MOMP (major outer membrane protein) of *Campylobacter jejuni*; a versatile pore-forming protein

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Abstract The great majority of trimeric porins of Gram-negative bacteria cannot be dissociated into monomers without disrupting their folded conformation. The porin of *Campylobacter jejuni*, however, displays two folded structures, a classical oligomer and a monomer resistant to detergent denaturation. We probed the transition of trimer to monomer using light scattering experiments and examined the secondary structures of these two molecular states by infra-red spectroscopy. The channel-forming properties of both trimer and monomer were studied after incorporation into artificial lipid bilayers. In these conditions, the trimer induced ion channels with a conductance value of 1200 pS in 1 M NaCl. The pores showed marked cationic selectivity and sensitivity to low voltage. Analysis of the isolated monomer showed nearly the same single-channel conductance and the same selectivity and sensitivity to voltage. These results indicate that the folded monomer form of *C. jejuni* MOMP displays essentially the same pore-forming properties as the native trimer.

Key words: Porin; Quaternary structure; Channel-forming property; Structure/function relationship

1. Introduction

Campylobacter jejuni is a Gram-negative bacterium which contains a major outer membrane protein (MOMP) with an apparent molecular weight of about 45 kDa [1,2]. Electron microscopic examination of the bacterial surface showed a regular lattice arranged in a hexagonal pattern covering the whole bacterium. This lattice is composed mainly of the MOMP [3]. Several roles have been ascribed to this protein: the structural organization of the outer membrane [3], pore activity [4] and more recently, adhesion to cultured cells [5].

In contrast to many porins of Gram-negative enterobacteria, the MOMP protein extracted from the outer membrane of *Campylobacter* by Triton X-100 EDTA showed no typical oligomeric pattern on SDS–polyacrylamide gels after solubilization at low temperature [4]. However, when the protein is extracted using mild conditions of solubilization in various detergents, it behaves as a trimeric structure [6]. The protein thus purified was reconstituted in artificial membranes and analyzed after two-dimensional crystallization. A trimeric protein with three prominent stain-filled indentations was observed suggesting a structural relationship with the family of the trimeric bacterial porins [7].

Structural studies have demonstrated that the functional properties of porins are related to their structural organization [8]. In a previous report, we showed that the MOMP isolated from *C. jejuni* was able to form either a folded monomer or a trimer depending on the SDS concentration used to solubilize it. Both molecular species demonstrated a predominantly β-sheet secondary structure [6], similar to other enterobacterial porins [9]. The conservation of the porin-like structure raised the question of the activity of these two molecular species.

Here, we describe the effect of SDS concentration on the assembly of the monomeric subunits in solution, as followed by light scattering experiments and infra-red spectroscopy. Planar lipid bilayer experiments were also used to study the channel-forming properties of the various porin states. Both trimers and (after dissociation) monomers were able to induce single channels in artificial bilayers, with almost identical conductance values and macroscopic properties (selectivity and voltage sensitivity).

2. Materials and methods

2.1. Protein purification

The purification of the porin from strain 85H was performed as previously described [6]. Bacterial cells were harvested and washed in 10 mM Tris–HCl, 1 mM EDTA (pH 7.2). After centrifugation, the cell pellet was washed in glycine–HCl (pH 2.2), in order to extract associated outer membrane proteins. The bacterial pellet was washed twice in 10 mM Tris (pH 7.2) in order to titrate the HCl and sonicated in the same buffer. After sonication, cell debris was removed by centrifugation ($6000 \times g$ for 30 min) and the total membrane fraction was recovered by ultracentrifugation ($100\,000 \times g$ for 1 h at 4°C).

