

## Isolation and Characterization of a Family of Porin Proteins from *Helicobacter pylori*

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Received 15 September 1994/Returned for modification 5 December 1994/Accepted 25 January 1995

**Two-dimensional gel electrophoresis was used to identify heat-modifiable outer membrane proteins, which were candidates for porins, from *Helicobacter pylori* membrane preparations. Four such proteins with apparent molecular masses of 48, 49, 50, and 67 kDa were isolated. The four proteins copurified together after selective detergent solubilizations followed by anion-exchange chromatography, and each protein was ultimately purified to homogeneity by gel purification. These proteins were then tested for pore-forming ability with a planar lipid bilayer model membrane system. All four proteins appeared to be present as monomers, and they formed pores with low single-channel conductances in 1.0 M KCl of 0.36, 0.36, 0.30, and 0.25 nS, respectively, for the 48-, 49-, 50-, and 67-kDa proteins which we propose to designate HopA, HopB, HopC, and HopD. N-terminal amino acid sequence analyses showed a high degree of homology among all four proteins, and it appears that these proteins constitute a family of related porins in *H. pylori*.**

*Helicobacter pylori* is a curved to spiral gram-negative bacterium that is an important gastroduodenal pathogen in humans. *H. pylori* is an etiological agent of active chronic gastritis, and the organism is associated with the development of peptic ulcers (8, 41). In addition, colonization by *H. pylori* has been linked to intestinal type gastric cancer (31). *H. pylori* infection is difficult to treat. Therapies to eradicate *H. pylori* usually require the use of combinations of antimicrobial agents such as the widely used triple therapy regimen of bismuth, metronidazole, and tetracycline or amoxicillin. However, while *H. pylori* is quite susceptible to these and a variety of other antimicrobial agents in vitro (40), this susceptibility is poorly correlated with in vivo efficiency. In many patients undergoing antibiotic therapy, *H. pylori* cannot be totally eradicated, and reoccurrence occurs (14, 23, 33).

A number of factors can influence the in vivo susceptibility of a bacterium to antibiotic therapy. One important factor is the site of the infection within the patient being targeted. In the case of *H. pylori*, its gastric mucosal niche probably contributes to the relative inefficiency of conventional antibiotic therapy. Another factor influencing in vivo antimicrobial susceptibility is the permeability of the bacterium by a given antimicrobial agent. In gram-negative bacteria, the outer membrane is a selective barrier between the cell and the external environment, and porin channels are the main diffusion routes across the outer membrane for hydrophilic molecules, including antibiotics (2, 32).  $\beta$ -Lactams as well as many small hydrophilic compounds primarily use porin channels to cross the outer membrane. Little is known about this important class of proteins in *H. pylori*, and although Tufano et al. (39) have proposed that a 30-kDa outer membrane protein may be a porin on the basis of gel migration properties, there have been no studies reporting either the ability of *H. pylori* proteins to form channels in lipid bilayers or the properties of the pores

participating in the permeability of the *H. pylori* outer membrane.

To better understand the permeability properties of this important human gastroduodenal pathogen, it is necessary to isolate and characterize *H. pylori* porin proteins. Here, by use of a planar lipid bilayer model membrane system, we provide the first characterization of the pore-forming ability of four *H. pylori* outer membrane proteins. All four proteins exhibited heat-modifiable gel migration and copurified with each other. In addition, automated Edman degradation showed that all four proteins had very similar amino-terminal sequences, while planar lipid bilayer analysis further showed that the four proteins exhibited similar channel behaviors. On the basis of these data, we suggest that these proteins may constitute a family of porin proteins in *H. pylori*, a family providing pores of relatively small channel size.

