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Antigenic Determinants of the OmpC Porin from *Salmonella typhimurium*

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The antigenic determinants of *Salmonella typhimurium* OmpC were investigated by the analysis of cyanogen bromide (CNBr)-generated porin peptides with antiporin monoclonal antibodies (MAbs). We identified six bands (f1 to f6) with estimated molecular masses of 35.5, 31.0, 25.0, 22.5, 13.8, and 10.0 kDa, respectively. In addition, two small fragments (f7 and f8; 3.0 to 6.0 kDa) were detected only infrequently. The OmpC monomer or its CNBr-generated peptides were electrophoretically transferred to a polyvinylidene difluoride membrane and then subjected to amino acid composition analysis and N-terminal sequencing. A comparison of the amino acid composition data with known compositions of *Escherichia coli* and *Salmonella typhi* OmpC showed some differences; however, the amino acid sequences of 71 residues identified in *S. typhimurium* showed 88 and 98% identity with OmpC from *E. coli* and *S. typhi*, respectively. The screening of CNBr peptides with the 12 anti-(*S. typhimurium*) OmpC MAbs by Western blot (immunoblot), in conjunction with the prediction of the OmpC folding pattern based on the known three-dimensional structure of *E. coli* OmpF, showed that four MAbs reacted with surface-exposed epitopes on loops L2, L8, and L4 to L7, four MAbs reacted with a region in the eyelet structure on loop L3, and four MAbs reacted with the buried epitopes on transmembrane β strands. The MAbs reacting with surface-exposed loops showed no cross-reaction with *E. coli* OmpC, whose sequence has diverged extensively from that of *S. typhi* and (probably) *S. typhimurium* OmpC only in regions of the externally exposed loops. In contrast, MAbs reacting with transmembrane β strands, whose sequence is strongly conserved, showed strong cross-reaction with *E. coli* OmpC. These results show that comparison with the *E. coli* OmpF structure predicts the folding pattern of *S. typhimurium* OmpC rather accurately and that evolutionary divergence in sequences is confined to the external loops. The possible roles of these surface-exposed and buried epitopes as potentially useful antigenic regions for diagnostic assays and vaccine development are discussed.

The outer membrane (OM) of *Salmonella typhimurium* and other gram-negative bacteria contains a family of pore-forming proteins called porins (22, 31). These proteins function as water-filled channels that allow passive diffusion of nutrients across the OM (31). Two general porins, OmpC and OmpF, and a third porin, PhoE, with preference for negatively charged solutes like phosphate, are produced by *Escherichia coli* K-12 and *S. typhimurium* LT-2 (22, 31). In addition, *S. typhimurium* also synthesizes a fourth general porin, OmpD (22, 31).

The structures of OmpF and PhoE from *E. coli* (7) and a porin from *Rhodobacter capsulatus* (43) have been resolved by X-ray crystallography. These proteins form hollow cylinders that consist of antiparallel β strands; long hydrophilic loops of irregular length and short β-hairpin turns connect these strands on the external and periplasmic surfaces of the OM bilayer, respectively (6, 7, 17, 18, 20, 30, 39, 43). The primary structure of porins varies significantly among gram-negative bacteria (12), but amphiphilic β strands (7 to 14 residues each) in the porin barrel are structurally conserved (12, 18). Generally, porins contain five or more surface epitopes, 6 to 25 residues in length (20, 33, 41, 42), that are partially obscured by the lipopolysaccharide (LPS) core and completely blocked by O-antigen sugars (2, 23). These proteins also contain conserved buried epitopes that are localized on the transmembranous β strands (18, 20, 33, 41).

