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Secondary Structure of the Outer Membrane Proteins OmpA of *Escherichia coli* and OprF of *Pseudomonas aeruginosa*

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When purified without the use of ionic detergents, both OmpA and OprF proteins contained nearly 20% α-helical structures, which disappeared completely upon the addition of sodium dodecyl sulfate. This result suggests that the proteins fold in a similar manner, with an N-terminal, membrane-spanning β-barrel domain and a C-terminal, globular, periplasmic domain.

OmpA is one of the major outer membrane proteins of *Escherichia coli*. It appears to exist as a monomer, and it shows very little pore-forming activity (16), apparently because only a small fraction of the OmpA population contains open channels (17). The accepted folding model of OmpA contains an N-terminal domain (residues 1 to 170) consisting of eight antiparallel β-strands, as well as a periplasmic C-terminal domain (residues 196 to 325) (8, 12). The two domains are connected by an Ala-Pro-rich hinge sequence. The proposed structure of the N-terminal domain is supported by a wealth of data, including data indicating the localization of phage-binding sites exclusively on segments predicted to correspond to the external loops connecting β-strands (8) and Raman spectroscopy data (19). However, the secondary structure of the C-terminal domain has so far received little attention.

Pseudomonas aeruginosa outer membrane contains OprF as a major protein. It is a homolog of *E. coli* OmpA (2) and similarly produces diffusion pores of low permeability (5, 9, 20). In contrast to OmpA, however, the entire length of OprF has been proposed to traverse the outer membrane as 18 β-strands (11).

We studied the secondary structure of these proteins, which were purified in the total absence of denaturing, ionic detergents, in view of these conflicting models of folding. OmpA was purified as follows. Cell envelope fraction from a 4-liter culture of HN705 (Δ*ompC ompF*::Tn*5*) (16) was first extracted twice with 50-ml portions of 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-Na buffer (pH 7.5)–3% octylpolyoxyethylene (POE) (Alexis Biochemicals, San Diego, Calif.) (10) and then extracted once with 50 ml of the same solution containing 1 M NaCl in addition to solubilize most of the cytoplasmic membrane proteins. The residue was then extracted with 20 ml of 20 mM HEPES buffer–10% octyl-POE–1 M NaCl, and the extract was fractionated by gel filtration on a column (1.5 by 92 cm) of Toyo Pearl 50F (TosoHaas, Montgomeryville, Pa.), equilibrated, and eluted with 0.1% dodecyl maltoside–0.4 M NaCl–10 mM HEPES buffer–3 mM NaN_3. When the circular dichroism (CD) spectrum was taken on a sample concentrated by ultrafiltration through an Amicon (Beverly, Mass.) PM-10 filter, it showed a strong ellipticity with two minima at 209 and 222 nm, characteristic of structures containing a significant fraction of α-helix. When curve fitting was used to estimate the secondary structure (1), OmpA appeared to contain 18% α-helix, 40% β-strand, 5% β-turn, and 25% unordered structure (Fig. 1). In contrast, OmpA prepared by solubilization of the outer membrane by sodium dodecyl sulfate (SDS) (16) showed a much weaker ellipticity with the minimum at 210 nm, indicative of significant β-structure but with little evidence for α-structure (Fig. 1). Finally, the N-terminal domain alone, prepared by trypsin digestion (12) of SDS-purified OmpA, also showed a spectrum characteristic of protein essentially consisting of β-strands (Fig. 1). (We believe that a larger magnitude of ellipticity found in SDS-treated OmpA [Fig. 1] is largely due to the contribution of unordered structure.) These results thus confirm the current folding model described above and suggest that the C-terminal domain contains a large fraction of α-helical structure. Our results do not support the recently proposed hypothetical model (15) in which the OmpA protein is assumed to traverse the membrane 16 times. The C-terminal domain appeared to be denatured in the presence of SDS. Indeed, addition of SDS to octyl-POE-purified OmpA at room temperature resulted in a rapid alteration of the CD spectrum, changing it, within a few minutes, to one resembling the spectrum of SDS-purified OmpA shown in Fig. 1 (data not shown).

