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A physical characterization of some detergents of potential use for membrane protein crystallization

P.A. Timmins*, M. Leonhard, H.U. Weltzien+, T. Wacker and W. Welte

*Institut Laue-Langevin, 156 X Centre de Tri, 38042 Grenoble Cedex, France, Institut für Biophysik und Strahlenbiologie, Albertstr. 23 and +Max-Planck-Institut für Immunbiologie, Stübeweg 51, 78 Freiburg, FRG

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Micellar solutions of lauryldimethylamine oxide, *n*-dodecyl-β-D-maltoside and 1-dodecanoylpropanediol-3-phosphorylcholine were studied by use of small-angle neutron scattering. These detergents have been selected due to their use as solubilizing agents for membrane proteins. LDAO was found to form a homogeneous, approximately spherical micelle with a radius of 20.7 Å and an M_r of 16 000. *N*-Dodecyl-β-D-maltoside forms an inhomogeneous micelle with a core of low scattering density surrounded by a shell of high scattering density. The data are in accord with a micelle forming an oblate ellipsoid and the disaccharide group pointing outward radially from the hydrophobic group. The semi-axes are 16.8 and 25.5 Å and the M_r is 66 000. 1-Dodecanoylpropanediol-3-phosphorylcholine forms a rather homogeneous, roughly spherical micelle. The radius is 24 Å, the M_r being 28 700. The data indicate a tangential packing of the phosphorylcholine head groups into a polar layer of 3–4 Å surrounding the micelle core. The use of these detergents as solubilizing agents during membrane protein crystallization is discussed.

Micelle; Neutron scattering; Membrane protein

1. INTRODUCTION

Several membrane proteins and complexes of proteins have recently been crystallized from their protein-detergent solutions [1–11]. There are indications that all these crystals are built up from mixed protein-detergent micelles [12–15]. It is therefore not surprising that the chemical structure of the detergent molecule, which ultimately determines the micellar properties, e.g. size and surface charge distribution of the micelle, intermicellar interactions as observed close to the phase boundary [16–21], interactions with proteins [22–27] and the tendency to form liquid crystalline phases at high concentrations [28], contributes to the conditions of crystallization. As all these micellar properties add unknown parameters to the crystallization process, it is desirable to determine some of them from an aqueous detergent solution, which is less complicated and more easily available than a protein-detergent solution. It then may be possible to find empirical rules to select a suitable detergent for solubilizing a membrane protein prior to a crystallization experiment.

Michel [13] has pointed out the importance of the size of the detergent belt, attached to the hydrophobic protein surface. The detergent could, for instance, cover hydrophilic amino acids which thus cannot take part in the interaction of two approaching protein-detergent micelles. The detergent may also inhibit the close approximation of hydrophilic groups by occupying excessive space around the protein. In order to overcome these difficulties, Michel [3,13] and Garavito and Jenkins [12] proposed adding small amphiphilic substances. These might either substitute the larger detergent at the moment of insertion of the protein micelle into the growing crystal [13] or insert into the micelle belt immediately upon addition to the protein-detergent solution. In both cases they could increase the curvature and decrease the size of the resulting mixed micelle and mixed micelle belt. However, some proteins are inactivated by

Correspondence address: P.A. Timmins, Institut Laue-Langevin, 156 X Centre de Tri, 38042 Grenoble Cedex, France

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small amphiphiles (unpublished). In order to solubilize these proteins it is necessary to use a solubilizing detergent which forms small enough micelle belts per se. The curvature of the detergent belt around the protein can be expected to correlate with the curvature of the pure detergent micelle, as it is determined by the area covered by the head group of the detergent and the conformation of the acyl chain [29,30]. Except for a few detergent molecules close to the protein surface, the latter two parameters can be expected to be similar to what they are in the micelle.

A powerful technique for studying the geometry of micelles is small-angle neutron scattering (SANS), as there is a large contrast between the polar groups, the hydrocarbon core and the solvent. This contrast may be varied by immersion in different H_2O/D_2O mixtures (review [31]).

