Transitional Steps in the Solubilization of Protein-Containing Membranes and Liposomes by Nonionic Detergent†

Ulrich Kragh-Hansen,‡ Marc le Maire,§ Jean-Pierre Nöel,‖ Tadeusz Gulik-Krzywicki,§ and Jesper V. Møller*,‡

Institutes of Biophysics and Medical Biochemistry, University of Aarhus, DK-8000 Aarhus C, Denmark, Centre de Génétique Moléculaire, Laboratoire propre du Centre National de la Recherche Scientifique, Associé à l'Université Pierre et Marie Curie (Paris VI), F-91198 Gif-sur-Yvette Cédex, France, and Service des Molécules Marquées, Centre d'Etudes Nucléaires de Saclay, F-91191 Gif-sur-Yvette Cédex, France

Received January 22, 1992; Revised Manuscript Received September 17, 1992

ABSTRACT: Membrane solubilization by dodecyl maltoside was studied, using Ca^{2+}-ATPase membranes and liposome preparations as prototypes of biological membranes. In equilibrium dialysis experiments, transition from saturable incorporation of monomeric detergent into the membrane to cooperative binding already occurred at a free detergent concentration about 50% of the cmc. This transition was discontinuous for unilamellar liposomes of dioleoylphosphatidylcholine, but gradual for Ca^{2+}-ATPase membranes and multilayered liposomes of sarcoplasmic reticulum lipid. Equilibrium detergent binding by Ca^{2+}-ATPase membranes (expressed on the basis of lipid content) was the same as for detergent binding by multilamellar liposomes of sarcoplasmic reticulum lipid. Equilibration involving cooperative binding was considerably delayed (for many days) if detergent was presented gradually to the membranous preparations in nonmicellar form by diffusion across the dialysis membrane, while equilibration of detergent occurred rapidly if detergent in micellar form was added directly to the membrane preparations. In contrast, equilibration was rapid in both directions if detergent was added at levels below that required to initiate cooperative binding. Detergent interaction resulted in a biphasic decrease in light scattering of Ca^{2+}-ATPase membranes. The first of these decreases coincided with the onset of cooperative binding, while the second one was associated with a decreased sedimentability during ultracentrifugation, i.e., with usual criteria of solubilization. The concentration at which this occurred corresponded to the level of free detergent at which lipid, after detergent solubilization, segregated from detergent after gel chromatography. By freeze–fracture electron microscopy, the decreases in light scattering were correlated with membrane reorganization, leading to the appearance of large holes in the vesicles before complete fragmentation into membrane sheets of diminishing size and solubilization of Ca^{2+}-ATPase as monomers and oligomers. The data indicate the existence of saturable uptake of nonmicellar detergent by the lipid phase of the membranes, followed by cooperative interaction, ultimately leading to solubilization. Rapid solubilization appears to require direct interaction of detergent micelles with membranes. Except for unilamellar liposomes of dioleoylphosphatidylcholine, our results are not consonant with an abrupt transition from the membranous to the detergent-solubilized state, but rather indicate a gradual mechanism of solubilization via fragmented vesicles, lamellar sheets, and intermediary solubilized forms.

Detergents are indispensable reagents in the solubilization and characterization of membrane proteins. Therefore, detailed information on their mode of interaction with biological membranes is essential. For pure lipid vesicles, it has been proposed that detergent interaction can be described by a three-stage process (Jackson et al., 1982; Lichtenberg et al., 1983; Møller et al., 1986; Paternostre et al., 1988): At low concentrations, detergent partitions into the lipid bilayer, not giving rise to solubilization (stage I). When membrane lipid has become saturated with detergent, a phase transition takes place by which detergent-saturated membrane lipid gradually is transformed into mixed micelles (stage II). This conversion reaches an end point which demarcates the beginning of stage III (complete solubilization). For biological membranes, containing both protein and lipid, the solubilization process is more complex, but it seems likely that the bulk of the membrane lipid is solubilized, together with integral membrane proteins, in essentially the same way as when present in pure liposomes. At the point of solubilization, integral membrane proteins are usually still covered with a layer of lipid which can only be removed by treatment with a substantially higher detergent concentration and/or by continuous lipid removal by, e.g., chromatographic procedures or sucrose density centrifugation (Helenius & Simons, 1975; de Foresta et al., 1989).

The primary purpose of this investigation has been to study in detail the transition from binding to solubilization of biological membranes by detergent as a function of the free (nonmicellar) concentration. Apart from a few studies on *n*-dodecyl octaethylene glycol monoether ($C_{12}E_8$)[1] [e.g., see Andersen et al. (1983) and le Maire et al. (1987)], this topic has received little attention. Using unilamellar vesicles of

† This work was supported by grants from the Danish Medical Research Council, the NOVO Nordic Foundation, the P. C. Petersen Foundation, and the Aarhus University Research Foundation.

‡ University of Aarhus.

§ Laboratoire propre du Centre National de la Recherche Scientifique, Associé à l'Université Pierre et Marie Curie.

‖ Centre d'Etudes Nucléaires de Saclay.

[1] Abbreviations: $C_{12}E_8$, *n*-dodecyl octaethylene glycol monoether; DDAO, *N*,*N*-dimethyldodecylamine *N*-oxide; DM, *n*-dodecyl β-D-maltoside; DOPC, dioleoylphosphatidylcholine; SR, sarcoplasmic reticulum; TX-100, Triton X-100; cmc, critical micellar concentration; csc, critical membrane solubilizing concentration of detergent; C_{sat}, detergent concentration for the transition between noncooperative and cooperative interactions of detergent in the membrane.

0006-2960/93/0432-1648$04.00/0

phosphatidylcholine, intermediary forms between the membranous and detergent-solubilized state have been described (Ollivon et al., 1988; Edwards et al., 1989; Vinson et al., 1989). It is clear that solubilization leads to cooperative binding of detergent to lipids (Goñi et al., 1986) and to delipidated membrane proteins (Makino et al., 1975; Robinson & Tanford, 1975; le Maire et al., 1983); therefore, it is of interest to follow binding of detergent during the solubilization process. Here we have chosen to study dodecyl maltoside (DM), a nonionic detergent which like other detergents useful for membrane protein studies ($C_{12}E_8$, Triton X-100, and DDAO) is characterized by an intermediate length of the hydrophobic moiety. DM has been used extensively for solubilization of cytochrome oxidase in active form (Suarez et al., 1984; Bolli et al., 1985), and along with the other detergents mentioned above has been found to have promising properties for solubilization of diverse membrane proteins (Lund et al., 1989; le Maire et al., 1992; de Foresta et al., 1992).

The properties of DM were studied with the aid of relatively simple membrane systems, viz., Ca^{2+}-ATPase membranes and liposomes, prepared from sarcoplasmic reticulum vesicles and pure lipid.[2] Our data indicate a number of new features in the interaction of nonionic detergents with these membranes. First, we find that after detergent addition only micellar detergent rapidly and cooperatively interacts with the membranes to produce solubilization while nonmicellar detergent primarily is taken up by the membranes by a saturable process. In addition, our data indicate that solubilization of the membranes by DM probably is a more gradual process than hitherto considered for nonionic detergents, comprising various steps of cooperative binding and fragmentation of membrane vesicles, before complete solubilization. This is similar to the mechanism previously suggested for solubilization of membranes with bile salt detergents (Small, 1971; Mazer et al., 1980).

EXPERIMENTAL PROCEDURES

Materials. Dioleoylphosphatidylcholine (DOPC) was obtained from Avanti Polar Lipids Inc., Birmingham, AL, and *n*-dodecyl β-D-maltoside (DM) was obtained from Biochemica Boehringer, Mannheim, Germany. Sarcoplasmic reticulum vesicles were prepared from rabbit skeletal muscles according to the method of de Meis and Hasselbach (1971). Purified and permeable ATPase vesicles (containing 0.58 μmol of phospholipid/mg of protein), unable to accumulate Ca^{2+}, were made by extraction of SR vesicles with a low concentration of deoxycholate [method 2 of Meissner et al. (1973)]. Lipid was isolated from SR by chloroform/methanol extraction (Folch et al., 1957). Multilamellar vesicles of SR lipid with and without inclusion of detergent were prepared as described by le Maire et al. (1987). Unilamellar liposomes (60–70-nm diameter) were prepared from cholate-solubilized DOPC by dialysis reconstitution as previously described (Heegaard et al., 1990).