### MATERIALS AND METHODS

**Isolation of membrane proteins.** *H. pylori* NTCC 11637 was grown at 37°C in an atmosphere of 10% CO<sub>2</sub> on brain heart infusion agar containing 1% hemoglobin (Acumedia, Baltimore, Md.). After an incubation period of 2 days, cells were harvested from 200 plates and resuspended in 20% sucrose with 50 mg of DNase I in 10 mM Tris-HCl (pH 8.0). The cells were then disrupted with a French pressure cell at 15,000 lb/in<sup>2</sup>. Broken cells were subjected to centrifugation in a sucrose gradient containing layers of 18 and 70% sucrose in 10 mM Tris-HCl (pH 8.0), and the total membrane fraction (850 mg) was collected and made up to 100 ml with distilled water. The membrane fraction was then pelleted at 150,000  $\times$  g, and the pellet was resuspended in 40 ml of 10 mM Tris-HCl (pH 8.0). Protein yields were assayed by a modified Lowry assay (35), with bovine serum albumin as a standard.

**Identification of porin proteins.** Two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was implemented to identify heat-modifiable proteins from the membrane fraction. Samples were run initially with a discontinuous buffer system (19) on 11% acrylamide separating gels with 4% stacking gels, and then vertical gel strips were excised, wrapped in cellophane, and heated to 95°C for 20 min in 0.375 M Tris-HCl (pH 8.6). The strips were placed horizontally on top of another separating gel (without a stacking gel) and electrophoresed in the second dimension. Gels were stained subsequently with Coomassie brilliant blue R250, and heat-modifiable proteins were visualized as proteins that appeared off-diagonal.

**Purification of the 49-kDa (HopB), 50-kDa (HopC), and 67-kDa (HopD) porin proteins.** Samples (100 mg) of membrane proteins were suspended to a final volume of 10 ml in 10 mM Tris-HCl (pH 8.0) with 1% Triton X-100 and 2 mM MgCl<sub>2</sub>. After brief sonication, the sample was centrifuged at 150,000  $\times$  g for 1 h. The supernatant was removed, and the pellet was resuspended by sonication in

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10 ml of Tris-HCl (pH 8.0) with 0.5% sodium lauryl sarcosinate and centrifuged again. The supernatant was removed, and the remaining pellet was extracted twice with 3.0% octyl polyoxyethylene (OPOE; Bachem Bioscience Inc., Philadelphia, Pa.) in 10 mM Tris-HCl (pH 8.0); pellets were resuspended by sonication, the samples were centrifuged at  $150,000 \times g$ , and the supernatants were saved.

Following detergent treatments, samples solubilized in 3% OPOE were further purified by fast protein liquid chromatography (FPLC) with a Mono-Q HR 5/5 anion-exchange column (Pharmacia) and a column buffer of 10 mM Tris-HCl (pH 8.0) containing 0.08% *N,N*-dimethyldodecylamine-*N*-oxide (Fluka Chemika, Ronkonkoma, N.Y.). Elution was performed with a salt gradient of 0 to 1 M NaCl in column buffer. FPLC fractions that were enriched with the heat-modifiable proteins of interest were collected. Final purification of these proteins, which copurified after FPLC, was accomplished by gel purification. Samples were solubilized at room temperature in solubilization mix (19) and loaded on SDS-11% polyacrylamide gels. After electrophoresis, bands corresponding to the proteins of interest were excised from the gel. The gel slices were crushed, and the pure proteins were eluted from the gel by soaking overnight in 0.5 ml of a buffer consisting of 10 mM Tris-HCl (pH 8.5), 1 mM EDTA, 150 mM NaCl, and 0.08% *N,N*-dimethyldodecylamine-*N*-oxide.

**Preparation of 48-kDa porin protein (HopA).** Strain NTCC 11637 cells were grown as described above except that chocolate blood agar was used instead of brain heart infusion agar with 1% hemoglobin. The medium variation was due to the use of different media by our two laboratories. However, no substantive variations were seen in the expression of HopA, -B, -C, and -D in cells grown on either medium (13). Cells were harvested in 10 mM Tris (pH 7.5), and total envelopes were prepared as described previously (9). The total membrane preparation (10 mg of protein per ml [final concentration]) was solubilized in 1.0% Brij 58-0.4 M NaCl in 20 mM Tris (pH 7.5) for 30 min at room temperature. Insoluble material was removed by centrifugation ( $160,000 \times g$ , 0.5 h, 4°C). The supernatant was dialyzed extensively against 20 mM Tris (pH 7.5). This sample was mixed with an equal volume of 0.125 M Tris (pH 6.8)-4% (wt/vol) SDS-20% (vol/vol) glycerol-10% (vol/vol) 2-mercaptoethanol, and the proteins were then separated by SDS-PAGE (12.5% total acrylamide). The segment of the unstained gel in which the HopA protein migrated (assessed by Western blotting [immunoblotting] using a monoclonal antibody specific for this antigen [9]) was excised, and the protein was eluted from the gel slice by suspension in water overnight at 4°C. The sample was concentrated with a 30-kDa-cutoff microconcentrator (Filtron Technology Corp., Northborough, Mass.). The purified protein was obtained after one further round of SDS-PAGE and elution. The purity of the sample was assessed by SDS-PAGE, and its identity was confirmed by Western blotting with a monoclonal antibody specific for the 48-kDa HopA protein (9).