The membrane pellets were first resuspended with 10 mM Tris (pH 7.6) containing sodium lauryl sarcosinate 0.1%. This low concentration of detergent allows solubilization of the inner membrane without disrupting the outer membrane vesicles of *Campylobacter* (data not shown). The outer membranes were then recovered by ultracentrifugation ($100\,000 \times g$, 1 h, 4°C). The supernatant was discarded and the pellet was resuspended in sodium-phosphate buffer (20 mM, pH 7.6) containing *n*-octyl-polyoxyethylene (octyl-POE, Bachem AG-Switzerland) at a final concentration of 1%. This leads to the specific recovery of porins in octyl-POE micelles, which were separated from insoluble material by ultracentrifugation ($100\,000 \times g$, 1 h, 4°C). After ion exchange chromatography and chromatofocusing on MonoQ and MonoP columns (Amersham-Pharmacia Biotech) respectively, the porin samples were extensively dialyzed against 20 mM NaPi (pH 7.6) 0.3% octyl-POE, 1% sodium azide, and stored at 4°C in this buffer.

2.2. Light scattering experiments

The oligomeric state of the porin was investigated by static and dynamic light scattering [10]. This was performed using a Brookhaven

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Instrument, as previously described [11]. The autocorrelation function of the scattered light intensity was analyzed using the method of cumulants [12] to determine the translational diffusion coefficient of the protein and an apparent hydrodynamic diameter was calculated using the Stokes–Einstein relation. Porin solutions were first dialyzed against 0.3% octyl-POE, 25 mM phosphate buffer (pH 7.0). Increasing SDS concentrations were obtained by adding small amounts of a 10% (w/v) SDS solution. All the solutions were filtered through Millipore filters (Millex-GV4, 0.22 mm filter unit) to remove dust particles and centrifuged 2 min at $2000 \times g$ to remove air bubbles.

2.3. ATR-FTIR spectroscopy

Attenuated total reflection FTIR (ATR-FTIR) was performed on an IFS28 (Bruker, Wissembourg) spectrophotometer using a Ge crystal. 500 scans were recorded on 0.35 ml samples at a resolution of 4 cm^{-1}. The protein concentration was 5 mg/ml in 0.3% octyl-POE, 10 mM KPO_4 pH 7.0. Aliquots of 6 mg SDS powder were added to a final concentration of 2%. Buffer spectra were subtracted from the protein spectra prior to the secondary structure analysis. Second derivative spectra were computed using the program OPUS/IR2 in order to assign the major bands in the amide I region.

2.4. Reconstitution in planar lipid bilayers

Solvent-free planar lipid bilayers were formed by the Montal and Mueller technique [13] modified by Saint et al. [14]. The current fluctuations were recorded using a BLM 120 amplifier (Biologic, Claix, France) and stored on a digital tape recorder (DTR 1202, Biologic, Claix, France). Currents and amplitude histograms were obtained from the stored signals using the Satori software from Intracell (Royston, UK). In voltage-gating experiments, doped membranes were subjected to triangular voltage sweeps at 1 mV/s. Transmembrane currents were fed to a Keitley amplifier (model 427). Current–voltage curves were recorded on an *X–Y* plotter.

Diphytanoylphosphatidylcholine (DPhPC, Avanti, Birmingham, USA) or asolectin IV-S from soybean (Sigma, St Quentin Fallavier, France) were used as lipids. The electrolyte solution was 1 M NaCl, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (pH 7.4) or 1 M KCl buffered for single-channel measurements and 0.2 M NaCl, 20 mM citric acid (pH 3) for voltage sensitivity experiments. The porin solubilized in different detergents was added to the measurement compartments (4 ml) in a symmetric manner, typically at 0.25–0.5 ng/ml for the single-channel experiments or 2.5–5 ng/ml for voltage-gating and selectivity experiments. The latter required establishment of a NaCl or KCl gradient through the lipid bilayer: from 0.1 M in the *cis* side to 1 M in the *trans* side. The zero-current potential was corrected by deducing the asymmetric potential due to the salt gradient.

3. Results

A concentrated solution of MOMP porin (5 mg/ml) was obtained via purification as described in Section 2. Each sample used in further experiments was checked by SDS–PAGE under conditions allowing discrimination between native, folded or fully denatured forms of the protein as previously described [6].

