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Synthesis of phospholipid-based detergents and their effects on the Ca^{2+}-ATPase of sarcoplasmic reticulum

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Abstract

Phospholipid molecules containing a cholate or hemisuccinylcholate moiety at the 2-position were synthesized as possible detergents for solubilization of membrane proteins. 1-Myristoleoyl-2-cholyl-*sn*-glycero-3-phosphatidylcholine ((C14:1,cholyl)PC) was found to solubilize sarcoplasmic reticulum vesicles at concentrations above its cmc of ca. 4 μg/ml. Effects of (C14:1,cholyl)PC on the activity of the Ca^{2+}-ATPase of sarcoplasmic reticulum were complex, as for other detergents. High concentrations (0.2 mg/ml) of (C14:1,cholyl)PC were able to displace phospholipids from the lipid/protein interface of the ATPase. Although under these conditions the activity of the ATPase was low, the ATPase was not denatured since activity could be regained by displacement of (C14:1,cholyl)PC with the detergent $C_{12}E_8$. 1-oleoyl-2-cholyl-*sn*-glycero-3-phosphatidylcholine ((C18:1,cholyl)PC) was found to have a very low water solubility, but this could be increased by the introduction of a hemisuccinyl group to give 1-oleoyl-2-(3α-hemisuccinyl)cholyl-*sn*-glycero-3-phosphatidylcholine ((C18:1,cholylCOOH)PC). This was able to solubilize and delipidate the Ca^{2+}-ATPase; the ATPase was stable in (C18:1,cholylCOOH)PC for long periods of time.

Keywords: Phospholipid; detergent; Sarcoplasmic reticulum; ATPase, Ca^{2+}-

1. Introduction

The first step in the purification of a membrane protein is generally to solubilize the membrane in a suitable detergent – that is, in a detergent which is harsh enough to solubilize the protein of interest in a form free from other membrane proteins but mild enough to maintain the protein in an active, native state [1]. Similarly, to obtain crystals of membrane proteins for X-ray crystallography, it is necessary to choose a detergent system in which the protein is both soluble and stable for a period of up to several weeks [2]. For some purposes it is important that the detergent have a high critical micellar concentration (cmc) so that it can be readily removed from the protein, for example by dialysis. However, if the intention is to study the properties of a membrane protein in a solubilised state or to crystallise the membrane protein, the cmc of the detergent will probably not be an important factor.

It seems likely that the stability of a membrane protein dissolved in detergent will be greatest the more closely the structure and physical properties of the detergent match the natural environment of the protein – the lipid bilayer. It has been shown that the activity of the Ca^{2+}-ATPase purified from skeletal muscle sarcoplasmic reticulum (SR) is dependent on the structure of the surrounding phospholipids. Thus, ATPase activity is highest for the ATPase reconstituted into bilayers of dioleoylphosphatidylcholine (di(C18:1)PC), and bilayers with shorter or longer fatty acyl chains support lower activities [3]. Cholate is widely used in the purification of the Ca^{2+}-ATPase from the SR membrane [4], but the ATPase has very low activity in cholate and other steroid-based detergents, and is unstable in such detergents [5]. The ATPase can be solubilized in stable form in detergents such as $C_{12}E_8$ or dodecyl maltoside, but only if a shell of phospholipids is maintained around the ATPase [5,6]. The ability of detergents and

Abbreviations: cmc, critical micellar concentration; SR, sarcoplasmic reticulum; (C14:1,cholyl)PC, 1-myristoleoyl-2-cholyl-*sn*-glycero- 3-phosphatidylcholine; (C18:1,cholyl)PC, 1-oleoyl-2-cholyl-*sn*-glycero-3-phosphatidylcholine; (C18:1,cholylCOOH)PC, 1-oleoyl-2-(3α-hemisuccinyl)cholyl-*sn*-glycero-3-phosphatidylcholine; di(Br_2C18:0)PC, 1,2-bis(9,10-dibromostearoyl)phosphatidylcholine.

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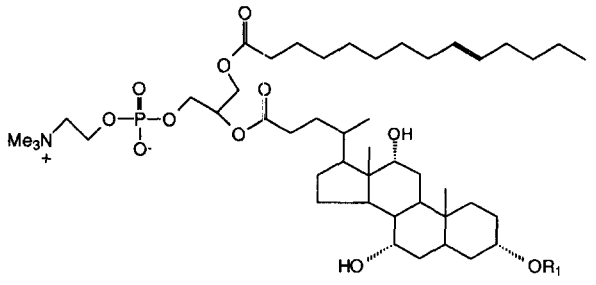

Fig. 1. Structures of the cholate-containing phospholipids. $R_1 = H$ or $COCH_2CH_2COOH$.

other molecules to bind at the lipid/protein interface of the ATPase and displace phospholipids can be assayed by a fluorescence quenching method [7]. If the ATPase is reconstituted into bilayers of 1,2-bis(9,10-dibromostearoyl)phosphatidylcholine (di(Br_2C18:0)PC)) then close contact between the bromine atoms in the lipid fatty acyl chains and the tryptophan residues in transmembrane α-helices of the ATPases, leads to quenching of tryptophan fluorescence. Displacement of di(Br_2C18:0)PC from the lipid/protein interface of the ATPase by detergent is detected as an increase in tryptophan fluorescence intensity [7].

