JOURNAL OF BACTERIOLOGY, Aug. 1995, p. 4266–4271 Vol. 177, No. 15
0021-9193/95/$04.00+0

Conformational Analysis of the *Campylobacter jejuni* Porin

JEAN-MICHEL BOLLA,[1]* ERWANN LORET,[2] MARTINE ZALEWSKI,[1] AND JEAN-MARIE PAGÈS[1]

UPR 9027, I. F. R. C1, Centre National de la Recherche Scientifique, 13402 Marseille Cedex 20,[1] and Laboratoire de Biochimie, Centre National de la Recherche Scientifique URA 1455, I. F. R. Jean Roche-Faculté de Médecine Nord, 13916 Marseille Cedex 20,[2] France

Received 30 January 1995/Accepted 28 May 1995

The major outer membrane protein (MOMP) of *Campylobacter jejuni* was purified to homogeneity by selective solubilization and fast protein liquid chromatography. The amino acid composition of the MOMP indicates the presence of cysteine residues. The amino-terminal sequence, determined over 31 residues, shows no significant homology with any other porin from gram-negative bacteria except in a discrete region. Immunocross-reactivity between *Escherichia coli* OmpC and the MOMP was analyzed, and a common antigenic site between these two porins was identified with an anti-peptide antibody. From circular dichroism and immunological investigations, the existence of a stable folded monomer, containing a high level of β-sheet secondary structure, is evident. Conformational analyses show the presence of a native trimeric state generated by association of the three folded monomers; the stability of this trimer is reduced compared with that of *E. coli* porins. This study clearly reveals that the *C. jejuni* MOMP is related to the family of trimeric bacterial porins.

The gram-negative bacterium *Campylobacter jejuni* is responsible for enteritis and diarrhea all over the world, in both industrialized and developing countries (41). Since 1970, this bacterium and other *Campylobacter* species have been routinely isolated from stools and other specimens. Numerous studies that have tried to elucidate the molecular mechanisms of pathogenicity have been done in several laboratories. Flagella play a role in mobility in the luminal mucosa, which appears to be one of the most important steps in the colonization of the host gut (14, 15, 33). Moreover, little is known about the structural and functional organization of the bacterial envelope.

Porins of gram-negative bacteria are involved in the diffusion of solutes through this envelope and are the most common way of communication between bacteria and media (16, 34). In *Escherichia coli*, the major outer membrane porins, OmpF and OmpC, have different pore sizes and their synthesis depends on external conditions (36). A previous report has established that the major outer membrane protein (MOMP) of *C. jejuni* presents the physicochemical properties of a porin (18). Although the stabilities of porin trimers are a peculiarity of these membrane pore proteins (32, 38), there are some divergent views on the quaternary structure of this MOMP (1, 18, 27). Recent studies of *E. coli* OmpA have reported a pore activity for this monomeric protein previously described as an architectural component of the bacterial envelope (36, 40). Similarly, a monomeric porin, showing noticeable homology with the OmpA sequence, was also isolated from *Pseudomonas aeroginosa* (2, 7). In addition, the electrophoretic migrations of these proteins are similarly modified by the temperature of solubilization (6, 7, 18). These results suggest that monomeric OmpA-like proteins form channels in the outer membranes of gram-negative bacteria (35).

The purification and determination of the amino acid composition and amino-terminal sequence of the MOMP isolated from a virulent strain of *C. jejuni* are presented here. By using specific conditions of solubilization, the trimeric form, in addition to the two monomeric forms (35 and 45 kDa) previously described (18), is clearly shown. Circular dichroism (CD) experiments show that the three states of this porin correspond to three different conformational organizations. A conserved epitope between *E. coli* OmpC and the MOMP is characterized. The antigenic profiles of the three MOMP conformational forms are compared by using three polyclonal antisera directed against the trimer, the denatured monomer, and the conserved peptide, respectively.

* Corresponding author. Mailing address: UPR 9027, CNRS, 31 Chemin Joseph-Aiguier, 13402 Marseille Cedex 20, France. Phone: (33) 91 16 44 89. Fax: (33) 91 71 21 24. Electronic mail address: J-M.Bolla@maccbm.cnrs-mrs.fr.

MATERIALS AND METHODS

Bacterial strain and culture conditions. *C. jejuni* 85H was obtained from M. Kervella (22). Cells were grown on Columbia agar medium under microaerobic conditions at 42°C for 48 h.

Purification of the MOMP. Bacterial cells were harvested and washed in 10 mM Tris-HCl–1 mM EDTA (pH 7.2) for 1 h at room temperature. After centrifugation, cell pellets were washed in glycine-HCl (pH 2.2) to eliminate associated outer membrane proteins (9, 23). Then the great majority of the MOMP recovered was associated with bacteria (data not shown). The bacterial pellet was washed twice successively in 10 mM Tris (pH 7.2) to titrate excess HCl and was sonicated in the same buffer. After sonication, cell fragments were removed by centrifugation (6,000 × *g* for 30 min) and the total membrane fraction was recovered by ultracentrifugation (100,000 × *g* for 1 h at 4°C).