3.1. Conformational studies using dynamic light scattering

The apparent hydrodynamic diameter of the porin/octyl-POE complex was found to be 10.4 ± 0.5 nm, assuming a spherical shape and a viscosity equal to that of water. Addition of increasing amounts of SDS induced a decrease in both the scattered light intensity and the apparent diameter (Fig. 1). The major changes, corresponding to about 30% for the diameter and 50% for the scattered light intensity, were observed in the range 0 to 1% SDS. The small decrease of diameter observed between 1% and 2.3% SDS was not due to structural changes of the protein (see below) but probably to an increasing number of detergent micelles, since a similar effect was observed upon increasing the octyl-POE concentration.

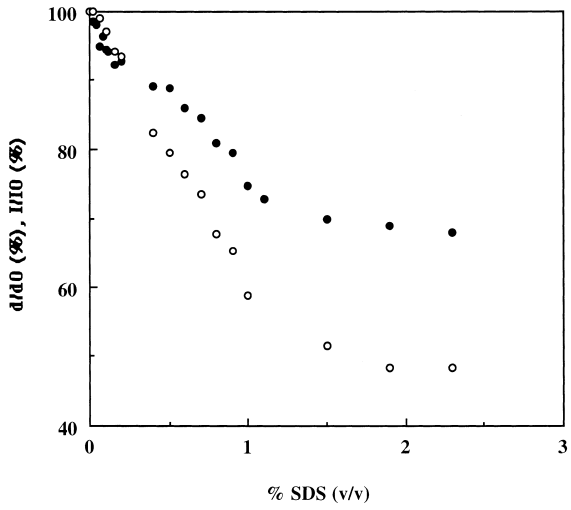

Fig. 1. Effect of SDS concentration on apparent diameter (●) and total scattered light intensity (○). Protein concentration: 3 mg/ml. Each value has been normalized to that obtained in the absence of SDS. The viscosity of water was used for all calculations. Intensities have not been corrected for dilution effects.

The ATR-FTIR measurements indicated the presence of similar amounts of β-strand conformation in both 0% and 2% SDS solution (Fig. 2). The major band is centered at 1630 cm^{-1} as observed for other porins (Fig. 2) [15]. The trimer spectrum was similar to that of the *Comamonas acidovorans* Omp32 porin [15] with shoulders attributed to β-strand, β-turns and loops, respectively (1648, 1665 1675 and 1690 cm^{-1}). Upon addition of SDS to a final concentra-

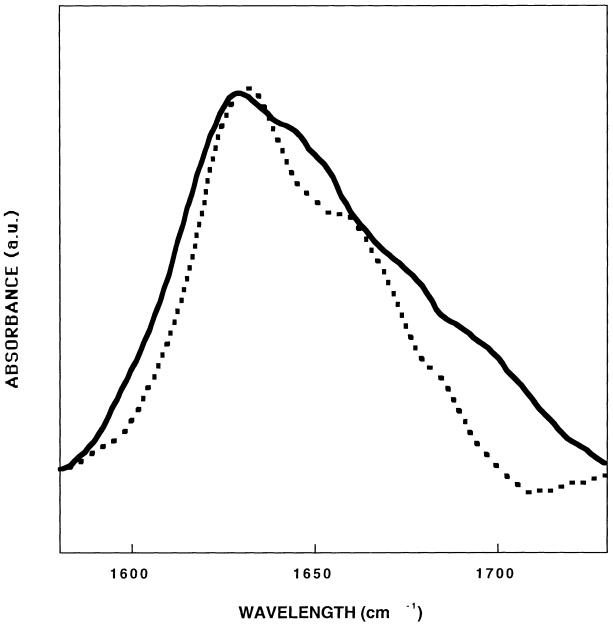

Fig. 2. Secondary structure analysis. ATR-FITR spectra in the amide region of MOMP in absence (solid line) and presence (dotted line) of 2% SDS. The buffer spectra (with or without SDS) were subtracted from the sample spectra. The band fitting analysis after deconvolution of the two spectra indicates the conservation of the β-sheet structure in the two forms. Changes in the conformation and the stability of the loop region may explain the minor changes in the spectra.

