An outer membrane PorB class 2 protein from Neisseria meningitidis has been overexpressed in Escherichia coli, isolated from inclusion bodies, and refolded in the presence of zwitterionic detergent. The purified recombinant and native (strain M986) counterparts exhibit most of the typical functional and structural properties that are characteristic of bacterial porins. Channel forming activity has been monitored by incorporating class 2 into reconstituted liposomes and measuring the permeation rates of various oligosaccharides through the proteoliposomes to derive a pore diameter of 1.6 nm. Structural studies employing a combination of spectroscopic and electrophoretic techniques reveal that recombinant and native class 2 are identical in terms of overall conformational stability. Both proteins form stable trimers in zwitterionic detergent and retain significant secondary and tertiary structure in the presence of SDS. The thermal unfolding of zwittergen-solubilized class 2 trimers (Ts = 88 °C) is reversible and characterized by solvent exposure of aromatic residues with concomitant disruption of tertiary and partial loss of secondary structures. SDS-induced destabilization and irreversible unfolding of the native trimeric assembly occurs at temperatures above 60 °C. Our physicochemical studies of PorB class 2 protein furnish significant insight regarding the structural and functional properties of this meningococcal outer membrane protein within the porin superfamily.

Porins are integral outer membrane proteins that function as molecular sieves in Gram-negative bacteria, mediating the aqueous diffusion of solutes through the water-filled channels derived from their unique folding/assembly (1–2). Although meningococcal outer membranes may contain as many as five major proteins, much of the observed serotype specificity can be attributed to PorB class 2 and class 3 proteins, both of which are mutually exclusive within different meningococcal strains and alleles from the porB gene (3). Studies on meningococcal outer membranes (3, 4) originally suggested that class 2 protein might function as a bacterial porin on the basis of noted similarities with Escherichia coli protein I (5). The strongest argument to date regarding porin function arises from comparative studies with other neisserial strains (i.e. gonococcal) for which equivalents of class 2 and class 3 proteins have been proposed (6). Evidence regarding the porin function of protein I from Neisseria gonorrhoeae has been furnished by Douglas et al. (7) and extended through further biophysical characterization (8–9). Meningococcal porin function has also been assessed in vivo by characterizing the relative sensitivity of native and mutant strains to hydrophobic antibiotics (10–11). A remarkable finding that appears exclusive to pathogenic neisserial strains (including the class 2-expressing serotypes) is the spontaneous transfer of porin molecules from the outer membrane of whole cells into artificial lipid bilayers (12) as well as target cell membranes (13–14).

The complete amino acid sequence of Neisseria meningitidis class 2 protein (M, 36,600) has been derived from cDNA sequence analysis (15). There is a relatively low sequence homology observed within the porin superfamily of proteins, yet intra- and interspecies correlation of structure and function reveals significant relatedness (16). Elucidation of the three-dimensional structures of bacterial porins from Rhodobacter capsulatus, E. coli, and Rhodopseudomonas blastica (17–20) obtained from x-ray crystallographic studies confirm the remarkable structural similarity within this family of proteins. Since high resolution structures are not available for the majority of porins isolated to date, indirect methods of analysis have been employed to glean basic structural information. These include epitope mapping, limited proteolysis of whole cells, and gene variability (21–22) in conjunction with sequence alignment and secondary structure predictions (16). Although a number of studies have attempted to characterize the structural stability of E. coli porins (23 and references contained therein), a comprehensive and systematic investigation of porins from different species is an essential prerequisite for identifying those forces and mechanisms governing the overall conformational stability of these outer membrane proteins.

Porin toxicity has generally impeded efforts to develop effective expression systems in heterologous cells (24). Nevertheless, recent studies have demonstrated that neisserial porins can be overexpressed at sufficiently high levels in E. coli as inclusion bodies (IBs)1 without impairing host cell viability (25). This unique advantage has enabled us to overexpress class 2 protein in E. coli and thereby conduct a comprehensive series of physicochemical studies designed to elucidate specific structural and functional properties of this bacterial porin.

1 The abbreviations used are: IBs, inclusion bodies; IPTG, isopropylthiogalactopyranoside; OMP, outer membrane protein; PAGE, polyacrylamide gel electrophoresis; rclass 2, recombinant class 2 protein; Z3-14, Zwittergen 3–14.
Consequently, the primary objectives of the present study are 2-fold, namely to 1) furnish the requisite physicochemical evidence that the recombinant porin retains specific conformational properties integral to full biological activity following detergent-assisted renaturation, and 2) assess the inherent functional and structural features of class 2 protein in the absence of homologous cell wall components and other nisserial outer membrane proteins. Such studies are especially relevant in the case of meningococcal porins from which limited structural and functional information have been derived primarily on the basis of indirect comparisons with porins from other bacterial strains.

**EXPERIMENTAL PROCEDURES**

**Overexpression of Mature Class 2 Protein in *E. coli***—Genomic DNA was isolated from Group B *N. meningitidis* nonencapsulated variant of strain M986 (serotype 2a) employing standard procedures and used as a polymerase chain reaction template for amplification of the class 2 protein gene as described elsewhere (25). The amplified product was cloned into *Nde*I and *Xho*I sites of the pET17b plasmid (Novagen, Inc.) that was used to transform competent *E. coli* DH5α. Plasmid DNA from selected DH5α clones was isolated and employed to transform *E. coli* BL21(DE3)-λopin libraries. The transformed bacteria were selected by carbenicillin expression and expressed in the presence of IPTG to a final concentration of 0.4 mM. The levels of class 2 protein expressed at various times after induction were monitored by SDS-PAGE of whole cell lysates followed by staining with Coomassie Brilliant Blue.