Radiolabeled [1-^{14}C]dodecyl β-D-maltoside was synthesized by the procedure of Rosevear et al. (1980) from [1-^{14}C]-dodecanol, obtained by reduction with lithium aluminum hydride of the ethyl ester of [1-^{14}C]dodecanoic acid which had been prepared by carbonation with [^{14}C]carbon dioxide of the Grignard reagent of 1-bromoundecane. The [1-^{14}C]-dodecanol (3.1 mmol; 55.8 mCi/mmol) was condensed with an equimolar amount of 2,3,4,6-tetraacetylglucopyranosyl bromide with silver carbonate (3.08 mmol) as the catalyst in dry dichloromethane (30 mL) in the presence of iodine (43 mg) and 4-Å molecular sieves (2.2 g). After standing for 20 h in darkness at room temperature, the product was checked by HPLC on an octadecylsilica gel [column Sup Rs ODS 2, Prolabo, France; solvent methanol/water (85:15)]. The product was dried by rotary evaporation and then dissolved in 37 mL of 0.01 N sulfuric acid in 90% aqueous acetone. After 30 min at room temperature, 30 μL of pyridine was added. The product was taken to dryness and checked by HPLC (same conditions as above). After purification of the product by liquid chromatography on Lichroprep Si 60 Merck (30 g) with hexane/ethyl acetate (70:30) as solvent, the product was deacetylated with 15 mL of methanol/triethylamine/water (20:1:10) for 23 h at room temperature. Finally, the product was purified by preparative HPLC on a Sup Rs ODS 2 column with methanol/water (85:15) as the solvent phase. The specific activity was 55.8 mCi/mmol and the yield 12.4% from [1-^{14}C]dodecanol. The radioactive purity was checked by thin-layer chromatography on silica with ethyl acetate/methanol (80:20) as the mobile phase and by high-performance liquid chromatography on Sup Rs ODS 2 [solvent methanol/water (85:15)].

Equilibrium Dialysis Studies. The experiments were carried out at pH 7.5 using media containing 0.01 M Tes, 0.1 M NaCl, and 0.1 mM $CaCl_2$ and with addition of sodium azide (1 mM) and gentamycin (20 μg/mL) to prevent bacterial growth. The interaction between DM and vesicles of Ca^{2+}-ATPase or liposomes was studied by the use of a Dianorm equilibrium dialyzer (Dianorm Geräte, München, Germany). Pieces of dialysis tubing (Visking type 18/32) were used as diaphragms to separate the chambers into two compartments. In cis experiments, a sample (usually 225 μL) of vesicles or serum albumin was added together with detergent to one of the compartments and dialyzed against the same volume of buffer without detergent and protein. In trans experiments, detergent was added to the lipid- and protein-free compartment. When the chambers had been filled, the apparatus was placed in a temperature-controlled water bath at 20 °C, and the cells were rotated for 3–12 days before withdrawal of samples for analysis.

The interaction between DM and Ca^{2+}-ATPase membranes was also studied by ordinary equilibrium dialysis in which vesicle containing solutions (0.5 mL), without detergent, were placed in small Visking dialysis bags and dialyzed against 50 mL of buffer, containing detergent at concentrations up to the cmc. As a consequence of the relatively large detergent-containing buffer reservoir, a virtually constant concentration of free detergent in the outside medium could be maintained during the whole experiment. In this way, it was possible to reduce problems that might be attributable to the low permeability of the dialysis membranes to detergent micelles in the Dianorm trans experiments. The cis type of experiment was performed in a similar manner by placing 0.5 mL of detergent- and protein-containing solution inside dialysis bags and dialyzing against 10 mL of buffer.

After dialysis, detergent concentrations in the various samples were measured by liquid scintillation counting. The radioactivity of solutions which had not been dialyzed was used to represent the known detergent concentration. The increment in the protein- and lipid-containing, dialyzed samples was used to calculate the concentration of bound detergent. We found that some detergent was lost during dialysis, presumably as the result of adsorption to the membrane and perhaps also to the cells, resulting in a recovery of DM of 88 ± 8%.

[2] For particular types of membranes, protein–protein interactions among integral membrane proteins (del Rio et al., 1991) or with cytoskeletal proteins (Hansen and Møller, unpublished results) have to be weakened before solubilization. These aspects of detergent solubilization are not covered here.

Column Chromatographic Experiments. The equilibrium between nonmicellar detergent and pure or mixed lipid/detergent micelles was also investigated by column chromatographic procedures according to principles previously described (Andersen et al., 1983). In the mixed micelle experiments, SR lipid was solubilized with DM detergent at a molar ratio of 1:(4.4–6.0), then applied, together with Blue Dextran and DTT, to a 0.5-m agarose (Bio-Rad, Richmond, CA) 1.5 × 85 cm column, and eluted with detergent-free buffer containing 0.01 M Tes, 0.1 M KCl, 0.1 mM $CaCl_2$, and 1 mM sodium azide which was also used for equilibration of the column before application of the sample. During passage through the column, nonmicellar detergent was left behind the micelles in the sample, resulting in the appearance of a micellar peak, followed by a plateau region, extending to the total volume (Figure 4A). The cmc was read at the midpoint of the plateau region. It has been our experience that this kind of experiment also serves as a convenient check on the purity of labeled detergents, since impurities tend to elute as a separate peak corresponding to the total volume of the column.

We also performed another type of gel chromatographic experiment in which we aimed to maintain a constant concentration of free detergent during chromatography. In these experiments, a fully solubilized lipid/detergent mixture, containing 1.3 μmol of SR lipid and 5.7 μmol of DM, was first dialyzed against 2–3 changes of 10 mL of detergent-free buffer [0.01 M Tes (pH 7.5), 0.1 M KCl, 0.1 mM $CaCl_2$, and 1 mM sodium azide]. The appearance of detergent in the dialysate was monitored so that dialysis could be stopped at a desired detergent to lipid molar ratio [(2.5–3.2):1], which we knew from the previously determined binding curve would be close to a critical concentration for solubilization with DM (free detergent concentration of 120–130 μM). The sample was then applied to an agarose 1.5-m column (1.5 × 85 cm), equilibrated with DM at the same concentration of nonmicellar detergent.

Other Methods. Protein concentrations were determined as previously described (Andersen et al., 1982), and phospholipids by the micromethod of Bartlett (1959). Changes in light scattering of Ca^{2+}-ATPase and SR membranes were measured on a Perkin-Elmer M-PF44A spectrofluorometer with both monochromators set at 600 nm. Stock solutions of detergent (47 mM) were added to ATPase membranes or SR vesicles (0.2–1 mg of protein/mL), suspended in 2 mL of buffer in a well-stirred cuvette. The effect of detergent was also investigated by centrifugation of 150 μL at 100000*g* in a Beckman Airfuge for 60 min, and solubilized protein and lipid resulting from this treatment were measured in the supernatant. Freeze–fracture of Ca^{2+}-ATPase membranes was performed after addition of dodecyl maltoside by standard procedures in the following way: small drops of the preparations cryoprotected with 30% glycerol (v/v) were deposited on thin copper holders and rapidly quenched in liquid propane. The samples were fractured (at −125 °C with a liquid nitrogen cooled knife in vacuum better than 10^{-6} torr) and replicated with Pt-C in a Balzers 301 freeze–etching unit. The replicas were cleaned with 2% SDS followed by distilled water and observed with a Philips electron microscope. All electron micrographs are positive images; i.e., platinum deposits appear dark. Care was taken to avoid the artifacts associated with this technique (Rash & Hudson, 1980).