Extraction of the porin from the membrane was carried out in six steps. Two extractions with 0.1% sodium lauryl sarcosinate in Tris buffer to solubilize the inner membrane were followed by four successive extractions with *n*-octylpolyoxyethylene (octyl-POE; Bachem AG-Switzerland) in 20 mM NaP_i (pH 7.6), leading to the specific recovery of the outer membrane porin associated with POE micelles (13, 24). After ion-exchange chromatography and chromatofocusing on a PBE74 column (Pharmacia) (13), porin samples were extensively dialyzed against 20 mM NaP_i (pH 7.6)–0.3% octyl-POE–1% sodium azide and stored at 4°C in this buffer before being used.

Preparation of antisera. Antiserum to the native porin was raised in rabbits by three successive injections of 100 μg of protein, subcutaneously on days 0 and 32 and intramuscularly on day 21. Animals were sacrificed on day 40. For the serum against denatured porin, samples were heated for 10 min at 96°C in 1% sodium dodecyl sulfate (SDS) before each injection.

SDS-PAGE and immunoblotting. Analyses of protein by SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotting were done as previously described (11, 22). In some experiments (see figure legends), the SDS concentrations were decreased to preserve the native conformation of this porin.

Determination of the amino terminus. After transfer of the MOMP (2 nmol) onto a polyvinylidene difluoride sheet, amino-terminal sequence analysis was performed by stepwise Edman degradation with an Applied Biosystems model 470A gas-phase sequencer.

Amino acid composition. Amino acid analyses were performed on a Beckman

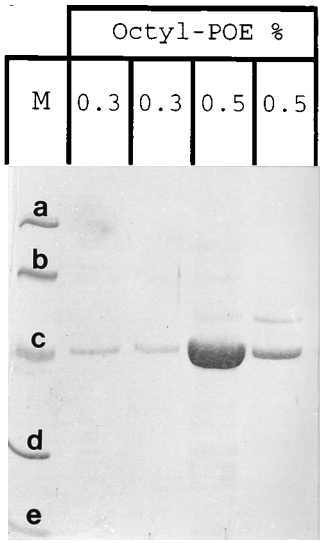

FIG. 1. SDS-PAGE analysis of outer membrane extracts. Similar aliquots of the four extractions obtained from 0.3 and 0.5% octyl-POE treatments were loaded on an SDS–10% polyacrylamide gel. After migration under standard conditions, proteins were stained by Coomassie blue. Lane M, molecular mass standards (a, 94 kDa; b, 67 kDa; c, 43 kDa; d, 30 kDa; e, 20 kDa).

model 6300 analyzer as previously described (29). Protein samples were hydrolyzed in 6 M HCl–1% phenol for 20 h at 110°C.

CD measurements. For UV CD measurements, samples were placed in 20 mM phosphate buffer (pH 7.6)–20 mM KF (28) that contained 0.3% octyl-POE. CD spectra in 50-μm-path-length cells were measured from 260 to 178 nm with a JOBIN-YVON (Longjumeau, France) UV CD spectrophotometer. Measurements were performed at 20°C, and CD spectra are reported as Δε per amide. The concentration ranged from 0.8 to 1 mg/ml and was determined on a Beckman amino acid analyzer. Light transmissions of samples in extreme UV (down to 175 nm) were checked with a SAFAS (Monte Carlo, Monaco) spectrophotometer.

RESULTS

Porin purification. A modification of the technique previously described by Garavito and Rosenbusch (13), which used octyl-POE for specific detergent extraction of native porin, was employed for the purification of the MOMP. In our first attempts, the protein was directly extracted from membrane vesicles after sonication of bacteria but the preparation was contaminated by other membrane proteins (data not shown).

To avoid contaminations, successive extractions were carried out. As previously reported, the treatment of intact bacteria with glycine-HCl at a low pH solubilized only weakly associated outer membrane proteins (9, 23). Sodium lauryl sarcosinate, currently used to selectively solubilize the inner membrane proteins of gram-negative bacteria, was used to eliminate this protein fraction. Outer membrane vesicles were incubated in 0.3% octyl-POE for 20 min at 4°C and centrifuged (100,000 × *g* for 1 h at 4°C). After a second run, two extractions were carried out with 0.5% octyl-POE. These steps produce a porin sample with very few contaminants (Fig. 1). The majority of the protein is extracted with 0.5% octyl-POE. As is the case for OmpF porin purification from *E. coli* (13), these successive treatments were used to obtain large amounts of protein. Ion-exchange chromatography and chromatofocusing were carried out with these four fractions before determination of the amino acid composition and before amino-terminal sequencing.

Amino acid composition. The amino acid composition of the MOMP was determined, and it was comparable to those of OmpC and OmpF (31). There are high-level Gly and Asx contents and reciprocally low-level Pro, His, and Met contents (Fig. 2). A striking difference was that two half-cysteine residues were detected, reflecting the presence of disulfide bridges. However, the migration of the MOMP was identical under reducing (50 mM dithiothreitol–1% 2-mercaptoethanol) and nonreducing conditions (without any reducing agent) during SDS-PAGE (data not shown). In addition, the Glx, Leu, and Tyr contents of these porins were also significantly different (Fig. 2).