**N-terminal amino acid sequence analysis of proteins.** Purified proteins were run on SDS-11% polyacrylamide gels and electrophoretically transferred to Immobilon membranes (24) with a Bio-Rad trans-blot cell. Membranes were stained with Ponceau S or Coomassie brilliant blue R250 to visualize the protein bands, which were then excised. Amino acid sequencing was performed with a model 475A sequenator (Applied Biosystems Inc., Culver City, Calif.). Phenylthiohydantoin derivatives were separated with an on-line analyzer (Applied Biosystems model 120A). Data handling was performed with an Applied Biosystems model 900 control data analysis module.

**Characterization of porins with a black-lipid bilayer apparatus.** The pore-forming ability of the purified heat-modifiable proteins was assessed in a model membrane system using planar lipid bilayers (4). Lipid bilayers made from 1.5% (wt/vol) oxidized cholesterol in *n*-decane were formed across a 0.2-mm<sup>2</sup> hole separating two compartments of a Teflon chamber containing a variety of salt solutions that were adjusted to pH 7.0 or 4.0 with 5 mM KH<sub>2</sub>PO<sub>4</sub> or to pH 2.0 with 1% CH<sub>3</sub>COOH. Calomel electrodes were implanted in each compartment, one connected to a voltage source and one to a current amplifier and chart recorder (with the output monitored on an oscilloscope). One to 10 ng of a protein sample, solubilized in 0.1% Triton X-100, was added to one of the compartments, and a voltage of 50 mV was applied across the lipid bilayer. Conductance increases were recorded, and average conductances for each purified protein were calculated for each of the salt solutions.

## RESULTS

**Identification of heat-modifiable membrane proteins.** The identification of porins normally involves analyses of outer membrane fractions. However, the outer membrane of *Campylobacter* species and *H. pylori* cannot be isolated free of inner membrane contamination by a variety of procedures (9, 29). For example, after sucrose gradient centrifugation, assays of succinic dehydrogenase and ketodeoxyoctonate showed a mixture of inner membrane and outer membrane markers in all fractions, even when as many as four different sucrose concentration steps were used in the gradient. Therefore, in this study, the whole membrane fraction was used to identify

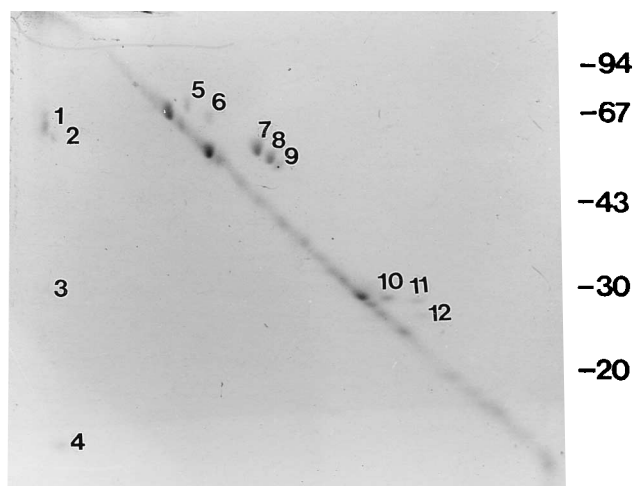


FIG. 1. Two-dimensional SDS-polyacrylamide gel stained by Coomassie blue identifying heat-modifiable proteins. Spots 1, 2, 3, and 4 appeared to have not entered the gel without heating and are assumed to be present as poorly soluble aggregates. Spots 1, 2, and 3, the last of which is barely visible, have molecular weights similar to those of urease subunits which have been shown to form high-molecular-weight aggregates (11). Spots 6, 7, 8, and 9 were the candidate porins purified in this study. Spots 4, 10, 11, and 12 have not been identified positively. Molecular masses (in kilodaltons) are indicated on the right.

