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Outer membrane protein A of *E. coli* folds into detergent micelles, but not in the presence of monomeric detergent

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Abstract

Outer membrane protein A (OmpA) of *Escherichia coli* is a β-barrel membrane protein that unfolds in 8 M urea to a random coil. OmpA refolds upon urea dilution in the presence of certain detergents or lipids. To examine the minimal requirements for secondary and tertiary structure formation in β-barrel membrane proteins, folding of OmpA was studied as a function of the hydrophobic chain length, the chemical structure of the polar headgroup, and the concentration of a large array of amphiphiles. OmpA folded in the presence of detergents only above a critical minimal chain length of the apolar chain as determined by circular dichroism spectroscopy and a SDS-PAGE assay that measures tertiary structure formation. Details of the chemical structure of the polar headgroup were unimportant for folding. The minimal chain length required for folding correlated with the critical micelle concentration in each detergent series. Therefore, OmpA requires preformed detergent micelles for folding and does not adsorb monomeric detergent to its perimeter after folding. Formation of secondary and tertiary structure is thermodynamically coupled and strictly dependent on the interaction with aggregated amphiphiles.

Keywords: β-barrel; critical micelle concentration; membrane protein folding; outer membrane protein A; porin; protein–detergent interaction; protein–lipid interaction

Integral membrane proteins are generally stable only in lipid bilayers or detergent micelles. Although detergents are widely used in membrane protein research for the purpose of protein solubilization, purification, characterization, and structure determination, the thermodynamics of membrane protein stabilization by detergents are less well understood. Mild detergents are often used to extract biomembranes and solubilize membrane proteins while preserving their native structure, mostly with retention of their functional properties. In contrast, strong ionic detergents such as SDS cause a complete disruption of the native structure, coupled to a complete loss of biological activity of most integral membrane proteins (Helenius & Simons, 1975; Tanford & Reynolds, 1976). Finding an appropriate detergent for the solubilization of a membrane protein is not only crucial for maintaining the activity of a protein, but also very important for solving protein structures by X-ray crystallography or solution phase NMR spectroscopy. Therefore, it is not surprising that numerous studies of protein–detergent interactions have been undertaken to elucidate the required detergent properties for preserving the native structure and function of membrane proteins (Makino et al., 1973, 1975; Robinson & Tanford, 1975; Le Maire et al., 1983; Lund et al., 1989; Eisele & Rosenbusch, 1990; Rosenbusch, 1990; Møller & Le Maire, 1993; Song & Gouaux, 1997). An interesting question regarding the stability of membrane proteins and the mechanism of protein–detergent interactions is whether an integral membrane protein folds first and then binds monomeric detergents on its hydrophobic perimeter or whether it requires detergent in a micellar aggregate to fold into its native structure. Thorough investigations of protein–detergent interactions with respect to the aggregation state of the detergent have been undertaken primarily with α-helical integral membrane proteins, for example cytochrome b_5 (Robinson & Tanford, 1975), and Ca^{2+}-ATPase (Møller & Le Maire, 1993), or serum lipoproteins (Makino et al., 1975; Bjerrum et al., 1980). Results obtained with serum albumin and cytochrome b_5 suggested that monomeric detergents (deoxycholate and Triton-X-100, re-

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Abbreviations: C_nE_4, alkyl-tetraethyleneglycol; C_n-glucoside, alkyl-β-D-glucoside; C_n-lysoPC, 1-acyl-2-hydroxy-*sn*-glycero-3-phosphocholine; C_n-maltoside, alkyl-β-D-maltoside; CD, circular dichroism; CMC, critical micelle concentration; C_nMe_2NO, N-alkyl-N,N-dimethylammonium-N-oxide; C_nP-choline; alkyl-phosphocholine; *di*C_nPC, 1,2 *di*acyl-*sn*-glycero-3-phosphocholine; *di*$C_{18(cis\ 9,10)}$PC, DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; EDTA, ethylenediaminetetraacetic acid; OmpA, outer membrane protein A; PAGE, polyacrylamide gel electrophoresis; SB-C_n, N-alkyl-N,N-dimethyl-3-ammonio-1-propane-sulfonate (Zwittergent-n); SDS, sodium dodecylsulfate; SUV, small unilamellar vesicle; UV, ultraviolet.

spectively) bind directly to these proteins and that preformed micelles are not required for productive detergent–protein interactions (Helenius & Simons, 1972; Makino et al., 1973; Nozaki et al., 1974; Robinson & Tanford, 1975). For cytochrome b_5, it was proposed that the protein could function as a nucleus for micelle formation at concentrations close to the critical micelle concentration (CMC) (Robinson & Tanford, 1975). Le Maire et al. (1983) suggested that detergent molecules could form a single monolayer torus around the hydrophobic segment of sarcoplasmic Ca^{2+}-ATPase. Theoretical considerations based on detergent binding experiments lead Møller and Le Maire (1993) conclude that detergents bind individually as monomers to the hydrophobic perimeter of Ca^{2+}-ATPase. Systematic studies on the interaction of detergents with β-barrel membrane proteins have focused mainly on conditions for crystal formation (Eisele & Rosenbusch, 1990). Thermodynamic and mechanistic studies on β-barrel membrane protein–detergent interactions are so far lacking.