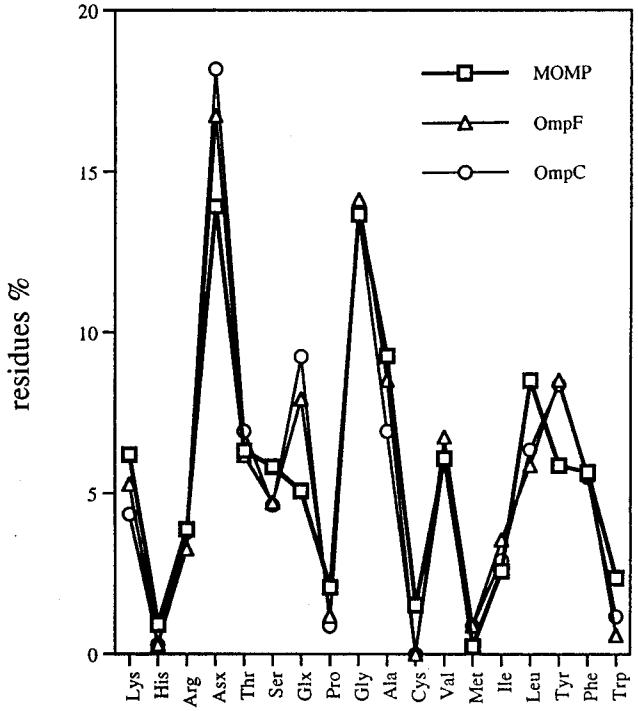

FIG. 2. Amino acid analyses of porins. The value for each residue is the mean of six experiments. The value for tryptophan is a theoretical calculation.

N-terminal amino acid sequence of the MOMP. The MOMP purified from *C. jejuni* 85H was sequenced over 31 residues from the N terminus by the Edman method in two independent experiments. Surprisingly, computer analysis presented no high homology with any other sequence from protein data banks (data not shown), and this sequence does not line up with the N termini of other porins (Fig. 3). However, a restricted comparison of the sequence showed one peptide, KDVD, which is conserved in *E. coli* OmpC (Fig. 3). Interestingly, this restricted homology was found in the first putative exposed loop of OmpC (19) and suggests the lack of the first β-strand. Although we cannot rule out proteolytic degradation of porin during purification, these results suggest a different folding of the N-terminal region of this porin compared with those of OmpC and OmpF.

Conformational analysis. Outer membrane porins are generally organized in trimers and very stable in SDS below 75°C, the temperature necessary to obtain monomers (32, 38). As previously reported, the *C. jejuni* MOMP presents porin activity in planar black lipid bilayers (18), although no trimeric organization has been clearly identified (1, 18, 27).

The conformation of the purified protein was analyzed under different conditions of solubilization. Under the usual analytical conditions, no trimeric conformation was observed,

```
MOMP            T P L E E A I K D V D V S . G V L R Y R Y D T G N F D K N F L N
ECOOMPC   (20)  L H Y F S D N K D V D G D Q T Y M R L G F K G E T Q V T D Q L T
ECOOMPF   (25)  K G N G E N S Y G G N G D M T Y A R L G F K G E T Q I N S D L T
STYOMPC   (20)  L H Y F S D D K G S D G D Q T Y M R I G F K G E T Q V N D Q L T
STYOMPF   (20)  L R Y F S D N A G D D G D Q S Y A R I G F K G E T Q I N D M L T
SMOMPC    (20)  L H Y F S S N N G V D G D Q S Y M R F G L R G E T Q I S D Q L T
PORIN H   (18)  R L L L K K E K D K R G D L M D N G S
BPPORIN   (34)  K Y N H S R F G M I N G V Q N G S R W G L R G T E D L G D G L Q
BAOMP     (25)  Y F Y I P G T E T C L R V H G Y V R Y D V K G G D D V Y S G T D
```

FIG. 3. Comparison and alignment of the N-terminal sequence of the MOMP with the N-terminal sequences of various bacterial porins. The conserved region in OmpC is underlined, and boldface type indicates the conserved amino acid residues in other porins. We have enclosed the β-strands of OmpF in boxes and indicated the Arg (**R**) residue located in the pore constriction zone previously shown by X-ray crystallography (4). Each number in parentheses indicates the position of the first residue in the corresponding sequence. The proteins used for this comparison were as follows (from top to bottom): the MOMP from *C. jejuni*, OmpC and OmpF porins from *E. coli* (ECOOMPC and ECOOMPF, respectively) (31), OmpC and OmpF porins from *Salmonella typhi* (STYOMPC and STYOMPF, respectively) (37; EMBL accession no. X73237), OmpC porin from *Serratia marcescens* (SMOMPC) (GenBank accession no. L24960), porin H from *Pasteurella multocida* (3), porin from *Bordetella pertussis* (BPPORIN) (26), and outer membrane porin from *Brucella abortus* (BAOMP) (10).

despite the mild condition of preparation (Fig. 4). A product of around 45 kDa was observed; it corresponded to the denatured form when the sample was heated to 96°C. After solubilization at room temperature, a unique band with an apparent molecular mass of 35 kDa, corresponding to the native monomer previously reported (18), was obtained. A high-molecular-weight product was observed when the SDS concentrations in the loading buffer, the gel, and the migration buffer were decreased (see the legend to Fig. 4). The apparent molecular mass of this band is estimated to be between 120 and 140 kDa. This result is in agreement with the calculated molecular mass of a trimer.