**Refolding and Purification Procedures**—Meningococcal class 2 protein expressed in *E. coli* was purified in accordance with the procedure of Qi et al. (25) incorporating several minor modifications. The overexpressed class 2 protein was isolated by resuspending and lysing the bacterial cells with a Stansted air-driven cell disrupter (Stansted Fluid Power Ltd.) in TEN buffer (50 mM Tris-HCl, 1 mM EDTA, 100 mM NaCl, pH 8.0) followed by centrifugation and isolation of the pellet containing class 2 protein aggregated in the form of inclusion bodies (IBs). After washing the pellet with 0.5% deoxycholate in TEN buffer, followed by two rinses with TEN buffer, the protein was solubilized by resuspending and sonicating the IBs in freshly prepared 8 mM urea solution for 5 min using a water bath sonicator. Refolding of class 2 protein into its native conformation was achieved by employing a detergent-assisted refolding procedure (25, 26). Equal volumes of urea-dissolved IBs and 10% Z-3-14 (Calbiochem) were combined and the final porin extract applied to a Sephacryl S-300 (5 × 100 cm) column (Pharmacia Biotech Inc.) equilibrated in a buffer comprised of 100 mM Tris-HCl, 200 mM NaCl, 10 mM EDTA, 20 mM CaCl₂, and 0.05% Z-3-14, pH 8.0. Fractions containing class 2 protein were identified by SDS-PAGE, pooled, and applied to a Hilo-Q-Sepharose HP ion exchange (2.6 × 20 cm) column (Pharmacia) equilibrated in 25 mM Tris-HCl, 200 mM NaCl, 1.0 mM EDTA, and 0.05% Z-3-14 pH 8.0. A gradient of 0.2–1.0 mM NaCl was applied and class 2 protein eluted as a single peak. Protein concentration was estimated based on a molar extinction coefficient of 45,800 M⁻¹ cm⁻¹ at 280 nm.

**Liposome Swelling Assay**—Liposome swelling of proteoliposomes prepared class 2 protein aggregated in the form of inclusion bodies (IBs). After washing the pellet with 0.5% deoxycholate in TEN buffer, followed by two rinses with TEN buffer, the protein was solubilized by resuspending and sonicating the IBs in freshly prepared 8 mM urea solution for 5 min using a water bath sonicator. Refolding of class 2 protein into its native conformation was achieved by employing a detergent-assisted refolding procedure (25, 26). Equal volumes of urea-dissolved IBs and 10% Z-3-14 (Calbiochem) were combined and the final porin extract applied to a Sephacryl S-300 (5 × 100 cm) column (Pharmacia Biotech Inc.) equilibrated in a buffer comprised of 100 mM Tris-HCl, 200 mM NaCl, 10 mM EDTA, 20 mM CaCl₂, and 0.05% Z-3-14, pH 8.0. Fractions containing class 2 protein were identified by SDS-PAGE, pooled, and applied to a Hilo-Q-Sepharose HP ion exchange (2.6 × 20 cm) column (Pharmacia) equilibrated in 25 mM Tris-HCl, 200 mM NaCl, 1.0 mM EDTA, and 0.05% Z-3-14 pH 8.0. A gradient of 0.2–1.0 mM NaCl was applied and class 2 protein eluted as a single peak. Protein concentration was estimated based on a molar extinction coefficient of 45,800 M⁻¹ cm⁻¹ at 280 nm.

**Circular Dichroism Spectroscopy**—The secondary and tertiary structures of native and recombinant class 2 protein were evaluated by CD spectroscopy in the far UV (180–250 nm) and near UV (250–350 nm) regions, respectively. Concentrated stock solutions of protein were dialyzed against buffer systems comprised of either 25 mM Tris-HCl, 200 mM NaCl, 1.0 mM EDTA, and 0.05% Z-3-14, pH 8.0, or 50 mM NaPO₄ and 0.05% Z-3-14, pH 7.5. Spectra of samples containing 1.0 mg/ml protein were recorded at 0.1-nm wavelength intervals on a Jasco model 710 CD spectropolarimeter employing a scan speed of 0.05 min and average reading time of 1 s. A plot of the four consecutive runs was accumulated, and the average spectra were stored. The temperature of the samples was maintained at either 25 or 95 °C through the use of water-jacketed 0.01- and 1.0-cm pathlength cells in the far and near UV, respectively.

**Fluorescence Spectroscopy**—Fluorescence measurements were performed on an SLM AMINCO-Bowman 8100 Series 2 spectrofluorometer. Fluorescence spectra of samples containing 100 μg/ml protein in 25 mM Tris-HCl, 200 mM NaCl, 1.0 mM EDTA, and 0.05% Z-3-14, pH 8.0, were recorded over the range of 200–500 nm on a Hewlett-Packard model 8453 UV/Vis spectrophotometer equipped with a diode array detector and a Peltier junction temperature-controlled sample compartment. Exposure of tryrosyl residues was determined by plotting second derivative peak–trough absorbance differences as a function of chemical denaturant concentration in accordance with the procedure of Ragone et al. (30). The ratio of a (A₂₉₅ − A₂₉₂) to b (A₂₉₄ − A₂₉₃) facilitates evaluation of tyrosyl exposure by eliminating the contribution of tryptophan residues that normally interferes in the analysis. N-Acetyltirosine and N-acetyltirosylphenylalanine (Sigma) were employed as model compounds at a molar ratio of 18:4 to approximate the total content of tyrosine and tryptophan residues in class 2 protein. The degree of tyrosyl exposure has been calculated using the relation (Equation 1):

\[
a = (r_u - r_r)(M_u - M_r)
\]

where \(r_u\) and \(r_r\) represent numerical values of the ratio \(a:b\) determined for the native (0.05% Z-3-14) and unfolded (6.0 mM GdnHCl) states, respectively, and \(r_r\) is the value of \(a:b\) obtained for the solution of model compounds in ethylene glycol. The latter reflects 0% exposure of aromatic residues since this solvent reportedly mimics the hydrophobic protein matrix (30).

**ThermoStability**—The conformational stability of class 2 protein was also investigated by monitoring temperature-dependent CD spectral changes in the far and near UV regions. These studies were performed by recording changes in the mean molar ellipticity at 217 and 290 nm during thermal denaturation over the temperature range of 25–95 °C. The reversibility of thermal unfolding/refolding was assessed by cooling the samples and rescanning CD spectra for both the native and renaturant proteins at 25 °C.