In the present work, we have investigated the modes of interaction of detergents with the outer membrane protein A (OmpA) of *Escherichia coli*. This protein forms a β-barrel composed of eight amphipathic antiparallel membrane-spanning β-strands (Pautsch & Schulz, 1998; see also Fig. 1). Denaturation in 8 M urea leads to a complete loss of all secondary and tertiary structure of OmpA (Schweizer et al., 1978) and urea-denatured OmpA can be successfully refolded into micelles formed of β-octylglucoside (Dornmair et al., 1990). These studies took advantage of the observation that if not boiled prior to electrophoresis, OmpA migrates on SDS polyacrylamide gels to different positions depending on the compactness of its structure. Native OmpA migrates with an apparent MW of ~30 kDa, whereas completely unfolded OmpA migrates as a ~35 kDa protein. Furthermore, it has been shown that membrane-incorporated and completely refolded OmpA (30 kDa form), but not its 35 kDa unfolded form, is partially protected from trypsin digestion by the membrane in the same way as native OmpA in the outer membrane of *E. coli*; a membrane-inserted 24 kDa fragment is produced under these conditions (Schweizer et al., 1978). Here, we have used this gel-shift assay to study the folding of OmpA into detergent as a function of the hydrophobic chain length, the chemistry of the headgroup, and the concentration of the detergent. In addition, we used circular dichroism (CD) spectroscopy to monitor the formation of secondary structure as a function of detergent concentration. Since unfolded OmpA also folds into lipid bilayers (Surrey & Jähnig, 1992; Kleinschmidt & Tamm, 1996), this protein serves as a suitable model to study membrane protein folding. The question of particular interest here is whether an aggregated state (physical property, pathway A or B in Fig. 1), certain functional groups (chemical properties), or both, of the amphiphiles are required for productive folding of OmpA. The chemical structures of the detergents used in this study are shown in Figure 2.

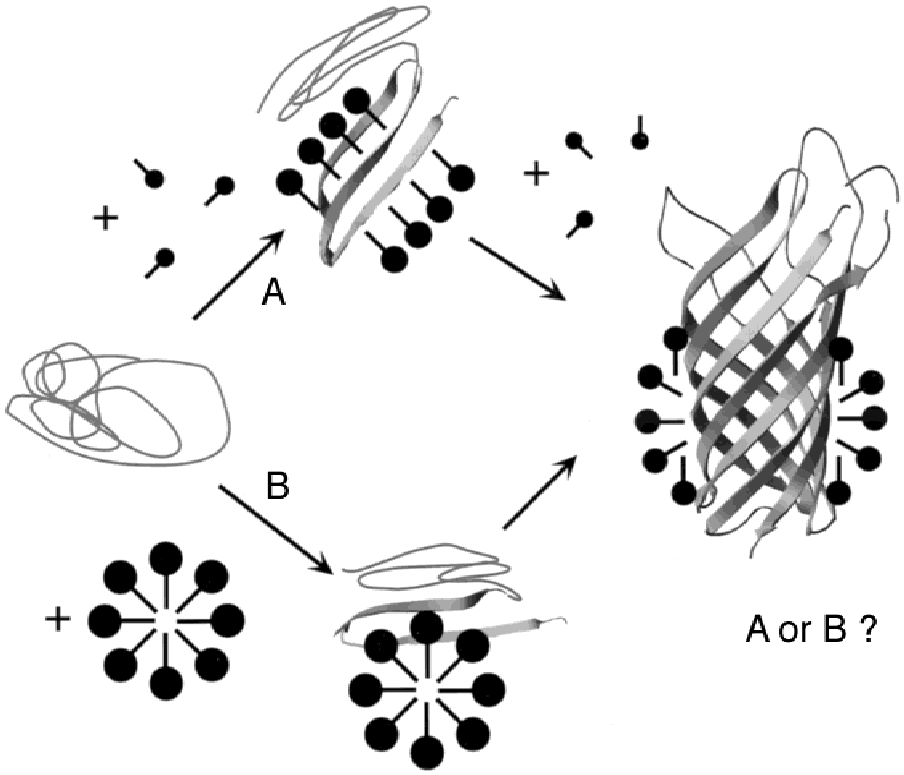

Fig. 1. Crystal structure of OmpA (from Pautsch & Schulz, 1998) and two possible modes of interaction with detergents. **A:** Binding of monomeric detergent to hydrophobic surfaces of prefolded OmpA. **B:** Folding of OmpA into preformed detergent micelle(s). The micelles are drawn in arbitrary sizes. They actually vary quite significantly (aggregation numbers ~16–140; MWs ~5,000–60,000) for the detergents used in this study. In some cases, more than one micelle will be needed to refold a single OmpA molecule and to form a mixed protein/detergent micelle. Therefore, the 1:1 stoichiometry shown is also arbitrary and may actually vary significantly in the various systems investigated.