The MOMP and *E. coli* OmpC common antigenic site. Antigenic cross-reactivity between the MOMP and *E. coli* porins had previously been investigated, and interestingly, only the OmpC porin was recognized by an antiserum specific to the MOMP (22). To characterize the conserved antigenic region between the MOMP and OmpC, various chimeric porins (12, 17) containing different OmpC domains were used (Fig. 5A). The serum prepared against denatured OmpC recognized OmpC, OmpF, and all of the chimeric proteins (Fig. 5B). In the case of anti-MOMP, only chimeric proteins harboring residues 149 to 236 of OmpC were recognized (Fig. 5C). This sequence contains three distinct OmpC-specific regions (19, 31). To localize the common epitope, antisera directed against three peptides encompassing these regions were prepared. The peptide, termed C3, corresponding to OmpC residues 209 to 223 induced the production of an antiserum which recognized both OmpC and the MOMP (data not shown). The other sera, specific to residues 152 to 169 and 197 to 211, respectively, recognized the OmpC porin, while no signal was obtained with the MOMP (39). These results indicate that the common antigenic epitope is located between residues 209 and 223 of OmpC.

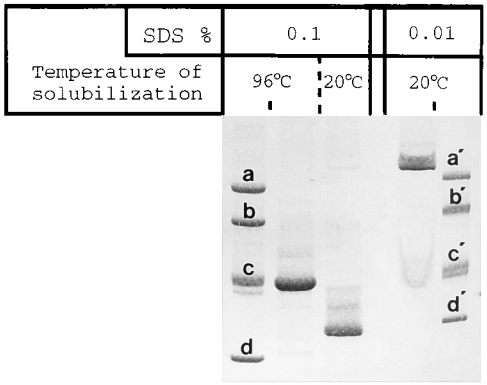

FIG. 4. SDS-PAGE analysis of this porin. The concentration of SDS in the loading buffer, gel, and migration buffer is either 0.1% (for standard conditions of SDS-PAGE) or 0.01% (to maintain the oligomeric assembly of the protein). The temperatures indicated correspond to the solubilization conditions used before the samples were loaded. Molecular mass markers are indicated as follows: a and a′, 94 kDa; b and b′, 67 kDa; c and c′, 43 kDa; d and d′, 30 kDa.

The immunoprofiles of the three conformational forms (trimer, 35-kDa product, and denatured monomer) were investigated by using antisera directed against the trimer, denatured monomer, and C3 peptide, respectively (Fig. 6). Serum D clearly recognized the denatured form, a weak signal was observed with the 35-kDa product, and no signal was obtained with the trimer (Fig. 6). Conversely, serum N mainly identified the native forms, the trimer and the 35-kDa product (Fig. 6). Interestingly, the anti-C3 serum detected only the denatured monomer (Fig. 6). The absence of a signal with the 35-kDa product suggests that this form corresponds to a folded monomer in which the C3 epitope is either masked by some intramolecular interactions or hidden within the conformation.

CD measurement. A CD spectrum of between 178 and 260 nm is directly proportional to the secondary structure content of the protein studied (21). Consequently, the conformational changes in a protein induced by different conditions of solubilization can be investigated by CD. Three different structures were evident for the *C. jejuni* MOMP, depending on the SDS concentration and temperature used for solubilization (Fig. 7). The available structural data indicate that the secondary structures of outer membrane pore proteins consist predominantly of β-strands which are susceptible to thermal treatment (8, 24, 38). The MOMP with and without SDS, corresponding to the 35- and 120- to 140-kDa products, respectively, showed very similar CD spectra, characterized by an n-to-π^* transition at 210 to 215 nm that is typical of β-pleated sheet structures (Fig. 7). Heating for 20 min at 96°C in the presence of SDS induced changes in the CD spectrum. The negative band at 210 to 215 nm disappeared, and conjointly, a new band was observed at 200 nm, which indicated alpha-helical structure formation typical of denatured proteins in SDS (8, 21). These CD profiles clearly show the differences in the secondary structures of these three MOMP forms, especially the two monomers. The