We have previously reported on the immunological relatedness of OmpC and OmpD porins from several *Salmonella* serotypes, including *S. typhimurium*, *Salmonella typhi*, and *Salmonella paratyphi* (37). The amino acid sequences of OmpC from *E. coli* (26) and *S. typhi* (33) are known; however, the three-dimensional structure of OmpC is not solved yet, and the *S. typhimurium* OmpC has not been sequenced either. We are interested in these proteins because of their potential role in diagnostic assays, in antibiotic resistance, and as immunogens for vaccination (3, 16, 21, 25, 28, 29, 34, 40, 41). Cyanogen bromide (CNBr)-cleaved peptides containing internal Met residues have been used to determine the specificity of monoclonal antibodies (MAbs) to porins (14, 20, 27, 35). In this study, we used anti-OmpC MAbs to define the surface and buried epitopes of *S. typhimurium* OmpC. Our results confirm that the OmpC protein folds in a way very similar to that of *E. coli* OmpF (7), as all surface-exposed epitopes were indeed localized to areas predicted to be external loops. The amino acid compositions of OmpC in *E. coli* (26), *S. typhi* (33), and *S. typhimurium* (this study) showed some differences, but the sequences of 71 residues identified by N-terminal analysis of CNBr-generated peptides were 88 and 98% identical with OmpC from *E. coli* and *S. typhi*, respectively.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The culture media, growth conditions, and *S. typhimurium* strains used in this study, including SH 5014 (*rfa* mutant), SH 7454, and SH 7457, have been described previously (37). The first strain expresses all three porins whereas the latter two express OmpD and OmpC proteins, respectively, as their sole porin when grown under highly osmotic conditions. *E. coli* JF 694, JF 701, and JF 703 (provided by K. Gehring) express PhoE, OmpF, and OmpC as their sole porins, respectively (11).

Isolation and purification of porins, OM, and LPS. The native porins (trimer)

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were isolated and purified by solubilization of bacterial cell envelopes in 1% sodium dodecyl sulfate (SDS)–0.5 M NaCl followed by size exclusion chromatography with Sephacryl S-200 (11). Denatured monomeric porin (monomer) was prepared by boiling the trimer at 100°C for 5 min in 1% SDS (20).

The OM was isolated by suspending the cell pellet in 10 mM HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), pH 7.4, and passing the suspension twice at 16,000 lb/in^2 through a French pressure cell (American Instrument Co.). OM fragments were then purified by sucrose density gradient centrifugation (38). LPS (R type) was isolated from *S. typhimurium* SH 7457 by the phenol-water extraction procedure of Galanos et al. (9).

Production of anti-OmpC MAbs. BALB/c mice were immunized with OmpC monomer, and cell fusion was carried out as described previously (37). Hybridomas were selected in hypoxanthine-aminopterin-thymidine medium (19). Culture fluids from wells with colonies were assayed by the enzyme-linked immunosorbent assay (ELISA) against porin monomers, trimers, OM, LPS, and whole cells. Hybridomas of interest were cloned by limiting dilution and injected in BALB/c mice for production of ascitic tumors (19). The class and subclass of MAbs were determined by ELISA with goat antisera against mouse heavy and light chains μ, γ_1, γ_{2a}, γ_{2b}, γ_3, κ, and λ (Fisher).

ELISA. ELISA was performed as described previously (37).

CNBr digestion of OmpC. Protein concentrations were measured with the Micro BCA protein assay reagent (Pierce), with bovine serum albumin as standard. Five milligrams of OmpC monomer was precipitated, washed with acetone, and digested with 250 mg of CNBr in 500 μl of 70% trifluoroacetic acid (10) by overnight incubation at room temperature.

Gel electrophoresis and Western blots (immunoblots). SDS-polyacrylamide gel electrophoresis (PAGE) was performed on either 11.5% polyacrylamide gels or 10 to 20% linear gradient gels; proteins were transferred to nitrocellulose paper, and immunoblots were performed as described previously (32, 37).

Amino acid composition and N-terminal sequencing. Purified OmpC or its CNBr peptides were separated by SDS-PAGE and transferred to Immobilon-P polyvinylidene difluoride (PVDF) membrane (Millipore). The transfer was carried out in 10 mM 3-[cyclohexylamino]-1-propanesulfonic acid buffer according to the procedure of Matsudaira (24). Protein bands on the PVDF were localized by staining with 0.1% amido black. The sections of the membrane containing the desired bands were excised, hydrolyzed with 6 N HCl, and analyzed in a Beckman model 6300 amino acid analyzer. For N-terminal sequencing, CNBr-generated peptides on PVDF were applied to a Beckman model LF3000G sequencer. The amino acid sequences of *S. typhimurium* OmpC were compared with those of *E. coli* (26) and *S. typhi* (33) with the Best Fit program of the University of Wisconsin Genetics Computer Group, Madison.