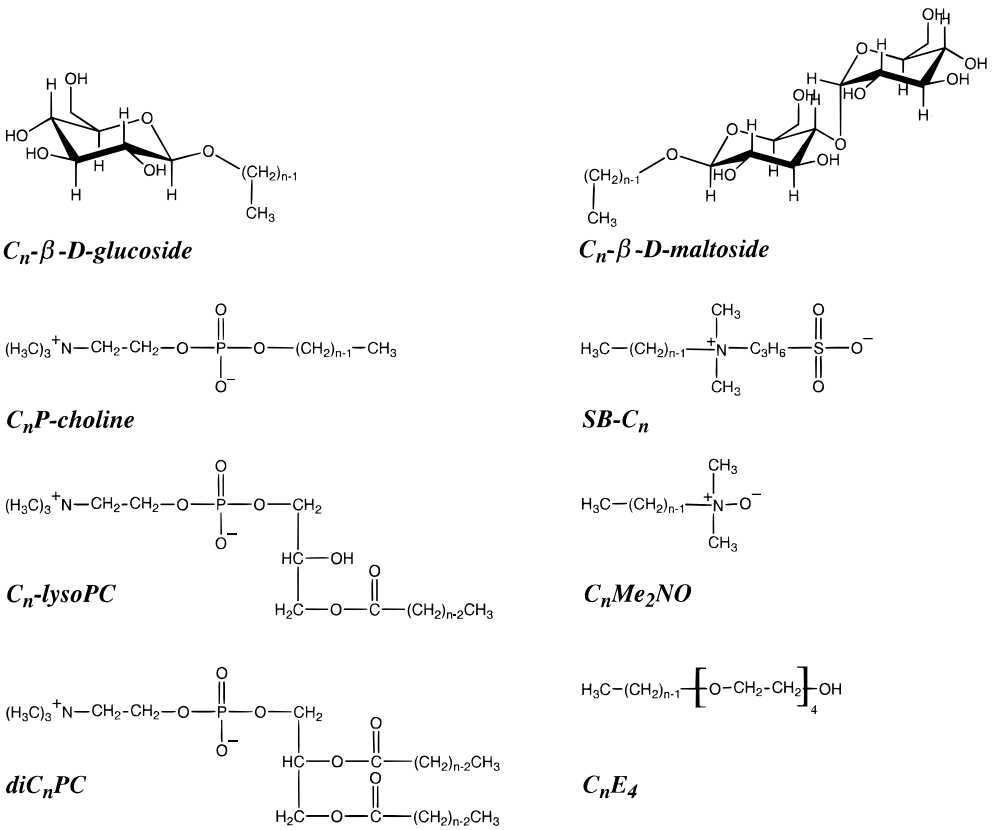

Fig. 2. Chemical structures of the detergents used in this study.

Results

Folding of OmpA in detergents of different chain lengths

We first investigated the folding of OmpA as a function of the alkyl chain length for different detergent species. We utilized the different migration behavior of unboiled samples of folded vs. unfolded OmpA in polyacrylamide gels as a folding assay (Schweizer et al., 1978). Appearance of the 30 kDa band has been previously correlated with formation of the native structure of OmpA. All experiments were performed at a fixed detergent/protein ratio of 800 mol/mol and a detergent concentration of 6.9 mM. Figure 3A shows the migration of OmpA incubated with alkyl-dimethylamine-N-oxides of increasing chain lengths from 6 to 13 carbon atoms. In these experiments, the folded form of OmpA appeared only when the length of the alkyl chain exceeded a lower limit, which was a C_{10}-chain for the alkyl-dimethylamine-N-oxides. Similar to the results shown in Figure 3A, tetraoxyethyleneglycols, glucosides, maltosides, and sulfobetaines exhibited similar chain length limits for folding of C_8, C_9, C_9, and C_{12}, respectively (data not shown). Based on these results, one might conclude that successful folding of OmpA requires a minimum hydrophobic chain length (C_8 to C_{12}), but not a specific polar headgroup structure. To test this hypothesis we performed additional studies with a series of single chain phosphocholines (C_nP-cholines, limit C_9, data not shown), lyso-phosphatidylcholines (lyso-PCs, Fig. 3B), and 1,2-diacyl-phosphatidylcholines (PCs, Fig. 3C). The data show that the addition of the glycerol group does not significantly alter the chain length limit of C_9 to C_{10} for folding, but that the second acyl chain in the phosphatidylcholines reduces this limit from C_{10} to C_7. Obviously, the chain length itself is not the limiting factor for successful folding of OmpA.

Because the critical micelle concentration (CMC) of amphiphiles also depends on the length of the hydrophobic chain, we suspected that the folding of OmpA might depend on the aggregation state of the amphiphiles. To test this hypothesis, we compared the ability of OmpA to fold in the different detergents with the CMCs of these detergents. Table 1 shows that, at a constant amphiphile concentration of 6.9 mM, the minimum hydrophobic chain length required for folding correlates well with the CMCs of the detergents. Most CMCs listed in Table 1 were taken from the literature, but some had to be interpolated using the known linear relationship between the logarithm of the CMC and the length of the hydrophobic chain as shown in Figure 4 (Tanford, 1980; Cevc & Marsh, 1987).