RESULTS

Figure 1A shows binding curves for dodecyl maltoside by Ca^{2+}-ATPase membranes after dialysis in the Dianorm apparatus for 3 days, the time most often used for equilibration

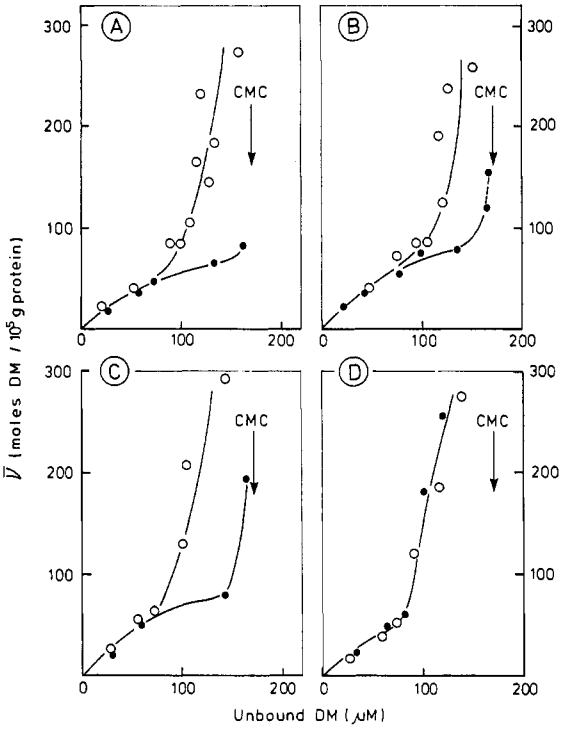

FIGURE 1: Binding of dodecyl maltoside by Ca^{2+}-ATPase membranes. (○) cis experiments; (●) trans experiments. (A, B) After Dianorm dialysis of Ca^{2+}-ATPase membranes for 3 and 12 days, respectively (initial detergent concentrations 0.17–1.7 mM; protein concentration 0.5 mg/mL). (C, D) After dialysis of Ca^{2+}-ATPase membranes in cellophane bags for 3 and 12 days, respectively. The bags in cis experiments initially contained 0.25 mg of protein together with 0.36–2.37 μmol of detergent per 0.5 mL which was dialyzed against 10 mL of detergent-free buffer. In trans experiments, the bags contained the same concentration of Ca^{2+}-ATPase membranes and were dialyzed against 50 mL of buffer, containing dodecyl maltoside in nonmicellar concentrations (0.028–0.176 mM) to ensure optimal conditions for equilibration of detergent across the cellophane membranes.

in these experiments. It is seen that in the cis situation (○), i.e., when detergent was added to the membranes in the same compartment, there is a steep, cooperative rise in binding above a concentration of unbound DM of 80–100 μM. In the trans experiment (●), binding initially follows the same course as in the cis samples, but above a concentration of 80–100 μM unbound DM, binding is considerably lower than in the cis experiment. In fact, there is only weak cooperative interaction of DM with the membranes under these conditions, as evidence of incomplete solubilization in the trans experiments (see below). In dialysis experiments with serum albumin, utilizing the Dianorm equipment, equilibrium is usually obtained with low molecular weight ligands within 24 h (Kragh-Hansen et al., 1990). However, in the presence of high concentrations of detergent, micellar forms predominate which are less easily equilibrated. Nevertheless, we found in experiments in which we examined the interaction of detergents with serum albumin under similar conditions as in Figure 1A that 3 days were sufficient for full equilibration between cis and trans samples above the cmc (data not shown). Therefore, the nonequilibration observed between the cis and trans samples in Figure 1A is probably not only attributable to impeded transfer of detergent micelles across the cellophane membranes.

In order to examine this situation in more detail, we extended the equilibration period in the Dianorm apparatus to 12 days (Figure 1B). This did not lead to any change in the binding isotherm in the cis experiment, while binding levels were increased in the trans samples above 100 μM unbound DM. Figure 1C,D shows experiments in which Ca^{2+}-ATPase membranes, suspended inside ordinary dialysis bags, were

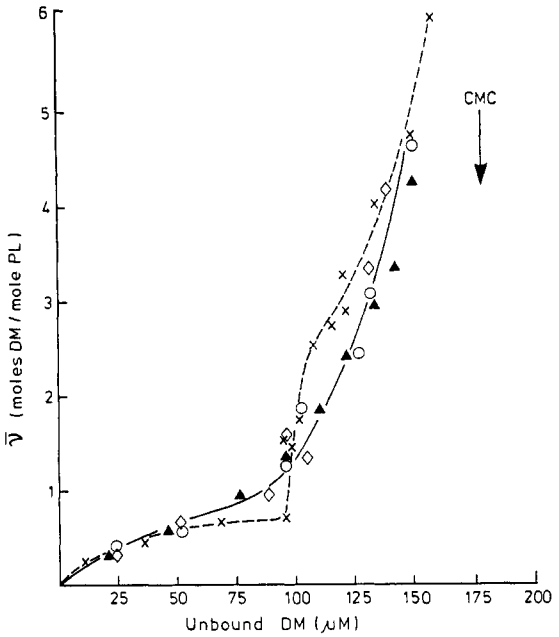

FIGURE 2: Binding of dodecyl maltoside by Ca^{2+}-ATPase membranes and liposomes. The continuous line is drawn for the Ca^{2+}-ATPase membrane (○) and sarcoplasmic reticulum extracted lipid with dodecyl maltoside added to (◊) or incorporated into the multilayered liposomes during their preparation (▲). The dashed line (× - - ×) shows the binding of dodecyl maltoside to unilamellar liposomes prepared from DOPC. The data were obtained as cis experiments with the Dianorm apparatus, using an equilibration period for 3 days. The Ca^{2+}-ATPase membrane and multilayered liposomes samples contained 0.24–0.32 mM phospholipid, and the dodecyl maltoside concentration was varied from 0.17 to 1.7 mM; for the unilamellar liposomes, the corresponding values were 0.39–0.55 mM phospholipid and 0.34–3.4 mM dodecyl maltoside.

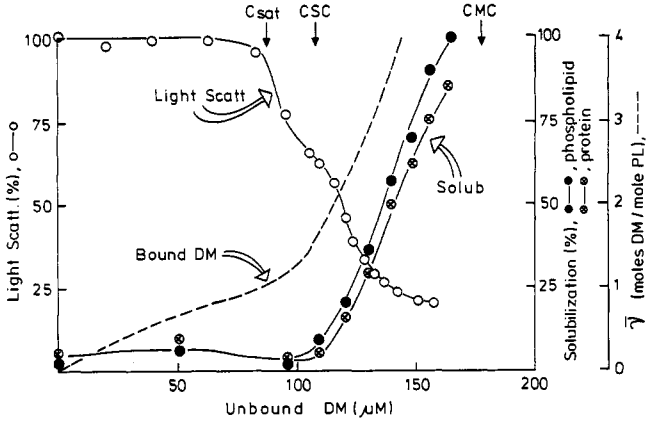

FIGURE 3: Effect of dodecyl maltoside addition on light scattering and sedimentability of Ca^{2+}-ATPase membranes. Changes in light scattering (○) were measured at 600 nm and 20 °C by addition of 5-μL increments of a stock solution of dodecyl maltoside (47 mM) to 2.5 mL of Ca^{2+}-ATPase membranes (0.5 mg of protein/mL) suspended in 0.01 M Tes/Tris buffer (pH 7.5), 0.1 M KCl, and 0.1 mM $CaCl_2$ in a well-stirred cuvette. Solubilization of Ca^{2+}-ATPase membranes (0.5 mg of protein/mL), suspended in the same buffer, was measured from the percentage of protein (⊗) and phospholipid (●) remaining in the supernatant after addition of detergent (0–2.84 mM) and centrifugation of 150-μL aliquots for 60 min at 100000*g* in the Beckman Airfuge. The dashed curve shows the binding of DM to the membranes, on the basis of the data of Figure 2. Characteristic transitions (C_{sat}, csc, and cmc; see text) are also indicated on the figure.