TABLE 1. Epitope specificity of anti-*S. typhimurium* MAbs

MAb[a]	Isotype	MAb reactivity							
		ELISA[b]				Western blot[c]			
		CT	CM	OM	WC	StyC	EcoC	EcoF	EcoE
CM104.1	IgM	+	+	+	+	+	−	−	−
CM61.9	IgG2b	+	+	−	−	+	−	−	−
CM51.1	IgG1	+	+	−	−	+	+	+	+
CM95.3[d]	IgG2a	−	+	−	−	+	+	+	+
CM44.19	IgM	+	+	+	+	+	−	−	−
CM206.11	IgM	+	+	+	+	+	−	−	−
CM73.3	IgG1	+	+	−	−	+	+	+	+
DM53.1	ND[e]	ND[e]	+	−	−	+	+	+	+
CM12.1	IgG2a	+	+	−	−	+	+	−	−
CM54.2	IgG1	+	+	−	−	+	+	−	−
CM88.1	IgG2a	−	+	−	−	+	+	−	−
CM190.2	IgG2b	+	+	−	−	+	+	−	+

[a] Purified porins, isolated from *S. typhimurium* SH 7457 (OmpC$^+$) or SH 7454 (OmpD$^+$), were used for immunizing mice; CM, OmpC monomer; DM, OmpD monomer.

[b] ELISA was performed as described in Materials and Methods. Antigens: CT, OmpC (trimer); CM, OmpC (monomer); OM, outer membrane from strain SH 5014, which expresses all three porins; WC, whole cells from strain SH 5014 with Ra-type LPS. Positive reactions were scored as weakly positive (+) or strongly positive (+) if the absorbances were greater than two times (but less than three times) the background or greater than three times the background, respectively.

[c] Cell envelopes from bacterial strains selectively expressing *E. coli* porins OmpC (JF 703), OmpF (JF 701), and PhoE (JF 694) and *S. typhi* OmpC (37) were lysed with SDS, subjected to SDS-PAGE, and transferred to nitrocellulose. The paper was blocked with 1% gelatin and cut into vertical or horizontal strips. The strips were incubated overnight with anti-porin MAb, washed, incubated with alkaline phosphatase-labeled goat anti-mouse immunoglobulin (Ig) for 3 h, washed, and developed with nitroblue tetrazolium-bromochloroindolyl phosphate. Reactions were scored as negative (−), weakly positive (+), or strongly positive (+) in comparison with the intensity of reaction with the immunogen porin and negative controls.

[d] CM95.3 was designated MAb 58 in our previous report (37).

[e] ND, not determined.

RESULTS AND DISCUSSION

MAb specificities. Ten previously unreported MAbs were raised to *S. typhimurium* OmpC (Table 1). In addition, one previously unreported MAb (DM53.1) was raised to the OmpD monomer. The test panel also included anti-OmpC MAb CM95.3, which recognized a buried epitope on the OmpC porin (Table 1) (37).

Specificities of the new MAbs were determined by their reactivity with purified porins, OM, and intact whole cells in ELISA and their immunoblot reactivity with either denatured whole cell or cell envelope lysates (Table 1). None of the MAbs in the panel reacted with LPS (data not shown). Three MAbs in the panel (CM44.19, CM104.1, and CM206.11) bound to OmpC monomer, trimer, OM, and whole cells; their epitopes are exposed on the cell surface (20, 37, 42). These determinants are probably sequential in nature since their recognition is not affected by denaturation of the protein. The remaining eight MAbs (CM12.1, CM51.1, CM54.2, CM61.9, CM73.3, CM88.1, CM190.2, and DM53.1) bound to purified OmpC but did not react with OM or intact whole cells; their epitope(s) is buried, either in the membrane bilayer or in the tertiary structure of OmpC. These epitopes may have a constrained conformation that limits their detection and binding on the cell surface (4). The globular surface domains of an OM protein may shield its other surface epitopes from MAb binding, and the failure of an antibody to recognize a periplasmic or external surface determinant does not conclusively identify this epitope as buried in the OM bilayer (36).