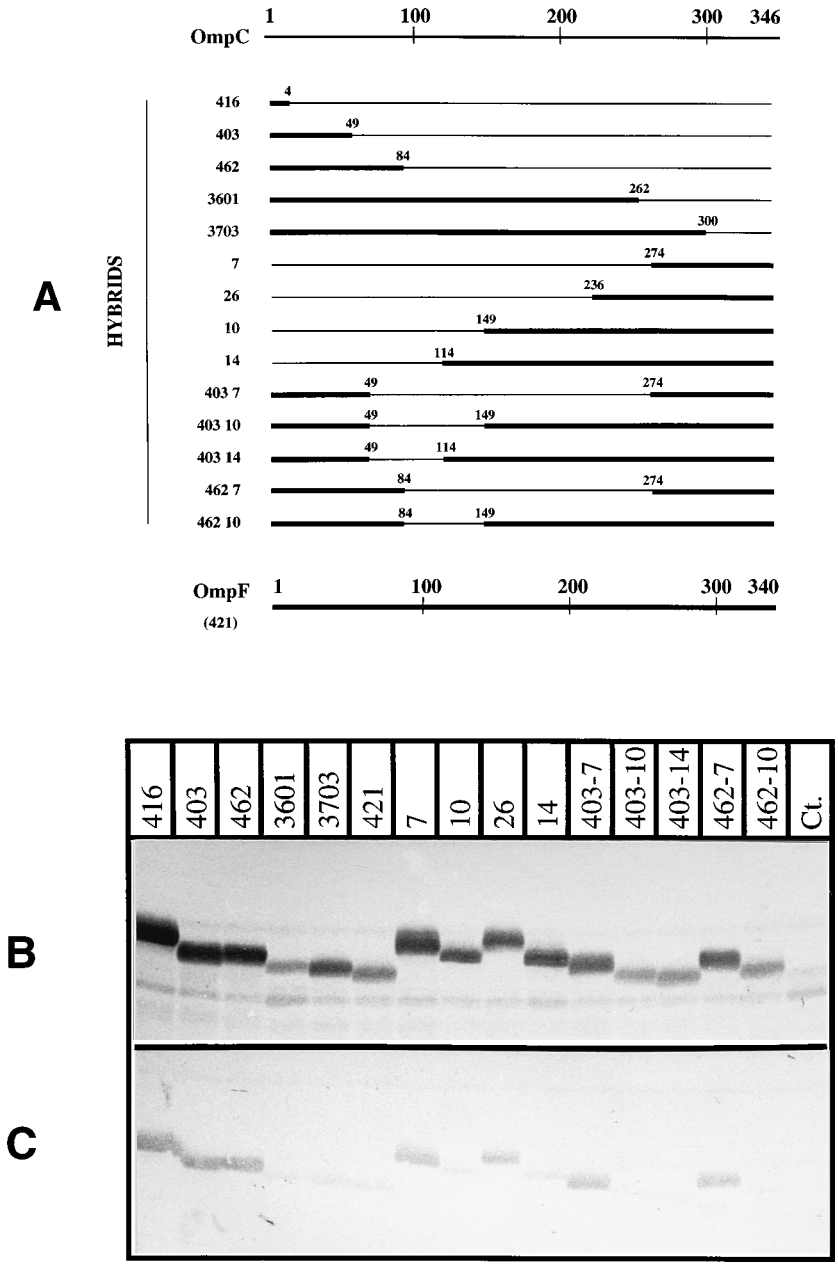

FIG. 5. Characterization of the common antigenic site. (A) Thin lines correspond to specific OmpC regions, and thick lines represent OmpF sequences in various OmpC-OmpF hybrids. Numbers indicate the first and/or last OmpC residues in chimeric porins. (B) Immunodetection with antiserum directed against denatured OmpC. Ct, *E. coli* cells devoid of porins. (C) Immunodetection with antiserum directed against the MOMP.

detection of β-sheet structures in the 35-kDa product agrees with the immunological results, suggesting a folded conformation (Fig. 6). In addition, these CD analyses validate the hypothesis of a native trimeric conformation built from three folded monomers.

DISCUSSION

In this paper, we have presented the purification and conformational analysis of the MOMP of *C. jejuni*. This protein was previously studied and characterized as a monomeric protein presenting a pore activity in planar black lipid bilayers (18). This is also the case for *E. coli* OmpA; it presents a pore activity associated with a monomeric conformation (40). Moreover, monomeric porin F from *P. aeroginosa* presents some sequence homologies with OmpA (2, 7). These results indicate the existence of monomeric porins that allow slow diffusion of small solutes across the bacterial outer membrane (35).

However, the conformational states of porins, analyzed in the presence of SDS at crucial temperatures, reflect the stabilities of these molecules under in vitro conditions. The use of various detergents and chaotropic agents during the extraction and analysis of intrinsic membrane proteins may have induced artificial dissociation of sensitive in vivo oligomeric configurations of these molecules. Consequently, the decreased stabilities of these porins, experimentally determined, are not di-

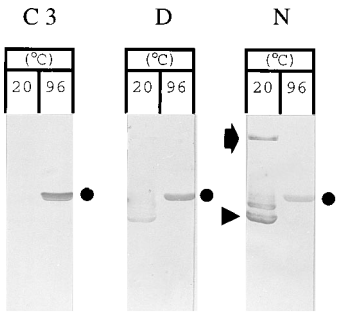

FIG. 6. Western blot (immunoblot) analysis of the different conformations of this porin. SDS-PAGE gels were run as indicated in the legend to Fig. 4. C3, anti-C3 serum; D, serum D; N, serum N. The arrow, triangle, and dots indicate the trimer, folded monomer, and denatured monomer, respectively.