**RESULTS**

**Overexpression of Recombinant Class 2 Protein (Rclass 2)**—Fig. 1, panel A, presents a schematic illustration of the plasmid construct that exhibits a size corresponding to 4.4 kilobase pairs and comprises the pET17b plasmid containing the gene for the recombinant class 2 protein encoded by the cloned mature class 2 gene. The level of class 2 protein expressed at various times after induction was monitored by SDS-PAGE of whole cell lysates followed by staining with Coomassie Brilliant Blue. The ratio of A₃₂₅ to A₂₉₄ (Eq. 1) facilitates evaluation of tyrosyl exposure by eliminating the contribution of tryptophan residues that normally interferes in the analysis. N-Acetyltirosine and N-acetyltirosylphenylalanine (Sigma) were employed as model compounds at a molar ratio of 18:4 to approximate the total content of tyrosine and tryptophan residues in class 2 protein. The degree of tyrosyl exposure has been calculated using the relation (Equation 1):

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**Overexpression of Recombinant Class 2 Protein (Rclass 2)**—Fig. 1, panel A, presents a schematic illustration of the plasmid construct that exhibits a size corresponding to 4.4 kilobase pairs and comprises the pET17b plasmid containing the gene

**Function and Stability of a Meningococcal PorB Protein**

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encoding the class 2 protein under control of the T7 promoter (25, 26). The expression levels of class 2 protein in E. coli BL21(DE3)DompA upon induction with IPTG is presented as a function of time in Fig. 1, panel B. Densitometric analysis indicates that maximal expression levels are achieved approximately 3 h following IPTG addition.

Refolding and Purification of Rclass 2—Overexpressed rclass 2 aggregates as insoluble IBs that are readily separated from the majority of E. coli proteins by centrifugation of the whole cell lysates. The protein pellet is washed with TEN buffer containing 0.5% deoxycholate to ensure that contaminants such as endotoxin are removed from the sample (31) since class 2 protein is not soluble in this detergent (3). Subsequent purification using a combination of gel filtration and ion exchange chromatography yields protein of 98% purity as evidenced by silver-stained gels and spectrophotometric analysis. Pyrogenic tests and limulus amebocyte lysate assays indicate that the purified protein is endotoxin-free. Fig. 2 furnishes a comparison of the purity levels for rclass 2 with respect to solubilized IBs.

Functional and Structural Characterization of N. meningitidis Class 2 Protein—To ascertain that the porin obtained by the renaturation procedure described above retains the structural features of native class 2 protein, several functional and physicochemical characteristics have been assessed. The results of these experiments including several novel aspects of class 2 protein as a bacterial porin are presented in the following sections.

SDS Resistance Properties of Relass 2 and E. coli OmpF Proteins—For comparison purposes and validation of the detergent-assisted refolding procedure reported in the present study, OmpF has been extracted from E. coli outer membranes under denaturing conditions (i.e., 8 M urea), refolded and purified in accordance with the experimental protocol employed for rclass 2 IBs. Fig. 3 depicts the typical SDS resistance behavior observed for oligomeric OmpF and reveals a striking similarity with rclass 2. These results are entirely consistent with the properties reported previously for the native E. coli porin (32). Permeability Conferring Activity of Class 2 Protein—Characterization of the pore-forming activity of porins by the liposome swelling assay requires accurate determination of the isosmotic concentration, particularly if pore sizes are to be determined (29). The oligosaccharide stachyose (M, 666) has been employed for determination of the isosmotic concentration to dextran-containing proteoliposomes prepared with either class 2 protein or OmpF, since this sugar does not permeate at significant rates through these protein channels (29, present study). Although protein-free liposomes exhibit no swelling in the presence of isosmotic solutions of l-arabinose (M, 150), both class 2 protein and OmpF promote significant permeability increases when inserted into the liposomes. Fig. 4 (upper panel) is a representative experiment depicting the time course of the swelling response.
Permeability rates of native and recombinant class 2 protein. Upper panel, changes in the optical density (A\textsubscript{405}) of proteoliposome suspensions prepared with rclass 2 (0.5 μg/μmol lipids) upon dilution into isosmotic solutions of the indicated sugars. The oligosaccharides rhamnose and N-acetylglucosamine have been omitted to improve overall clarity in the narrow molecular weight range between galactose and sucrose. Lower panel, solute-dependent permeation rates of proteoliposomes prepared with native class 2 (open circles), rclass 2 (closed circles), and E. coli OmpF (dashed line, open triangles). The values are normalized to the permeation rate of L-arabinose and plotted on logarthmic and linear (inset) scales. The oligosaccharides employed in the study include arabinose (M\textsubscript{r} 150), galactose (M\textsubscript{r} 180), rhamnose (M\textsubscript{r} 182), N-acetylglucosamine (M\textsubscript{r} 221), sucrose (M\textsubscript{r} 342), raffinose (M\textsubscript{r} 342), rhamnose (M\textsubscript{r} 342), raffinose (M\textsubscript{r} 342), raffinose (M\textsubscript{r} 342), and stachyose (M\textsubscript{r} 504).

Assessing Rclass 2 Conformation by Trypsin Resistance Assays—Trypsin digestion was performed to study the conformational properties of class 2 protein under different experimental conditions and compared with those of E. coli OmpF (Fig. 5). Panel A depicts the results of trypsin digestion for class 2 and OmpF under the indicated pre-digestion conditions, followed by incubation with SDS-loading buffer at 25 °C. Both class 2 and OmpF exhibit typical trimeric migration patterns (designated T), and preincubation with SDS and thermal treatments employing E. coli OmpF as a reference standard (32). The resultant trypsin-digested samples were incubated with SDS loading buffer at 25 °C (panel A) or 95 °C (panel B) prior to electrophoresis on a 8–16% gradient gel. Arrows indicate the positions of trimeric (T) and monomeric (M) species.

Decrease in optical density at 450 nm of dextran-containing class 2 proteoliposomes in isosmotic solutions of various oligosaccharides. In the present study, the permeability rates of rclass 2 are similar regardless of the detergent in which the porin samples are resuspended prior to proteoliposome preparation. Identical rates are obtained for rclass 2 prepared in either 0.05% Z 3-14 or 1% octylglucoside (data not shown). In both cases, the final detergent concentrations in the proteoliposome preparation are sufficiently below the established interference level of 100 μg/μmol phospholipids for this assay (29). Fig. 4 (lower panel inset) compares the diffusion rates of various sugars as a function of solute molecular weight. Analysis of the relative permeability rates to L-arabinose in the logarithmic plot of the data (Fig. 4, lower panel) reveals similar behavior for native and rclass 2 protein channels. Using the values corresponding to 10% of the L-arabinose diffusion rate (i.e. F (0.1 Ara)) as an index of the pore size (29), the M\textsubscript{r} (0.1 Ara) for OmpF and class 2 protein are approximately 280 and 400, respectively (Fig. 4, lower panel). A nonlinear least squares fit of the data to Renkin’s equation (33) yields a pore radius of approximately 0.8 nm for this particular PorB class 2 protein.