Identity of CNBr fragments. Since CNBr cleaves polypeptides at the carboxy-terminal end of Met residues (10), complete digestion of *S. typhi* (and possibly *S. typhimurium*; see below) OmpC with CNBr would produce four peptides: amino acids (aa) 1 to 36, 37 to 121, 122 to 321, and 322 to 357 (Fig. 1), with calculated molecular weights of 4,057, 9,484, 21,579, and 4,111, respectively. However, we detected at least six bands (fragments) with estimated molecular masses of 35.5 (f1), 31.0 (f2), 25.0 (f3), 22.5 (f4), 13.8 (f5), and 10.0 (f6) kDa in CNBr digests of *S. typhimurium* OmpC (data not shown). Of these, f4(122-321) and f6(37-121) were the only complete digestion products identified in our gel system. The smallest fragments, f7(1-36) and f8(322-357) (each 3 to 6 kDa), were detected only infrequently, because they ran at the dye front under the electrophoresis conditions used (27). This suggests that fragments f1, f2, f3, and f5 resulted from incomplete digestion of OmpC, which was confirmed by the sequence alignment (see below). The partial cleavage of proteins with CNBr, as observed in this study, has been reported for other OM proteins also (10, 20, 27, 35); it occurs because of the incomplete cleavage of Met-Ser or Met-Thr sequences which can form homoserine and are therefore not cleaved (10, 27).

Electrophoretically separated peptides of *S. typhimurium* were transferred onto PVDF membrane, and the fragments f1, f3, f4, f5, and f6 were excised and sequenced. The sequences of the first 10 to 35 aa were identified, aligned, and compared with the known sequences of OmpC from *S. typhi* (33) and *E. coli* (26) (Fig. 1). From their N-terminal sequences and molecular weights, fragments f1, f3, f5, and f6 were unambiguously identified. Fragments f2, f4, f7, and f8 did not give distinct N-terminal sequence, but their identities were deduced from their relative electrophoretic mobilities and the process of elimination (Fig. 1).