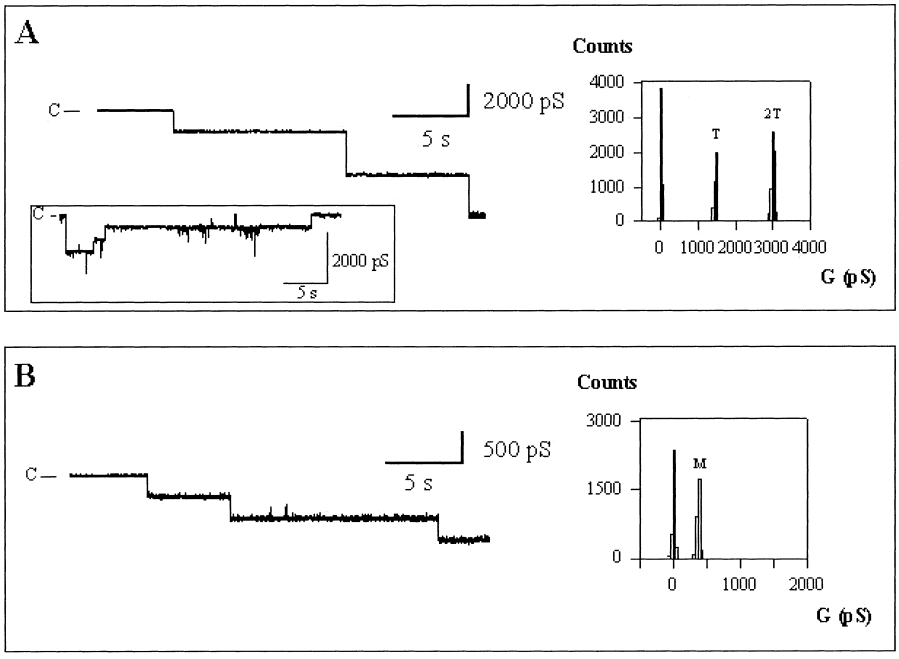

Fig. 3. Single-channel behavior of the *C. jejuni* MOMP. A: Single-channel activity, in 1 M KCl, induced by the octyl-POE-solubilized MOMP. The recording shows the incorporation of trimers at an applied potential of −100 mV. The associated histogram measures two single-channel conductance levels of 1500 (T) and 3000 pS (2T). The insert presents the opening of a trimer under an applied voltage and its closure as three successive monomers. B: Single-channel activity of the SDS-solubilized MOMP, in 1 M NaCl, under a −40 mV potential. The associated histogram shows a conductance level of 380 pS (M). (T) and (M) denote the trimer and the monomer conductances respectively and (C) denotes the closed state.

tion of 2%, the main band remained highly similar while the shoulders observed at lower SDS concentrations were not evidenced. The main band and the two shoulders were assigned to well-ordered and distorted β-structures, respectively.

3.2. Functional assays using planar lipid bilayers

The pore-forming activity of the porin was studied using two experimental conditions: in 0.3% octyl-POE and in 1% SDS.

Octyl-POE-solubilized MOMP caused well-isolated single events and allowed measurements of the porin single-channel conductance. Fig. 3A shows the current variation resulting of the incorporation of MOMP in a preformed bilayer. From different experiments carried out in 1 M KCl with various purified samples, two major conductance values were determined: 510 pS and a higher conductance value of 1500 pS (see associated histogram of Fig. 3A) which results from the incorporation of an oligomer. These values could thus be attributed to the monomer and the trimer, respectively. The insert of Fig. 3A unambiguously shows the opening of a trimer (1500 pS) and its closure as three successive monomer closures of 510 pS. In 1 M NaCl, conductance values of 400 pS and 1200 pS were determined (Table 1).

In the case of the SDS-solubilized MOMP, the preparation showed single-channel activity with a mean conductance value about 380 pS in 1 M NaCl (Fig. 3B and associated histogram). In order to determine whether or not the heat-modified form was active, the SDS-solubilized MOMP was heated to 100°C for 15 min before reconstitution. Addition of this heat-denatured porin to a bilayer did not result in increasing current when voltage was applied.

