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Genetic Identification of the Pore Domain of the OmpC Porin of *Escherichia coli* K-12

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We have isolated and characterized 31 mutations in the *ompC* gene which allow *Escherichia coli* to grow on maltotriose (Dex$^+$) in the absence of the LamB and OmpF porins. These *ompC*(Dex) mutations include single-base-pair substitutions, small deletions, and small insertions. DNA sequence analysis shows that all of the alterations occur within the coding region for the first 110 amino acids of mature OmpC. The 26 independent point mutations repeatedly and exclusively alter residues R37, R74, and D105 of mature OmpC. In each case, a charged amino acid is changed to an uncharged residue. Biochemical and physiological tests suggest that these alterations increase the size of the pore channel. Starting with three different *ompC*(Dex) strains with alterations affecting R74, we isolated mutants that could grow on maltohexose (Hex$^+$). These mutants each contained a second alteration in the *ompC* gene involving residues R37, D105, or R124. The combined effects on pore function of the two mutations appear to be additive. These experiments suggest that we have identified the important residues of OmpC peptide involved in pore function. On the basis of these mutations and general rules for membrane protein folding, a model for the topology of the OmpC protein is proposed.

The outer membrane of the gram-negative bacterium *Escherichia coli* K-12 provides the first permeability barrier of the cell. Small hydrophilic molecules cross this structure by diffusing through water-filled channels formed by the two major porins, OmpF and OmpC (18). These proteins also serve as receptors for various bacteriophages, facilitate transport of colicins, and play a role in membrane integrity through their interaction with other membrane components such as lipopolysaccharides and peptidoglycans (for a review, see reference 21).

The DNA sequences for the *ompF* and *ompC* genes are known (9, 15), and the primary amino acid sequences for the proteins are available. These proteins show a high degree of homology to each other and to other porin proteins both at the DNA level and at the protein level (3, 15).

An extensive structural analysis of the porins, predominantly OmpF, has been done by using biochemical approaches such as circular dichroism, infrared spectroscopy, X-ray diffraction, and high-resolution electron microscopy (25). The following facts are generally accepted with regard to the porin structure. (i) The porins exist as trimers. (ii) They are rich in β-sheet structures which are oriented roughly perpendicular to the plane of the membrane. (iii) The porins protrude a little on both sides of the membrane. (iv) They interact with both the peptidoglycan and the lipopolysaccharide moiety of the outer membrane. These studies provide a general model for the anatomy of the porins but do not provide information regarding the locations of functional domains.

One means of defining functional domains of porin proteins is the use of mutants with specific defects. This approach has been very effectively used to study the topology of LamB (6, 8), a maltoporin which, in addition to forming a general pore like OmpF and OmpC, specifically facilitates diffusion of maltose and maltodextrins (30). Experiments using in vivo-constructed hybrid *ompF-ompC* or *ompC-ompF* (16, 22) and *ompC-phoE* or *phoE-ompC* (28, 29) genes provided a rough approximation of the positions of the phage receptor and pore domains for these proteins. We have previously characterized several *ompC* mutants which suggest participation of residue 74 (R74) of the mature OmpC protein in pore function (13, 14).

To define more precisely the pore domain of the OmpC protein, we have characterized 31 independent *ompC* mutants with altered pore properties. Our studies with these mutants suggest that the pore domain resides in the first one-third of the protein and that charged residues are important in determining channel properties of the pore. In addition, we have developed a genetic system which allows us to easily recombine chromosomal *ompC* mutations onto a low-copy-number plasmid (pSC101 replicon).

MATERIALS AND METHODS

Media and chemicals. Minimal (M63) and Luria broth were prepared as described previously (27). Defined maltodextrins (maltotriose to maltoheptose) were purchased from Boehringer Mannheim Biochemicals and used at a final concentration of 1 mM in both liquid and agar medium. Maltodextrin was purchased from Pfanstiehl Laboratories Inc. and was further purified as described previously (13). [^{14}C]maltose and [^{35}S]ATP were purchased from Amersham Corp. Antibiotic disks were purchased from Difco Laboratories. Restriction enzymes and DNA sequencing kits were purchased from New England BioLabs.