dialyzed against a large external reservoir to permit equilibration to proceed below the cmc throughout the whole experiment (see Experimental Procedures). Although transfer under these conditions proceeded more quickly than in the Dianorm apparatus, equilibration was still incomplete after 3 days (Figure 1C). On the other hand, it was complete after 12 days (Figure 1D). Note that during the different experimental conditions of Figure 1A–D the binding curves for DM in the cis experiments were unchanged within the limit of experimental variation. Thus, the cis data, obtained either with the Dianorm apparatus or in a more conventional dialysis equilibration experiment, approximate the thermodynamic equilibrium existing between free detergent and the membrane components. The more rapid equilibration observed in the cis than in the trans experiments may be ascribed to the initial addition of DM in predominantly micellar form to the membranes when high detergent concentrations were used. By contrast, in the trans situation, membranes are always exposed to nonmicellar detergent due to the impermeability of the membranes to micelles. This will retard equilibration, but according to the experiments with serum albumin not to the extent observed in Figure 1B,C. Accordingly, the cooperative reaction between nonmicellar detergent and membranous material is also slow, requiring the use of longer equilibration times than is necessary with serum albumin. On the other hand, at low initial concentrations of detergent, not resulting in cooperative binding, equilibration in both directions is rapid, resulting in concordant results between cis and trans after a few days.

Figure 2 shows that there is no difference between the binding level of DM by Ca^{2+}-ATPase membranes (○, expressed on a detergent to lipid basis) and by multilayered liposomes of SR lipid (▲, ◊). No difference was observed whether DM had been incorporated into SR liposomes already during their preparation (▲) or added after their formation (◊). The same result was previously obtained for the interaction of such preparations with $C_{12}E_8$, suggesting availability of the detergents (by flip-flop) to both sides of the membrane (le Maire et al., 1987). However, unilamellar liposomes of DOPC behaved differently. This preparation (× in Figure 2), in contrast to the preparations derived from SR, exhibited a very sharp, discontinuous transition, leading to cooperative interaction at 95 μM, corresponding to 53% of the cmc for pure detergent. Below this transition hyperbolic uptake of DM by the membrane is observed, which approaches a saturation level of 0.6 mol of detergent/mol of lipid, a level somewhat lower than that observed for Ca^{2+}-ATPase and SR lipid. This means that the uptake of detergent by the lipid phase cannot be appropriately described by a partition coefficient, but is a saturable process; i.e., the affinity of detergent for the membrane is decreased by the presence of other detergent molecules in the membrane. Above the transition, the binding isotherm of the unilamellar liposomes was vertical (strongly cooperative) until ≃2 mol/mol of lipid. By further addition of detergent, the free concentration of DM gradually approached that observed for Ca^{2+}-ATPase and SR liposomes.

Cooperative Binding and Solubilization. Previous data on solubilization of membranes have most often been obtained either by observation of changes in light scattering during detergent addition or by determination of changes in the sedimentability of detergent-treated, membranous material by centrifugation. Figure 3 shows how these changes are related to the free detergent concentration in the interaction of DM with Ca^{2+}-ATPase membranes. A remarkable feature is that the decrease in light scattering during the solubilization process takes place in two phases, separated by a small plateau. The start of the first decrease in light scattering corresponds to the concentration of free detergent demarcating the onset of cooperative binding of detergent by the membranes (87 μM) which we have termed C_{sat}. However, there is an interesting difference between the light scattering and cen-

trifugation data. After centrifugation for 60 min at 100000*g*, solubilization does *not* occur during the first decrease in light scattering; instead, solubilization is seen to correspond perfectly to the second stage of the decrease in light scattering starting at 107 μM unbound DM. This breakpoint we have termed csc (*c*ritical *s*olubilization *c*oncentration) as a phenomenological parameter to characterize the onset of membrane solubilization, according to usual criteria.

Analogous findings of biphasic decreases in light scattering were obtained also with other detergents ($C_{12}E_8$ and TX-100, data not shown). In all cases, the second phase of light-scattering decrease invariably was associated with solubilization, according to the criterion of nonsedimentability. On the other hand, for unilamellar liposomes, the decrease in light scattering was monophasic and was preceded by a rise, caused by incorporation of detergent into the membrane. With this preparation, solubilization took place at 95 μM, corresponding to the vertical part of the binding isotherm (Figure 2). Accordingly, in this case C_{sat} = csc, while C_{sat} < csc for solubilization of Ca^{2+}-ATPase membranes. The reasons for these characteristic differences between a biological, protein-containing membrane and a pure lipid membrane are considered under the Discussion.

Chromatographic Experiments. The reversibility of the events taking place during solubilization was studied after application of detergent-solubilized material to gel chromatographic columns and elution in the absence of detergent. Figure 4A shows the elution profiles after application of samples to an agarose 0.5-m column of (a) DM micelles, containing saturating amounts of SR lipid (prepared at a molar ratio of detergent to lipid of 6:1) or (b) pure DM micelles. In both cases, a micellar peak emerges at positions which by comparison with water-soluble standards (le Maire et al., 1986) correspond to a Stokes radius of 3.2 nm for the mixed micelles and 2.7 nm for pure micelles (which in Figure 4A is represented by the curve with the dashed line). Thus, the mixed micelles only have a slightly larger size than pure DM micelles. The micellar peaks are followed by plateau regions which extend to the total volume of the column. These regions represent free detergent that was in equilibrium with the micellar peak during passage through the column, but which was left behind, due to the small size of the unassociated detergent molecules. It is seen that in the presence of lipid the concentration of free detergent is lower than after application of pure micelles (approximately 80%), consistent with the expected decrease in the thermodynamic activity of detergent in mixed micelles (Helenius & Simons, 1975), but much higher than the csc for unilamellar liposomes, i.e., solubilization in the forward direction. During passage through the column, part of the phospholipid will form unilamellar liposomes (le Maire et al., 1978; Levy et al., 1990) that segregate from micellar DM, due to supersaturation of the mixed micelles with phospholipid, caused by the delayed elution of nonmicellar detergent in the plateau[3] (Figure 4A). Figure 4B shows what happens by application of SR lipid, barely solubilized by DM, when applied to the same column, at a molar ratio of phospholipid to DM of 1:4.5. This results in two remarkable effects: (i) The nonmicellar concentration, while originally at the same level after application to the column as in Figure 4A, is drastically