Dependence of OmpA folding on the detergent concentration

To rigorously prove that OmpA requires an aggregated substrate, i.e., detergent micelles, for folding, we measured folding as a function of detergent concentration at constant protein concentration for decyl-maltoside and dodecyl-phosphocholine. The CMCs of the two detergents are 1.8 and 1.5 mM, respectively. Folding experiments were carried out at detergent/protein ratios of 400, 200, 100, and 50 mol/mol, respectively (Fig. 5). Clearly, OmpA folded only when the detergent concentrations were above their

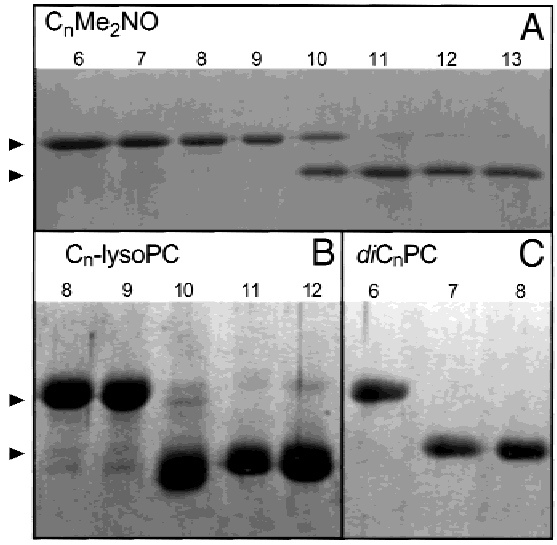

Fig. 3. SDS-PAGE showing the folding of OmpA into (**A**) alkyl-dimethylamine-N-oxides, (**B**) lyso-phosphatidylcholines, and (**C**) phosphatidylcholines of different hydrophobic chain lengths. The lengths of the hydrophobic alkyl chains are indicated on top of each lane. 8.6 μM OmpA was reacted with an 800-fold molar excess of detergent in borate buffer (20 mM borate, pH 10.0). Incubation was stopped by addition of SDS after 12 h at 20 °C. Samples were not boiled before loading onto the gels. The upper arrow in each gel indicates the unfolded 35 kDa form, the lower arrow the native 30 kDa form of OmpA. The minimal hydrophobic chain lengths required for refolding at 6.9 mM detergent are 10, 10, and 7 carbon atoms for alkyl-dimethylamine-N-oxides, lyso-phosphatidylcholines, and phosphatidylcholines, respectively.

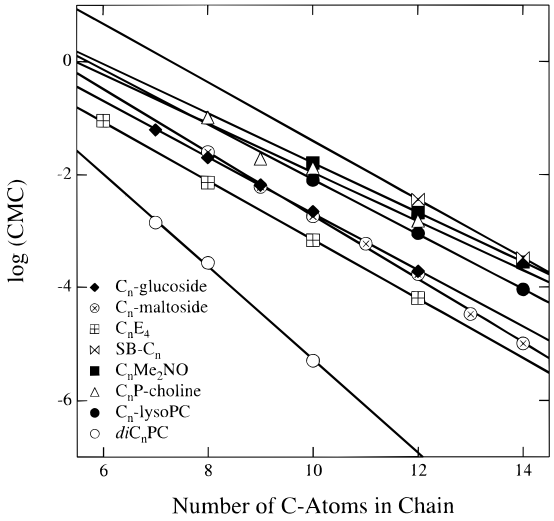

Fig. 4. Logarithm of the critical micelle concentrations of detergents and lipids used in this study plotted as a function of the number of carbon atoms in the hydrophobic chains. Linear interpolation and extrapolation were used to calculate some of the CMCs shown in Table 1.

respective CMCs. Similarly, OmpA folded in C_{10}-glucoside (CMC = 2.2 mM) at a detergent concentration of 3.4 mM, but only to about 40% at 1.7 mM (data not shown). In a complementary experiment with C_8-lysoPC, we increased the detergent/protein ratio to determine whether the unsuccessful folding experiments carried out at a detergent/protein ratio of 800 (Fig. 3B) would result in folding. Indeed, OmpA folded in 172 mM C_8-lysoPC whose CMC is estimated from Figure 4 to be about 80 mM (data not shown). In addition to demonstrating that detergent micelles are required for folding of OmpA, these results also indicate that the protein does not measurably perturb the CMC of the detergents investigated.

CD spectroscopy at different detergent concentrations

To follow the development of secondary structure as OmpA folded into detergent micelles, we recorded CD spectra as a function of

Table 1. *Correlation between the minimum hydrophobic chain length required for refolding of OmpA and the CMCs of the corresponding detergents and lipids*[a]

Detergent or lipid	CMC (mM) at n = 7	8	9	10	11	12	14	Refolding at 6.9 mM surfactant
C_n-glucoside	*65*	20	**6.5**	2.2	*0.62*	0.19	*0.02*	**9**
C_n-maltoside	*91*	25	***6***	1.8	0.59	0.17	0.01	**9**
C_nE_4	*26*	**7.2**	*2.4*	0.68	*0.20*	0.04	*5.6×10^{-3}*	**8**
SB-C_n	*1,430*	*435*	*132*	*38*	*12*	**3.6**	0.33	**12**
C_nMe_2NO	*334*	*122*	*44.7*	**16**	*5.7*	2.1	0.28	**10**
C_nP-choline	*220*	102	19	**13**	*3.9*	1.5	*0.2*	**10**
C_n-lysoPC	*240*	*78*	*26*	**8**	*2.6*	0.9	0.09	**10**
diC_nPC	**1.4**	0.27	0.029	0.005	*7.6×10^{-4}*	*1.2×10^{-4}*	*2.7×10^{-6}*	**7**