Fig. 5. SDS-PAGE analysis of trypsin-digested recombinant class 2 protein and E. coli OmpF. Aliquots (1 μg) of rclass 2 protein (lane 1) or E. coli OmpF (lane 4) incubated with 0.1 μg of tosylphenylalanyl chloromethyl ketone-treated trypsin for 30 min at 37 °C. Lanes 1 and 4 represent the controls of rclass 2 and E. coli OmpF in the absence of SDS. Lanes 2 and 3 correspond to trypsin-digested rclass 2 protein following preincubation with 1% SDS at either 25 or 95 °C, respectively. Lanes 5 and 6 represent identical SDS and thermal treatments employing E. coli OmpF as a reference standard (32). The resultant trypsin-digested samples were incubated with SDS loading buffer at 25 °C (panel A) or 95 °C (panel B) prior to electrophoresis on a 8–16% gradient gel. Arrows indicate the positions of trimeric (T) and monomeric (M) species.
Function and Stability of a Meningococcal PorB Protein

TABLE I

Class 2 protein regions assigned as putative extracellular loops

Predicted extracellular loops appear in bold face, and the flanking transmembrane β-strand domains are italicized (16, 21). Segments assigned as periplasmic turns have been omitted to improve overall clarity. Tyrosine residues contained within the exposed loops are underlined. The position of a trypsin cleavage site (●) is noted in loop I (6; present study). Solvent exposure of tyrosyl residues is deduced from second derivative analysis of the rclass 2 UV spectrum according to the protocol of Ragone et al. (30). The native state of class 2 protein (400 μg/ml) has been determined in a buffer system comprised of 25 mM Tris-HCl, 0.2 mM NaCl, 1.0 mM EDTA, and 0.05% Z-3–14 (pH 8.0). The resultant r values (see “Experimental Procedures”) are compared with those obtained for model compounds of tyrosine and tryptophan at a molar ratio of 18:4 (Y:W) in ethylene glycol (0% exposure) and 6.0 M Gdn-HCl (100% exposure). ND, not determined.

<table>
<thead>
<tr>
<th>Loop number</th>
<th>Amino acid sequence</th>
<th>Whole cells</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>VTVLYGTDKAGVEYRVDAGTYR[1]AQGGKSKATQIADFGSKGFK</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>II</td>
<td>MKIQLQLEKQAIAGTNSSGWRQSFGLKQGF</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>AGINLNTVLKQGGDNKAMEGSGTEDVLGALTIRVES REISVRYDSP</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IV</td>
<td>AGFSGSVQYVEPONANDVYKHTKSSRESYHAGLYE</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V</td>
<td>FPGQAGSYFVKYADNTAERVVANTANHPKVDKQVRWVAGYD</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>VI</td>
<td>LVYSVAGQVMKAAKNVEVSQGGKGKHEQTG VAAATAYRFG</td>
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<td>-</td>
</tr>
<tr>
<td>VII</td>
<td>VSYAHSFKAVSVKDNSQYDQ VIVGADVDF</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>VIII</td>
<td>TSALVSAGWLQKQGGAKQKVEQT ASMGDLRHIF</td>
<td>-</td>
<td>-</td>
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Predicted number of exposed tyrosyl residues

<table>
<thead>
<tr>
<th>Amino acid sequence</th>
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a Assigned based on secondary structure predictions (16, 21).
b Antibody binding studies (21).
c Limited proteolysis (6).
d Limited proteolysis (present study).
e Indirect and deduced from the findings in a, b, and c.
f Tyrosyl exposure (present study).

g in N. meningitidis whole cell extracts (6). Sequence analysis of trypsin-digested class 2 samples indicates that a 2.6-kDa peptide is shed from the N terminus (6), since two species corresponding to the intact and cleaved (i.e. at Lys-24) sequences are detected (refer to Table I).

Secondary Structure of Native and Rclass 2 Protein—The far UV CD spectrum of a protein is a diagnostic probe of secondary structure and facilitates determination of specific structural features that comprise the native conformation. Fig. 6, panel A presents the far UV CD of recombinant class 2 protein that is essentially identical to that of its native counterpart in Fig. 6, panel C. The far UV CD spectra are consistent with those obtained for other proteins that retain characteristic structural features of porins (23, 32, 34–36). The minima observed at 217 nm may be correlated with the native conformation of class 2 protein that is comprised predominantly of β-pleated sheet structures. Although secondary structure prediction methods have been applied primarily to the analysis of soluble proteins (37), recent advances in the development of deconvolution algorithms and the general availability of CD spectral data bases have enabled accurate determination of structural components for membrane proteins (38). Deconvolution analysis of the far UV CD spectra employing the convex constraint algorithm for secondary structure estimation (38) reveals that native and rclass 2 contain approximately 38% β-sheet structure, a value entirely consistent with that predicted solely from the amino acid sequence.

Tertiary Structure of Native and Rclass 2 Protein—Near UV CD spectroscopy may be employed to detect asymmetry in the environment of aromatic residues and thereby monitor changes in the tertiary structure of proteins. The near UV CD spectrum is sensitive to minor structural perturbations in protein conformation that are not always manifested in conventional absorption spectra. Visual comparison of the near UV CD spectra of native and rclass 2 proteins (Fig. 6, panels B and D, respectively) reveals a unique profile characterized by a broad positive ellipticity over the wavelength range of 240 to 280 nm. The two well defined ellipticities observed at 284 and 290 nm reflect the asymmetric environment of the tyrosyl and tryptophanyl residues in the native oligomeric state.