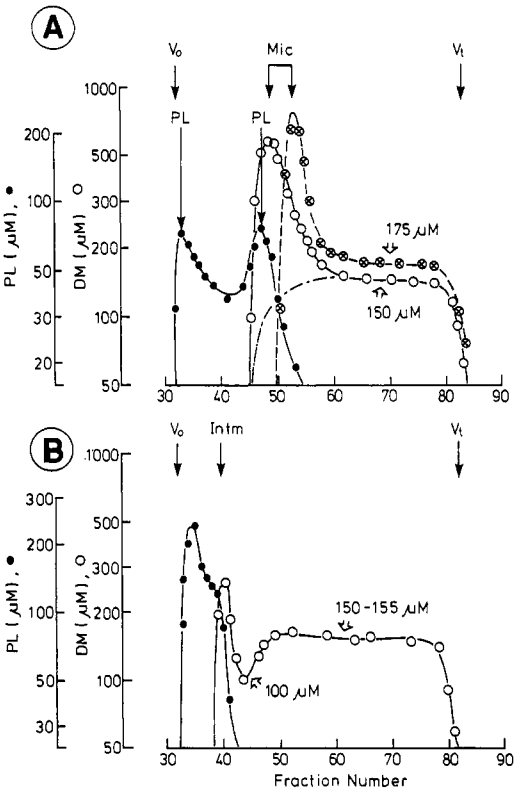

FIGURE 4: Gel chromatography of DM micelles and mixed micelles of phospholipid and DM. Panel A shows the result of application of 1 mL of (a) 2.6 μmol of SR phospholipid, solubilized by 15 μmol of DM, or (b) pure DM (15 μmol) to an agarose 0.5-m column (1.5 × 85 cm). In Panel B, 2.6 μmol of SR phospholipid, solubilized by 11.4 μmol of DM, was applied to the same column. The column was equilibrated and eluted with 0.01 M Tes (pH 7.5), 0.1 M KCl, 0.1 mM $CaCl_2$, and 1 mM NaN_3. (○) DM concentrations in the eluted fractions, in the experiments with application of mixed micelles; (●) phospholipid concentrations in the same experiments. The dashed line in panel A (⊗ - - -⊗) shows the concentration profile after application of *pure* DM, while the composite dashed curve (– • –) tentatively illustrates the variation of free detergent concentration across the micellar peak after application of DM and phospholipid. V_0 = void volume, V_t = total volume (measured with dithiothreitol), Mic = elution position of micellar peaks, and Intm = intermediary forms. Note that ordinate scales are logarithmic to adapt them for illustration of both the height of micellar peaks and the much smaller changes in nonmicellar concentrations in the plateau regions. Numerical values for the concentrations of nonmicellar detergents in the plateau regions are indicated on the figure.

decreased during the latter stages of the elution process from 150 to 100 μM, producing a trough. (ii) The formation of a peak of phospholipid and detergent, eluting close to the void volume of the column, is seen. This peak is larger than that of a typical mixed micelle and probably represents an intermediary, solubilized form (labelled Intm on Figure 4B).

In order to explore in more detail the transition state detected in Figure 4B, we performed supplementary experiments under equilibrium conditions (not shown) in which we first by dialysis reduced the level of detergent of a barely solubilized sample (molar ratio of phospholipid to DM of 1:4) to a level corresponding to that present in the trough in Figure 4 (100–150 μM unbound detergent). These samples were then applied to a 1.5-m agarose column, equilibrated with detergent at a concentration corresponding to the free concentration of DM in the dialyzed sample, as calculated from the binding curve (Figure 2). In one experiment, a sample applied at 130 μM eluted at K_D = 0.23, which was the same position as for thyroglobulin (R_s = 8.6 nm). The K_D of a sample processed at 120 μM DM was 0.10, significantly larger than thyroglobulin, but smaller than that of vesicles typical for those

[3] In addition, a diffusion zone develops, corresponding to the ascending limb of the micellar peak where the concentration of free detergent changes from zero (in fractions eluting before the micellar peak) to reach the value seen in the plateau region [a tentative illustration of the transition zone is shown by the composite (– • –) broken curve in Figure 4A]. A low concentration of free detergent in the front probably also accounts for the slight displacement toward the void volume of the phospholipid peak associated with the DM micelles.

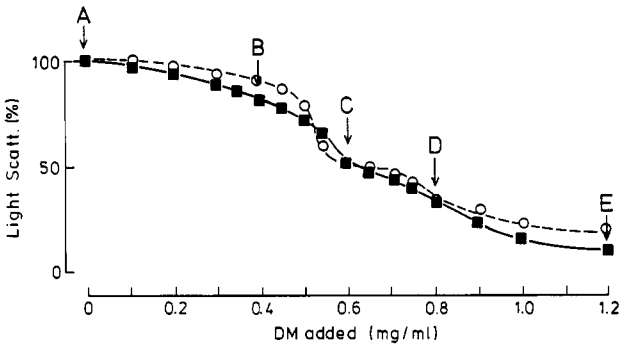

FIGURE 5: Effect of dodecyl maltoside addition on light scattering of sarcoplasmic reticulum. Changes were measured at 600 nm by addition of 4- or 8-μL increments of a stock solution of dodecyl maltoside (25 mg/mL) to 2 mL of sarcoplasmic reticulum (1 mg of protein/mL) suspended in 7 mM Tes (pH 7.5), 70 mM KCl, 0.07 mM $CaCl_2$, and 30% glycerol (v/v) at 20 °C in a well-stirred cuvette. Vesicles were equilibrated with the glycerol-containing buffer for at least 3 h before detergent addition. The entire solubilization curve was obtained within 25 min. Arrows A–E correspond to sample aliquots (10 μL) which were taken out for immediate freezing less than 30 s after a step of detergent addition and light-scattering measurement; representative freeze–fracture electron micrographs of replicas of these samples are shown as panels A–E in Figure 6. (■—■) SR vesicles; (O- - -O) Ca^{2+}-ATPase membranes.

reconstituted from nonionic detergents (Ueno et al., 1984; Cornelius & Skou, 1984). These results are evidence of a gradual change in size occurring during the transition from the fully solubilized to the nonsolubilized state.

Freeze–Fracture Electron Microscopy. To study membrane solubilization by DM in more detail, we have used freeze–fracture electron microscopy to obtain an idea about the nature of the intermediary forms arising from DM during solubilization. These experiments were performed in the presence of 30% glycerol (v/v) as a cryoprotectant. Furthermore, in addition to Ca^{2+}-ATPase membranes, we used SR vesicles in these experiments, because of the more regular structure and uniform orientation of protein particles of the latter preparation (Andersen et al., 1983). Figure 5 shows that differences in the light-scattering properties of these two preparations are minor: SR vesicles, in contrast with Ca^{2+}-ATPase membranes, experience a slight decrease in light scattering, before C_{sat}, while Ca^{2+}-ATPase after maximal solubilization retains a slightly higher light scattering. This is probably due to a fraction of nonsolubilizable protein always present in these preparations; compare, e.g., in Figure 3 the slightly lower solubilization of protein than of the lipid. Also note that in Figure 5 light scattering was plotted as a function of *added* detergent, rather than of unbound detergent, as in Figure 3, since we do not know the detergent binding curve in the presence of glycerol. For both SR vesicles and Ca^{2+}-ATPase membranes, the characteristic biphasic decrease in light scattering was retained in the presence of glycerol. We rapidly froze small aliquots of membrane samples after addition of detergent to produce the characteristic transitions (see arrows labeled A–E in Figure 5 with pictures of representative freeze–fractured replicas of SR vesicles shown in Figure 6A–E). Figure 6A is a control showing the usual appearance of SR vesicles of diameter 100–200 nm without addition of detergent (smooth convex fracture faces and concave fracture faces crowded with intramembranous particles of Ca^{2+}-ATPase). In Figure 6B, 0.4 mg of DM was added per milligram of SR protein (free [DM] $\simeq C_{sat}$); this amount of detergent did not change the vesicular nature of the SR preparation. However, there was an increased tendency for SR vesicles to be cleaved with the formation of rings (see arrows in Figure 6B). These rings are produced by fractures cutting perpendicularly through the membrane and not at the interface between the two leaflets of the bilayer, as is usually the case. This certainly reflects a weakening of membrane structure produced by insertion of the detergent molecules,[4] which previously also was seen after addition of $C_{12}E_8$ to SR vesicles (Andersen et al., 1983). In this case, we concluded on the basis of a thorough quantitative analysis that addition of detergent up to the point of saturation was not accompanied by statistically significant changes in particle size, vesicle size, and particle density.

The largely intact appearance of the membranes in Figure 6B at C_{sat} contrasts with Figure 6C where the amount of DM added corresponds to csc. In the latter replica, a number of membrane sheets are visible in addition to vesicles. These sheets appear either flat or nearly flat on the surface of the replica or, when the sheet is cut perpendicular to the surface, as rows of particles. Some vesicles appear intact at first sight, but two points are interesting to note: large holes are sometimes visible in the vesicles (left arrow in Figure 6C). The holes can also be seen as interruptions in the ring structure (double arrows in Figure 6C). Finally, the uniform sidedness of the particles is no longer obvious: particles are sometimes present on convex fracture faces, and empty concave fracture faces are also visible. At higher [DM] (0.8 mg/mL, Figure 6D), vesicles are no longer present, except in very rare instances, but are replaced by small membrane pieces, either seen as sheets or seen as rows of particles as in Figure 6C. At this stage, some particles in solution are also visible. Finally, at 1.2 mg/mL DM, the membranes are entirely solubilized (Figure 6E); membrane sheets are absent, and single particles, with a diameter of about 100 Å, or small aggregates, consisting of 2–4 particles, are present (see arrows in Figure 6E). These particles represent Ca^{2+}-ATPase monomers and oligomers with bound detergent and some lipid, while pure detergent micelles and mixed phospholipid micelles are invisible by freeze–fracture electron microscopy (le Maire et al., 1981).