Here we report on the synthesis of a series of phospholipids in which one of the fatty acyl chains of a phospholipid molecule has been replaced by cholate or hemisuccinyl-cholate (Fig. 1). We show that these lipids have detergent properties, and that they can solubilize the ATPase in stable form.

2. Materials and methods

2.1. General materials and methods

Dioleoylphosphatidylcholine (di(C18:1)PC), 1-oleoyl-2-hydroxy-*sn*-glycero-3-phosphatidylcholine and 1-myristoleoyl-2-hydroxy-*sn*-glycero-3-phosphatidylcholine were obtained from Avanti Polar Lipids. Cholic acid was from Sigma. 1,3-Dicyclohexylcarbodiimide, 4-dimethylaminopyridine, *t*-butyldimethylsilyl chloride, and succinic anhydride were from Aldrich.

Di(C18:1)PC was brominated to give 1,2-bis(9,10-dibromostearoyl)phosphatidylcholine (di(Br_2C18:0)PC)) by the method described in East and Lee [7].

ATPase was purified from skeletal muscle sarcoplasmic reticulum as described in East and Lee [7]. ATPase activities were measured at 25° C in 40 mM Hepes/KOH, pH 7.2, 100 mM KCl, 5 mM $MgSO_4$, 2.1 mM ATP and maximally stimulating Ca^{2+} [7]. Lipid reconstitutions were performed as described in Starling et al. [8]. Phospholipid (10 μmol) in buffer (400 μl; 10 mM Hepes/Tris, pH 8.0, containing 15% sucrose, 5 mM $MgSO_4$, 5 mM ATP and 12 mg/ml cholate) was sonicated to clarity in a bath sonicator. ATPase (1.25 mg) in a volume of 20–30 μl was then added and left for 15 min at room temperature and 45 min at 5° C to equilibrate before being diluted with buffer (2 ml; 20 mM Hepes/Tris, pH 7.2, 100 mM KCl, 5 mM $MgSO_4$) and stored on ice until use.

Fluorescence measurements were made by diluting 10-μl aliquots of the reconstitution mixture into 2.5 ml of buffer (20 mM Hepes/Tris, pH 7.2, 100 mM KCl, 5 mM $MgSO_4$, 100 μM Ca^{2+}) and recording the tryptophan fluorescence using an SLM-Aminco 8000C fluorometer, with excitation and emission wavelengths of 295 and 330 nm, respectively, at 25° C. Light scatter at 90° was recorded using the SLM-Aminco 8000C fluorometer with excitation and emission monochromators set at 500 nm.

Fluorescence quenching in mixtures of brominated and non-brominated lipids was fitted to the equation:

$$F/F_o = F_{mix} + (F_o - F_{min})/(1 - f_{Br})^n \quad (1)$$

where F_o and F_{min} are the fluorescence intensities for the ATPase reconstituted with pure non-brominated or brominated lipid, respectively; F is the fluorescence intensity in the lipid mixture when the mol fraction of brominated lipid is x_{Br} and the fraction of sites at the lipid/protein interface occupied by brominated lipids is f_{Br}; and n, representing the number of lipid sites around an average tryptophan residue, has a value of 1.6 [7]. The fraction of sites occupied by brominated lipid is related to x_{Br} by

$$f_{Br} = x_{Br}/(x_{Br} + K(1 - x_{Br})) \quad (2)$$

where K is the binding constant for the brominated lipid relative to that for the non-brominated lipid [9].