Bacterial strains, plasmids and bacteriophages. All strains were derived from MC4100 [F$^-$ *araD139* Δ(*argF-lac*)*U169* *rpsL150* *relA1* *flbB5301* *ptsF25* *deoCl* *thi-1* *rbsR*] (5). MCR106 is MC4100 Δ*lamB106* (7), DME553 is MCR106 Δ*ompF80* (13), and RAM105 is DME553 *zei-06*::Tn*10* (13). All Dex$^+$ mutants were derived from RAM105. Hex$^+$ mutants were derived from various Dex$^+$ mutants (see below for details).

The majority of the *ompC* mutants used in this study exhibited increased sensitivity to ampicillin (Ap) and did not yield Ap^r transformants when transformed with pMAN006 (Ap^r OmpC$^+$), a low-copy-number plasmid (pSC101 replicon) (12). The mutants did not show increased sensitivity to kanamycin. To overcome the increased sensitivity to ampi-

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cillin, the *Bam*HI-*Hin*dIII piece carrying the Km^r determinant from pNEO (P-L Biochemicals, Inc.) was inserted into pMAN006 in several steps (data not shown), resulting in pRAM1006 (Km^r Amp^r OmpC$^+$). Hy2 and SS4 are OmpC-specific phages (1).

Mutant isolation and genetic techniques. Spontaneous mutants able to grow on maltodextrins (Dex$^+$) were isolated starting with strain RAM105 as described previously (13). The *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG)-induced Dex$^+$ mutants were isolated by plating approximately 10^9 mutagenized cells from an overnight culture on M63 medium containing maltodextrin as the sole carbon source. Isolated colonies were picked after 72 h and purified twice by streaking on M63 medium containing maltodextrins. The NTG-induced mutants which were able to grow on maltohexose (Hex$^+$) were selected on M63 maltohexose medium and purified by being streaked twice on the selective medium. Standard genetic techniques, such a P1 transduction, NTG mutagensis, and transformation procedures, were done as described by Silhavy et al. (27). The genetic location of the mutations conferring the Dex$^+$ and Hex$^+$ phenotypes was determined by testing linkage to the *zei06*::Tn*10* marker which is 55% linked by P1 cotransduction to the *ompC* locus (13).

Bacteriological and biochemical techniques. Growth, antibiotic, and detergent sensitivity tests were done as described previously (2, 13). Whole-cell envelope fractions were prepared from 10-ml cultures as described by Morona and Reeves (17). Proteins were analyzed on linear sodium dodecyl sulfate (SDS)-polyacrylamide gels (11% acrylamide) as described previously (11). Plasmid DNA was prepared by the method of Ish-Horowicz and Burk (10).

Cloning and DNA sequencing of the mutant *ompC* alleles. The mutant *ompC*(Dex) alleles were recombined onto pRAM1006 as described below. The *ompC* gene was then subcloned into M13mp19 and sequenced by the dideoxy method of Sanger et al. (26) as described previously (13).

RESULTS

Isolation and DNA sequence analysis of the *ompC*(Dex) mutants. In order for *E. coli* to grow on maltodextrins larger than maltotriose, the LamB maltoporin is required (30). Strains which lack both LamB and OmpF can grow on maltose but are unable to grow on maltotriose (14). We have previously reported *ompC* mutations which allow growth on maltodextrin in the absence of LamB and OmpF (13). All of these mutations affect residue 74 of mature OmpC. To determine whether other OmpC residues can be altered to give a Dex$^+$ phenotype, we characterized an additional 7 spontaneous mutants and 18 NTG-induced *ompC*(Dex) mutants.