Similar results were obtained by addition of DM to Ca^{2+}-ATPase membranes (not shown). Despite the somewhat heterogeneous picture of detergent solubilization emerging from the freeze–fracture replica, we may conclude that the first phase of light-scattering decrease for DM is associated with a reshuffling and reorganization of membrane constituents, leading to partial formation of large sheets. During the second phase of the light-scattering decrease, vesicle breakdown to sheets is virtually complete, and these gradually decrease in size, before full solubilization is obtained.

DISCUSSION

The present data provide the basis for a detailed description of the solubilization of membranes as a function of the free detergent concentration of DM, as a representative of a nonionic detergent of medium chain length. They suggest that for a protein-containing membrane at least four different phases can be distinguished as shown schematically in Figure 7: up to a certain concentration (C_{sat}), which is appreciably lower than the cmc, detergent is taken up by the membranes by a process which is saturable and leads to no major change in vesicle morphology (phase a). Above C_{sat}, detergent cooperatively interacts with the membranes, gradually producing changes in membrane morphology (phases b and c). Phase b is mainly accompanied by changes in membrane

[4] An interesting possibility is that ring formation is the result of partial interdigitation of the phospholipid alkyl chains: when the membrane becomes loaded with the C_{12} alkyl chain detergent, this induces a shortening of the membrane thickness, due to the disparity in hydrocarbon length compared with that of SR lipid. Therefore, a partial interdigitation of the alkyl chains of phospholipids could occur, and this in turn could reduce the probability of cuts in the middle of the bilayer.

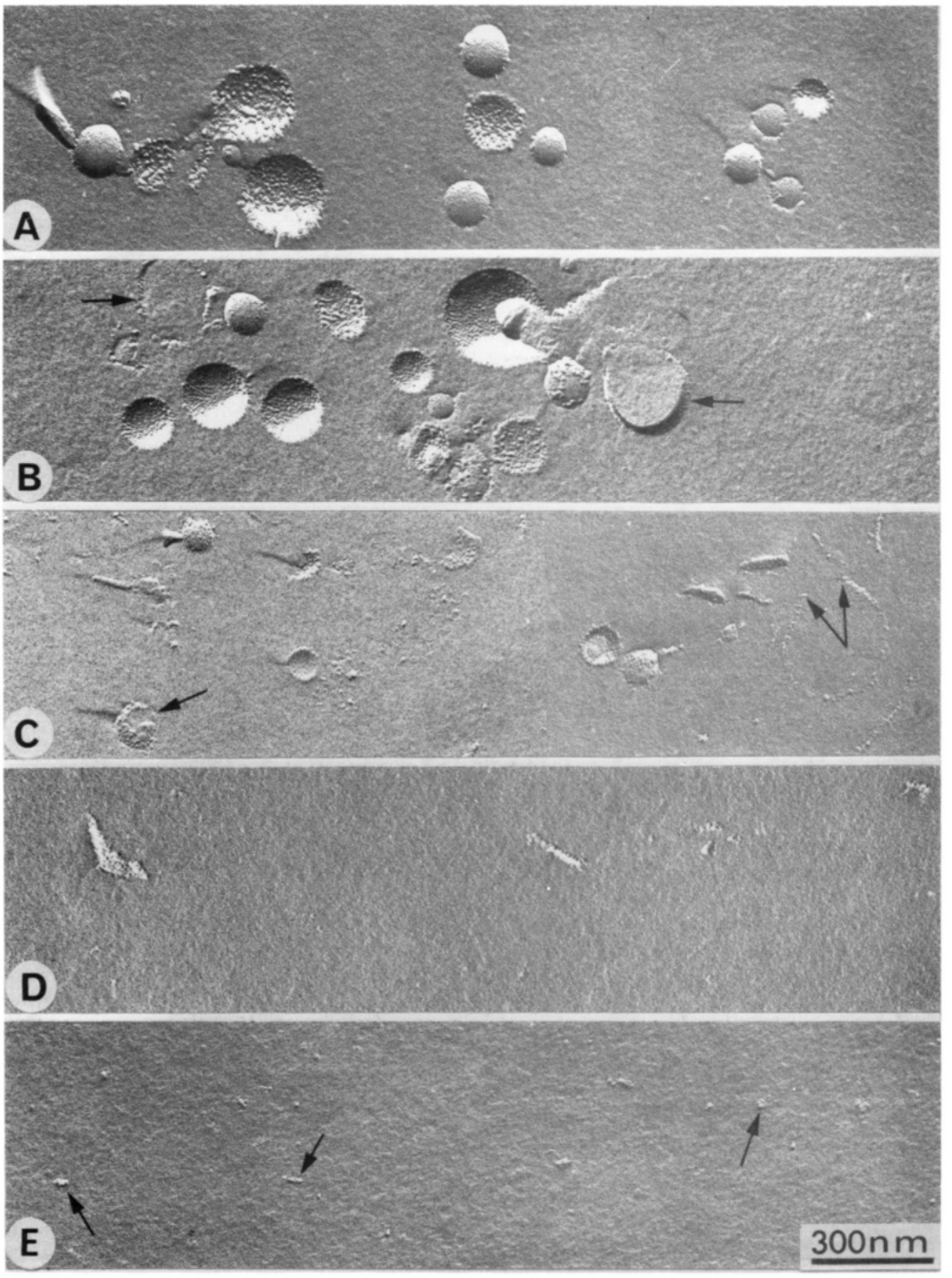

FIGURE 6: Representative pictures of sarcoplasmic reticulum obtained by addition of increasing amounts of dodecyl maltoside (B–E) to SR vesicles, followed by freeze–fracture and Pt–carbon replication at high magnification (60000×). Picture A shows the appearance of the vesicles without addition of detergent. The samples were prepared as described in the legend of Figure 5 and contained no detergent (A), 0.4 mg/mL DM (B), 0.6 mg/mL DM (C), 0.8 mg/mL DM (D), or 1.2 mg/mL DM (E), added to 1 mg of SR protein/mL. Arrows in panel B show vesicles cut as rings; arrows in panel C point toward holes in vesicles; arrows in panel E indicate solubilized Ca^{2+}-ATPase oligomers, see text. Bar, 300 nm.

organization, leading to partial conversion of vesicles to membrane sheets; this is associated with a first phase decrease in light scattering (Figure 5), but not by solubilization according to centrifuge criteria (Figure 3). During phase c, vesicle breakdown is complete, and membrane sheets gradually decrease in size. This results in a decrease in ultracentrifugal sedimentability as well as a further decrease in light scattering. Finally, in phase d, the membrane is solubilized as mixed lipid–detergent micelles and as complexes of detergent and protein, usually with some boundary lipid attached (de Foresta et al., 1989). This occurs close to but below the cmc for pure detergent. Especially by the use of bile salts as solubilizing agents, evidence has previously been obtained for the existence of intermediary forms during the solubilization process (Mazer et al., 1980; Schurtenberger et al., 1985). These forms are assumed to represent bilayer disks of varying sizes, surrounded at the periphery by a double layer of bile steroid (Small, 1971; Mazer et al., 1980; Fromherz et al., 1986). In a similar way, we propose that nonionic detergents during solubilization seal the hydrophobic edges of chopped bilayer disks with micellar structures (see inset to Figure 7) as previously suggested (Lasic, 1988; Fromherz et al., 1986).