2.2. Synthesis of 1-oleoyl-2-cholyl-sn-glycero-3-phosphatidylcholine and 1-myristoleoyl-2-cholyl-sn-glycero-3-phosphatidylcholine

To a solution of cholic acid (0.62 g, 1.5 mmol) in dry *N,N*-dimethylformamide (30 ml) was added *t*-butyldimethylsilylchloride (1.1 g, 7.3 mmol) followed by imidazole (1.04 g, 15.3 mmol). The reaction mixture was stirred under dry nitrogen at 40° C for 5 h, cooled to room temperature, diluted with a saturated solution of NaCl (80 ml) and extracted with diethyl ether (40 ml × 3). The combined extracts were washed with 1 M aqueous HCl (40 ml × 3) and distilled water (40 ml × 3). Concentration in vacuo gave crude tri-*O*-*t*-butyldimethylsilyl-*t*-butyldimethylsilyl cholate (1.26 g) as a viscous liquid. This was dissolved in 60 ml of a 3:1 (v/v) mixture of methanol and tetrahydrofuran, mixed with a solution of Na_2CO_3 (1.2 g) in water (15 ml), and stirred for 2 h. The mixture was concentrated in vacuo to half its volume, diluted with a saturated solution of NaCl (50 ml), cooled to 0° C, and acidified to pH 5 with dilute aqueous HCl. The mixture was then extracted with diethyl ether (50 ml × 3). The combined extracts were washed with a saturated solution of NaCl, and dried. Tri-*O*-*t*-butyldimethylsilyl cholic acid (0.95 g) was obtained in 87% yield.

1-Oleoyl-2-hydroxy-*sn*-glycero-3-phosphatidylcholine (75 mg, 0.144 mmol) and tri-*O*-*t*-butyldimethylsilyl cholic acid (160 mg, 0.213 mmol) were dissolved in chloroform (10 ml) which had been purified by passage through a column packed with activated, basic aluminium oxide powder (10 g). Dicyclohexylcarbodiimide (215 mg, 1.04 mmol) and 4-dimethylaminopyridine (95 mg, 0.78 mmol) were added. The mixture was stirred at room temperature for 48 h under dry nitrogen in the dark, and the reaction was stopped by addition of methanol (10 ml). The crude product was purified by flash column chromatography on a column (22 mm i.d.) packed with 32 g of silica gel (E. Merck, particle size 0.040–0.063 mm) with chloroform/methanol/concentrated ammonium/water (5:3:0.3:0.15, v/v) as eluant. Pure 1-oleoyl-2-(tri-*O*-*t*-butyldimethylsilyl)cholyl-*sn*-glycero-3-phosphatidylcholine (88 mg) was obtained, running as a single spot on TLC, in a yield of 49%.

The *t*-butyldimethylsilyl protecting groups were removed by overnight treatment with 10 ml of acetic acid/water (2:1, v/v, pH ~ 1.5), and flash chromatography was used as described above to prepare pure 1-oleoyl-2-cholyl-*sn*-glycero-3-phosphatidylcholine ((C18:1, cholyl)PC) (42 mg), in a yield of 48%. The sample was stored as a solution in chloroform/methanol (2:1, v/v) at −20° C.

1-Myristoleoyl-2-cholyl-*sn*-glycero-3-phosphatidylcholine ((C14:1,cholyl)PC) was synthesized in the same way.

All structures were confirmed by FT-NMR (60 MHz, Hitachi R-1500), mass spectrometry, and infra-red spectrometry (Pye Unicam SP3-200).

2.3. Synthesis of 1-oleoyl-2-(3α-hemisuccinyl)cholyl-sn-glycero-3-phosphatidylcholine

(C18:1,cholyl)PC (60 mg, 0.066 mmol), succinic anhydride (30 mg, 0.3 mmol) and 4-dimethylaminopyridine (50 mg, 0.41 mmol) were dissolved in dichloromethane (5 ml). The reaction mixture was stirred at room temperature under dry nitrogen for 48 h, and the reaction was stopped by addition of methanol (10 ml). The crude product was purified by column chromatography as described before, to obtain pure 46 mg of 1-oleoyl-2-(3α-hemisuccinyl)cholyl-*sn*-glycero-3-phosphatidylcholine ((C18:1, cholyl-COOH)PC) in a yield of 63%. The sample was stored as a solution in chloroform/methanol (2:1, v/v) at −20° C.

3. Results

3.1. Synthesis of the detergents

The synthetic route adopted was the esterification of cholic acid to 1-acyl-2-hydroxy-*sn*-glycero-3-phosphatidylcholine using dicyclohexylcarbodiimide and 4-dimethylaminopyridine. A variety of protecting groups for the hydroxyl groups on cholic acid were tried, and the most successful was found to be the *t*-butyldimethylsilyl group. Reaction of cholic acid with *t*-butyldimethylsilyl chloride lead to derivitization of the carboxyl group and the three hydroxyl groups; a free carboxyl group was regenerated by reaction with Na_2CO_3. Following linkage to the lysophosphatidylcholine, the protecting groups on the hydroxyls were removed by overnight treatment with acetic acid. The compounds were purified by silica gel chromatography, and obtained in ca. 50% yield based on the starting lysophosphatidylcholine.