Experiments were then carried out to determine the ionic selectivity of MOMP after solubilization in the different detergents. After installation of a salt gradient (0.1 M/1 M, *cis*/*trans*), a resulting reversal potential allowed the estimation, from the application of the Hodgkin–Goldman–Katz equation [18], of the P_K/P_{Cl} and P_{Na}/P_{Cl} ratios (Table 1). These results demonstrated a cationic selectivity irrespective of the method used during solubilization.

When reconstituted in asolectin bilayer bathed by 1 M NaCl (pH 7.4) solution, the octyl-POE-solubilized MOMP did not exhibit voltage-gated behavior in the range of 0 to ±200 mV, similarly to the trimeric *Escherichia coli* OmpC which shows no closing tendency at voltages under 200 mV [16]. For the monomeric state, i.e. MOMP solubilized in 1% SDS, voltage ramps could not be applied beyond ±100 mV

Table 1
Effect of solubilization conditions on single-channel conductance and selectivity of the MOMP porin

Sample	Single-channel conductance (pS)		Selectivity	
	in 1 M NaCl	in 1 M KCl	P_{Na}/P_{Cl}	P_K/P_{Cl}
MOMP in 0.3% octyl-POE	400 ± 30/1200 ± 50	510 ± 40/1500 ± 60	9.7 ± 0.7	9 ± 0.8
MOMP in 1% SDS	380 ± 20	N.D.[a]	8.2 ± 0.7	N.D.[a]

Conductance values and selectivity ratios are given for MOMP reincorporation in a DPhPC membrane bathed by buffered electrolyte solutions.
[a]N.D., NaCl was used as the only salt in order to prevent sample precipitation (by KDS precipitation) for SDS-solubilized MOMP.

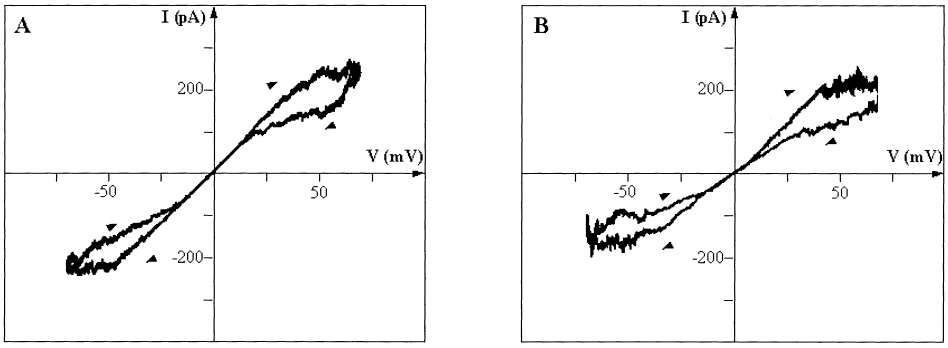

Fig. 4. Macroscopic current–voltage (*I–V*) curves of the *C. jejuni* MOMP. A: I–V curves of octyl-POE-solubilized MOMP doped membrane. B: *I–V* curve of SDS-solubilized MOMP doped membrane. Subphase: 0.2 M NaCl, 20 mM citric acid pH 3 with 5 ng/ml of MOMP. Voltage ramps: ±70 mV at 1 mV/s.

without disrupting the membrane, and conductance was ohmic in this potential range. The critical voltage, above which porin channels close, can be lowered by the use of low ionic strength or acidic pH buffers [14,17]. Therefore the octyl-POE-solubilized MOMP was assayed with a 0.2 M NaCl solution at pH 3 bathing the lipid bilayer. After equilibration, the macroscopic conductance was high and ohmic in the −50 to +50 mV range, and when the absolute voltage was increased, a current reduction in both quadrants was observed (Fig. 4A). These experimental conditions, however, did allow a test of the voltage-gating sensitivity of the SDS-solubilized MOMP. The macroscopic current versus voltage plot is shown in Fig. 4B. In this case, voltages above ±40 mV caused closure of the MOMP monomeric porin channels.