To determine the exact nature of the mutations, we sequenced the *ompC* gene from each mutant. Previously, we have sequenced *ompC*(Dex) mutations by subcloning the *Hin*dIII chromosomal fragment that carries the *ompC* gene (13). During the course of these experiments, we noted that Dex$^+$ strains which carried the *ompC* plasmid pRAM1006 segregated faster-growing colonies on maltodextrin medium. We reasoned that the larger colonies arose due to the recombination of the chromosomal *ompC*(Dex) allele onto the plasmid, thereby increasing the expression of the Dex$^+$ allele by increasing the gene dosage. To test this, we prepared plasmid DNA from the larger colonies and transformed it into an *ompC* deletion strain, RAM191, selecting for Km^r. All of the Km^r transformants acquired both a Dex$^+$ and a large-colony phenotype. This confirmed that the plasmid had acquired the *ompC*(Dex) allele and that the presence of the *ompC*(Dex) allele on the plasmid conferred increased growth ability. Plasmid (pRAM1006) DNA prepared from an *ompC* wild-type strain (RAM105) yielded Km^r transformants which were Dex$^-$. The segregation of larger Dex$^+$ colonies required the following conditions. (i) The plasmid carried the *ompC* gene. (ii) The chromosomal *ompC* allele conferred a Dex$^+$ phenotype. (iii) The strain was *recA*$^+$ (data not shown). Together, the results strongly suggest that the plasmid *ompC*(Dex) alleles resulted from recombination with the chromosomal *ompC*(Dex) allele. The *ompC*(Dex) alleles were sequenced as described in Materials and Methods. A summary of the sequence data is shown in Table 1.

TABLE 1. *ompC*(Dex) mutations

Mutation[a]	No. of isolates	Mutagen
CgT to CaT transition (R37 to H)	1	NTG
cGT to tGT transition (R37 to C)	4	NTG
cGT to gGT transversion (R74 to G)[b]	1	None
cGT to aGT transversion (R74 to S)[b]	7	None
cGT to tGT transition (R74 to C)	11	NTG
GaC to GgC transition (D105 to G)	2	NTG
GTGGCT or GTGGCA insertion (V-A insert after R74)[c]	3	None
Deletion from W103 to F110	2	None

[a] The substituted base in a codon is shown in lowercase. Amino acids are given in single-letter codes and shown in parenthesis. The number after the amino acid is the number of the residue in the mature OmpC protein.
[b] A mutant of this type has been described previously (13).
[c] The GTGGCT insert was found in two isolates, and the GTGGCA insert was found in one isolate.

The frequency and types of mutations obtained were influenced by the mutagenic procedure used. Spontaneous mutations were of three types: transversions (C to A and C to G), small insertions, and small deletions. The transversions were identical to *ompC*(Dex) mutations described previously (13) and resulted in the arginine at position 74 of mature OmpC being changed to a serine (R74 to S) or a glycine (R74 to G) residue. The insertion mutants contained 6-base-pair DNA sequences of GTGGCT or GTGGCA which resulted in valine and alanine residues being inserted between amino acids R74 and V75 of mature OmpC (V-A insert). The two deletion mutations were identical and removed residues W103 (tryptophan) through F110 (phenylalanine) of mature OmpC (ΔW103 to F110). The preliminary characterization of one of the deletion mutants (PLB3256) has been reported previously (2).

The NTG-induced mutations were all transitions (C to T, G to A, and A to G) (Table 1). The C-to-T transitions were the predominant type of alteration (15 of 18 transitions). The 18 NTG-induced *ompC*(Dex) mutations identified three residues (R37, R74, and D105) in the OmpC protein that can be altered to confer a Dex$^+$ phenotype.

Characterization of the *ompC*(Dex) mutants. One mutant of each alteration type (Table 1) was selected for further analysis. To determine the effect of these alterations on OmpC protein levels, envelope fractions were prepared and analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 1). The parent and mutant strains produced similar amounts of the OmpC protein. Several mutants produced OmpC protein with altered mobility. For example, the R37-to-C, D105-to-G, and ΔW103-to-F110 mutations resulted in a slight increase in mobility, whereas the R37-to-H alteration de-