[a] At a given detergent concentration (6.9 mM), the minimum chain length (last column) for successful refolding of OmpA correlates with the CMC of the surfactant. The CMCs closest to 6.9 mM are shown in boldface for each class of surfactant. OmpA refolds only close to or above the CMC. CMCs shown in roman type are from the literature: C_n-glucosides n = 8, 10 from Rosen (1989), n = 7–9 and C_n-maltosides n = 8–12 from Anatrace Corp. (Maumee, Ohio) Catalog; C_6E_4 from Shinoda (1978); C_8E_4 from Zulauf (1990); $C_{10}E_4$ and $C_{12}E_4$ from Rosen (1989); SB-C_{12} from Goenne and Ernst (1978); SB-C_{14} from Bailyes et al. (1983); C_nP-choline n = 8–10, 12 from Anatrace Corp. Catalog; n-alkyl-dimethylammonium-N-oxides n = 10, 12, 14 from Shinoda (1978); C_n-lysoPC n = 10, 12, 14 from Helenius et al. (1979); diC_nPC: n = 7, 8, 10 from Tanford (1980), n = 9 from Tausk et al. (1974). All other CMCs shown in italics were estimated from the plots of log(CMC) vs. the hydrophobic chain length (see Fig. 5).

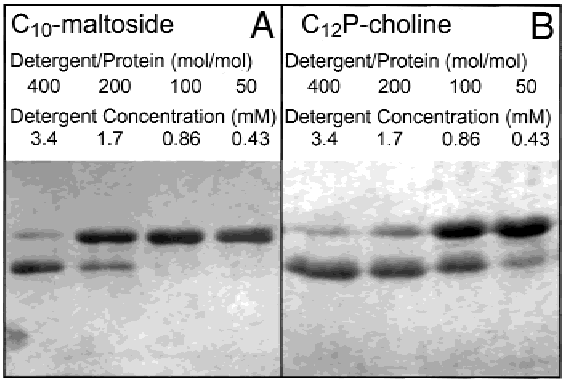

Fig. 5. Folding of OmpA in solutions of different concentrations of (**A**) decyl-maltoside and (**B**) dodecyl-phosphocholine. Detergent concentrations were 3.4, 1.7, 0.86, and 0.43 mM, resulting in molar detergent/lipid ratios of 400, 200, 100, and 50, respectively. All other experimental conditions are as described in the caption to Figure 3.

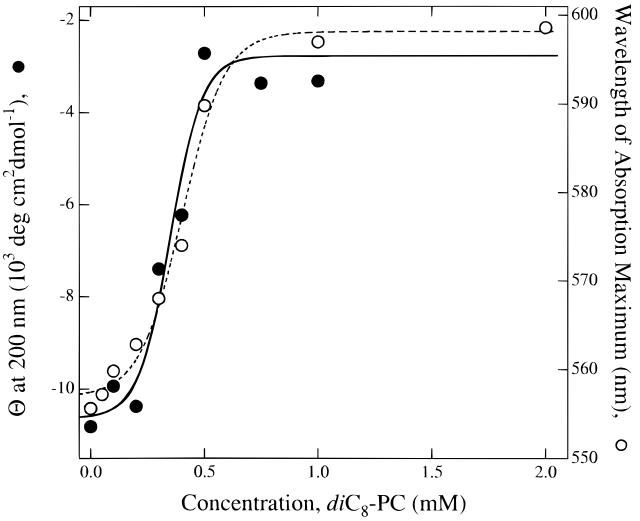

Fig. 7. Correlation of secondary structure formation of OmpA with the formation of detergent micelles of dioctanoyl-PC. Filled circles: mean residue molar ellipticity at 200 nm of 17.1 μM OmpA titrated with dioctanoyl-PC. Open circles: wavelength of the absorption maximum of Coomassie blue as a function of dioctanoyl-PC concentration (CMC assay). The same buffer (20 mM borate, pH 10.0) and temperature (20 °C) conditions were used in both sets of experiments.

detergent concentration. Figure 6 shows far-UV CD spectra of OmpA in 8 M urea, detergent-free buffer, and in *di*C_8-PC at 0.3 and 10 mM. The spectrum in 8 M urea is typical for a random coil. The CD spectrum of OmpA diluted into borate buffer (to a residual urea concentration of 20 mM) indicates that the protein adopts a mixture of secondary structures, including helix, sheet, and random coil. These spectra are quite similar to those reported by Surrey and Jähnig (1992). When 0.3 mM *di*C_8-PC was added, the CD spectrum was qualitatively similar to that in buffer, indicating only a small change in secondary structure. However, increasing the detergent concentration to 10 mM, i.e., well above its CMC, resulted in a very different CD spectrum, indicative of a much larger content of β-structure. We found that the formation of secondary structure, mainly β-sheet, was complete after about 6–10 min. Spectra taken 10 min after initiation of the folding reaction were indistinguishable from those of samples that were incubated over night. The spectra of the refolded protein were very similar to those of the native protein reported by Sugarawa et al. (1996). The spectral changes upon unfolding are most evident at ~200 nm. Therefore, we monitored the mean residue molar ellipticity at this wavelength as a function of *di*C_8-PC concentration. Figure 7 shows a titration with *di*C_8-PC in the range from 0 to 2 mM detergent. The mean residue molar ellipticities show a sigmoidal transition with a mid-point at 0.37 mM *di*C_8-PC. The data point at 10 mM *di*C_8-PC (Fig. 6), which had an ellipticity of -2×10^3 deg cm^2 dmol^{-1} at 200 nm, was included in this fit. Micelle formation of *di*C_8-PC was independently measured by an assay that monitors a red shift of the absorption maximum of Coomassie Blue (BB R-250) upon binding of the dye to aggregated, but not to monomeric detergent. Again, a sigmoidal curve was recorded with a transition from monomeric to aggregated detergent occurring at 0.40 mM, in reasonably good agreement with the published CMC of 0.27 mM of *di*C_8-PC (Tanford, 1980). The close superposition of the two transition curves provides further strong support for our conclusion that OmpA folds into detergent micelles, but not in the presence of monomeric detergent.