Multilamellar SR lipids showed similar binding characteristics of smooth transitions and binding levels as Ca^{2+}-ATPase membranes when expressed in terms of moles of detergent bound per mole of lipid (Figure 2). This indicates

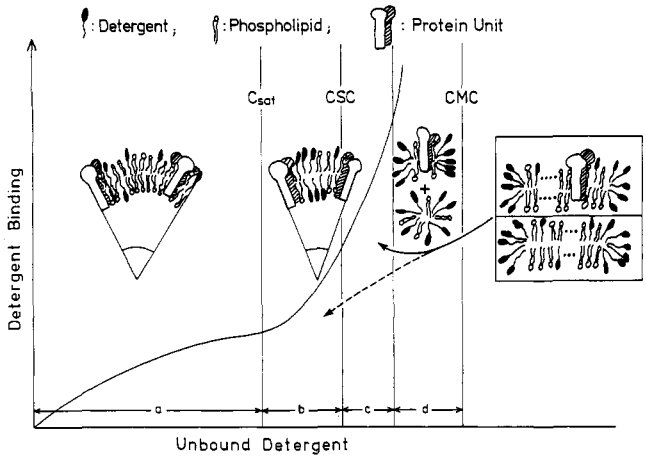

FIGURE 7: Model for the various phases leading to solubilization of protein-containing membranes and multilayered liposomes as a function of free detergent concentration. In phase a, detergent is noncooperatively taken up by the lipid phase; in phase b, above C_{sat}, detergent molecules cooperatively interact in the membrane, producing fragmentation as membrane sheets, but no solubilization of the vesicles; in phase c, at csc, lipid and protein units (monomers, protomers, or oligomers) start to become solubilized as small membrane sheets or bilayer-containing complexes, sealed at the edges by micellar detergent structures (see inset). For unilamellar liposomes made with a single lipid, C_{sat} = csc (see text). In phase d, mixed lipid/detergent micelles and detergent-solubilized protein units, covered by any remaining lipid and detergent, are formed.

that the lipid is the major detergent binding component of the membranes, as previously found for $C_{12}E_8$ (Andersen et al., 1983). This initial solubilization results in the formation of fairly large structures (depicted as bilayer disks with sealed edges in Figure 7) eluting close to the void volume of a 1.5-m agarose column (cf. Figure 4B). By further addition of detergent, these structures are converted to globular mixed micelles of smaller size corresponding to phase d in Figure 7. The binding of DM by unilamellar DOPC liposomes was characterized by sharper transitions, and here the initial conversion of the membranes to a solubilized state occurred at a constant concentration of DM, corresponding to about 50% of the cmc for pure micelles, essentially in agreement with the I–III stage hypothesis for detergent solubilization (Lichtenberg, 1985; Paternostre et al., 1988; Levy et al., 1990; Almog et al., 1990) (see the introduction). The reason why the I–III stage formulation does not apply to the other membrane systems studied probably must be sought in heterogeneity, both in terms of composition (protein versus lipid, the presence of various lipid classes in SR lipid) as well as in terms of size and morphology (multilayered liposomes). These forms of heterogeneity may result in different breakpoints for solubilization of either individual vesicles or components, accounting for the smoother transitions. For the Ca^{2+}-ATPase membranes, the protein component probably resists solubilization of the whole membrane, resulting in the formation of large membrane sheets as an intermediary stage (see Figure 6C,D).

Visualization of intermediate detergent–lipid forms arising during solubilization has been performed by cryotransmission electron microscopy of frozen–hydrated samples after addition of octyl glucoside (Vinson et al., 1989), $C_{12}E_8$ (Edwards, 1991), and Triton X-100 (Edwards et al., 1989) to egg yolk lecithin liposomes. In these systems, a pronounced rise in light scattering preceded the formation of mixed micelles, especially when small unilamellar vesicles were used. In some studies, increased light scattering during detergent addition has been attributed to vesicle fusion (Paternostre et al., 1988; Lasch et al., 1990; Edwards et al., 1989). However, Vinson et al. (1989), Edwards (1991), and Walter et al. (1991) propose increased light scattering to result from formation of very elongated structures (threads), seen by cryotransmission electron microscopy of these preparations. These structures were observed together with open lipid bilayer membranes and lamellar sheets. The elongated structures, which probably would have passed unnoticed in our freeze–fracture analysis, are presumed to represent cylindrical micelles of lipid and detergent that are formed before complete solubilization as globular micelles. However, in our experiments, phase c invariably was associated with a decrease in light scattering. Thus, we have no evidence for the formation of detergent–lipid structures with a marked asymmetry such as cylindrical micelles, except if we consider the plateau at csc (the region between C and D in Figure 5) to represent a local maximum in the light-scattering curve at the transition between phases b and c. On the other hand, our freeze–fracture data give direct evidence for the formation of vesicles with holes and lamellar sheets during the solubilization process. Recently, Lacapère et al. (1992), by the use of atomic force microscopy, after treatment of Ca^{2+}-ATPase membranes with $C_{12}E_8$ to produce 3D crystals, also observed the presence of other structures which gave the impression of being perforated sheets of bilayers.

Our data also provide information on the events taking place during incorporation of detergent into membranes (phase a), corresponding to the use of detergents in perturbation studies (Andersen et al., 1983; Champeil et al., 1986; de Foresta et al. 1992). The only evidence of structural changes in this case are indications of increased fluidity (Andersen et al., 1983) and a possible interdigitation of phospholipid hydrocarbon in the middle of the membrane, resulting in formation of rings by freeze–fracture (see footnote 4). Binding data corresponding to phase a have been described in terms of a partition coefficient, i.e., as being *proportional* to the free detergent concentration, until the saturation limit is reached [e.g., see Schurtenberger et al. (1985) and Paternostre et al. (1988)]. Our data show that detergent uptake in the membrane under these conditions is a saturable process which only can be considered to be proportional to the free concentration at low levels of detergent incorporation into the membrane. During solubilization, this mode of uptake is replaced by cooperative interaction, resulting in the initial formation of intermediary structures before complete solubilization. A natural consequence of this view is the existence of a range of free concentrations of detergent in thermodynamic equilibrium with these forms.

A surprising feature of the present data was the slowness with which nonmicellar detergent above C_{sat} equilibrated with the membranes to produce cooperative interaction (phases b and c). Apparently, cooperative interaction, while thermodynamically favored above a certain level of detergent in the membrane, has slow kinetic characteristics by nonmicellar detergent molecules. This contrasts to the rapidity of formation of detergent micelles from detergent monomers in aqueous solution. Probably, detergent–detergent interactions in the membrane are hindered by competing interactions with phospholipid hydrocarbon chains and by other factors such as a high viscosity, geometric constraints, etc. A particular aspect of the concentration dependence of detergent uptake is the possibility pointed out by Lichtenberg (1985) of a cooperative interaction across the membrane being dependent on flip-flop of the detergent molecules across the membrane. If flip-flop occurs with a low frequency, this could be rate-limiting for cooperative interaction. However, we previously found flip-flop of $C_{12}E_8$, even at very low, noninteracting concentrations of $C_{12}E_8$, to proceed quickly across liposomal

membranes [see le Maire et al. (1987)]. In addition, we found in the present study that during the ordinary dialysis procedure the detergents equilibrated quickly when added below the cmc to preformed multilayered liposomes. In the cis experiment, the important factor for rapid membrane solubilization with these detergents appears to be contingent on direct interaction of *micelles* with membranes, despite the energy barrier toward fusion of micelles with membranes that is assumed to exist (Smit et al., 1990).

The presence of energy barriers during the solubilization process may account for the slow kinetics with which some detergents like Lubrol WX and Tween 20, also when added in micellar form, solubilize lipid membranes (de Foresta et al., 1989). A thorough understanding of these processes will be useful not only for membrane solubilization studies but also for the use of detergents for other purposes like reconstitution, permeabilization, and perturbation studies of biological membranes.