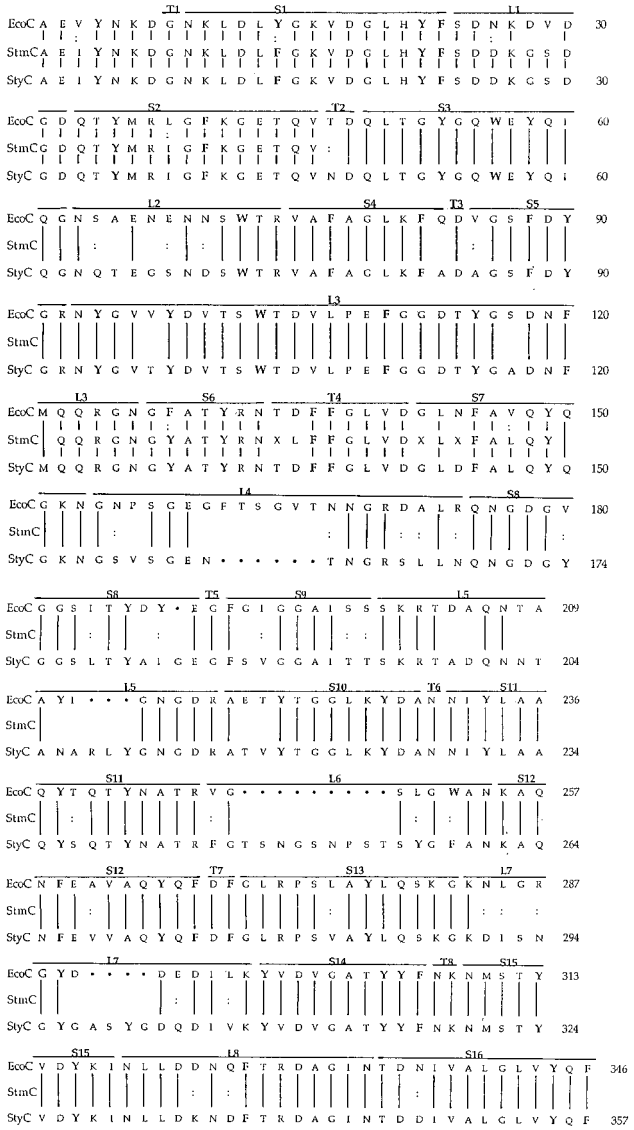

FIG. 1. Alignment of OmpC sequences with folding predictions. N-terminal sequences of the *S. typhimurium* OmpC (middle line) were compared with known amino acid sequences of *E. coli* OmpC (upper line) and *S. typhi* OmpC (bottom line). Boldface dots indicating deletion of amino acid residues and extra sequences are included in the *E. coli* and *S. typhi* proteins to obtain the best-fit alignment (Best Fit algorithm; Genetics Computer Group). Residues of identity (|) and similarity (:) between the proteins are shown; aromatic residues are indicated by boldface letters. The externally exposed loops (L1 through L8), periplasmic turns (T1 through T8), and membrane-spanning β strands (S1 to S16) were predicted by a comparison of the OmpC sequence with the three-dimensional structure of *E. coli* OmpF (7, 18). The amino acid sequences are shown with the conventional one-letter codes: A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine; X, unknown.

The 71 amino acids identified in *S. typhimurium* OmpC were aligned and compared with the known sequences of OmpC from *S. typhi* (33) and *E. coli* (26) (Fig. 1). Residues of identity and similarity among these proteins and aromatic residues which tend to flank the membrane-spanning β strands were identified. Since the three-dimensional structure of OmpC is unknown at this time, the prediction of its exposed loops (L1 through L8), periplasmic turns (T1 to T8), and membrane-spanning β strands (S1 to S16) was made (Fig. 1) by comparing the sequences of OmpC with that of *E. coli* OmpF (7, 18). Six of these sequences (L1, L2, L4, L5, L6, and L7) correspond to the regions with pronounced differences between *E. coli* and *S. typhi* proteins (33). As expected, *S. typhimurium* OmpC, on the basis of the 71 amino acid residues identified in this study, is more closely related to OmpC from *S. typhi* (98%) than to that from *E. coli* (88%). Similarly, the amino acid composition of *S. typhimurium* OmpC, despite some differences in the number of Glx, Gly, and Tyr residues, showed considerable homology with OmpC from *S. typhi* (Table 2). These results are consistent with the highly conserved nature of the OmpC gene within various *Salmonella* serotypes (34).

TABLE 2. Amino acid compositions of OmpC porin from *Salmonella* spp. and *E. coli*[a]

Amino acid	No. of residues/mole[b]		
	S. typhimurium[c]	*S. typhi*[d]	*E. coli*[d]
Asx	63	64	63
Thr	25	27	24
Ser	21	22	16
Glx	35	29	32
Pro	3	3	3
Gly	53	49	47
Ala	27	26	24
Val	20	19	21
Met	3	3	3
Ile	11	11	10
Leu	24	21	22
Tyr	24	31	29
Phe	19	20	19
His	1	1	1
Lys	14	16	15
Arg	11	12	13
Cys	ND[e]	0	0
Trp	ND[e]	3	4

[a] Purified *S. typhimurium* OmpC was electrophoresed by SDS-PAGE, and the protein was transferred to PVDF as described in Materials and Methods. The blotted monomer band was excised, hydrolyzed with 6 N HCl, and analyzed in a Beckman model 6300 amino acid analyzer.

[b] Without the signal peptide.

[c] Average value from three analyses, rounded to the nearest whole number. Estimate is based on 354 aa in *S. typhi* OmpC, without taking into account the loss of Trp during hydrolysis.

[d] Compositions of OmpC from *S. typhi* and *E. coli* were taken from the work of Puente et al. (33) and Mizuno et al. (26), respectively.