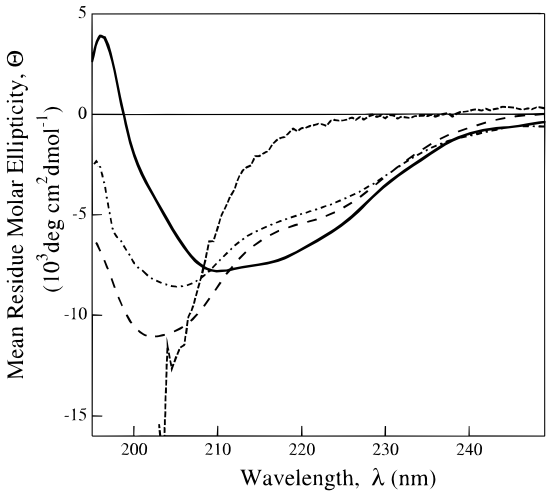

Fig. 6. Circular dichroism spectra of OmpA in 8 M urea (short dashed line), in detergent-free buffer (20 mM borate, pH 10.0, long dashed line), and in *di*octanoyl-PC at concentrations of 0.3 mM (dashed-dotted line), and 10 mM (solid line) in 20 mM borate buffer, pH 10.0. Background spectra of respective solutions without protein were subtracted. OmpA was 17.1 μM in a volume of 100 μL. Spectra were recorded 10 min after diluting unfolded OmpA (in 8 M urea) 12.3-fold with the prepared solutions.

Discussion

OmpA has become a popular model for studying the folding of integral membrane proteins, particularly those that form transmembrane β-barrels. Folding of OmpA was previously studied mainly in the presence of lipid bilayers, but only to a limited extent in detergent micelles. In the present work, we have critically evaluated the respective roles of the hydrophobic chain length, the headgroup chemistry, and the physical state of aggregation of the

detergent on the folding of OmpA. Folding was monitored at the level of secondary structure formation by CD spectroscopy and at the level of tertiary structure formation by a SDS-PAGE gel-shift assay. Under all conditions investigated, the requirements for secondary and tertiary structure formation were the same (even if in kinetic experiments the two levels of structure did not always appear simultaneously). Thermodynamically, secondary and tertiary structures were formed in a two-state all-or-none process as demonstrated by the sharpness of the transition presented in Figure 7. Our results clearly show that amphiphiles (detergents or lipids) must be present in an aggregated state to support folding of OmpA (pathway B in Fig. 1). OmpA folded into detergent micelles only above their respective CMCs. Absolute detergent or protein concentrations and detergent/protein ratios were irrelevant parameters for folding of OmpA. The hydrophobic chain length of the detergents also had no direct influence on the folding behavior of this integral membrane protein. This result raises interesting questions about how the detergents pack around the hydrophobic perimeter of the β-barrel. It appears that a model with a bilayer-type torus around the protein is unreasonable, at least for the shorter chain lipids because of an excessive hydrophobic mismatch between the bilayer thickness and the hydrophobic length of the β-barrel, which is about 30 Å (Pautsch & Schulz, 1998). A monolayer or prolate ellipsoid arrangement of detergent on the hydrophobic protein surface seems to be a more realistic model. Such a model was previously proposed for detergent complexes with the sarcoplasmic reticulum Ca^{2+}-ATPase (Møller & Le Maire, 1993). However, these authors suggested that monomers of detergent assembled one-by-one on the hydrophobic surface of the Ca^{2+}-ATPase, which is clearly not the case for the refolding of OmpA.

Because of the strict correlation between folding and micelle formation, we may tentatively reach the reverse conclusion, i.e., that the dependence of refolding of OmpA on detergent concentration measures the CMC of that detergent. Accepting this reciprocity, we may further conclude from the very similar transition curves that we obtain in the presence and absence of protein (see e.g., Fig. 7) that OmpA does not change the CMCs of the detergents investigated in this study to any significant degree. This by itself is a remarkable new finding that to our knowledge has not been previously reported in the literature. The result is remarkable because many of the micelles of this study are smaller than the membrane-inserted β-barrel portion of the protein (19 kD; the aggregation numbers of the detergents range between approximately 16 and 140 and their aggregate molecular masses between approximately 5 and 60 kD). Therefore, two or more micelles will have to coalesce in some cases to form larger structures in the mixed protein/detergent micelles. Since the CMC is a measure of the difference of the chemical potentials of the surfactants in the monomeric and aggregated forms, we must conclude that the chemical potentials of a given surfactant are similar in the pure and mixed detergent micelles.