Exposure of Tyrosyl Residues—Second derivative analysis of UV spectra employing the method of Ragone et al. (30) reveals that approximately 9% of the tyrosine residues in rclass 2 (i.e. 1–2 out of 18 tyrosines; Table I) may be exposed to solvent in detergent solution. Significantly, these solution features are similar to the structural arrangement observed in the native membrane-embedded state. Although secondary structure predictions suggest that six tyrosine residues are located in the long loop stretches, not all appear to be surface-exposed as indicated by antibody binding in whole cell assays (refer to Table I). Three tyrosine residues are located in putative loops I and V that are assumed to be surface-exposed in vivo (21). Therefore, our estimation obtained for the purified protein in detergent solution is generally consistent with the findings of whole cell assays.

Conformational Stability of Class 2 Protein, SDS Resistance as a Function of Temperature—Fig. 7 presents the temperature-dependent SDS resistance profiles of the wild type (strain M986, serotype B:2a) N. meningitidis detergent extract, where class 2 protein appears as a fraction of the isolated OMPs. Lower migration profiles characteristic of trimeric species are observed at temperatures below 70 °C, above which only monomeric species are detected. The resultant data presumably reflect tight protein-protein and perhaps peptidoglycan-protein interactions, which may stabilize the oligomeric conformation of these proteins. Fig. 8 depicts the behavior of refolded and purified rclass 2 in SDS-PAGE as a function of temperature and preincubation with either 0.5% Z-3–14 or 0.5% SDS. Fig. 8, panel A presents the results of SDS-PAGE conducted on rclass 2 samples containing 0.5% Z-3–14 and preincubated at the specified temperatures. Only trimeric species are detected under such conditions signifying that rclass 2 possesses the ability to maintain or revert to the trimeric state in the presence of zwitterionic detergent. Fig. 8, panels B and C, presents SDS-PAGE of rclass 2 samples preincubated with 0.5% SDS. Fig. 8, panel B, demonstrates the effects of incubation at the indicated...
temperatures for 5 min followed by cooling for 1 min, whereas Fig. 8, panel C represents 2 weeks’ storage at 4 °C following the treatments performed in Fig. 8, panel B. Although SDS resistance is not observed above 60 °C following immediate cooling (Fig. 8, panel B), some reversibility is noted even at 65 °C when heated samples are incubated at 4 °C for 2 weeks. Nevertheless, the trimer:monomer ratios are reduced upon long term storage in SDS even at lower temperatures (Fig. 8, panel C). The apparent rclass 2 transition temperature of ~60 °C in 0.5% SDS is somewhat lower than that observed for the neisserial outer membrane proteins (Fig. 7), which may be attributed to the fact that the neisserial preparation contains other outer membrane components such as class 1 protein as part of the SDS-resistant pool migrating as trimeric species in SDS-PAGE.

**Thermal Stability of Class 2 Protein**—Temperature-dependent CD profiles of rclass 2 protein have been recorded at 217 and 290 nm to selectively monitor the disruption of secondary and tertiary structure, respectively. The thermal denaturation profiles presented in Fig. 9, panels A and B, exhibit transition midpoints of 88 °C, and rescans of the samples after cooling to 25 °C reveal greater than 95% reversibility (Fig. 9, panels C and D, dotted lines). Thermal unfolding of rclass 2 protein in the presence of 0.05% Z 3-14 promotes a reversible loss of secondary structure and partial disruption of overall secondary structure (Fig. 9, panel C, dashed line) that is paralleled by the disruption and partial conversion of β-sheet conformation to an α-helical-like structure (Fig. 9, panel D, dashed line). The reversible perturbation of tertiary structure may be correlated with a temperature-induced unfolding of class 2 protein involving solvent exposure of aromatic residues, a finding that is corroborated by measurement of intrinsic fluorescence as a function of temperature (Fig. 11, panel A). The recovery of ellipticity in the far and near UV CD spectra indicates that the native conformation is fully regained upon cooling. The reversibility observed in spectroscopic studies of rclass 2 is also evident in SDS-PAGE analysis, whereby only trimeric species are detected upon heating samples in zwitterionic detergent to 95 °C and cooling prior to gel loading (Fig. 8, panel A). These results indicate that thermal denaturation of zwittergen-containing class 2 protein solutions is characterized by a reversible unfolding transition involving complete loss of tertiary structure and partial disruption of overall secondary conformation.

**Temperature and SDS-induced Conformational Changes in Relcase 2**—Spectral changes of class 2 protein as a consequence of exposure to SDS or 6 M Gdn-HCl have been examined by CD and fluorescence spectroscopy. The far UV CD spectrum of rclass 2 acquired at 25 °C in the presence of 6 M Gdn-HCl (Fig. 10, dashed line) is characteristic of the denatured or random coil conformation, whereas SDS-treated rclass 2 retains secondary structural features common to the native trimeric state. The latter phenomenon is a common finding among porins and is assumed to characterize the partially unfolded monomeric states (23, 32, 35). Such species also experience trypsin sensitivity as demonstrated in the trypsin digestion experiments presented in Fig. 5.

The fluorescence spectrum of rclass 2 in 0.05% Z 3-14 at 25 °C exhibits a maximum intensity at 340 nm upon excitation at 295 nm (Fig. 11, panel A, solid line). There is a temperature-dependent change in the fluorescence signal that is characterized by a slight red-shift (340–345 nm) and approximately 80% reduction in intensity at 95 °C (Fig. 11, panel A, dashed line). Samples of SDS-treated rclass 2 retain 85% of the native fluorescence intensity at 25 °C (Fig. 11, panel B, solid line) albeit with a slight blue shift (340–336 nm). The thermally induced
changes are more drastic when the protein is incubated at 95 °C in the presence of SDS (Fig. 11, panel B, dashed line) as evidenced by a 90% reduction in peak intensity accompanied by a significant red-shift (336–351 nm). The overall degree of thermal reversibility differs dramatically in the absence or presence of denaturants. Specifically, 100% of the fluorescence signal is recovered after cooling rclass 2 samples in zwitterionic detergent (Fig. 11, panel A, dotted line), whereas only 50% recovery occurs in the presence of SDS (Fig. 11, panel B, dotted line). The fluorescence intensity decreases and the spectrum is characterized by a significant red-shift (i.e. 340–355 nm) in the presence of 6.0 M Gdn HCl (Table II) as the tryptophan residues are further exposed to solvent in the unfolded state.