3.2. Effects of (C14:1,cholyl)PC

The effects of the detergents on light scatter by SR vesicles was used as a convenient measure of their ability to solubilize membrane proteins [6,10]. SR vesicles (12 μg protein/ml) were incubated for ca. 5 min with (C14:1,cholyl)PC. Solubilization starts at ca. 4 μg/ml and light scatter reaches a limiting value at 10 μg/ml (Fig.

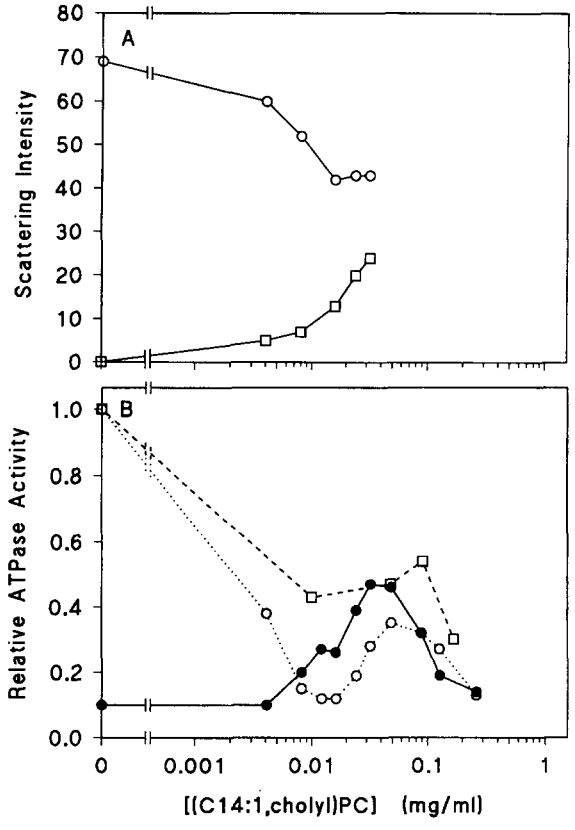

Fig. 2. Effect of (C14:1,cholyl)PC on the Ca^{2+}-ATPase. (A) Light scatter was measured for (C14:1,cholyl)PC in the absence (□) or presence (○) of SR (12 μg protein/ml) in 20 mM Hepes/Tris, pH 7.2, 100 mM KCl, 5 mM $MgSO_4$, 100 μM Ca^{2+}, 25° C. (B) Effects of (C14:1,cholyl)PC on ATPase activity measured at pH 7.2, 2.1 mM ATP, 5 mM Mg^{2+}, 100 mM KCl: (□), purified ATPase; (○), SR in the presence of A23187 (4 μg/ml); (●) SR in the absence of A23187. ATPase activities are calculated relative to that for SR vesicles in the presence of A23187 and absence of detergent.

Table 1
Effect of (C14:1,cholyl)PC on the tryptophan fluorescence intensity of the Ca^{2+}-ATPase

System	Relative fluorescence intensity
di(C18:1)PC	1
di(C18:1)PC + (C14:1,cholyl)PC (0.2 mg/ml)	1
di(Br_2C18:0)PC	0.5
di(Br_2C18:0)PC + (C14:1,cholyl)PC (0.2 mg/ml)	0.85

The ATPase was reconstituted with di(C18:1)PC or di(Br_2C18:0)PC at a molar ratio of phospholipid to ATPase of 1000:1 and diluted into buffer (20 mM Hepes/Tris, pH 7.2, 100 mM KCl, 5 mM $MgSO_4$, 100 μM Ca^{2+}) to a protein concentration of 2.5 μg/ml. Tryptophan fluorescence intensity was measured with excitation and emission wavelengths of 295 and 330 nm, respectively.

2A). Light scatter from (C14:1,cholyl)PC alone is observed at concentrations greater than ca. 10 μg/ml (Fig. 2A), so that at concentrations greater than this it is likely that (C14:1,cholyl)PC forms large aggregates in water.

The effect of (C14:1,cholyl)PC on the activity of the Ca^{2+}-ATPase is shown in Fig. 2B. In the absence of the Ca^{2+} ionophore A23187 the activity of the ATPase in sealed SR vesicles is limited by the accumulation of high concentrations of Ca^{2+} within the vesicle [11]. As shown, ATPase activity under these conditions first increases on addition of (C14:1,cholyl)PC at concentrations greater than 4 μg/ml, and then decreases above 50 μg/ml. The increase in ATPase activity at concentrations above 4 μg/ml (C14:1,cholyl)PC is consistent with the initiation of solubilization, as suggested by the measurements of light scatter (Fig. 2A).