prospective *H. pylori* porins. *H. pylori* cells were grown at 37°C in 10% CO<sub>2</sub> on brain heart infusion agar with 1% powdered hemoglobin. The medium and growth conditions stated were used because cells grew as well under these conditions as cells grown on serum- or blood-supplemented medium in a microaerophilic atmosphere. Growth on the hemoglobin-containing medium was also more cost effective and convenient. The membrane proteins from these cells were analyzed for heat-modifiable electrophoretic behavior by two-dimensional SDS-PAGE. At least 12 spots appeared off-diagonal and were identified as heat-modifiable proteins (Fig. 1). Four such proteins were prominent species in the membrane preparation and were selected as porin candidates. The proteins were named HopA, HopB, HopC, and HopD, with gel migrations corresponding to molecular masses of 37, 38, 39, and 55 kDa, respectively, before heating, which modified to apparent molecular masses of 48, 49, 50, and 67 kDa after heating at 95°C for 20 min.

**Isolation and purification of porin proteins.** A detergent solubilization scheme was developed to selectively solubilize and separate the heat-modifiable proteins from other membrane proteins. The 49-kDa HopB and 50-kDa HopC proteins were specifically targeted since they were prominent species and were in a size range typical for porins (26). Ten different detergents were tested at different concentrations to evaluate which would be best suited to selectively solubilize the proteins of interest. It was determined that the best method would involve a series of sequential solubilizations with different detergents. Extraction of membranes with Triton X-100 followed by sodium lauryl sarcosinate resulted in selective solubilization of the HopA, -B, -C, and -D proteins, with minimal loss. HopB, -C, and -D were also highly enriched in the 3% OPOE-soluble fraction, although HopA was not abundant (Fig. 2, lane 2). The sample solubilized in 3% OPOE was used for anion-exchange chromatography, and after one passage through a Mono-Q HR 5/5 anion-exchange column, HopB, -C, and -D coeluted together with a 50-kDa protein which was not heat modifiable (Fig. 2, lane 3). While this 50-kDa protein appeared not to be

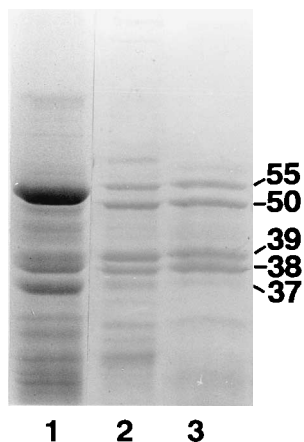


FIG. 2. SDS-polyacrylamide gel of partially purified membrane proteins stained by Coomassie blue. Proteins (15 µg) were loaded onto gels after solubilization in solubilization-reduction mix at 23°C for 10 min. Lanes: 1, total membrane proteins solubilized by 1% Brij 58; 2, membrane proteins solubilized in 3% OPOE after sequential solubilizations in Triton X-100 and sodium lauryl sarcosinate; 3, FPLC partially purified sample after Mono-Q HR 5/5 anion-exchange chromatography. Molecular masses (in kilodaltons) of the HopA (37-kDa), HopB (38-kDa), HopC (39-kDa), HopD (55-kDa), and 50-kDa proteins in their non-heat-modified forms are indicated on the right.

a porin, it did appear to have surface-exposed epitopes and to be conserved in *H. pylori* (9).

Additional purification attempts with subsequent passages through anion-exchange, cation-exchange, or chromatofocusing columns resulted in no further separation of these four proteins. Gel purification with SDS-polyacrylamide gels was therefore used to purify each protein to homogeneity. After cutting bands out of the gels and eluting them, the proteins still appeared to maintain their structural integrity. Each pure protein, when run on an SDS-polyacrylamide gel without heating in solubilization buffer prior to electrophoresis, migrated with apparent molecular weights identical to those seen for the unheated proteins prior to gel purification, and the pure proteins were still capable of being modified by heating (Fig. 3).