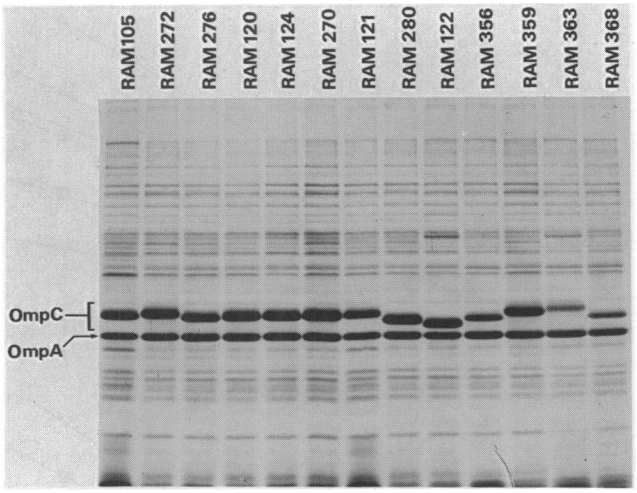

FIG. 1. SDS-polyacrylamide gel electrophoresis analysis of whole-cell envelopes from the *ompC*(Dex) and *ompC*(Hex) mutants. The alterations present in the OmpC peptide are as follows: RAM105, wild type; RAM272, R37 to H; RAM276, R37 to C; RAM120, R74 to S; RAM124, R74 to G; RAM270, R74 to C; RAM121, V-A insert; RAM280, D105 to G; RAM122, ΔW103 to F110; RAM356, R74 to S and D105 to G; RAM359, R74 to S and R37 to H; RAM363, V-A insert and D105 to G; RAM368, R74 to G and D124 to C.

creased the mobility of the OmpC protein (Fig. 1). Alterations at R74 (R74 to S, R74 to C, and R74 to G) had no detectable effect on mobility (Fig. 1).

Strains carrying the various *ompC*(Dex) mutations were tested for their sensitivity to antibiotics and SDS (Table 2). The three substitutions at R74 (G, S, and C) had similar effects with regards to sensitivity of the strains to β-lactams and chloramphenicol. In contrast, the two substitutions at residue R37 produced different effects; the R37-to-C substitution resulted in a greater increase in sensitivity to β-lactams and chloramphenicol than did the R37-to-H change (Table 2). The D105-to-G substitution produced an effect very similar to that of the R37-to-C substitution, with the exception of its sensitivity to chloramphenicol (Table 2). All the *ompC*(Dex) point mutants had a similar increase in their sensitivities to SDS. It should be noted that although the point mutants became sensitive to SDS, they remained unaltered in their sensitivities to rifampin and erythromycin. The insertion mutant had a sensitivity pattern for β-lactams and chloramphenicol very similar to that of the R37-to-C point mutant. However, it showed a much greater increase in its sensitivity to SDS than any other point mutant and exhibited slight sensitivity to erythromycin. The ΔW103-to-F110 deletion mutation conferred sensitivity to all compounds tested (Table 2).

Growth rates and maltose uptake assays. We tested the ability of the *ompC*(Dex) mutants to grow in liquid minimal medium containing glucose, maltose, maltotriose, maltotetrose, maltopentose, or maltohexose as the sole carbon source (Fig. 2). These sugars were provided at a very low concentration (1 mM), such that diffusion across the outer membrane was rate limiting for growth (19, 20). The parent strain, RAM105 (LamB$^-$ OmpF$^-$ OmpC$^+$), grew slowly on maltose and was unable to grow on larger maltodextrins. The *ompC* point and insertion mutations increased the growth rate on maltose and allowed growth on maltotriose and, to a lesser extent, on maltotetrose. These mutants were unable to grow on maltopentose or larger maltodextrins (Fig. 2). There were two exceptions; RAM272 (R37 to H) was unable to grow on maltotetrose, and RAM276 (R37 to C) was able to grow on maltopentose but not on maltohexose (Fig. 2). The deletion mutant, RAM122 (ΔW103 to F110), grew on all the sugars tested with growth rates very similar to that of MC4100, a LamB$^+$ strain (Fig. 2). However, its growth rate on maltoheptose was much slower than that of MC4100 (data not shown).