How then does OmpA fold into preformed detergent micelles? We imagine that this occurs by a process that is similar to that observed for the folding of OmpA into lipid bilayers (Kleinschmidt & Tamm, 1996; Kleinschmidt et al., 1999). In our previous kinetic work, we were able to distinguish between three structurally different intermediates on the folding pathway, which were each characterized by different degrees of polypeptide chain penetration into the lipid bilayer. Most secondary but no tertiary structure was completed in the early folding intermediates with mainly surface-located polypeptide chains. A similar mechanism might occur when OmpA uses the interface of detergent micelles as a catalyst for folding. However, folding into micelles is much faster (complete after a few minutes) than folding into lipid bilayers (minutes to hours), which may be a result of the faster dynamics of lipids (or detergents) in micelles compared to lipids in bilayers. A possible explanation for the requirement of an aggregated substrate may be that OmpA needs to form hydrogen bonds between neighboring β-strands to form the β-barrel and to incorporate it into a hydrophobic environment such as a detergent micelle or a lipid bilayer. The low average hydrophobicity of -0.4 to -0.6 of amphipathic β-strands compared to that of about 1.6 for hydrophobic helices of α-helical proteins (Vogel & Jähnig, 1986) and the alternating occurrence of hydrophobic and hydrophilic residues in the amino acid sequences of β-barrel membrane proteins require a shielding of all polar groups including unsatisfied backbone hydrogen bonds from the hydrophobic environment of the lipid bilayer or detergent micelle. In this context, it is interesting that the chemical composition of the interface, i.e., the polar headgroup structure, apparently has no effect on the folding of OmpA. While this is definitely true for folding at equilibrium as studied here, we cannot exclude at the present time the possibility that interfacial properties play a role in the kinetics of OmpA folding into detergent micelles. Nevertheless, headgroup structures as diverse as phosphocholines, sulfobetaines, ammoniumoxides, polyethyleneglycols, and mono- and disaccharides did not seem to drastically affect structure formation of OmpA. However, it should be noted that these are all zwitterionic or uncharged detergents. OmpA does not refold into the harsher charged detergents, even though it has long been known from the gel shift assays (as employed in this work) that some kind of compact structure is preserved even in SDS at room temperature.

Materials and methods

Materials

OmpA was purified from the outer membrane of *E. coli* as described (Kleinschmidt & Tamm, 1996). 1,2-diacylphosphatidylcholines and 1-acyl-2-hydroxyphosphatidylcholines with chain lengths of 6 to 14 carbon atoms were from Avanti (Alabaster, Alabama). Alkylsulfobetaines, SB-C_n, $n = 8$, 10, 12, 14, 16, and alkyl-sucroses were obtained from Calbiochem (San Diego, California). Alkyl-glucosides (*n*: 7–10, 12), alkyl-maltosides (*n*: 8–14, 16), and alkyl-phosphocholines (*n*: 8–10, 12) were from Anatrace (Maumee, Ohio), *n*-alkyl-N,N-dimethylamine-N-oxides were from Fluka (Ronkonkoma, New York). Alkyl-tetraethyleneglycols, C_nE_4, (*n*: 6,8,10) were from Bachem (Philadelphia, Pennsylvania). Coomassie Brilliant Blue R-250 was from Sigma (St. Louis, Missouri).

Folding and membrane insertion detected by SDS-PAGE

8.6 μM protein (1.5 μL in 8 M urea, 10 mM borate, pH 10, 2 mM EDTA) was incubated with an 800-fold molar excess of detergent or lipid in 20 μL of borate buffer (H_3BO_3/NaOH, 10 mM, pH 10.0, containing 2 mM EDTA) at 20 °C for 12 h if not described otherwise. All incubation experiments were done in parallel for detergents with the same headgroup, but different chain lengths, and with protein from the same stock solution to ensure proper comparisons in dependence of the hydrophobic chain length. Incubation was stopped by addition of 20 μL of

0.125 M Tris buffer, pH 6.8, containing 4% SDS, 20% glycerol and 10% 2-mercaptoethanol at room temperature. SDS-PAGE was performed as described (Weber & Osborne, 1964; Laemmli, 1970), but samples were not boiled in SDS. Refolding experiments were carried out at pH 10 because a yield close to 100% was achieved at this pH. Similar experiments conducted at pH 7 or 8 yield about 80 to 90% refolded protein and lead to the same general conclusions.

Folding monitored by CD spectroscopy

Far-UV CD measurements were performed at 20 °C on a Jasco 720 CD spectrometer using a 0.1 mm thermostated cuvette. Five scans were accumulated for each spectrum with a response time of 2 s, a bandwidth of 1 nm, and a scan speed of 10 nm/min from 190 to 250 nm (205–250 nm in presence of 8 M urea). Background spectra without protein were subtracted. The protein concentration was 17 μM.

Coomassie blue (BB R-250) dye assay for determination of CMCs

Estimation of detergent CMCs was performed according to a method developed by C. Snook and M. Wiener (unpubl. results). The method utilizes a red shift of the absorption maximum of Coomassie Brilliant Blue R-250 from 555 nm in the absence to 595 nm in the presence of detergent micelles. Ten μL of a 10 mM solution of Coomassie blue in borate buffer were added to 1 mL detergent solution and UV spectra were recorded on a Hitachi UV spectrometer from 500 to 650 nm. Background spectra in the absence of Coomassie blue were subtracted.

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References

Bailyes EM, Newby AC, Siddle K, Luzio JP. 1983. Solubilization and purification of rat liver 5′-nucleotidase by use of a zwitterionic detergent and a monoclonal-antibody immunoadsorbent. *Biochem J 203*:243–251.

Bjerrum OJ, Bhakdi S, Rieneck K. 1980. Monitoring of detergent binding to amphiphilic proteins by means of micelles containing the hydrophobic dye sudan black B. *J Biochem Biophys Methods 3*:355–366.