For the purified ATPase present as unsealed membrane fragments, or for the ATPase in SR vesicles in the presence of the Ca^{2+} ionophore A23187, three phases can be distinguished. ATPase activity decreases in the range of (C14:1,cholyl)PC concentrations from 0 to 10 μg/ml; this effect has been observed with a range of other detergents, and has been referred to as the perturbation phase [6,12]. At slightly higher concentrations of (C14:1,cholyl)PC up to 50 μg/ml, activity increases; this phase parallels solubilization of the ATPase (Fig. 2A). Finally, at higher concentrations, ATPase activity falls, more or less in parallel under all conditions. The effect of high concentrations of (C14:1,cholyl)PC is not due to denaturation of the ATPase since addition of an excess of the detergent $C_{12}E_8$ (0.2 mg/ml) to the ATPase after several hours in (C14:1,cholyl)PC leads to an increase in activity to the level observed in $C_{12}E_8$ alone (data not shown).

To test for the ability of (C14:1,cholyl)PC to displace phospholipids from around the ATPase, we studied the effect of (C14:1,cholyl)PC on the tryptophan fluorescence intensity of the ATPase reconstituted with di(Br_2C18:0)PC. Reconstitution was performed by mixing the purified ATPase in cholate with phospholipid at a molar ratio of phospholipid to ATPase of 1000:1 followed by a 500-fold dilution into buffer to drop the concentration of cholate below its cmc and thus reform membrane fragments containing the ATPase and the bromine-containing phospholipid [7]. As shown in Table 1, reconstitution of the Ca^{2+}-ATPase with di(Br_2C18:0)PC reduces tryptophan fluorescence intensity by ca. 50%. Addition of (C14:1,cholyl)PC at 0.2 mg/ml to the ATPase reconstituted with di(C18:1)PC had no effect on tryptophan fluorescence. However, addition of (C14:1,cholyl)PC to the ATPase reconstituted with di(Br_2C18:0)PC resulted in a 70% increase in fluorescence intensity, consistent with displacement of di(Br_2C18:0)PC from around the ATPase by (C14:1,cholyl)PC.

3.3. Effects of (C18:1,cholyl)PC

(C18:1,cholyl)PC was sparingly soluble in water, and addition of (C18:1,cholyl)PC to SR vesicles failed to solubilize the membrane, as determined from changes in light scatter. We studied the effects of (C18:1,cholyl)PC on the activity of the ATPase by reconstitution, using cholate as detergent, as described under Materials and methods. The purified ATPase in cholate was mixed with (C18:1,cholyl)PC also dissolved in cholate, at a molar ratio of (C18:1,cholyl)PC to ATPase of 1000:1, giving a molar ratio of (C18:1,cholyl)PC to endogenous lipid of ca. 30:1. The sample was then diluted 500-fold into buffer to reform a membrane system containing the Ca^{2+}-ATPase and, predominantly, (C18:1,cholyl)PC. The ATPase activity for the ATPase reconstituted with (C18:1,cholyl)PC was 90% of that reconstituted with di(C18:1)PC (data not shown).

The fluorescence intensity for the ATPase reconstituted with mixtures of di(Br_2C18:0)PC and (C18:1,cholyl)PC is shown in Fig. 3. The fluorescence intensity increases with increasing content of (C18:1,cholyl)PC in the mixtures, indicating that (C18:1,cholyl)PC can bind at the lipid/pro-

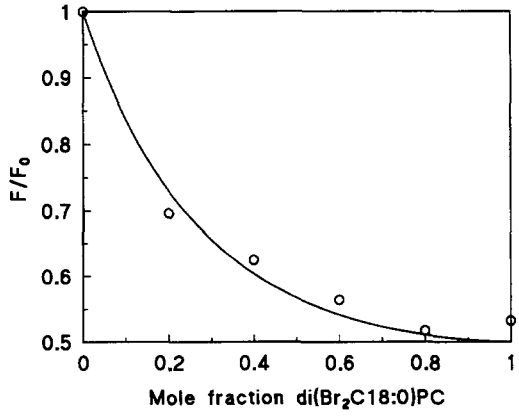

Fig. 3. Tryptophan fluorescence intensity for the ATPase reconstituted in mixtures of di(Br_2C18:0)PC and (C18:1,cholyl)PC at a molar ratio of total phospholipid to ATPase of 1000:1. Fluorescence intensities are expressed relative to the value (F_0) observed for the ATPase reconstituted in (C18:1,cholyl)PC alone. The solid line shows a simulation calculated using Eqs. (1) and (2), with $K = 0.4$.