The increased permeability of the *ompC*(Dex) mutants to various compounds (Table 2) and the extended growth rates on defined maltodextrins (Fig. 2) suggest that these mutants have functionally larger OmpC pores. To further test this, [^{14}C]maltose uptake rates were determined. This assay is, in principle, similar to the growth experiments described above

TABLE 2. Antibiotic sensitivities of the *ompC*(Dex) mutants

Strains	Mutations[a]	Sensitivity to[b]:						
		A10	P10	C5	CR30	SDS	RA5	E15
Parent strain								
RAM105	OmpF$^-$ OmpC$^+$ LamB$^-$	14	0	8	12	0	8	0
ompC(Dex) mutants								
RAM272	R37 to H	18	8	12	12	11	8	0
RAM276	R37 to C	22	14	16	16	13	8	0
RAM120	R74 to S	19	10	14	13	13	8	0
RAM124	R74 to G	20	10	14	14	14	7	0
RAM270	R74 to C	20	10	14	13	14	8	0
RAM280	D105 to G	23	15	12	17	11	8	0
RAM121	V-A insert	21	13	16	15	20	8	8
RAM122	ΔW103 to F110	23	17	18	18	22	11	12
ompC(Hex) mutants								
RAM356	R74 to S, D105 to G	23	15	17	17	13	8	0
RAM359	R74 to S, R37 to H	22	14	15	16	13	8	0
RAM363	V-A insert, D105 to G	24	16	18	17	20	9	9
RAM368	R74 to G, R124 to C	23	15	17	16	14	7	7

[a] Mutations are shown as changes in amino acid residues indicated by single-letter codes.
[b] Numbers show the zone of growth inhibition (mm). Abbreviations (concentrations): A10, ampicillin (10 μg); P10, penicillin (10 U); C5, chloramphenicol (5 μg); CR30, cephalothin (30 μg); SDS, sodium dodecyl sulfate (1 mg); RA5, rifampin (5 μg); E15, erythromycin (15 μg).

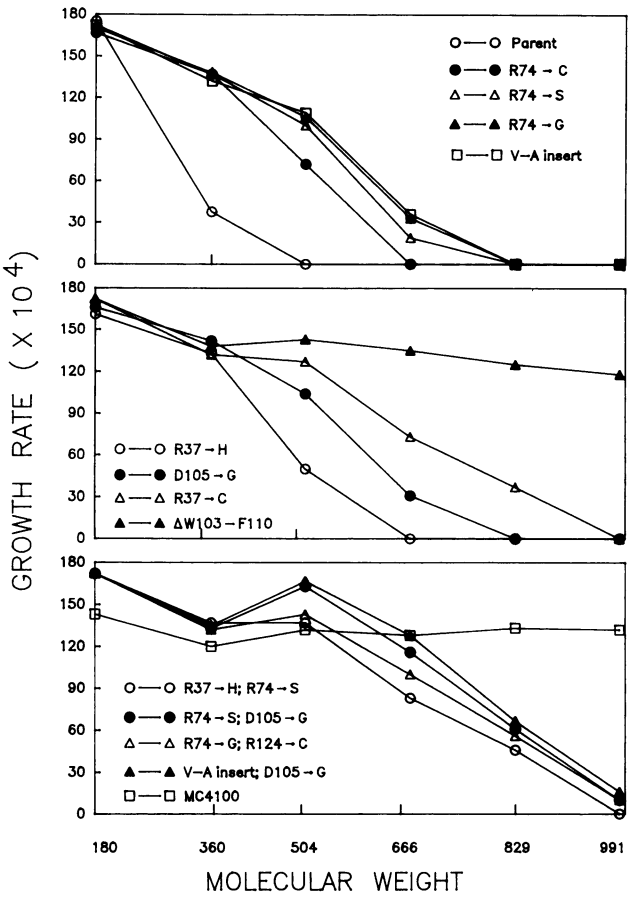

FIG. 2. Growth rates of the various mutants on defined maltodextrins of different molecular weights. Growth rates were determined as described previously (13). The parent and mutational alteration are shown in each panel. The substrates (glucose [molecular weight, 180]; maltose [molecular weight, 340]; maltotriose [molecular weight, 504]; maltotetrose [molecular weight, 666]; maltopentose [molecular weight, 829]; and maltohexose [molecular weight, 991]) were provided at a concentration of 1 mM. Growth rates are represented as the inverse of the doubling time in minutes. MC4100 is a LamB$^+$ OmpF$^+$ OmpC$^+$ strain.