We have used SANS to study micellar sizes of three selected detergents: lauryldimethylamine oxide (LDAO), *N*-dodecyl-β-D-maltoside (C12MS) and 1-dodecanoylpropanediol-3-phosphorylcholine (ES12H). From their chemical structure, the hydrophobic parts of these three molecules are of similar size. Differences in the size of the micelles thus should be due to the different polar groups. The present results give indications on the conformation of the polar groups in the micelle which may have consequences for crystallization experiments.

LDAO has been used for the crystallization of bacterial reaction centers and light-harvesting complexes [3,7,8]. It has thus gained a reputation as a detergent for crystallization. However, it inactivates a number of labile protein complexes (unpublished). C12MS, in contrast, is mild and has been used successfully for the crystallization of a photosystem I complex [9,11]. ES12H [32] is less well known and its ester bond hydrolyses within weeks in certain buffers (unpublished). It is an attractive detergent, as it is a 2-deoxylysophosphatidylcholine. Its micelle thus provides a chemical environment for membrane proteins which is very similar to a lipid bilayer.

2. MATERIALS AND METHODS

2.1. *Preparation of the detergent solutions*

LDAO ('purum') was purchased from Fluka (Neu Ulm, FRG) and used without further treatment. C12MS was purchased from Boehringer Mannheim and used without further treatment. ES12H was synthesized as described in [33–37].

A 10% (w/v) stock solution of each of these detergents was prepared by adding 100 mg detergent to 1 ml distilled H_2O. 1% (w/v) solutions of each detergent were made from these stock solutions by dilution with the desired D_2O/H_2O (v/v) mixture. For background measurements, the same amount of water was diluted with the desired D_2O/H_2O mixture.

For neutron scattering experiments, the samples were contained in standard quartz spectrophotometer cells (Hellma, FRG). For D_2O concentrations above 0.40 volume fraction D_2O, 400 μl were placed in cuvettes with a path length of 2.0 mm; for D_2O concentrations below 0.4 volume fraction, 200 μl were put in 1.0 mm cuvettes.

2.2. *Small-angle neutron scattering*

Scattering curves were measured on either the D11 [38] or D17 instrument at the high-flux reactor of the ILL Grenoble. In each case the detector is a 64 × 64 cm BF_3 multidetector. The sample-to-detector distance was 2 m for D11 and 2.85 m for D17. The incident wavelength (λ) was 10 Å with $\Delta\lambda/\lambda$ = 8% (for D11) or 10% (for D17). For each detergent the scattering curve was measured for the sample dissolved in 6 different H_2O/D_2O mixtures as well as for the H_2O/D_2O mixtures alone. Neutron transmission measurements were carried out to determine the precise D_2O/H_2O content of the solution and to verify that each sample and background had the same H_2O/D_2O content. The scattering curves were corrected for a non-uniform response of the detector and put on an absolute scale by dividing by the scattering of a 1 mm thick sample of H_2O. The samples were thermostatted at 16°C for ES12H and for C12MS and at 20°C for LDAO. The beam cross-section was 70 mm^2. Measuring times varied from about 10 min per spectrum for samples with high D_2O content to 1 h for those with low D_2O content. All spectra were circularly averaged about the beam direction, corrected for buffer scattering and normalized to the scattering of water using standard programs [39]. The resulting curves were plotted as $\ln I(Q)$ vs Q^2 (Guinier plot) from which are obtained the radius of gyration and the intensity at zero angle I_0. For each detergent the highest D_2O content was measured as a function of detergent concentration over the range 1.0–0.1% (w/v). No change in radius of gyration or I_0/C was observed. We therefore conclude that interference effects are negligible at the concentrations used in the experiments. Match points of the detergent and of head groups and alkyl chains were calculated from the scattering length densities of the constituent atoms and the molar volumes obtained via specific volume measurements. The volumes of alkyl chains were estimated using the formula $V(Å^3) = 27.4 + 26.9n_c$, where n_c is the number of C atoms in the alkyl chain [40].