In light of our CD and fluorescence studies, we may conclude that the overall conformation of class 2 protein is relatively unaffected by the presence of SDS at 25 °C, whereas irreversible changes occur when SDS-treated samples are exposed to temperatures above 60 °C. Nevertheless, these conformational changes reflect partial unfolding of class 2 protein when compared with the effect of 6 M Gdn HCl that induces complete denaturation. Table II furnishes a summary of the results obtained for physicochemical characterization of class 2 protein in the present study.

**DISCUSSION**

Porins are rather unusual proteins in terms of their extensive β-pleated sheet structure. Spectroscopic studies (23, 32, 34–36) extended further by elucidation of porin crystal structures (17–20) have clearly indicated that porins consist almost entirely of β-sheets. Considering the relatively low sequence homology observed within this family of proteins, a general consensus among structural characteristics is the presence of a barrel motif. Although porins function as transmembrane proteins, these molecules are distinct in view of their high polarity and unusual stability. Porins are also unique in terms of their resistance to extremes of pH and proteases (36), both of which are vital properties particularly for enterobacteria to survive in the intestinal tract (18 and references contained therein). A limited number of studies have attempted to characterize the physicochemical properties of porins derived from *N. meningitidis*. Some functional and structural features ascribed to *N. meningitidis* class 2 and class 3 proteins have resulted from indirect evidence by comparing their sequence/structure relatedness with other neisserial strains, primarily gonococcal (15). The isolation and purification of these proteins represents a difficult challenge as native bacterial porins are often co-purified (11, 39). Consequently, the expression of such proteins in heterologous systems may facilitate characterization of the inherent properties for each isolated class of porin in the absence of interferences from other contaminant OMPs.

Expression of Porins in Heterologous Cells—An extensive review of the literature reveals that efforts to develop effective expression systems for the production of recombinant porins are somewhat restricted. Several studies have reported the expression of *Haemophilus influenzae* type b porin in the membrane of insect cells using the baculovirus system (40) and in *Bacillus subtilis* (41) in an attempt to obtain a final product free of lipopolysaccharide. This latter complication poses significant difficulties when *E. coli* is used as the expression system. Moreover, investigators have reported that both gonococcal and meningococcal class 2 proteins are lethal if expressed in *E. coli* (24). In the present study, we have employed a strategy reported recently by Qi et al. (25) whereby large amounts of porins are accumulated without significant deleterious effects to the host cells. Similar procedures have also been described for *H. influenzae* P2 (26) and proven useful for the overexpression and accumulation of porins as IBs. This strategy affords an additional advantage by eliminating the majority of *E. coli* contaminant proteins in the supernatant following centrifugation. The suitability of the present procedure has been confirmed recently by the successful crystallization of *R. blastica* porin, obtained through use of an identical expression system in *E. coli* followed by subsequent isolation and refolding from IBs (42).

Detergent-assisted Refolding of Class 2 Protein—The use of amphiphilic molecules to facilitate proper refolding of bacterial porins has proven efficient in terms of basic criteria for characterization of refolding. The use of Z 3-14 as the detergent of choice in porin studies has been documented by a significant number of investigators since it is assumed to promote the efficient refolding of these proteins in the absence of membrane components, while retaining the native properties of mem-

![FIG. 7. SDS-PAGE analysis of class 2 outer membrane proteins extracted from *N. meningitidis* and preincubated with SDS as a function of temperature.](http://www.jbc.org/)

**Fig. 7.** SDS-PAGE analysis of class 2 outer membrane proteins extracted from *N. meningitidis* and preincubated with SDS as a function of temperature. Aliquots of partially purified class 2 outer membrane proteins incubated with 0.5% SDS at the indicated temperatures for 5 min and cooled to 25 °C prior to electrophoresis. The arrows indicate positions of trimeric (T) and monomeric (M) species. Class 2 protein appears as the second band from the top of the group designated as monomeric species (~40 kDa). The remaining monomeric species conceivably represent class 1 (top band ~42 kDa) and classes 4 and 5 (two bottom bands).

![FIG. 8. SDS-PAGE analysis of recombinant class 2 protein as a function of temperature and detergent.](http://www.jbc.org/)

**Fig. 8.** SDS-PAGE analysis of recombinant class 2 protein as a function of temperature and detergent. Aliquots (5 µg) of purified rclass 2 incubated at the indicated temperatures for 5 min and cooled to 25 °C prior to electrophoresis. Panel A, preincubation of rclass 2 with 0.5% Z 3-14; panel B, preincubation of rclass 2 with 0.5% SDS; panel C, SDS-PAGE of rclass 2 samples stored for 2 weeks at 4 °C following the temperature-dependent study in panel B.
brane-extracted porins (25, 26, 43). Significantly, we have obtained unequivocal evidence that both the secondary and tertiary structures of rclass 2 protein refolded from an 8M urea solution employing the detergent-assisted protocol described herein are identical to the native counterpart as demonstrated by the superimposable far and near UV CD spectra (refer to Fig. 6). Crystallographic studies of E. coli porins furnish additional evidence that detergent molecules bind to the so-called hydrophobic zones that most likely interact with the outer membrane lipid components in vivo (44). Detergent-assisted refolding procedures have also been proposed for soluble proteins (45) based on their ability to counteract aggregation and thereby facilitate proper refolding.

Characterization of Rclass 2 Protein—The literature does not contain sufficient empirical studies on the biophysical properties of neisserial (particularly meningococcal) OMPs, although numerous reports refer to these proteins on the basis of a presumed equivalence with their gonococcal analogs (25). The biochemical properties of rclass 2 protein from N. meningitidis expressed in E. coli and accumulated as IBs resemble those of a large number of OMPs, with respect to its electrophoretic migration pattern in SDS-PAGE and inherent structural features. The temperature-dependent SDS-PAGE profiles observed for this and other OMPs is a characteristic consequence of the high thermodynamic stability of such proteins.

Biological Activities of Native and Rclass 2 Protein—The functionality of porins may be assessed by several techniques including measurements of conductivity and liposome swelling (1, 2, 9, 29). Studies conducted by Frasch and Mocca (4) originally postulated that class 2 protein resembled bacterial porins on the basis of electrophoretic migration pattern in SDS-PAGE and inherent structural features. The temperature-dependent SDS-PAGE profiles observed for this and other OMPs is a characteristic consequence of the high thermodynamic stability of such proteins.