ACKNOWLEDGMENTS

We are grateful to Mrs. E. Dørge for assistance with the binding experiments, to Mr. J. Delvallée, Service des Molécules Marquées, CEA, for help with the synthesis of ^{14}C-labeled dodecyl maltoside, and to Mr. J. C. Dedieu and Mr. C. Granchamp, Centre de Génétique Moléculaire, CNRS, Gif-sur-Yvette, for their help in the freeze–fracture and electron microscopy experiments. We also thank Drs. Philippe Champeil, Alain Sanson, and Stephane Orlowski, Service de Biophysique, CEA, Saclay, and Michel Ollivon, URA, CNRS 1218, Université de Paris-Sud, for their help and many stimulating discussions.

REFERENCES

Almog, S., Litman, B. J., Wimley, W., Cohen, J., Wachtel, E. J., Barenholz, Y., Ben-Shaul, A., & Lichtenberg, D. (1990) *Biochemistry 29*, 4582–4592.

Andersen, J. P., Møller, J. V., & Jørgensen, P. L. (1982) *J. Biol. Chem. 257*, 8300–8307.

Andersen, J. P., le Maire, M., Kragh-Hansen, U., Champeil, P., & Møller, J. V. (1983) *Eur. J. Biochem. 134*, 205–214.

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466–468.

Bolli, R., Nalecz, K. A., & Assi, A. (1985) *Arch. Biochem. Biophys. 240*, 102–116.

Champeil, P., le Maire, M., Andersen, J. P., Guillain, F., Gingold, M., Lund, S., & Møller, J. V. (1986) *J. Biol. Chem. 261*, 16372–16384.

de Foresta, B., le Maire, M., Orlowski, S., Champeil, P., Lund, S., Møller, J. V., Michelangeli, F., & Lee, A. (1989) *Biochemistry 28*, 2558–2567.

de Foresta, B., Henao, F., & Champeil, P. (1992) *Eur. J. Biochem. 209*, 10023–10034.

del Rio, E., González-Manñus, Gurtubay, J.-I. G., & Goñi, F. (1991) *Arch. Biochem. Biophys. 291*, 300–306.

de Meis, L., & Hasselbach, W. (1971) *J. Biol. Chem. 246*, 4759–4763.

Edwards, K. (1991) *Vesicle–surfactant interactions: effect of non-ionic surfactants on structure and leakage of small unilamellar vesicles, Acta Universitatis Upsaliensis*, Almqvist & Wiksell, Stockholm, Sweden.

Edwards, K., Almgren, M., Bellare, J., & Brown, W. (1989) *Langmuir 5*, 473–478.

Folch, J., Lee, M., & Stanley, G. H. S. (1957) *J. Biol. Chem. 266*, 497–509.

Fromherz, P., Röcker, C., & Rüppel, D. (1986) *Faraday Discuss. Chem. Soc. 81*, 39–48.

Goñi, F. M., Urbaneja, M.-A., Arrondo, J. L., Alonso, A., & Durrani, A. A. (1986) *Eur. J. Biochem. 160*, 659–665.

Heegaard, C. W., le Maire, M., Gulik-Krzywicki, T., & Møller, J. V. (1990) *J. Biol. Chem. 265*, 12020–12028.

Helenius, A., & Simons, K. (1975) *Biochim. Biophys. Acta 415*, 29–79.

Jackson, M. L., Schmidt, C. F., Lichtenberg, D., Litnau, B. J., & Albert, A. D. (1982) *Biochemistry 21*, 4576–4582.

Kragh-Hansen, U., Minchiotti, L., Brennan, S. O., & Sugita, O. (1990) *Eur. J. Biochem. 193*, 169–174.

Lacapère, J.-J., Stokes, D. L., & Chatenay, D. (1992) *Biophys. J. 63*, 303–308.

Lasch, J., Hoffmann, J., Omelyanenko, W. G., Klibanov, A. A., Torchilin, V. P., Binder, H., & Gawrisch, K. (1990) *Biochim. Biophys. Acta 1022*, 171–180.

Lasic, D. D. (1988) *Biochem. J. 256*, 1–11.

le Maire, M., Lind, K. E., Jørgensen, K. E., Roigaard, H., & Møller, J. V. (1978) *J. Biol. Chem. 253*, 7051–7060.

le Maire, M., Møller, J. V., & Gulik-Krzywicki, T. (1981) *Biochim. Biophys. Acta 643*, 115–125.

le Maire, M., Kwee, S., Andersen, J. P., & Møller, J. V. (1983) *Eur. J. Biochem. 129*, 525–532.

le Maire, M., Aggerbeck, L. P., Monteilhet, C., Andersen, J. P., & Møller, J. V. (1986) *Anal. Biochem. 154*, 525–535.

le Maire, M., Møller, J. V., & Champeil, P. (1987) *Biochemistry 26*, 4803–4810.

le Maire, M., Garrigos, M., & Møller, J. V. (1992) in *Technologies on protein studies and purification* (Briand, Y., Doinel, C., Gagnon, J., & Faure, A., Eds.) Vol. 5, pp 75–85, G.R.B.P., Villebon sur Yvette, France.

Levy, D., Gulik, A., Seigneuret, M., & Rigaud, J.-L. (1990) *Biochemistry 29*, 9480–9488.

Lichtenberg, D. (1985) *Biochim. Biophys. Acta 821*, 470–478.

Lichtenberg, D., Robson, R. J., & Dennis, E. A. (1983) *Biochim. Biophys. Acta 737*, 285–304.

Lund, S., Orlowski, S., de Foresta, B., Champeil, P., le Maire, M., & Møller, J. V. (1989) *J. Biol. Chem. 264*, 4907–4915.

Makino, S., Woolford, J. L., Tanford, C., & Webster, R. E. (1975) *J. Biol. Chem. 250*, 4327–4332.

Mazer, N. A., Benedik, G. B., & Carey, M. C. (1980) *Biochemistry 19*, 601–615.

Meissner, G., Conner, G. E., & Fleischer, S. (1973) *Biochim. Biophys. Acta 298*, 246–269.

Møller, J. V., le Maire, M., & Andersen, J. P. (1986) in *Progress in Protein–Lipid Interations* (Watts, A., & de Pont, J. J. H. H. M., Eds.) Vol. 2, Chapter 5, Elsevier Biochemical Press, Amsterdam, The Netherlands.

Ollivon, M., Eidelman, O., Blumenthal, R., & Walter, A. (1988) *Biochemistry 27*, 1695–1703.

Paternostre, M. T., Roux, M., & Rigaud, J.-L. (1988) *Biochemistry 27*, 2668–2676.

Rash, J. E., & Hudson, C. S., Eds. (1980) in *Freeze–fracture: Methods, Artifacts and Interpretation*, Raven Press, New York.

Robinson, N. C., & Tanford, C. (1975) *Biochemistry 14*, 369–378.

Rosevear, P., Van Aken, T., Baxter, J., & Ferguson-Miller, S. (1980) *Biochemistry 19*, 4108–4115.

Schurtenberger, P., Mazer, N. A., & Kanzig, W. (1985) *J. Phys. Chem. 89*, 1042–1049.

Small, D. M. (1971) in *The Bile Acids: Chemistry, Physiology and Metabolism* (Nair, P. P., & Kritchevsky, D., Eds.) Plenum Press, New York and London.

Smit, B., Hilbers, P. A. J., Esselink, K., Rupert, L. A. M., van Os, N. M., & Schlijper, A. G. (1990) *Nature 348*, 624–625.

Suarez, M. D., Revsin, A., Narlock, R., Kempner, E. S., Thompson, D. A., & Ferguson-Miller, S. (1984) *J. Biol. Chem. 259*, 13791–13799.

Ueno, M., Tanford, C., & Reynolds, J. A. (1984) *Biochemistry 23*, 3070–3076.

Vinson, P. K., Talmon, Y., & Walter, A. (1989) *Biophys. J. 56*, 669–681.

Walter, A., Vinson, P. K., Kaplun, A., & Talmon, Y. (1991) *Biophys. J. 60*, 1315–1325.