2.3. *Determination of the specific volume*

The specific volume $\bar{v}$ of LDAO, C12MS and ES12H was determined by use of a densitometer (DMA 02C, Anton Paar, Graz). The density of the solution was calculated from the shift of the proper frequency of a v-shaped glass tube upon filling with the detergent solution.

3. RESULTS

3.1. *C12MS*

Fig.1 shows Guinier plots for the detergent in

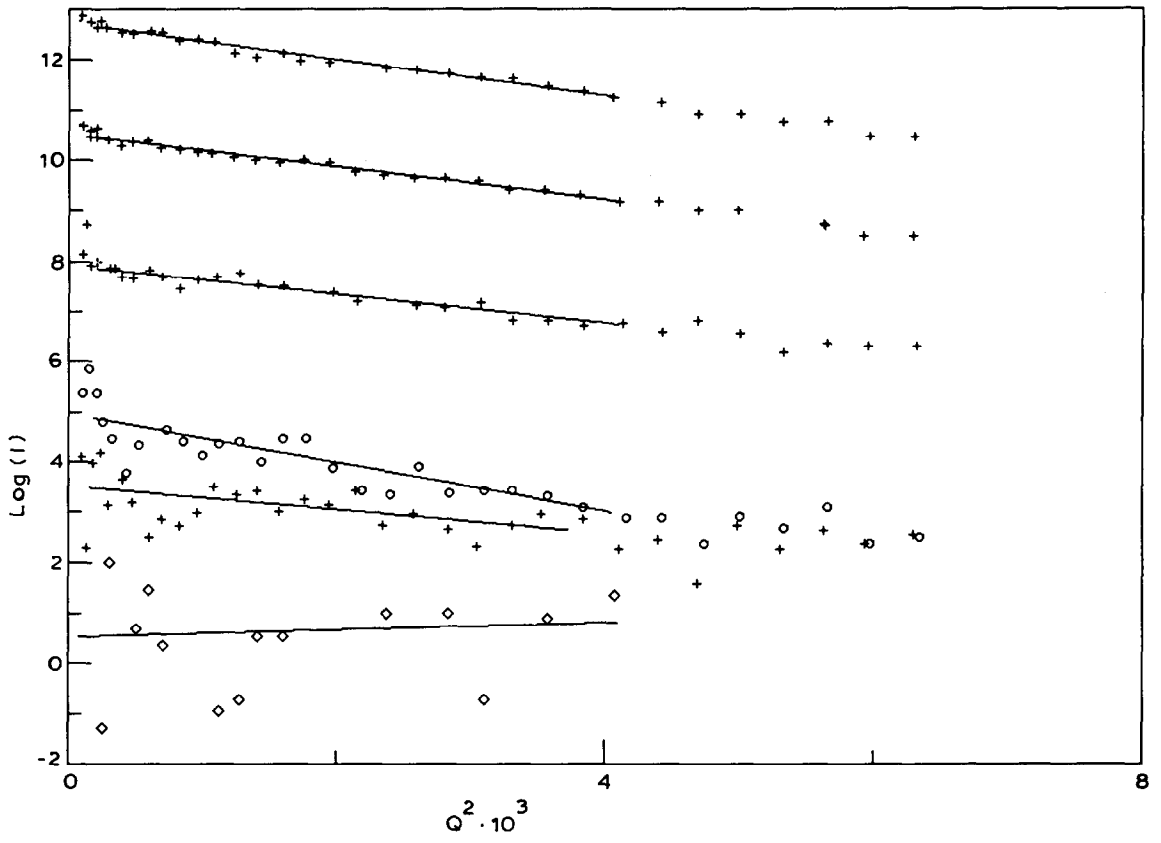

Fig.1. Guinier plots from 1% solutions of C12MS in various H_2O/D_2O mixtures. The D_2O content is indicated alongside each curve. All curves are on the same scale.