Cevc G, Marsh D. 1987. *Phospholipid bilayers. Physical principles and models*. New York: John Wiley & Sons.

Dornmair K, Kiefer H, Jähnig F. 1990. Refolding of an integral membrane protein. OmpA of *Escherichia coli*. *J Biol Chem 265:*18907–18911.

Eisele JL, Rosenbusch JP. 1990. *In vitro* folding and oligomerization of a membrane protein. Transition of bacterial porin from random coil to native conformation. *J Biol Chem 265*:10217–10220.

Goenne A, Ernst R. 1978. Solubilization of membrane proteins by sulfobetaines, novel zwitterionic surfactants. *Anal Biochem 87*:28–38.

Helenius A, McCaselin DR, Fries E, Tanford C. 1979. Properties of detergents. *Methods Enzymol 56*:734–749.

Helenius A, Simons K. 1972. The binding of detergents to lipophilic and hydrophilic proteins. *J Biol Chem 247*:3656–3661.

Helenius A, Simons K. 1975. Solubilization of membranes by detergents. *Biochim Biophys Acta 415*:133–170.

Kleinschmidt JH, den Blaauwen T, Driessen A, Tamm LK. 1999. Outer membrane protein A of *E. coli* inserts and folds into lipid bilayers by a concerted mechanism. *Biochemistry 38*:5006–5016.

Kleinschmidt JH, Tamm LK. 1996. Folding intermediates of a β-barrel membrane protein. Kinetic evidence for a multi-step membrane insertion mechanism. *Biochemistry 36*:12993–13000.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature 227*:680–685.

Le Maire M, Kwee S, Andersen JP, Møller. 1983. Mode of interaction of polyoxyethyleneglycol detergents with membrane proteins. *Eur J Biochem 129*:525–532.

Lund S, Orlowski S, de Foresta B, Champeil P, le Maire M, Møller JV. 1989. Detergent structure and associated lipid as determinants in the stabilization of solubilized Ca^{2+}-ATPase from sarcoplasmic reticulum. *J Biol Chem 264*:4907–4915.

Makino S, Reynolds JA, Tanford C. 1973. The binding of deoxycholate and Triton X-100 to proteins. *J Biol Chem 248*:4926–4932.

Makino S, Woolford JL, Tanford C, Webster RE. 1975. Interaction of deoxycholate and of detergents with the coat protein of bacteriophage. *J Biol Chem 250*:4327–4332.

Møller JV, Le Maire M. 1993. Detergent binding as a measure of hydrophobic surface area of integral membrane proteins. *J Biol Chem 268*:18659–18672.

Nozaki Y, Reynolds JA, Tanford C. 1974. The interaction of a cationic detergent with bovine serum albumin and other proteins. *J Biol Chem 249*:4452–4459.

Pautsch A, Schulz GE. 1998. Structure of the outer membrane protein A transmembrane domain. *Nature Struct Biol 5*:1013–1017.

Robinson NC, Tanford C. 1975. The binding of deoxycholate, Triton X-100, sodium dodecyl sulfate, and phosphatidylcholine vesicles to cytochrome b_5. *Biochemistry 14*:369–378.

Rosen MJ. 1989. *Surfactants and interfacial phenomena*, 2nd ed. New York: John Wiley & Sons.

Rosenbusch JP. 1990. The critical role of detergents in the crystallization of membrane proteins. *J Struct Biol 104*:134–138.

Schweizer M, Hindennach M, Garten W, Henning U. 1978. Major proteins of the *Escherichia coli* outer cell envelope membrane. Interaction of protein II with lipopolysaccharide. *Eur J Biochem 82*:211–217.

Shinoda K. 1978. *Principles of solution and solubility*. New York: Marcel Dekker Inc.

Song L, Gouaux JE. 1997. Membrane protein crystallization: Application of sparse matrices to the alpha-hemolysin heptamer. *Methods Enzymol 276*: 60–74.

Sugarawa E, Steiert M, Rouhani S, Nikaido H. 1996. Secondary structure of the outer membrane proteins OmpA of *Escherichia coli* and OprF of *Pseudomonas aeruginosa*. *J Bacteriol 178*:6067–6069.

Surrey T, Jähnig F. 1992. Refolding and oriented insertion of a membrane protein into a lipid bilayer. *Proc Natl Acad Sci USA 89*:7457–7461.

Tanford C. 1980. *The hydrophobic effect: Formation of micelles and biological membranes*, 2nd ed. New York: John Wiley & Sons.

Tanford C, Reynolds JA. 1976. Characterization of membrane proteins in detergent solutions. *Biochim Biophys Acta 457*:133–170.

Tausk RJM, Karmiggelt J, Oudshoorn C, Overbeek JTG. 1974. Physical chemical studies of short-chain lecithin homologues. I. Influence of the chain length of the fatty acid ester and of electrolytes on the critical micelle concentration. *Biophys Chem 1*:175–183.

Vogel H, Jähnig F. 1986. Models for the structure of outer-membrane proteins of *Escherichia coli* derived from Raman spectroscopy and prediction methods. *J Mol Biol 190*:191–199.

Weber K, Osborne M. 1964. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J Biol Chem 244*:4406–4412.

Zulauf M. 1990. Detergent phenomena in membrane protein crystallization. In: Michel H, ed. *Crystallization of membrane proteins*. Boca Raton: CRC Press. pp 53–88.