A separate protocol was developed to purify the 48-kDa heat-modifiable HopA protein since it was poorly extracted by

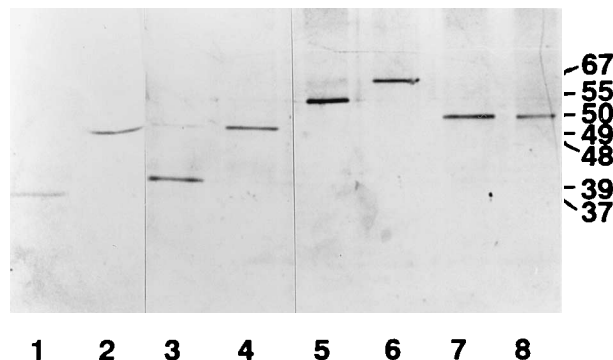


FIG. 3. Silver stained SDS-polyacrylamide gel showing heat modifiability of gel-purified porin proteins. The HopA (37-kDa), HopB (38-kDa), and HopD (55-kDa) proteins which were not heated prior to loading are shown in lanes 1, 3, and 5, respectively. These same proteins heated for 20 min at 95°C prior to loading are seen with apparent molecular masses of 48, 49, and 67 kDa in lanes 2, 4, and 6, respectively. The 50-kDa protein which was not heat modifiable is shown unheated (lane 7) and heated (lane 8). Molecular masses (in kilodaltons) of protein markers are indicated on the right.

48 kDa (HopA)	E	N	D	G	V	F	M	S	V	G	Y	Q	I	G	Q	A	V	Q	Q	V				
49 kDa (HopB)	E	E	D	G	G	F	M	T	F	G	Y	E	L	G	Q	V	V	Q	Q	V	X	N	P	G
50 kDa (HopC)	E	D	D	G	G	F	F	T	V	G	Y	Q	L	G	Q	V	M	Q	D	V	Q	N	P	G
67 kDa (HopD)	E	D	N	G	P	E	V	A	A	G	Y	Q	D	G	E	S	S	Q	M	V				

50 kDa Protein V T Y E V H G D F I N F S K V G F N H  
(not heat modifiable)

FIG. 4. N-terminal amino acid sequence comparisons. Identical residues are boxed. The single-letter amino acid code is utilized. X, not definitely determined.

3% OPOE. This involved selective extraction of membranes into 1% Brij 58, leading to a significant enrichment of this protein relative to OPOE extracts (Fig. 2, lane 1). The protein was further purified by two rounds of SDS-PAGE and elution (Fig. 3, lane 1) and was still heat modifiable after this treatment (Fig. 3, lane 2).

**N-terminal amino acid sequence comparisons.** Automated Edman degradation allowed the amino-terminal sequences to be obtained for all four of the isolated heat-modifiable proteins as well as for the non-heat-modifiable 50-kDa protein that copurified with HopB, -C, and -D after FPLC. The results shown in Fig. 4 show that the amino-terminal 20 to 24 residues of the four heat-modifiable proteins displayed strong homology to each other, while the 50-kDa non-heat-modifiable protein showed virtually no sequence similarity with the heat-modifiable proteins. A comparison with known sequences by use of the basic local alignment search tool system (1) showed no homology to the sequences of other porin proteins, and searches of GenPept, SWISS-PROT, and PIR data banks failed to reveal other homologs.