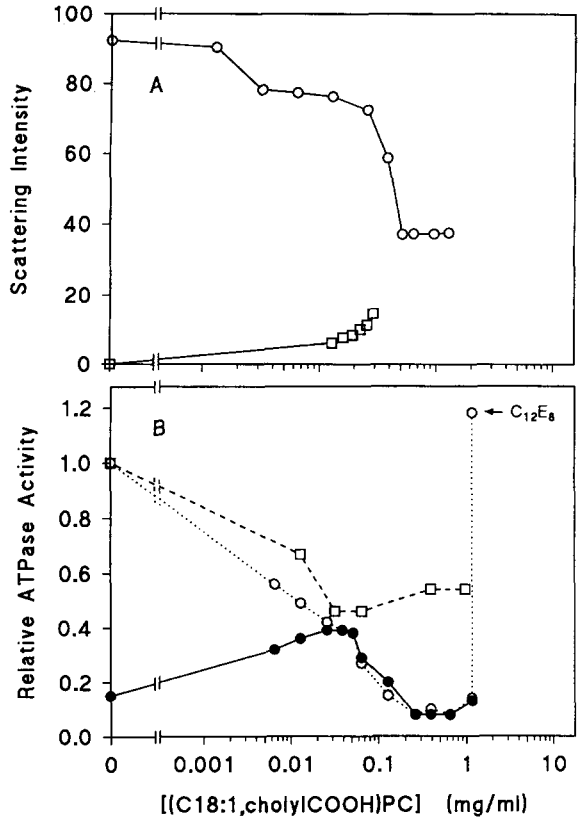

Fig. 4. Effect of (C18:1,cholylCOOH)PC on the Ca^{2+}-ATPase. (A) Light scatter was measured for (C18:1,cholylCOOH)PC in the absence (□) or presence (○) of SR (12 μg protein/ml) in 20 mM Hepes/Tris, pH 7.2, 100 mM KCl, 5 mM $MgSO_4$, 100 μM Ca^{2+}, 25° C. (B) Effects of (C18:1,cholylCOOH)PC on ATPase activity measured at pH 7.2, 2.1 mM ATP, 5 mM Mg^{2+}, 100 mM KCl: (□), purified ATPase; (○), SR in the presence of A23187 (4 μg/ml); (●) SR in the absence of A23187. At the marked point, $C_{12}E_8$ was added to a final concentration of 0.2 mg/ml.

tein interface, displacing di(Br_2C18:0)PC. The data can be fitted to Eqs. (1) and (2) assuming a value for F_{min} of $0.5 \times F_0$, with a value of the binding constant K for (C18:1,cholyl)PC relative to di(Br_2C18:0)PC of 0.4.

3.4. Effects of (C18:1,cholylCOOH)PC

Addition of (C18:1,cholylCOOH)PC to SR vesicles resulted in a reduction in light scatter, with a small change at ca. 20 μg/ml and a larger change at concentrations above 200 μg/ml (Fig. 4A). Light scatter from (C18:1,cholylCOOH)PC alone is low and increases linearly with concentration up to 0.3 mg/ml. Effects on ATPase activity were generally rather similar to those observed with (C14:1,cholyl)PC. Low concentrations of (C18:1,cholylCOOH)PC decreased ATPase activity for SR vesicles in the presence of A23187 but increased activity in the absence of A23187. Higher concentrations caused inhibition for SR vesicles both in the absence and presence of A23187 (Fig. 4B). Effects on the purified ATPase were broadly similar to those seen for SR vesicles in the presence of A23187, but with a decreased level of inhibition in the initial phase. Again, inhibition is not due to denaturation of the ATPase, since addition of 0.2 mg/ml $C_{12}E_8$ reverses the effect of (C18:1,cholylCOOH)PC on the ATPase activity of SR in the presence of A23187 (Fig. 4B). The reason why, at high concentrations of (C18:1, cholylCOOH)PC, the ATPase activity of the purified ATPase is greater than that of SR vesicles in the presence of A23187 is unclear, but could be due to partition of A23187 into detergent micelles, if solubilization of the SR vesicles is incomplete.

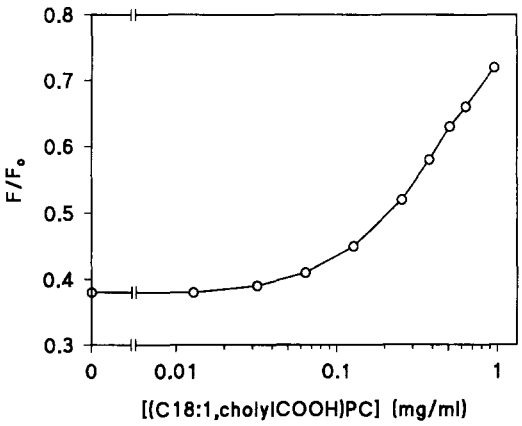

Fig. 5. Effect of (C18:1,cholylCOOH)PC on the tryptophan fluorescence intensity for the ATPase reconstituted in di(Br_2C18:0)PC at a molar ratio of di(Br_2C18:0)PC to ATPase of 1000:1. The final protein concentration was 2.5 μg/ml and the buffer was 20 mM Hepes/Tris, pH 7.2, 100 mM KCl, 5 mM $MgSO_4$, 100 μM Ca^{2+}