[e] ND, not determined.

MAb reactivity with CNBr fragments. The OmpC protein from *S. typhimurium* was digested with CNBr, electrophoresed, and transferred to nitrocellulose. The fragments were analyzed for their immunoreactivity with 11 anti-OmpC MAbs and 1 anti-OmpD MAb. Each antibody recognized at least one product of CNBr digestion, and all MAbs (except CM104.1) reacted strongly with the f3 band (Table 3). The remaining 11 MAbs in the panel were classified into four distinct groups on the basis of their immunoreactivity with CNBr peptides in Western blot (Table 3; see Fig. 3).

Group I contained a single MAb (CM61.9) that reacted with band f3 (see Fig. 3); weak reactivity of CM61.9 seen infrequently for f1 and f2 was nonspecific, similar to that of negative controls, and never reproducible. Failure of this antibody to bind f1, f2, or f4 indicates that the epitope recognized by this MAb is localized to a region between residues 322 and 357 on the carboxy-terminal peptide f8 (Fig. 2). This antibody did not react with OM or intact whole cells (Table 1). Nevertheless, the absence of cross-reaction with *E. coli* OmpC (Table 1)

TABLE 3. Reactivity of antiporin MAbs with CNBr peptides of *S. typhimurium* OmpC[a]

MAb	Reactivity with peptide						Probable epitope[b]
	f1	f2	f3	f4	f5	f6	
CM104.1	−	−	−	−	−	+	L2
CM61.9	−	−	+	−	−	−	L8
CM51.1	+	+	+	−	−	−	TM
CM95.3	+	+	+	−	−	−	TM
CM44.19	+	+	+	+	−	−	L4 to L7
CM206.11	+	+	+	+	−	−	L4 to L7
CM73.3	+	+	+	+	−	−	TM
DM53.1	+	+	+	+	−	−	TM
CM12.1	+	+	+	+	+	+	L3
CM54.2	+	+	+	+	+	+	L3
CM88.1	+	+	+	+	+	+	L3
CM190.2	+	+	+	+	+	+	L3

[a] Purified porin was digested with CNBr and separated by SDS-PAGE on a 10 to 20% gradient gel, and the peptides were transferred to nitrocellulose. The paper was cut into strips which were incubated with individual MAbs, washed, and amplified with goat anti-mouse immunoglobulin. The strips were then washed and developed with alkaline phosphatase-labeled rabbit anti-goat immunoglobulin–nitroblue tetrazolium-bromochloroindolyl phosphate. Reactions were scored as indicated in footnote *c*, Table 1.

[b] L1 to L8, externally exposed loops; TM, transmembrane β strands, as predicted in the OmpC model (Fig. 1).

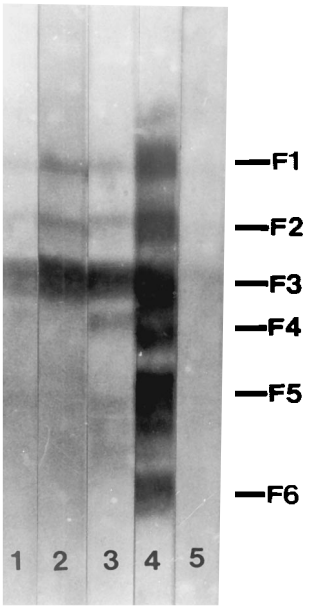

FIG. 3. Western immunoblot of anti-porin MAbs with CNBr-generated peptides of *S. typhimurium* OmpC. The CNBr peptides were separated by SDS-PAGE and immunoblotted (see Table 3, footnote *a*). Strips 1, 2, 3, and 4 were probed with CM61.9, CM95.3, DM53.1, and CM190.2, respectively; these represent the immunoreactivity pattern observed with MAb groups I through IV, respectively (see text for explanation of MAb groupings and for identification of CNBr bands); strip 5 was incubated with ascites fluid from the cell fusion partner P3x63-Ag.8.653.

indicates that the epitope recognized by this antibody must be located on loop L8 because L8 is the only region on this fragment where the sequences of *S. typhi* (and possibly *S. typhimurium*) differ from those in *E. coli* (Fig. 1). The expected binding of this and group II MAbs (see below) to f8 could not be shown directly; we assume that this peptide ran at the dye front under the electrophoresis conditions used.