FIG. 9. Effects of thermal denaturation on the circular dichroism spectra of recombinant class 2 protein. Thermal denaturation profiles of rclass 2 protein recorded at 290 nm (panel A) and 217 nm (panel B) to monitor the disruption of tertiary and secondary structure, respectively. The extent of thermal reversibility is illustrated in the temperature-dependent CD spectra of rclass 2 (1.0 mg/ml) in the near UV (panel C) and far UV (panel D). The solid line represents the rclass 2 spectrum in 50 mM NaPO₄ and 0.05% Z-3-14, pH 7.5, at 25 °C; the dashed line is the protein spectrum following thermal denaturation at 95 °C, and the dotted line is the renatured protein spectrum after cooling to 25 °C.

have generally supported the conjecture that class 2 is a pore-forming protein. In this regard, a close relatedness is observed between the N. meningitidis and N. gonorrhoeae strains, the latter of which has been subjected to a more detailed functional analysis (8). There is sufficient evidence for a possible role of these porins during intracellular invasion processes (12, 13). The ability of class 2 protein to insert into foreign membranes may likely represent one of the mechanisms of virulence adopted by this and other neisserial porins as postulated recently (14).

Ulmr et al. (43) have proposed an erythrocyte permeability assay (i.e. hemoglobin release) as a probe of porin function for N. meningitidis class 2 protein, although the authors acknowledge that such activity is enhanced in the presence of zwittergen. In attempting to reproduce this assay, we have concluded that the hemolytic response is due primarily to destabilization of the erythrocyte membrane in the presence of protein-micelle complexes rather than an accurate measure of porin function. This observation has been corroborated by conducting control experiments employing octylglucoside as the detergent of choice. Significantly, the false positive response measured for zwittergen-containing rclass 2 in hemolytic assays is not observed in the presence of octylglucoside, although the native trimeric conformation and functionality of the protein is retained under both conditions as demonstrated in this study through the liposome swelling assay.

Nikaido (2) has suggested that the variability and conflicting results noted when comparing studies of porin function may be attributed to the different approaches and techniques employed in such investigations. Although there is no conclusive data in the literature regarding the pore size of N. meningitidis porins, indirect evidence suggests that class 2 and class 3 proteins are related to N. gonorrhoeae porins (15). Pore sizes in
the range of 1.1 nm have been determined for the latter on the basis of single channel conductance measurements in planar lipid bilayer membranes (8). Nevertheless, preliminary studies employing the liposome swelling assay have revealed that these porins produce larger channels (7). In the present study, inspection of the permeability rates of class 2 protein as a function of solute molecular weight predicts a pore diameter of approximately 1.6 nm, a value slightly wider than that assigned for E. coli OmpF (29; present study). Since the liposome swelling assay is regarded as a more reliable technique for the unequivocal determination of pore size (2), our results represent the first accurate quantitative estimate derived for this bacterial strain. Moreover, expression and purification of class 2 protein affords an additional advantage in terms of examining the intrinsic permeability properties of this bacterial porin in the absence of potential interferences arising from co-purified meningococcal OMPs exhibiting porin activity.

Structural Characterization of Class 2 Protein—Our physicochemical characterization of PorB class 2 protein represents the first comprehensive experimental determination of the basic structural features of neisserial porins since only topology models are available to date. Deconvolution analysis of the far UV CD spectra reveals that both native and rclass 2 exhibit a typically high content of β-sheet structure (~38%) that is characteristic of other porins. Inspection of the near UV CD spectra reveals a unique profile consisting of a broad positive ellipticity, presumably arising from the combined contribution of tyrosyl tryptophanyl residues. The latter is particularly evident when comparing the near UV CD spectra of the PorB class 2 and class 3 proteins with that of H. influenzae P2 porin. The similarity in spectral features observed for class 2 and class 3 proteins differs markedly from the near UV CD spectrum of H. influenzae P2 porin that lacks tryptophan residues and exhibits a negative molar ellipticity.1

In accordance with structure prediction models (21), there is sufficient evidence in the present study to suggest that the same regions predicted as the putative cell surface loops are also exposed to solvent in detergent solution. Likewise, we have estimated that approximately 9% of tyrosines (i.e. 1–2 residues) are solvent-exposed in the native state. Fluorescence studies reveal that a limited number of aromatic residues are exposed to solvent in the native state. Specifically, Gdn-HCl-induced unfolding of rclass 2 protein is characterized by a significant reduction in the fluorescence intensity coupled with a concomitant red shift that is indicative of tyrosyl and tryptophanyl residue exposure (refer to Table II).

Limited proteolysis has been used widely as a tool in the elucidation of protein topology. Porins are particularly resistant to proteolysis. A fingerprint for class 2 protein as studied in whole cells is the presence of a trypsin cleavage site at Lys-24, yielding a polypeptide that is 2.4 kDa shorter than the mature protein (6). Both native and rclass 2 exhibit identical trypsin digestion patterns in detergent solution, which is also characterized by incomplete reaction, irrespective of relative enzyme/protein concentrations or incubation time. As a result, two species are detected by SDS-PAGE, one exhibiting the normal apparent molecular weight of class 2 protein and a less abundant form migrating at reduced size (~2–3 kDa smaller). The intriguing finding that trypsin digestion is limited to a fraction of the total polypeptide molecules present in solution has also been obtained for whole cell assays (4, 6), which implies that the tertiary/quaternary structures of porins in vivo are attained with protein-micelle complexes in vitro. Incomplete digestion suggests that individual subunits within the trimer experience different degrees of exposure and accessibility to enzyme digestion. Moreover, the trimer appears to remain stable since the trypsin-digested class 2 samples retain SDS-resistant properties at room temperature (refer to Fig. 5, panel A).

The overall results are therefore consistent with a structural model in which the solution conformation of rclass 2 in Z 3-14 is similar to that of whole cells in terms of the number of aromatic amino acids as well as the trypsin-cleavage sites exposed in the native trimeric state (21; Table I). These structural features suggest that detergent molecules span hydrophobic regions of the protein in a similar fashion to the topology observed in vivo within the outer membrane. Studies on tyrosyl residue accessibility of E. coli porins by chemical modification with low molecular weight probes or spectrophotometric titration as a function of pH have also provided evidence that a very limited number of tyrosines are exposed to solvent in detergent solutions (46).