4. Discussion

Our previous biochemical studies showed that the *C. jejuni* MOMP was able to behave either as a monomer or as a trimer depending on the detergent used during solubilization [6,7]. We demonstrate here that the two molecular structures of this porin are both functional.

The transition of the trimer to folded monomer was followed by dynamic light scattering measurements. In the absence of SDS, the size of the porin/octyl-POE complex, about 10.4 nm, was in agreement with that of bacterial porin trimers observed in two-dimensional [7] and three-dimensional crystals [19]. The binding of SDS molecules to the porin/detergent complex, by increasing the negative charge of the complex, favored repulsive interactions and induced the dissociation of porin trimers into monomers, as previously observed [6]. The similar amount of β-sheet structure observed from the infra-red study with the monomer and trimer forms, as well as the smaller size of the monomer (7.4 nm) determined by light scattering experiments, suggest that dissociation was not accompanied by unfolding of monomers, in agreement with a previous UV-CD study [6]. The weak changes observed in the secondary structure may be attributed to rearrangements of the long external loops upon SDS addition, especially for those involved in the monomer–monomer interface [20].

The reconstitution of the *Campylobacter* porin in planar lipid bilayers allowed to the observation of two main conductance values depending on the solubilization conditions. The octyl-POE sample generated a set of ion channels with conductance values of 1200 and 400 pS corresponding to the trimeric and monomeric porins, respectively. The replacement of these mild detergents by SDS only permitted observation of the monomeric form (380 pS). Taking into account these data, the *C. jejuni* MOMP exhibits two distinct functional structures: the trimer with a large single-channel conductance value (1200 pS) close to that of the *E. coli* OmpC trimeric porin [21,22] and the free monomer with a conductance value of 380 pS similar to that of the *Campylobacter coli* porin, for which no oligomerization has been observed [23]. Both monomeric and trimeric states display nearly the same cationic selectivity and when the salt concentration and pH of the electrolyte were decreased (0.2 M NaCl, pH 3), they were observed to close in the potential range of ±40–50 mV.

Interestingly, dissociation of oligomeric structures of porins has also been reported for two porins, the porin of *Rhodopseudomonas sphaeroides* [24] and the D2 porin from *Pseudomonas aeroginosa* [25]. In these cases, EDTA or SDS treatments, abolishing subunit interactions, did not modify the general folding of the molecule and preserved the pore-forming activity as determined by liposome swelling assays.

Trimerization of native porins in the outer membrane of Gram-negative bacteria may be an ultimate step necessary to (i) protect the functional monomeric unit from degradation [27], and (ii) organize the voltage and pressure sensitive unit on the cell surface [31–33]. As reported by van Gelder and Tommassen [26], a folded monomer of PhoE can be observed, provided the monomer–monomer interactions are weakened by a single amino acid substitution in the protein. The main structural differences between the *C. jejuni* porin and the classical trimeric porins may be the strength of the inter-chain bonds [6], illustrated by the differences observed in 2D reconstitution crystals of OmpF and MOMP [7]. In the case of the *E. coli* OmpF porin, various loops (mainly L2, L3 and L4) contribute to the integrity of the trimer via numerous hydrophilic interactions [20]. The MOMP sequence from *C. jejuni* strain K22 [5] however, shows no significant homology with OmpF, supporting the idea of significant divergence in functional pore organization. Moreover, in vivo, in addition to subunit interactions, lipopolysaccharide (LPS) plays an important role during membrane assembly of porin trimers [27–29]. Differences in LPS structure between *C. jejuni* and *E. coli* might also contribute to differences in the threshold of porin trimer stability [7,30].

In conclusion, our results show that the trimer and the folded monomer forms of *C. jejuni* MOMP are able to pro-

mote pore-forming activity depending on the solubilizing detergent. More information is required to come to a better understanding of the equilibrium between monomers and trimers of porins. The fact that both monomers and trimers of the *C. jejuni* porin are functional makes it a good model system for further studies of the interactions involved in the oligomerization reaction.

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