Group II MAbs (CM51.1 and CM95.3) reacted with bands f1, f2, and f3 (Table 3; Fig. 3), indicating that the epitope(s) recognized by these MAbs is likely localized on a buried region between residues 122 and 321 (Fig. 2). This region contains membrane-spanning β strands S6 to S14 (Fig. 1), and since these antibodies cross-react with *E. coli* OmpC, OmpF, and PhoE (Table 1), their epitope(s) may be present on any one of these conserved strands (18). Group III MAbs (CM44.19, CM206.11, CM73.3, and DM53.1) reacted with bands f1, f2, f3, and f4. The surface-specific MAbs in this group (CM44.19 and CM206.11) do not cross-react with *E. coli* porins (Table 1), and therefore, the epitopes recognized by these antibodies are probably located on the externally exposed loops (L4 through L7 [Fig. 1]) on peptide f4 (aa 122 to 321 [Fig. 2]). On the other hand, buried epitopes recognized by CM73.3 and DM53.1 are likely present on the conserved transmembranous strands (S6 through S14 [Fig. 1]) on peptide f4.

Group IV MAbs (CM12.1, CM54.2, CM88.1, and CM190.2) bound CNBr fragments f1 through f6 (Table 3; Fig. 3), indicating that the epitope recognized by this panel of MAbs is likely localized at or near residue 121 (Fig. 2). This residue is within the eyelet-forming loop, L3 (7, 39), which folds into the barrel, leading to the inaccessibility of the epitope on the cell

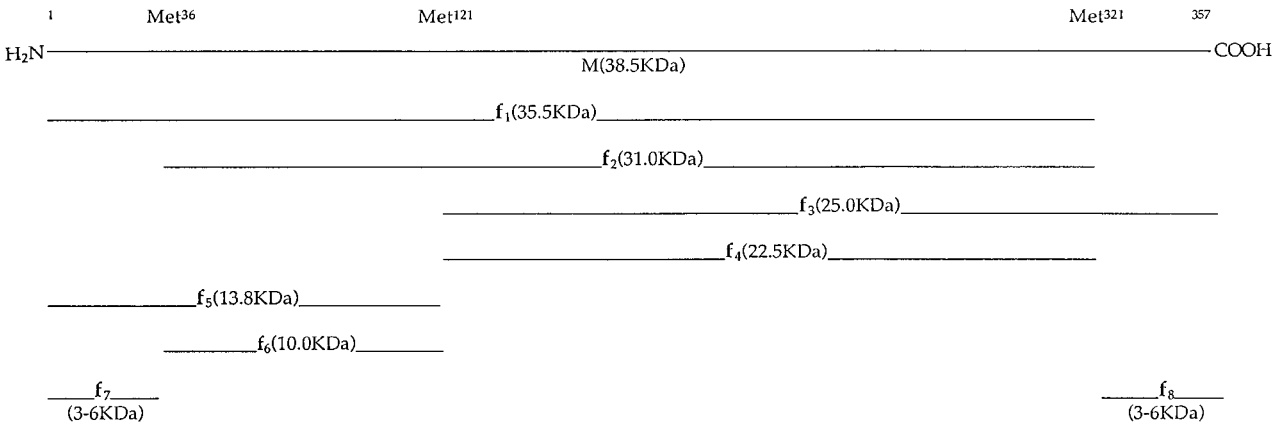

FIG. 2. Alignment of CNBr peptides of *S. typhimurium* OmpC. The peptides (f1 to f8) were aligned with the mature OmpC sequence of *S. typhi* (33) by using the molecular weight estimates of CNBr fragments from SDS-PAGE, and their primary amino acid sequences were deduced from N-terminal sequencing. See text for the identification of fragments. M, monomer.