rectly related to intrinsic properties, such as pore activity, antigenicity, or receptor functions for bacteriocins and phages. Recent results in our laboratory (11) show that the stabilities of *E. coli* OmpF trimers depend on the presence of a strategic region encompassing residues 115 to 144. In the case of an amino acid exchange between OmpF and OmpC in this region, a specific monoclonal antibody directed against the trimer indicates the presence of the native forms in the outer membrane while the stability of the molecule is drastically reduced. Determined conditions of solubilization (temperature and SDS concentration) are necessary to maintain the trimeric configuration (11). A similar effect on trimer stability is conferred by punctual substitution in the pore lumen, which modifies the channel properties without changing outer membrane localization (12, 20, 25). In the case of the porin of *C. jejuni*, a trimeric form of the porin is clearly observed under mild conditions of solubilization. When SDS concentrations exceed 0.05%, only a monomeric form, detected by the anti-C3 antiserum, is evident. In addition, CD analysis clearly indicates the presence of β-sheet structure in the 35-kDa monomer. This product corresponds to a folded conformation in which the C3 epitope, used as the conformational reporter, is masked (Fig. 8). The

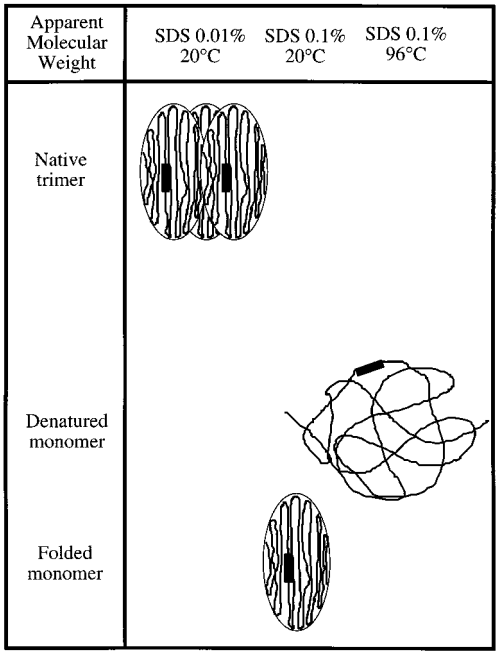

FIG. 8. Presentation of the three conformations of the *C. jejuni* porin. The black rectangle indicates the C3 epitope.

trimeric state, exhibiting the β-organization reported for *E. coli* porins (4, 8), is built by the association of three folded monomers, with ultimate reorganization of the monomer structure occurring as the final step in porin assembly (Fig. 8). By analogy to the *E. coli* porins (4), we propose that each subunit constitutes a β-barrel containing a pore.

It is interesting that the presence of a folded monomer during *E. coli* PhoE assembly has been previously reported (5). The existence of such a folded form suggests that the 35-kDa product of *C. jejuni* may be a conformational remembrance of

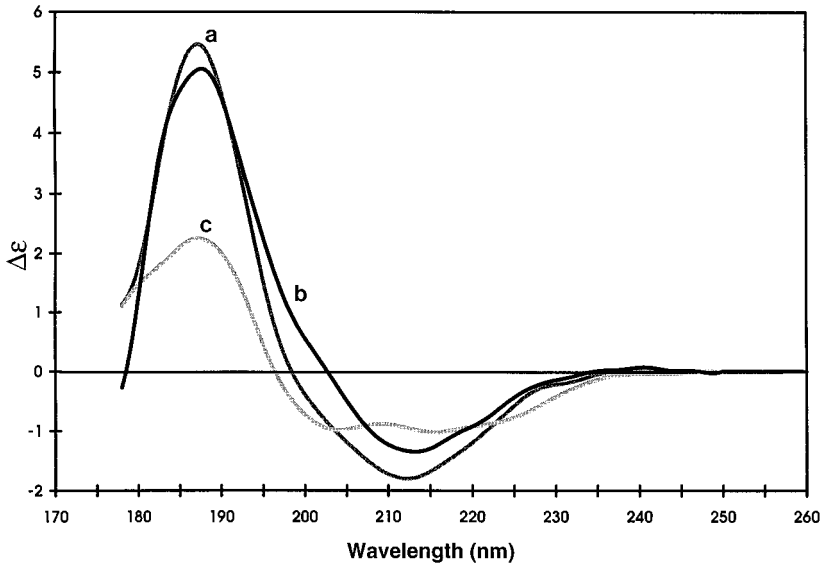

FIG. 7. UV CD spectra of the MOMP under the following conditions: 20 mM phosphate buffer (pH 7.6)–20 mM KF that contained 0.3% octyl-POE (a), the same buffer supplemented with 2% SDS (b), and the same conditions listed for b with heating for 20 min at 96°C (c). Results are expressed in moles per centimeter per amide base.

a transient intermediate of MOMP assembly. In the case of the *C. jejuni* MOMP, the stability of this folded monomer could probably be increased by putative intrasubunit disulfide bridges. To support this hypothesis, it is important to take into account the demonstration that disulfide bonds play a strategic role in the thermal stability of the *E. coli* LamB porin (30). Crystallographic studies of this new type of porin should be very informative about an alternative possibility of protein assembly in the outer membranes of gram-negative bacteria.

Porins are involved in the entry of β-lactam antibiotics (34), and our knowledge concerning this process is a strategic point of antibiotherapy. In this regard, analysis of the outer membrane pore proteins appears to be a very promising approach. Cloning and sequencing of the gene coding for the MOMP are in progress to better understand the organization of this porin.