water containing various volume fractions (x) of D_2O (0, 0.18, 0.36, 0.54, 0.72 and 0.9). From these plots the values of I_0 and R_g are obtained as a function of contrast. Fig.2 shows $\sqrt{I_0}$ (normalized to unit concentration, thickness and neutron transmission) as a function of D_2O/H_2O content. This plot is linear as expected for a monodisperse solution and indicates a contrast match point of $x = 0.23 \pm 0.01$. The specific volume of C12MS was determined to be 0.837 cm^3/g. Using this value, a match point of $x = 0.217$ can be calculated, in good agreement with the experimental value.

The value of I_0/c can be used to calculate the micelle molecular mass [31,41]. This is best done using the value in H_2O where assumptions concerning hydrogen exchange and partial specific volume have the least effect. Using the partial specific volume, we obtain a micelle molecular mass of 66 kDa corresponding to an aggregation number of 130.

The radii of gyration are obtained from the slopes of the plots in fig.1. The data in high D_2O concentration show only a small difference in radius of gyration, whereas at low contrast this variation is much more marked. Indeed, at $x = 0.18$ the slope is positive, indicating an imaginary radius of gyration. This variation of R_g can be quantified by plotting R_g vs the inverse mean contrast [42]. The mean contrast is defined as the difference between the scattering length density at the contrast match point and that of a given D_2O/H_2O mixture. This plot is shown in fig.3 and its positive slope indicates that the micelle consists of a core of low scattering density surrounded by a shell of higher scattering density [42]. The point at which the fitted straight line intersects the ordinate gives the radius of gyration at 'infinite contrast'. We do not attempt here to interpret this parameter as corresponding to the radius of gyration of the homogeneously filled envelope of the particle. This

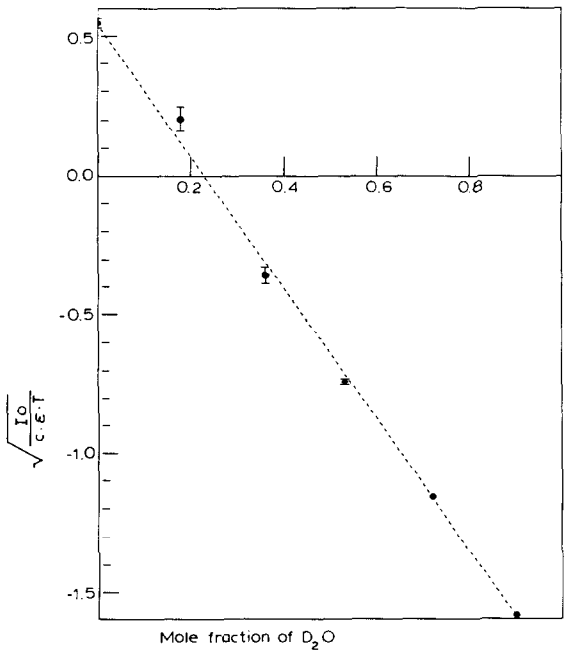

Fig.2. Variation of $\sqrt{I(0)}$ normalised to sample thickness, transmission and concentration of C12MS as a function of D_2O mole fraction (x) in solvent. Linear regression indicates a match point of $x = 0.23$.

