on the lipid concentration of the membrane (Table 1) and with evidence showing that integral membrane proteins interact more strongly with lipids having hydrophobic domains of dimensions comparable to those of naturally occurring phospholipids (Riegler, 1985). If the interaction of DHPC with membrane proteins is indeed weak, it follows that intrinsic membrane lipids will remain associated with membrane proteins. Such a preferential association of integral membrane proteins with their intrinsic membrane lipids is probably responsible for the stabilization of the native protein conformation and retention of enzymatic activities in the solubilized state.

**Mechanism of Membrane Solubilization with DHPC; Interaction of DHPC with Lipid Bilayers.** Assuming that DHPC interacts weakly with integral membrane proteins, the mechanism of membrane solubilization by DHPC is therefore postulated to involve primarily the interaction of DHPC with the lipid part of the membrane. If DHPC is inserted into a lipid bilayer, it is envisaged to exert a wedge-like effect on the neighboring lipids mainly due to its bulky polar group and its short hydrocarbon chains. This leads to membrane destabilization at relatively low DHPC concentra-

tions. For instance, BBMV are solubilized at a DHPC concentration of 15 mM, corresponding to a detergent/protein wt ratio of 1.6 (Figure 1A; Table 1). For comparison the detergent/protein wt ratios at which BBMV are solubilized by Triton X-100 and sodium cholate were 5 and 10, respectively, 3 and 6 times higher than for DHPC (Boffelli and Hauser, unpublished observation). This is good evidence for the remarkable dispersing power of short-chain PCs. The main role of the DHPC molecules in the mixed micelles is to solubilize the complex consisting of intrinsic membrane lipids and protein(s) in water. This is supposedly accomplished by DHPC, effectively minimizing the energetically unfavorable edge or hydrophobic effect due to exposure of the hydrocarbon chains of intrinsic membrane lipids to water. The short-chain DHPC seems to be structurally well suited to adequately shield the hydrocarbon chains of the intrinsic lipids from water contacts. We postulate that this kind of lipid-lipid interaction contributes significantly to the minimizing of the free energy of mixed lipid-protein micelles. The sum of DHPC-lipid interactions discussed above is then responsible for the effective solubilization of biological membranes by DHPC, and the retention of the native protein structure in the resulting lipid-protein micelles is attributable to a weak DHPC-protein interaction, as discussed above. The particular environment of the protein in the mixed micelles may lead to the observed stimulation or reduction of enzyme activities. The principle underlying the preservation of the native protein structure is the inability of DHPC to displace intrinsic membrane lipids from integral membrane proteins. Only at very high concentrations, DHPC competes successfully with intrinsic membrane lipids for integral membrane proteins, leading to the removal of intrinsic membrane lipids from these proteins and to protein denaturation. The complete delipidation of integral membrane proteins in the course of solubilization has been suggested before to be accompanied with loss in enzymatic activities (Hesketh et al., 1976; Anholt et al., 1981; Ochoa et al., 1983; Fukuda et al., 1990).

A study of the interaction of DHPC with liposomes made of isoelectric phospholipids (DPPC, ditetradecyl PC) and negatively charged ones (1,2-dipalmitoyl-*sn*-phosphatidylserine, 1,2-dilauroyl-*sn*-phosphatidate) also supports the interpretation of a predominant DHPC-lipid interaction. DHPC interacts readily with liquid crystalline bilayers of DPPC, 1,2-dipalmitoyl-*sn*-phosphatidylserine, and 1,2-dilauroyl-*sn*-phosphatidate, all in the form of unsonicated liposomes. Complete solubilization of the large multilamellar and unilamellar liposomes was observed at DHPC/phospholipid mole ratios of 0.25 (Kessi, 1994). Solubilization of the phospholipid bilayers occurs at a relatively low total DHPC concentration compared to other detergents (Womack et al., 1983). This is further evidence for the excellent dispersing power of DHPC. It is stressed that the solubilization of phospholipid bilayers by DHPC requires the phospholipid to be in the liquid crystalline state. The interaction of DHPC with crystalline bilayers or bilayers in the gel state is greatly decreased (Kessi, 1994), and under these conditions the solubilization of phospholipid bilayers is correspondingly reduced or abolished.

**Protein Purification and Membrane Reconstitution.** Sucrase-isomaltase solubilized from rabbit small intestinal BBMV with 40 mM DHPC was purified to homogeneity using a two-step purification procedure (Figure 7A). The recovery of the sucrase activity of the purified protein was significantly improved compared to that reported by Sigrist et al. (1975) for sucrase-isomaltase solubilized with Triton X-100.

The two-step purification of the bacteriochlorophyll-containing proteins of *R. rubrum* yields a complex of reaction centers and light-harvesting peptides which is virtually free of other chromatophore proteins (Figure 7B).

The two representative examples of protein purification demonstrate that the activity of the proteins is well preserved during the purification steps. We conclude that integral membrane proteins solubilized by DHPC should lend themselves well to standard purification procedures.

The cmc of DHPC is relatively high and similar to that of bile salts. The high cmc enables us to effectively remove the monomeric DHPC by dialysis and gel filtration. Due to this property and its character of a mild detergent ensuring the preservation of the three-dimensional protein structure and enzymatic activity, DHPC is particularly well suited for the reconstitution of integral membrane proteins into simple phospholipid bilayers. From the data presented we conclude that DHPC is very good and compared to most other detergents even superior for solubilizing and purifying integral membrane proteins in their active form.

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