**Black-lipid bilayer analysis of proteins.** All four heat-modifiable proteins that were isolated showed porin activity in a lipid bilayer model membrane system. Stepwise increases in conductance across the planar lipid bilayer membrane due to incorporation of single channels were seen only upon the addition of protein in 0.1% Triton X-100 solution and not with protein-free detergent solutions alone (Fig. 5). Channels were observed when as little as 1 to 2 ng of pure protein was added to a chamber containing approximately 6 ml of salt solution. Single-channel conductance measurements in all salt solutions were distributed about a single mean for each of the proteins

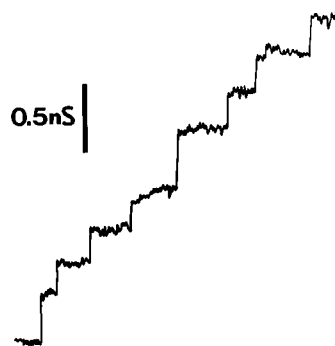


FIG. 5. Stepwise conductance increases for the HopB protein. Conductance increases were recorded upon addition of 10 ng of protein to the aqueous phase (1.0 M KCl) bathing a planar lipid bilayer. Each stepwise increase in conductance represents the incorporation of a pore-forming protein molecule into the lipid bilayer.

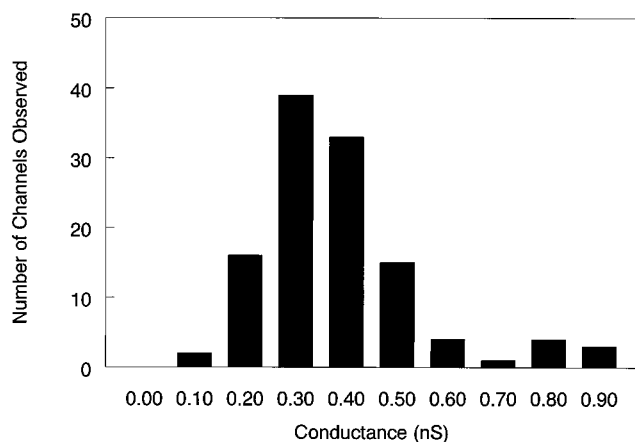


FIG. 6. Histogram of conductance measurements for the HopB protein. The frequency of insertion of channels of each size was charted to enable a determination of the average single-channel conductance for a specific protein. This was done for each protein in each salt solution used, but only the HopB protein in 1.0 M KCl (pH 7.0) is represented here.

(e.g., Fig. 6), and all four porins had similar mean conductances (Table 1). The average single-channel conductance for HopA was 0.36 nS in 1.0 M KCl, but measurements with other salt solutions were not performed. The 50-kDa protein, which was not heat modifiable but which copurified with the porin proteins, showed no pore-forming ability. A linear relationship was observed between the salt concentration and conductance, indicating that these *H. pylori* porins form water-filled channels similar to other porins such as *Escherichia coli* OmpF. The low conductances observed with CH<sub>3</sub>COOK as the mobile salt were consistent with a weak selectivity for anions over cations for the *H. pylori* porins. HopB and HopC proteins showed marked decreases in single-channel conductance at lower pH, while HopD appeared to be unaffected.

## DISCUSSION

Porins are proteins with monomer molecular weights normally ranging from 25 to 50 kDa that form channels which facilitate the diffusion of small water-soluble molecules across the bacterial outer membrane. Porins may be classified into two types, nonspecific porins with large channels (0.6 to 2.3 nm in diameter), which allow nonspecific diffusion of substrates, and specific porins which have substrate binding sites (27). Porins possess a high proportion of  $\beta$ -sheet structure, which traverses the membrane in a tightly packed  $\beta$ -barrel organization (3), and this makes them relatively resistant to denaturation by SDS at low temperature but not at higher tempera-

TABLE 1. Average single-channel conductance measurements

Salt	HopB		HopC		HopD	
	Conductance (nS)	<i>n</i> <sup>a</sup>	Conductance (nS)	<i>n</i>	Conductance (nS)	<i>n</i>
0.3 M KCl (pH 2.0)	0.12	98	0.11	105	0.09	172
1.0 M KCl (pH 2.0)	0.10	66	0.20	42	0.24	36
1.0 M KCl (pH 4.0)	0.23	106	0.31	127	0.25	149
1.0 M KCl (pH 7.0)	0.36	265	0.32	210	0.24	298
3.0 M KCl	0.68	104	0.64	194	0.66	182
1.0 M LiCl	0.20	97	0.24	158	0.27	128
1.0 M CH <sub>3</sub> COOH	0.11	90	0.13	158	0.09	136