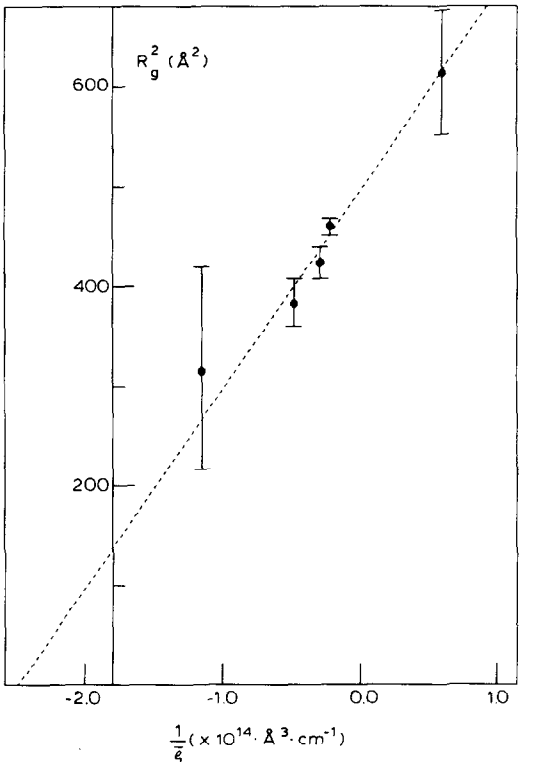

Fig.3. Plot of R_g^2 vs inverse contrast (Stuhrmann plot) for C12MS.

can lead to serious errors in estimation of the particle size when the exchangeable hydrogens are not homogeneously distributed [43] as is precisely the case in this micelle where all exchangeable hydrogens are found in the maltoside head group. However, we can estimate from this plot the radius of gyration of the micelle core when the maltoside head group is matched out. This we calculate to be at $x = 0.495$ and therefore estimate the core to have a radius of gyration of ~19 Å. If the micelle were spherical, it would then have a radius of 25.1 Å, which is considerably greater than the length of a fully extended dodecyl chain [40]. Moreover, if we assume a volume of 350.2 Å^3 for the dodecyl chain, the resulting core volume would imply an aggregation number of ~190 whereas from I_0 measurement we find 130. We therefore conclude that the micelles must be nonspherical. A reasonable alternative would be an ellipsoid in which at least one axis were determined by the dodecyl chain length. The aggregation number of 130 can be accounted for either by a prolate ellipsoid of dimensions 16.7 × 16.7 × 39.0 Å or an oblate ellipsoid of dimensions 16.8 × 25.5 × 25.5 Å. The latter would have an R_g of 17.8 Å and the former of 20.2 Å. These are to be compared with an R_g of ~19 Å obtained experimentally when the disaccharide is matched out. The thickness of the head group shell can now be calculated such as to predict the correct R_g when the core is matched out (close to 0% D_2O). The experimental value is 24.5 ± 2 Å. We find that any shell surrounding the prolate ellipsoidal core has a very large R_g and that only oblate ellipsoids are consistent with our observations. A shell of thickness 10 Å along the minor axis and 3 Å along the major axis fits with the observed radii of gyration.

3.2. *LDAO*

Scattering curves for LDAO were measured for $x = 0.0$, 0.35, 0.50, 0.70 and 0.87. Radii of gyration and I_0 were obtained from Guinier plots (not shown). The matchpoint was determined as $x = 0.57 \pm 0.03$.

The specific volume of LDAO was determined

to be 1.134 cm^3/g. Using this value, a match point of $x = 0.55$ can be calculated, in good agreement with the experimental value.

The value of I_0/c can be used to calculate the micelle molecular weight. We used here the value of $I(0)/c$ in 87% D_2O as that in H_2O is very poorly determined, since it is close to the match point. Using the partial specific volume above we obtain a micelle molecular mass of 15858 Da corresponding to an aggregation number of 69 monomers. It should be noted that at high D_2O content the molecular mass determination is very sensitive to errors in the peptide specific volume, i.e. a 1% error leads to a ~2% error in molecular mass. The variation of the radii of gyration with contrast is shown in the Stuhrmann plot (fig.4). Two of the low contrast points with very large errors are not included in this plot but all values are listed in the inset. From this plot we measure the radius of gyration at infinite contrast to be 16.0 Å. As LDAO contains no exchangeable hydrogen atoms, this corresponds to the radius of gyration of the micelle envelope. If the micelle were spherical it would then have a radius of 20.7Å which is close to the estimated fully extended length of the LDAO molecule (20.3 Å). The volume of the micelle envelope would be 35041 $Å^3$ which could accommodate 69 LDAO molecules plus approx. 180 H_2O molecules. We therefore conclude that the LDAO micelle is close to spherical.