ACKNOWLEDGMENTS

We are pleased to acknowledge P. Berche for stimulating advice. We thank S. Mizushima and M. Kervella for the generous gift of *Campylobacter* strains and OmpF-OmpC hybrids. We gratefully acknowledge D. Fourel, K. Kelle, and M. Malléa for helpful discussions; Jacques Bonnicel for amino-terminal sequencing; and Razika Oughideni for technical assistance in amino acid analysis. We thank M. Green for carefully reading the manuscript.

This work was supported by the Centre National de la Recherche Scientifique and the Institut National de la Santé et de la Recherche Médicale (CRE 930610).

REFERENCES

1. **Blaser, M. J., J. A. Hopkins, R. M. Berka, M. L. Vasil, and W.-L. L. Wang.** 1983. Identification and characterization of *Campylobacter jejuni* outer membrane proteins. Infect. Immun. **42:**276–284.
2. **Chen, R., W. Schmidmayr, C. Krämer, U. Chen-Schmeisser, and U. Henning.** 1980. Primary structure of major outer membrane protein II* (OmpA protein) of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **77:**4592–4596.
3. **Chevalier, G., H. Duclohier, D. Thomas, E. Schechter, and H. Wroblewski.** 1993. Purification and characterization of protein H, the major porin of *Pasteurella multocida*. J. Bacteriol. **175:**266–276.
4. **Cowan, S. W., T. Schirmer, G. Rummel, M. Steiert, R. Ghosh, R. A. Pauptit, J. N. Jansonius, and J. P. Rosenbusch.** 1992. Crystal structures explain functional properties of two *E. coli* porins. Nature (London) **358:**727–733.
5. **de Cook, H., R. Hendriks, T. de Vrije, and J. Tommassen.** 1990. Assembly of an *in vitro* synthesized *Escherichia coli* outer membrane porin into its stable trimeric configuration. J. Biol. Chem. **265:**4646–4651.
6. **Dornmair, K., H. Kiefer, and F. Jähnig.** 1990. Refolding of an integral membrane protein. J. Biol. Chem. **265:**18907–18911.
7. **Duchêne, M., A. Schweizer, F. Lottspeich, G. Krauss, M. Marget, K. Vogel, B.-U. von Specht, and H. Domdey.** 1988. Sequence and transcriptional start site of the *Pseudomonas aeruginosa* outer membrane porin protein F gene. J. Bacteriol. **170:**155–162.
8. **Eisele, J.-L., and J. P. Rosenbusch.** 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.
9. **Fauchère, J.-L., M. Kervella, A. Rosenau, J.-M. Pagès, and C. Fendri.** 1992. In vitro study of virulence factors of enteric *Campylobacter* spp., p. 168–175. *In* I. Nachamkin, M. J. Blaser, and L. S. Tompkins (ed.), *Campylobacter jejuni*: current status and future trends. American Society for Microbiology, Washington, D.C.
10. **Ficht, T. A., S. W. Bearden, B. A. Sowa, and L. G. Adams.** 1989. DNA sequence and expression of the 36-kilodalton outer membrane protein gene of *Brucella abortus*. Infect. Immun. **57:**3281–3291.
11. **Fourel, D., A. Bernadac, and J.-M. Pagès.** 1994. Involvement of exposed loops in trimeric stability and membrane insertion of *Escherichia coli* OmpF porin. Eur. J. Biochem. **222:**625–630.
12. **Fourel, D., S. Mizushima, A. Bernadac, and J.-M. Pagès.** 1993. Specific regions of *Escherichia coli* OmpF protein involved in antigenic and colicin receptor sites and in stable trimerization. J. Bacteriol. **175:**2754–2757.
13. **Garavito, M., and J. P. Rosenbusch.** 1986. Isolation and crystallization of bacterial porin. Methods Enzymol. **125:**309–328.
14. **Grant, C. C. R., M. E. Konkel, W. Cieplak, Jr., and L. S. Tompkins.** 1993. Role of flagella in adherence, internalization, and translocation of *Campylobacter jejuni* in nonpolarized and polarized epithelial cell cultures. Infect. Immun. **61:**1764–1771.
15. **Guerry, P., S. M. Logan, S. Thornton, and T. J. Trust.** 1990. Genomic organization and expression of *Campylobacter* flagellin genes. J. Bacteriol. **172:**1853–1860.
16. **Hancock, R. E. W.** 1987. Role of porins in outer membrane permeability. J. Bacteriol. **169:**929–933.
17. **Hikita, C., Y. Satake, H. Yamada, T. Mizuno, and S. Mizushima.** 1989. Structural and functional characterization of the OmpF and OmpC porins of the *E. coli* outer membrane. Res. Microbiol. **140:**177–190.
18. **Huyer, M., T. R. Parr, R. E. W. Hancock, and W. J. Page.** 1986. Outer membrane porin protein in *Campylobacter jejuni*. FEMS Microbiol. Lett. **37:**247–250.
19. **Jeanteur, D., J. H. Lakey, and F. Pattus.** 1991. The bacterial porin superfamily: sequence alignment and structure prediction. Mol. Microbiol. **5:**2153–2164.