<sup>a</sup> *n*, number of channel forming events observed.

tures. Porins therefore display different mobilities when separated at low or high temperatures. Two-dimensional gel analysis involving the electrophoresis of an unheated *H. pylori* membrane sample in the first dimension and then heating the samples at 95°C prior to separation in the second dimension showed that an unusually large number of heat-modifiable proteins exist in *H. pylori*. In this study, proteins migrating with apparent molecular masses of 37, 38, 39, and 55 kDa which were heat modifiable to 48, 49, 50, and 67 kDa, respectively, were chosen as possible porin candidates. These proteins possessed molecular masses in an appropriate range for typical porin proteins, although 67 kDa is at the high end of the scale (cf. *Pseudomonas stutzeri* 65-kDa NosA protein [20]). In addition, the 45- to 50-kDa size range was similar to that of porins isolated in *Campylobacter* species (28). However, the *Campylobacter* porins are more readily identifiable than the *H. pylori* porins because they constitute the predominant species in SDS-PAGE of both whole-cell lysates and membrane preparations (21, 22). In the case of the HopA, -B, -C, and -D *H. pylori* porins, the individual proteins are present in a lower copy number than the *Campylobacter* porins and do not constitute the predominant species in equivalent preparations. Also, in contrast to the *Campylobacter* porins (21, 22), these *H. pylori* porins appear not to be peptidoglycan associated, as tested by the classical Rosenbusch procedure (9, 34).

Many porins exist as trimers (26), and denaturation of the trimers to the monomeric form results in a loss of porin activity. However, the porins of *Campylobacter* species are functional as monomers (17, 28). Since the last step in the purification of the Hop porins was extraction from SDS-polyacrylamide gels, these *H. pylori* proteins would appear to fit into this latter subclass of porins. Nevertheless, the HopA, -B, -C, and -D *H. pylori* porins differ from the *Campylobacter* porins with respect to the size of the channels they produce. The Hop porin single-channel conductances in 1.0 M KCl of 0.24 to 0.36 nS are markedly lower than those found for the *Campylobacter coli* (0.53 nS), *Campylobacter jejuni* (0.82 nS), and *Campylobacter rectus* (0.49 and 0.60 nS) porins (17, 18, 28). The relatively small channel size indicated by the low conductance measurements (5) is comparable to that of porins isolated from other antibiotic-resistant bacteria, such as the small-channel form of OprF from *Pseudomonas aeruginosa* and a porin from *Pseudomonas cepacia*, which have single-channel conductances in 1.0 M KCl of 0.34 and 0.23 nS, respectively (30, 42). Because the small channel size would restrict passage of hydrophilic antibiotics, this property of *H. pylori* porins might contribute to the in vivo antibiotic insensitivity of this organism.

The small single-channel conductance measurements were also consistent with a possible role for these proteins as substrate-specific channels. Channels with substrate specificity have pore sizes which are generally about an order of magnitude smaller than general diffusion pores such as OmpF of *E. coli* (1.8 nS). For example, *P. aeruginosa* protein OprP (0.25 nS), which is phosphate specific (15, 16), OprB (0.035 nS), which is a glucose-selective porin from *Pseudomonas putida* (36), and the *E. coli* porins LamB (0.16 nS) and Tsx (0.01 nS), which are maltodextrin and nucleoside specific, respectively (6, 7), all demonstrate very low single-channel conductances in model membrane studies. The decreased channel sizes for specific porins are probably due to a constriction of the pore to form the specific binding site. However, there is to date no evidence for substrate specificity for the *H. pylori* porins described here. The HopB, -C, and -D porins showed substantially reduced conductance when 1.0 M CH<sub>3</sub>COOK rather than 1.0 M KCl was used as a salt solution. CH<sub>3</sub>COO<sup>-</sup> is a large

anion, and apparently, restricted conductance of this large anion may indicate a channel selectivity for anions, especially since there was no decrease in conductance in experiments with  $\text{Li}^+$ , which is usually highly hydrated and thus present as a large cation. Alternately, these data may reflect the small size of the channel which could restrict diffusion of acetate. Decreases in conductance were also observed for the HopB and, to a lesser extent, the HopC protein at acidic pH. These data may reflect a change in protein conformation with a drop in pH, or they may be partially due to membrane instability at a low pH. Considering that *H. pylori* exists in the acidic environment of the stomach for some period of time, any alteration in channel function or structure corresponding to a change in pH may be significant in the survival of the organism.