3.3. *ES12H*

Scattering curves from ES12H solutions were measured in D_2O/H_2O mixtures of $x = 0.0$, 0.37, 0.53, 0.72 and 0.90. The intensity at zero angle gives a match point of $x = 0.14 \pm 0.01$.

The specific volume of ES12H was determined to be 1.012 cm^3/g. The experimental match point is in good agreement with the calculated value of 13.2% using $\bar{v}$. The micelle molecular mass calculated from I_0 in H_2O is 28.7 kDa in very good agreement with a determination using sedimentation equilibrium [44]. This corresponds to 68 ES12H monomers.

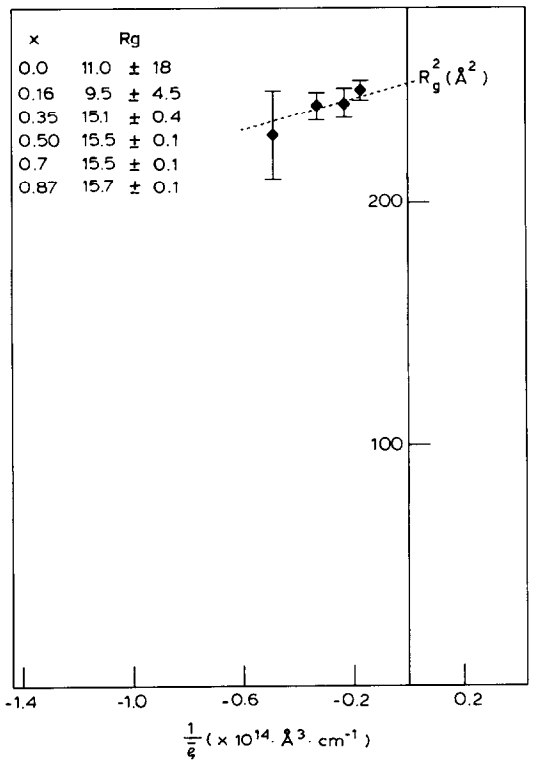

Fig.4. Plot of R_g^2 vs inverse contrast (Stuhrmann plot) for LDAO. Linear regression gives R_∞ = 16 Å.

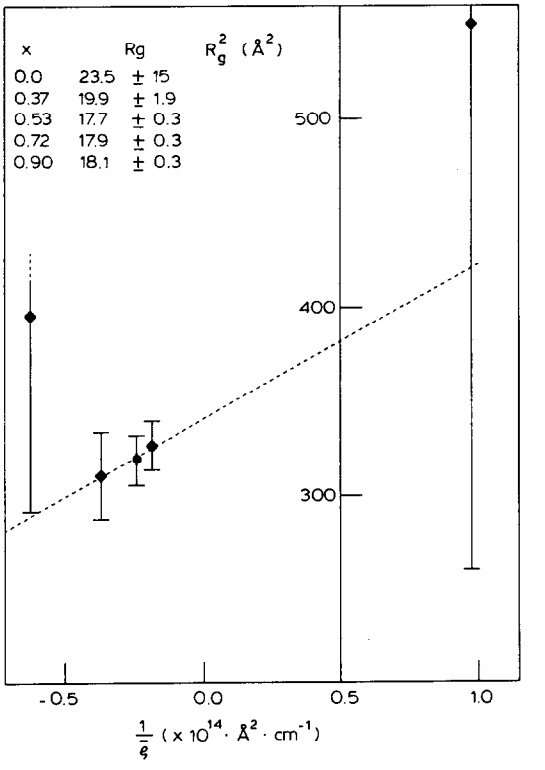

Fig.5. Plot of R_g vs inverse contrast (Stuhrmann plot) for ES12H. Linear regression gives R_∞ = 18.5 Å.