The secondary structure of the C-terminal domain, however, appeared to be rather stable in the absence of SDS. As judged by the ellipticity at 220 nm, little change in secondary structure was detected in octyl-POE up to 60°C (Fig. 2). However, there was a drastic decrease in the magnitude of ellipticity at 80°C, presumably reflecting the denaturation of the C-terminal domain. The secondary structure of the N-terminal domain can be probed by the intrinsic fluorescence emission from tryptophan residues, as all of the five tryptophan residues in OmpA are located in this domain. The emission spectra showed little change even when the octyl-POE-purified OmpA was heated at 100°C for 5 min, showing that the N-terminal domain is much more stable against high temperature than the C-terminal domain (Fig. 2). The N-terminal domain remained undenatured even in SDS at up to 80°C (Fig. 2), and indeed it took heating at 100°C in SDS to denature this domain, as judged by the severe quenching of the fluorescence (Fig. 2) and a slight red shift in the emission spectrum (not shown). Although the ellipticity at 220 nm changed little after heating at 100°C in SDS (Fig. 2), the shape of the CD spectrum was altered conspicuously to resemble more an unordered structure (not shown), again suggesting the denaturation of the N-terminal domain.

These results indicate that although the N-terminal, transmembrane domain of OmpA is exceptionally stable, as is true for other β-barrel-type proteins like OmpF and LamB, the

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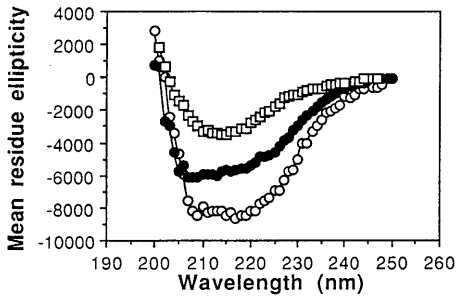

FIG. 1. CD spectra of OmpA preparations. OmpA purified in octyl-POE (○) was measured in 3% octyl-POE–10 mM HEPES-Na (pH 7.5). OmpA purified in SDS (●) was measured in 0.1% SDS–10 mM HEPES-Na (pH 7.5). The N-terminal domain (□) was obtained by trypsinization of the SDS-purified OmpA, as described in the text. Spectra were obtained with an Aviv model 62DS CD spectrometer and were corrected for backgrounds obtained with appropriate detergent-buffer mixtures. Mean residue ellipticity is in degrees times square centimeters divided by decimoles.

C-terminal periplasmic domain is less stable and is easily denatured by SDS. This explains the unusual behavior of OmpA during SDS-polyacrylamide electrophoresis, often called heat modifiability (4). OmpA dissolved in the SDS-containing sample buffer at room temperature is denatured only in the C-terminal domain and migrates faster than a fully denatured OmpA protein because the N-terminal domain retains its compact, folded structure. When OmpA is heated in SDS at 100°C, the N-terminal domain also becomes denatured, and the expanded conformation of this form presumably slows down its

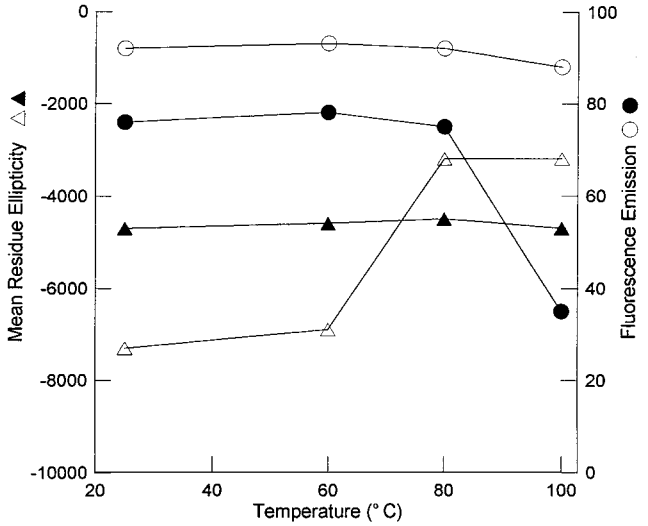

FIG. 2. Heat-induced conformational changes in OmpA. Conformational changes were monitored by CD and by intrinsic tryptophan fluorescence, techniques that mainly indicate the structural alterations in the C-terminal and N-terminal domains, respectively (see text). CD spectra were measured by heating the samples in the cuvette after equilibrating them at the indicated temperatures for 5 min. The 100°C sample was heated in a boiling-water bath for 5 min. The spectra were recorded between 200 and 250 nm, but only the mean residue ellipticity values at 220 nm are shown. Fluorescence emission spectra were recorded between 310 and 370 nm, but only the intensity at 340 nm is plotted. In this case, samples were equilibrated at each temperature for 5 min and spectra were measured immediately thereafter at room temperature. Open symbols represent OmpA purified in octyl-POE and heated in 0.3% octyl-POE, and filled symbols represent OmpA purified in SDS and heated in 0.1% SDS. The detergent solutions in both cases contained in addition 0.1 M NaCl and 10 mM Tris-HCl, pH 8.0. Fluorescence emission values are in percent; mean residue ellipticity values are in degrees times square centimeters divided by decimoles.