surface. Loop L3 contains 1 1/2 turns of α-helix, contributes to the constriction of the pore, and is responsible for ion selectivity (7). As seen in Fig. 1, sequence around residue 121 is well conserved, and these MAbs cross-react with *E. coli* OmpC, as predicted. Finally, CM104.1, a surface-specific antibody, bound to band f6 (aa 37 to 121) only, indicating that its epitope is localized into an externally exposed loop on this peptide. Since this antibody does not react with *E. coli* OmpC, it is very likely that its epitope is located on L2, given the divergence of this region between *E. coli* and *S. typhi* and nearly total conservation of sequences in other regions within f6. We do not know the reason(s) for the failure of this antibody to recognize band f1, f2, or f5; we assume that its antigenic site was either destroyed or modified (1) by the CNBr used in the digestion process, although the immunoreactivity of the other two surface-specific antibodies (CM44.19 and CM206.11) was not affected by this treatment.

We have thus identified a surface-exposed epitope on peptides f4 (CM44.19 and CM206.11; aa 122 to 321; loops L4 to L7), f6 (CM104.1; aa 37 to 121; loop L2), and f8 (CM61.9; aa 322 to 357; loop L8) (Fig. 1 and 2). The remaining MAbs in our panel recognize buried epitopes which appear to be localized to transmembranous β strands on peptide f4 (CM51.1, CM73.3, CM95.3, and DM53.1; aa 122 to 321; S6 to S14) or adjacent to residue 121 on loop L3 (CM12.1, CM54.2, CM88.1, and CM190.2) (Fig. 1 and 2). Although the present data allow us to only crudely localize the MAb-specific epitopes, we can, nevertheless, draw significant conclusions from the MAb reactivity patterns observed in this study. First, *S. typhimurium* and *S. typhi* are immunologically similar as evidenced by the strong cross-reactivity of all 12 MAbs in the panel with these two organisms (Table 1). This is consistent with our earlier report (37) and with the recent observations of Puente et al. (34) that the *ompC* gene is highly conserved within various *Salmonella* serotypes and that *S. typhi* and *S. typhimurium* genes exhibit 100% homology. Second, some of the MAbs in the panel did not cross-react with *E. coli* porins (Table 1); their epitopes appear to be localized in external loops (L1 through L8 according to our OmpC model). These results first suggest that the folding model of OmpC, based on the structure of *E. coli* OmpF (7), is likely to be essentially correct. They further confirm and strengthen the earlier conclusions drawn by several investigators (7, 18, 30, 39) that the evolutionary divergence of sequences in enterobacterial porins is confined to the externally exposed loops. Third, group IV MAbs recognize an epitope that is located at or near residue 121 within the eyelet-forming loop, L3 (7, 39). However, this epitope is not accessible on the cell surface (Table 1), indicating that the three-dimensional structure of the porin is essential for an accurate interpretation of MAb reactivity. The hydropathy analysis can be misleading since we know that the transmembrane strands can be quite hydrophilic because of the alternation of hydrophobic and hydrophilic residues (6, 7, 18, 30).

Murine salmonellosis, caused by *S. typhimurium*, has traditionally been used as a model for the study of human typhoid disease induced by *S. typhi* (21, 25, 28, 40). The porins from the two organisms appear to be immunologically (37) and structurally (34) related. Therefore, the identification of surface and buried epitopes on the porin molecule, and antibodies that recognize them, is important. These epitopes, or synthetic peptides mimicking such epitopes, may be useful for diagnostic assays, structure-function relationships, and vaccine developments (3, 16, 20, 21, 25, 28, 29, 34, 40, 41). A surface epitope that is antibody accessible and conserved may provide broad cross-protection against related bacterial species (8), whereas a variable epitope may provide species- or strain-specific protection (13, 16). The *S. typhi* OmpC sequence contains eight regions which, on the basis of the model structures of enterobacterial porins (18, 20, 30, 39), correspond to the surface-exposed domains of the porin (33). These are likely to be good candidates for a vaccine against *Salmonella* infections. Furthermore, these epitopes may provide attractive sites for insertion and expression of foreign genes in *Salmonella* strains (5, 15, 34).

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