Again the R_g varies only slightly with contrast as shown in the Stuhrmann plot (fig.5) and insert. The R_g at infinite contrast is 18.5 Å which in the case of a spherical particle corresponds to a radius of 23.9 Å. This micelle envelope would then, in addition to 68 ES12H molecules, be able to accommodate about 300 H_2O molecules. The variation of R_g with contrast is compatible with a head group layer of 3–4 Å thickness.

4. DISCUSSION

The small variation in radius of gyration of LDAO with contrast indicates that the micelle is rather homogeneous. We find a radius of gyration of 16 Å and a micellar mass of 16 kDa. The micelle appears to be almost spherical with a radius of 20.7 Å, close to the length of an extended dodecyl chain. Due to its small nonionic polar group, one would expect LDAO to have a small surface area per amphiphile and consequently to form relatively big micelles [30]. As this is not the case, we suppose that the amine oxide group is hydrated. We calculated some three water molecules per amine oxide group.

The scattering length density of the micelle of C12MS is inhomogeneous with a core of low density surrounded by a shell of higher density. The micellar mass is only 66 kDa, much smaller than that of a spherical micelle with a radius calculated from the radius of gyration of the hydrophobic core. The micelle must therefore be nonspherical.

The thickness of the maltoside shell of 3–10 Å indicates that the disaccharide group is roughly pointing radially outward from the hydrophobic micelle core.

The scattering length density of the micelle of ES12H appears homogeneous with a radius of gyration of 18.4 Å. As in the case of LDAO, the micellar mass of 28.7 kDa can be packed roughly into a sphere with a radius of 23.9 Å as calculated from the R_g value. According to geometrical packing arguments [29,30], increasing the area per head group of an amphiphile will favour the formation of spherical micelles as compared to prolate or oblate ellipsoids. At first glance, the mass of the head group could be expected to correlate with the area per head group. The molecular masses of the head groups of LDAO, C12MS and ES12H are 60, 340 and 180 Da, respectively. One thus would expect C12MS and ES12H to form highly curved micelles while LDAO should form a micelle of smaller curvature. However, it turns out that the ES12H micelle resembles the LDAO micelle more than that of C12MS and that the former two are nearly spherical, while the latter deviates from the spherical form. The mere comparison of the masses of the head groups does not take into account the conformation and hydration of the head groups, which will contribute to the area per head group. As the phosphorylcholine head group and the amine oxide head group are smaller than the maltoside group, the greater area per head group in ES12H and in LDAO as compared to C12MS micelles can be achieved only by spreading the phosphorylcholines out into a thin shell on the hydrophobic core and a stronger hydration of the amine oxides as compared to the maltosides.

In phospholipid membranes, the orientation of the phosphorylcholine head group has been investigated using several techniques. Hanai et al. [45] argued for an orientation parallel to the membrane surface on the basis of the isoelectric behaviour of phosphorylcholine vesicles in an electric field. This matching of the negatively charged phosphate group and of the positively charged tetramethylammonium group was also concluded from NMR data [46–48] by the finding that the trimethylamine group bends back to the phosphate group so that a charge matching is realized within the head group. A series of X-ray and neutron diffraction studies confirmed this view and showed that an additional charge matching is effected by a special in-plane arrangement of neighbouring head groups [49–54] (review [55]). ES12H was investigated by a single-crystal structure analysis [54] as well as by an NMR study [44]. According to the latter work, the conformation of the phosphorylcholine head group in the crystal must be similar to that in the micelle. In the micelle, however, the propanediol group, which influences the relative orientations of the polar group and of the hydrocarbon group, possesses an even distribution of rotational conformations about the two C–C bonds. This is in contrast to the glycerol backbone of diacylglycerols in solution [47]. In diacylglycerols, two neighbouring hydrocarbon chains are linked together. The hydrophobicity of these hydrocarbon chains favours a parallel alignment by chain stacking and thus influences the rota-