Conformational Stability of Class 2 Protein—It is often desirable to employ a combination of physicochemical techniques when characterizing the conformational stability of a protein since each technique examines a subset of biochemical and physical properties (47). In accordance with previous studies on other porin molecules, elucidation of the physicochemical characteristics of class 2 is complicated by the fact that the overall stability of this oligomeric protein is dictated by a combination of factors including the energetics of folding, subunit association, and protein-detergent association interactions. Nevertheless, a primary focus of the present study is to conduct an initial

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1 C. A. S. A. Minetti, M. S. Blake, and D. P. Remeta, unpublished observations.
thermodynamic characterization of the conformational stability of class 2 protein employing chemical and thermal denaturation techniques. The trimeric conformation of class 2 protein is resistant to both thermal and chemical denaturation, and therefore, only drastic conditions such as treatment with 6 M Gdn\textsubscript{HCl} or heat in the presence of SDS are sufficient to promote complete unfolding/dissociation of class 2 protein. Although our results reflect the structural properties of this protein \textit{in vitro}, it is conceivable that class 2 protein \textit{in vivo} possesses an extraordinary ability to resist harsh conditions such as extremes of pH, temperature, and the presence of proteases. These findings are striking considering the fact that meningococci do not normally encounter such adverse conditions in the host system during infection as compared with enteric bacteria. We have obtained preliminary evidence suggesting that neisserial porins (\textit{e.g.} class 1/class 2) may assemble as heterotrimers \textit{in vivo}. Moreover, our physicochemical studies indicate that although the PorB class 2 and class 3 proteins are equally virulent and functionally equivalent, both serotypes are clearly distinct in terms of their overall conformational stability (48).

\textit{E. coli} OmpF has been studied extensively with respect to its

![Figure 11](image-url)

**FIG. 11.** \textbf{Fluorescence spectra of recombinant class 2 protein as a function of temperature and detergent.} Panel A, fluorescence spectrum of rclass 2 protein (100 \mu{g/ml}) at pH 8.0 in Buffer A (25 mM Tris-HCl, 200 mM NaCl, 1.0 mM EDTA, and 0.05% Z 3–14). The solid line represents the rclass 2 spectrum recorded at 25 °C, the dashed line is the sample spectrum at 95 °C, and the dotted line is the spectrum at 25 °C following thermal denaturation. Panel B, fluorescence spectra of rclass 2 protein in Buffer A + 0.5% SDS, pH 8.0, at 25 °C (solid line), 95 °C (dashed line), and following thermal denaturation at 25 °C (dotted line).

**TABLE II**

Conformational stability of class 2 protein as a function of chemical and thermal denaturation

<table>
<thead>
<tr>
<th>Treatment\textsuperscript{a}</th>
<th>Temperature\textsuperscript{b}</th>
<th>SDS resistance</th>
<th>Fluorescence spectrum\textsuperscript{c}</th>
<th>Circular dichroism spectra\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C</td>
<td></td>
<td>\lambda\textsubscript{max} (nm)</td>
<td>\textit{F}_rel</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>+</td>
<td>340</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>+</td>
<td>340</td>
<td>100</td>
</tr>
<tr>
<td>SDS</td>
<td>25</td>
<td>+</td>
<td>336</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>–</td>
<td>351</td>
<td>45</td>
</tr>
<tr>
<td>Gdn\textsubscript{HCl}\textsuperscript{f}</td>
<td>25</td>
<td>–</td>
<td>355</td>
<td>20</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Control, rclass 2 protein dissolved in 25 mM Tris-HCl, 0.2 mM NaCl, 1.0 mM EDTA, 0.05% Z 3–14 adjusted to pH 8.0 (Buffer A); SDS, rclass 2 protein in Buffer A containing 1% SDS; Gdn\textsubscript{HCl} = rclass 2 protein in Buffer A containing 6.0 mM Gdn\textsubscript{HCl}.

\textsuperscript{b} Analysis following incubation at the indicated temperatures and cooling to 25 °C prior to loading onto the gel or recording the spectrum.

\textsuperscript{c} The excitation wavelength (\lambda\textsubscript{ex}) is 295 nm; \lambda\textsubscript{max} (nm) = wavelength of maximum fluorescence intensity; \textit{F}_rel, relative fluorescence intensity at \lambda\textsubscript{max} expressed as the percent of rclass 2 native fluorescence in Buffer A.

\textsuperscript{d} Far UV CD, control, 38% \beta-sheet; SDS/heat, \alpha-helical-like conformation; Gdn\textsubscript{HCl}, random coil; \lambda\textsubscript{min} (nm), wavelength of minimum ellipticity; (\theta)\textsubscript{217}, relative ellipticity expressed as the percent of rclass 2 native ellipticity at 217 nm in Buffer A.

\textsuperscript{e} Near UV CD, (\theta)\textsubscript{290}, relative ellipticity expressed as the percent of rclass 2 native ellipticity at 290 nm in Buffer A.

\textsuperscript{f} Gdn\textsubscript{HCl} (6 mM) treatment induces complete unfolding to a random coil conformation.
trimeric stability in SDS-PAGE. Preincubation with SDS at ambient temperatures is characterized by the occurrence of a predominant band at an apparent molecular mass of 68 kDa indicative of oligomeric species. This phenomenon has been described for E. coli (32, 34, 36) and a significant number of other bacterial porins (including class 2 protein in the present study), although not all appear to share this characteristic property. As an illustrative example, H. influenzae P2 does not exhibit such behavior. The instability of this porin may explain the immunodominance associated with the putative loop III property. As an illustrative example, other bacterial porins (including class 2 protein in the present study) exhibit such behavior. The instability of this porin may explain the immunodominance associated with the putative loop III property. As an illustrative example, other bacterial porins (including class 2 protein in the present study) exhibit such behavior.

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REFERENCES

Structural and Functional Characterization of a Recombinant PorB Class 2 Protein from *Neisseria meningitidis*: CONFORMATIONAL STABILITY AND PORIN ACTIVITY

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