20. **Jeanteur, D., T. Schirmer, D. Fourel, V. Simonet, G. Rummel, C. Widmer, J. P. Rosenbusch, F. Pattus, and J.-M. Pagès.** 1994. Structural and functional alterations of a colicin-resistant mutant of OmpF porin from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **91:**10675–10679.
21. **Johnson, W. C., Jr.** 1985. Circular dichroism and its applications. Methods Biochem. Anal. **31:**61–163.
22. **Kervella, M., J.-L. Fauchère, D. Fourel, and J.-M. Pagès.** 1992. Immunological cross-reactivity between outer membrane pore proteins of *Campylobacter jejuni* and *Escherichia coli*. FEMS Microbiol. Lett. **99:**281–286.
23. **Kervella, M., J.-M. Pagès, Z. Pei, G. Grollier, M. J. Blaser, and J.-L. Fauchère.** 1993. Isolation and characterization of two *Campylobacter* glycine-extracted proteins that bind to HeLa cell membranes. Infect. Immun. **61:**3440–3448.
24. **Kleffel, B., R. M. Garavito, W. Baumeister, and J. P. Rosenbusch.** 1985. Secondary structure of a channel-forming protein: porin from *E. coli* outer membranes. EMBO J. **4:**1589–1592.
25. **Lakey, J. H., E. J. A. Lea, and F. Pattus.** 1991. *ompC* mutants which allow growth on maltodextrins show increased channel size and greater voltage sensitivity. FEBS Lett. **278:**31–34.
26. **Li, Z. M., J. H. Hannah, S. Stibitz, N. Y. Nguyen, C. R. Manclark, and M. J. Brennan.** 1991. Cloning and sequencing of the structural gene for the porin protein of *Bordetella pertussis*. Mol. Microbiol. **5:**1649–1656.
27. **Logan, S. M., and T. J. Trust.** 1983. Molecular identification of surface protein antigens of *Campylobacter jejuni*. Infect. Immun. **42:**675–682.
28. **Loret, E., P. Georgel, W. C. Johnson, Jr., and P. S. Ho.** 1992. Circular dichroism and molecular modelling yield a structure for the complex of human immunodeficiency virus type 1 trans-activation response RNA and the binding region of Tat, the trans-acting transcriptional activator. Proc. Natl. Acad. Sci. USA **89:**9734–9738.
29. **Loret, E., E. Vives, P. S. Ho, H. Rochat, J. Van Rietschoten and W. C. Johnson, Jr.** 1991. Activating region of HIV-1 Tat protein: vacuum UV circular dichroism and energy minimization. Biochemistry **30:**6013–6023.
30. **Luckey, M., R. Ling, A. Dose, and B. Malloy.** 1991. Role of a disulfide bond in the thermal stability of the LamB protein trimer in *Escherichia coli* outer membrane. J. Biol. Chem. **266:**1866–1871.
31. **Mizuno, T., M.-Y. Chou, and M. Inouye.** 1983. A comparative study on the genes for three porins of the *Escherichia coli* outer membrane. J. Biol. Chem. **258:**6932–6940.
32. **Nakamura, K., and S. Mizushima.** 1976. Effects of heating in dodecyl sulfate solution on the conformation and electrophoretic mobility of isolated major outer membrane proteins from *E. coli* K-12. J. Biochem. **80:**1411–1422.
33. **Newell, D. G., and H. McBride.** 1985. Investigation on the role of flagella in the colonization of infant mice with *Campylobacter jejuni* and attachment of *Campylobacter jejuni* to human epithelial cell lines. J. Hyg. **95:**217–227.
34. **Nikaido, H.** 1989. Outer membrane barrier as a mechanism of antimicrobial resistance. Antimicrob. Agents Chemother. **33:**1831–1836.
35. **Nikaido, H.** 1994. Porins and specific diffusion channels in bacterial outer membranes. J. Biol. Chem. **269:**3905–3908.
36. **Nikaido, H., and M. Vaara.** 1987. Outer membrane, p. 7–22. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
37. **Puente, J. L., V. Alvarez-Scherer, G. Gosset, and E. Calva.** 1989. Comparative analysis of the *Salmonella typhi* and *Escherichia coli ompC* genes. Gene **83:**197–206.
38. **Rosenbusch, J. P.** 1974. Characterization of the major envelope protein from *Escherichia coli*. J. Biol. Chem. **249:**8019–8029.
39. **Simonet, V., M. Malléa, D. Fourel, J.-M. Bolla, and J.-M. Pagès.** Crucial domains in the porin organization are conserved in *Enterobacteriaceae*. Submitted for publication.
40. **Sugawara, E., and H. Nikaido.** 1992. Pore-forming activity of OmpA protein of *Escherichia coli*. J. Biol. Chem. **267:**2507–2511.
41. **Walker, R. I., M. B. Caldwell, E. C. Lee, P. Guerry, T. J. Trust, and G. M. Ruiz-Palacios.** 1986. Pathophysiology of *Campylobacter* enteritis. Microbiol. Rev. **50:**81–94.