The effect of the addition of (C18:1,cholylCOOH)PC on the tryptophan fluorescence intensity of the ATPase reconstituted with di(Br_2C18:0)PC is shown in Fig. 5. A marked increase in fluorescence intensity is observed at concentrations greater than ca. 200 μg/ml, the concentra-

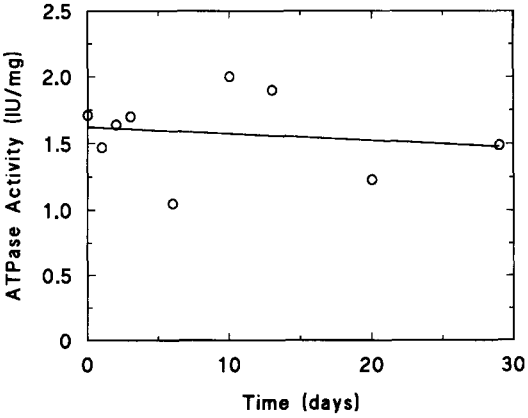

Fig. 6. Long-term stability of the Ca^{2+}-ATPase in (C18:1,cholylCOOH)PC. The ATPase (2.8 mg/ml) was solubilized in 5 mg/ml (C18:1,cholylCOOH)PC in 10 mM K-Mops, 100 mM KCl, 3 mM $MgCl_2$, 22 mM $CaCl_2$, 3 mM NaN_3, 5 mM DTT, 20% glycerol and 2 μg/ml 2,6-di-*tert*-butyl-*p*-cresol, pH 6.0 and kept under nitrogen at 4° C. Samples (2.8 μg protein) were taken at the given time, diluted 240-fold into buffer, and assayed for ATPase activity at 25° C under standard conditions.

tion causing a significant decrease in light scatter (Fig. 4A).

The effect of (C18:1,cholylCOOH)PC on the long-term stability of the ATPase was tested. The ATPase was solubilized in 5 mg/ml (C18:1,cholylCOOH)PC in 10 mM K-Mops, 100 mM KCl, 3 mM $MgCl_2$, 22 mM $CaCl_2$, 3 mM NaN_3, 5 mM DTT, 20% glycerol and 2 μg/ml 2,6-di-*t*-butyl-*p*-cresol, pH 6.0 and kept under nitrogen at 4° C. Samples were then taken, diluted into buffer, and assayed for ATPase activity at 25° C. As shown, activity was stable for up to 30 days (Fig. 6).

4. Discussion

In crystals of the photosynthetic reaction centre grown from solution in *N,N*-dimethyldodecylamine-*N*-oxide (LDAO), detergent molecules form a disordered shell around the protein [13]. The Ca^{2+}-ATPase of sarcoplasmic reticulum is unstable in polar detergents such as LDAO, but has been shown to be stable in nonionic detergents such as $C_{12}E_8$ for long periods of time [14,15]. A detailed study of the effect of $C_{12}E_8$ on the function of the Ca^{2+}-ATPase has shown a correlation between solubilization, displacement of phospholipids from around the ATPase, and activity [6]. Low concentrations of $C_{12}E_8$ resulted in perturbation of the structure of the ATPase, a decreases in ATPase activity, and displacement of a few phospholipid molecules from around the ATPase, but without solubilization. At higher concentrations of $C_{12}E_8$, solubilization occurred but without displacement of further phospholipids; an increase in ATPase activity was observed. Further addition of $C_{12}E_8$ to the solubilized ATPase led to displacement of phospholipids from the ATPase, a change correlated with a decrease in ATPase activity [6].

The activity of the Ca^{2+}-ATPase is markedly dependent on the structures of the phospholipids surrounding it in the membrane, with the lipid supporting highest activity being di(C18:1)PC, with phospholipids with shorter or longer fatty acyl chains or different headgroups supporting lower activities [3]. Some activity is, however, observed even in the presence of phospholipids of very abnormal structure. Thus, the ATPase activity for the ATPase reconstituted into bilayers of 1–oleoyl-2-(cholesteryl hemisuccinyl)-phosphatidylcholine, a phospholipid in which one of the fatty acyl chains was replaced by cholesterol, was 20% of that in di(C18:1)PC [16]. The maximal level of phosphorylation of the ATPase by ATP was the same if reconstituted with the cholesteryl-containing phospholipid or with di(C18:1)PC, implying that the lower activity in the cholesteryl-containing phospholipid followed from the slowing of some step in the reaction pathway for the ATPase and not from protein denaturation [16].