The lack of homology between the amino-terminal sequence of these *H. pylori* porin proteins and those of other porins is not surprising, because porins often show varied sequences in this region of the molecule. However, the HopA, -B, -C, and -D porins possessed amino-terminal sequences which were clearly homologous to each other. This combined with their solubilization properties and similar channel behavior is strong evidence that they are members of a family of four closely related porins. The copurification of HopB, -C, and -D suggests that they are quite similar in their biophysical properties. However, HopA appears to be somewhat different because of its poor solubility in 3% OPOE. HopA also appears to be the immunodominant member of the family (9). Indeed, when membranes were used to raise monoclonal antibodies, a total of 27 antibodies were raised against this protein. One was raised against the 50-kDa HopD protein, and none was raised against HopB and -C. Three of the monoclonal antibodies to HopA reacted with cell surface-exposed epitopes, one being strain specific, while the other two recognized the equivalent protein in only one of the other 15 strains of *H. pylori* tested, two of which were fresh clinical isolates (9, 10). The surface-exposed regions of HopA therefore appear not only to differ from surface-exposed regions of HopB, -C, and -D, but also to vary from strain to strain. The differences may be due to different expression levels of these proteins in each strain, but culture conditions and the number of passages of the strain may also play some role in expression. The monoclonal antibody to the surface-exposed epitope on HopC was not reactive with HopA, -B, or -D (9), although it did react with the equivalent protein in all *H. pylori* strains tested. No antigenic cross-reactivity was shown with these monoclonal antibodies nor with the porin of the phylogenetically related *C. coli*. Instead, the *Campylobacter* porin carries epitopes which are cross-reactive with a 31-kDa outer membrane protein of *H. pylori* (9). This protein may be the same one as the 30-kDa protein recently reported by Tufano et al. (39), who, on the basis of heat-modifiable behavior, suggested that this protein may be a porin. The 31-kDa protein was present in the 3% OPOE and 1% Brij fractions isolated in this study but was not enriched by these extraction procedures, suggesting that it has solubilization characteristics different from those of the HopA, -B, -C and -D proteins. This together with the significantly smaller subunit  $M_r$  and the antigenic cross-reactivity it shares with *Campylobacter* porins suggests that it represents a second class of *H. pylori* porin (12).

The Hop family of porins probably influences nutrient uptake by *H. pylori* and presumably plays an important role in the ability of this pathogenic bacterium to colonize and persist in its gastroduodenal niche. In addition, certain porins have been reported to have various other roles in pathogenesis (25, 37, 38). Recently, Tufano et al. (39) provided evidence that their 30-kDa heat-modifiable outer membrane protein has a variety of immunobiological activities, modifying the behavior of poly-

morphonuclear leukocytes and inducing the release of some cytokines from human lymphocytes-monocytes. Studies are now under way to determine whether the Hop porins may also contribute to the pathogenesis of *H. pylori* infection. In addition, because *H. pylori* is such a widespread pathogen, it is critical that a simple, rapid, and reliable means to completely eradicate the organism from the body be found. It is hoped that by studying porins in *H. pylori*, insight into the general physiology of the outer membrane of *H. pylori* will be gained. Of special interest is information regarding mechanisms by which specific molecules, including antibiotics, penetrate the outer membrane of the organism, and this knowledge should have a direct impact on antibiotic therapy.

#### ACKNOWLEDGMENTS

This work was supported by the Canadian Bacterial Diseases Network and by grant R01AI29927-01A2 from U.S. Health and Human Services to T.J.T. The support of Astra Pharma Inc. is also acknowledged. M.M.E. was the recipient of a scholarship from the Natural Sciences and Engineering Research Council of Canada.

Sandy Kielland is thanked for amino-terminal amino acid sequence analysis.

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