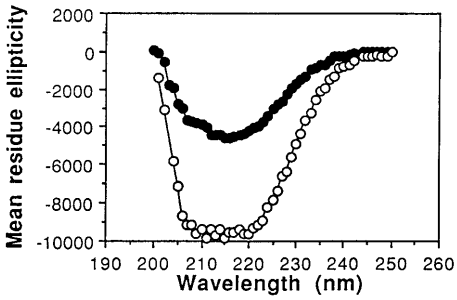

FIG. 3. CD spectra of OprF preparations. OprF purified in the absence of ionic detergents (○) was measured in 0.1% dodecyl maltoside–10 mM HEPES-Na (pH 7.5). OprF purified in SDS (●) was measured in 0.1% SDS–10 mM HEPES-Na (pH 7.5). For other conditions, see the legend to Fig. 1.

migration in the cross-linked gel matrix. It is also not surprising that when the N-terminal domain alone was expressed from a truncated *ompA* gene, the truncated protein migrated more slowly during SDS-polyacrylamide electrophoresis than expected for its molecular weight, unless it was heated in SDS at 100°C (12): the stable N-terminal domain is not denatured at all in SDS at room temperature, resulting in the binding of few SDS molecules, and the protein can migrate only slowly because it carries few negative charges. Our results also suggest that experiments involving denaturation and renaturation of OmpA should be designed carefully. For example, OmpA denatured in urea was reported to be renatured by insertion into octylglucoside micelles or phospholipid vesicles (18). However, the CD spectra of OmpA claimed to have been renatured are very similar to that of our OmpA half-denatured by SDS (Fig. 1). It has also been our experience that, starting from totally denatured OmpA, it is difficult to renature the C-terminal domain, although the N-terminal domain can be rapidly renatured, as shown by others (18). It is not clear why the denatured OmpA preparation used for Raman spectroscopy (19) finally folded to contain 13% α-helix; possibly the use of lipopolysaccharide bilayers helped the folding process (13), or the lengthy dialysis could have facilitated the correct folding.

P. aeruginosa OprF protein has been reported to contain only small amounts (9%) of α-helix (14). However, this value was obtained by using a preparation purified in the presence of SDS. We therefore purified OprF entirely in the absence of SDS as follows. The outer membrane isolated from a 1.5-liter culture of strain PAO1 (6) was first extracted with 30 ml of 0.1% Lubrol PX–0.4 M NaCl–10 mM Tris-HCl (pH 8.0) and then with 15 ml of 0.008% dodecyl maltoside–10 mM Tris-HCl (pH 8.0) to remove other proteins. The residue was extracted with 5 ml of 68 mM octyl-β-D-glucoside–5 mM EDTA–10 mM Tris-Cl (pH 8.0). The extract was fractionated by gel filtration as described for OmpA above, except that the eluting buffer contained Tris-Cl buffer, pH 8.0, instead of HEPES buffer. OprF-containing fractions were pooled, dialyzed against 0.2% Lubrol PX–1 mM EDTA–10 mM Tris-Cl (pH 8.0)–3 mM NaN_3. The sample was then fractionated in a high-performance liquid chromatography apparatus with a DEAE-5PW column (7.5 by 75 mm; Bio-Rad), which was eluted by a 0 to 0.5 M gradient of NaCl in 10 mM Tris-Cl (pH 8.0)–1 mM EDTA–0.2% Lubrol PX. The OprF peak was refractionated to remove traces of contaminants by using the gel filtration setup used earlier. The final OprF preparation displayed much α-helical structure, as shown by the characteristic negative peaks at 209 and 220 nm in the CD spectrum (Fig. 3). From the spectrum, OprF appeared to contain 18% α-helix, 40% β-strand, 12%

β-turn, and 30% unordered form. In contrast, the CD spectrum of OprF, purified in SDS as described earlier (20), showed a characteristic β-strand-rich pattern with a much weaker ellipticity (Fig. 3), similar to the results of earlier workers (7). We emphasize that the α-helix-containing domain of OprF is quite labile, and the mere use of Sarkosyl (*N*-lauryl sarcosinate, an anionic detergent) to remove the inner membrane component (3) was sufficient to denature it completely even if the subsequent extraction was carried out exclusively with nonionic detergent (results not shown). The conformation of the native OprF, containing much α-helical structure, is not consistent with the 18-β-strand model of this protein proposed earlier (11) and in fact suggests a folding pattern similar to that of OmpA. This conclusion is consistent with the observation that the homology between OmpA and OprF is especially strong in the C-terminal region (2). It is also consistent with the heat-modifiable behavior of OprF (5, 7). At present, it is not clear how the various pieces of evidence that were thought to favor the 18-transmembrane-strand model can be reconciled with our new data.

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