tional conformation of the glycerol C–C bonds [47]. In contrast, in the ES12H micelle, the hydrocarbon chains are independent of each other. There will be no relation between chain packing and head group conformation. The hydrocarbon chains will be oriented on average radially within the spherical micelle. The tendency of the phosphorylcholine groups to be hydrated and the hydrophobicity of the hydrocarbon core will result in an energetically optimized structure characterized by phosphorylcholine head groups providing the most effective separation of water from the hydrocarbon core by a tangential arrangement of the head group which gives the largest surface area per head group. The thickness of such a head group shell can be expected to be approx. 7.5 Å [55].

In an SANS study, Lin et al. [56] have investigated micelles of dihexanoylphosphorylcholine (DHPC) which show some remarkable differences vs ES12H micelles. This micellar structure is represented by a prolate ellipsoid, is inhomogeneous with respect to the scattering length density and possesses a polar shell with a thickness of between 6 and 10 Å. On the basis of the aforementioned NMR study of both substances [47], we suppose that the connection between neighbouring hydrocarbon chains and their tendency to adopt the most effective hydrophobic packing by chain stacking in DHPC fixes the glycerol C–C bond rotation angles to a value which increases the inclination of the phosphorylcholine head group to the micellar surface, similarly to that found in the crystal structure of dimyristoylphosphatidylcholine [55]. The resulting smaller area per phosphorylcholine head group will favour a prolate ellipsoid as compared to a spherical micelle. Another consequence will be a less perfect matching of opposite electrical charges as compared to ES12H, so that the polar shell has to accommodate more hydration water. This conformational difference explains why the radius of the DHPC micelle is similar to that of the ES12H micelle, although the length of the alkyl chains in the former is only half of the length in the latter.

Our results would be consistent with an ES12H micelle where the dodecanoylpropyl ester moiety extends radially in a spherical micelle and the phosphatidylcholine head group lies almost parallel to the micelle surface. In contrast, maltoside head groups pointing outward from the micelle center result in a less dense packing of the head group, favouring oblate ellipsoids and a thicker polar shell around the micelle.

The three detergents studied here differ with respect to their ability to preserve membrane proteins in their native conformation. Several membrane protein complexes are quickly denatured by LDAO, among them the oxygen-evolving photosystem (PS) II complex (unpublished) and sarcoplasmic Ca^{2+}-ATPase (unpublished). In all cases where we compared ES12H and C12MS, both were equivalent in preserving activities of dissolved membrane proteins and better than LDAO. This is surprising in view of the zwitterionic nature of ES12H, since other zwitterionic detergents tend to be very denaturing to these proteins. The matching of the two charges in ES12H by its head group conformation might resolve the apparent contradiction. We also tested an ether derivative of propanediol-3-phosphorylcholine, ET12H [32]. The ester in all cases of labile proteins was more conserving than the ether. Ca^{2+}-ATPase and the PS II complex were inactivated by the ether, but not by the ester (unpublished). We thus suppose that this charge matching is more perfect in ES12H than in ET12H. This may be due to a different rotational conformation of the -C-O-CH_2-CH_2- bonds of the ether as compared to the -CO-O-CH_2-CH_2- bonds of the ester.

The synthetic lysolecithins thus could be of use as solubilizing detergents for crystallization experiments with labile membrane proteins. Two disadvantages should, however, also be mentioned. The ester bond has a tendency to hydrolyse within several weeks. Furthermore, in crystallization experiments with membrane proteins we occasionally observed ES12H crystals when Ca^{2+} was present in the solution.

As SANS studies can help to clarify the role of the micelle belt in membrane protein crystallization, we are currently studying the influence of various additives on micelles which are present in real crystallization experiments.

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