Here we report on the detergent properties of phospholipids containing a cholate or hemisuccinyl cholate moiety instead of a fatty acyl chain at the 2 position of phosphatidylcholine (Fig. 1). The water solubility of these molecules was found to depend on the length of the fatty acyl chain at position 1. Addition of (C14:1,cholyl)PC to sarcoplasmic reticulum reduced light scatter (Fig. 2A), an indication of solubilization. In general, it has been found that the onset of solubilization occurs at a concentration of detergent close to but slightly lower than the detergent cmc in water [17,18]. The cmc for (C14:1,cholyl)PC would then be ca. 4 μg/ml (Fig. 2A). The initiation of solubilization at this concentration is also consistent with the observed increase in ATPase activity for sealed SR vesicles in the absence of Ca^{2+} ionophore (Fig. 2B).

As with other detergents such as $C_{12}E_8$, three stages could be recognised in the effects of (C14:1,cholyl)PC on the activity of the ATPase in unsealed preparations (Fig. 2B). At low concentrations, below its cmc, (C14:1,cholyl)PC inhibited the ATPase. This perturbing effect has been attributed to binding of detergent to a limited number of sites on the ATPase, with a reduction in the rate of some slow step [6]. Further addition of detergent initiates solubilization of the SR with an increase in ATPase activity; the reason for the increase in activity has not been established [6]. Finally, (C14:1,cholyl)PC at concentrations above 40 μg/ml inhibits ATPase activity (Fig. 2B). At these high concentrations, (C14:1,cholyl)PC delipidates the ATPase as shown by the increase in tryptophan fluorescence intensity for the ATPase reconstituted with di(Br_2C18:0)PC (Table 1).

(C18:1,cholyl)PC was found to be very poorly soluble in water, and addition of (C18:1,cholyl)PC to SR vesicles had no effect on light scatter (data not shown). We conclude that (C18:1,cholyl)PC is too hydrophobic to act as a detergent. The nature of the aggregates formed by (C18:1,cholyl)PC in water are unknown, but a bilayer type structure is likely. Effects of (C18:1,cholyl)PC on the ATPase could be determined by reconstitution, using cholate as detergent. ATPase activities for the ATPase reconstituted with (C18:1,cholyl)PC were comparable to those in di(C18:1)PC (data not shown). Measurement of fluorescence quenching for the ATPase reconstituted with mixtures of (C18:1,cholyl)PC and di(Br_2C18:0)PC allowed the measurement of the relative binding constant of (C18:1, cholyl)PC to the ATPase. As shown in Fig. 3, the data was consistent with a value for the binding constant of (C18:1,cholyl)PC relative to di(Br_2C18:0)PC of 0.4. The relative binding constant determined for 1-oleoyl-2-(cholesteryl hemisuccinyl)phosphatidylcholine was 0.6 [16], suggesting that the hydroxyl groups present on the steroid ring had relatively little effect on the strength of interaction with the ATPase.

In order to increase the water solubility of the steroid-containing phospholipids, we introduced a hemisuccinyl group (Fig. 1). As shown in Fig. 4 this was able to solubilize SR vesicles at concentrations above ca. 0.2 mg/ml. At these concentrations, it delipidated the ATP-

ase, as shown by its effects on tryptophan fluorescence intensity for the ATPase reconstituted with di(Br_2C18:0)PC (Fig. 5). ATPase activities were low at high concentrations of (C18:1, cholylCOOH)PC (Fig. 4). The low activity was not due to denaturation, since addition of $C_{12}E_8$ to displace (C18:1,cholylCOOH)PC from around the ATPase led to recovery of activity (Fig. 4). As shown in Fig. 6, the ATPase is stable in a solution of 5 mg/ml (C18:1, cholylCOOH)PC for up to 30 days at 4° C, in the presence of glycerol, DTT, and 2,6-di-*t*-butyl-*p*-cresol, molecules shown by Pikula et al. [15] to stabilize the ATPase in other detergent systems.

We conclude that (C14:1,cholyl)PC and (C18:1,cholylCOOH)PC can act as detergents and solubilize the Ca^{2+}-ATPase. At high concentrations they are able to displace phospholipids from around the ATPase. Although ATPase activities are low at high concentrations of these detergents, the ATPase is stable for long periods of time. ATPase activities are comparable to those observed for the ATPase reconstituted in 1-oleoyl-2-(cholesteryl hemisuccinyl)phosphatidylcholine [16] so that the activity of the ATPase presumably reflects effects of the presence of a sterol ring in the shell of phospholipid molecules bound at the lipid/protein interface.

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