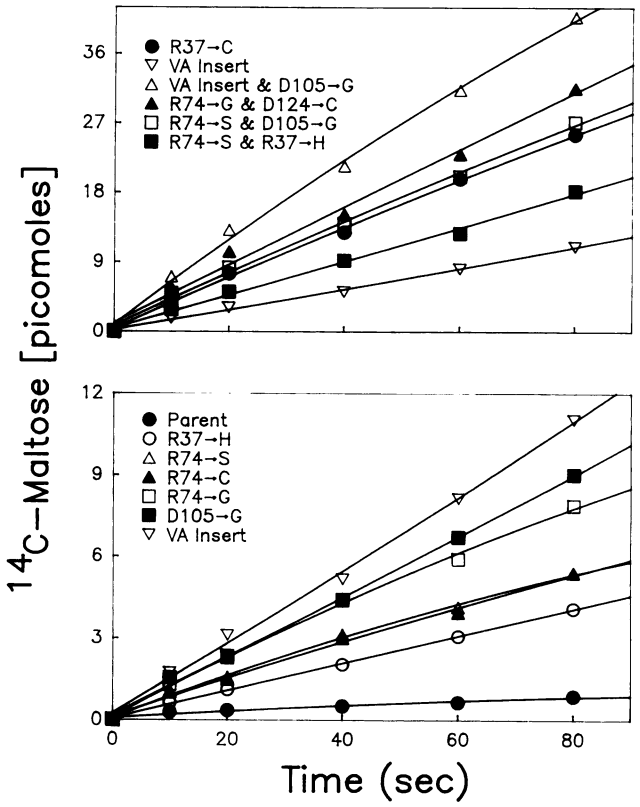

FIG. 3. [^{14}C]maltose uptake assays on the *ompC* mutants. Assays were done as described in Materials and Methods. [^{14}C]maltose was provided at a concentration of 3 μM. The parent strain was RAM105. The alterations in the various mutants are shown in the upper left of each panel. The *y* axis scale is different in the two panels; the upper panel has an extended scale to accommodate the Hex$^+$ mutants. For comparison, the V-A insert mutant is shown in both panels. Note that the R37-to-C mutant is shown in the upper panel only.

in that when [^{14}C]maltose is provided at micromolar concentrations, the rate at which it is taken up is a measure of the rate at which it crosses the outer membrane (4). All of the mutants were more efficient at taking up [^{14}C]maltose than was the parent strain, RAM105 (Fig. 3). More importantly, the relationship of the various mutants to each other in this regard was comparable to that obtained in the growth experiments, i.e., those with the fastest uptake rates had the fastest growth rates.

Isolation and characterization of *ompC*(Hex) mutants. With the exception of the deletion mutant, all the *ompC*(Dex) strains were unable to grow on maltohexose (Fig. 2). Starting with three of the *ompC*(Dex) strains (RAM120, RAM124 and RAM121), we isolated mutants that could grow on maltohexose (Hex$^+$). The *ompC*(Hex) mutants were obtained from NTG-mutagenized cultures at a frequency of approximately 10^{-10}. Control experiments with nonmutagenized cultures failed to yield Hex$^+$ mutants. Selection for the Hex$^+$ phenotype, starting with the parent strains (RAM105), yielded mutants which were very difficult to characterize. These mutants were either mucoid or genetically unstable or lysed during overnight growth in the rich medium; consequently, they were not characterized further. Thirty Hex$^+$ mutants were mapped by P1 transductions as described in Materials and Methods. In every case, the Hex$^+$ phenotype mapped to the *ompC* gene (data not shown). To confirm that the original *ompC*(Dex) mutation was still present and to determine the exact nature of the Hex$^+$ alteration, we recombined the chromosomal *ompC*(Hex) allele onto pRAM1006 and sequenced the *ompC* gene for six of the mutants. In each case, the *ompC* gene carried two alterations.

Of the four independent mutants derived from RAM120 (R74 to S), three carried a second substitution of D105 to G, and the remaining mutant contained a second substitution of R37 to H. The Hex$^+$ mutants derived from RAM124 (R74 to G) and RAM121 (V-A insert) had additional changes of R124 to C and D105 to G, respectively (Table 2). Note that in five of the six mutants, the second alteration was identical to a previously identified *ompC*(Dex) mutation (Table 1). The only exception was the R124-to-C change.

We determined the level of the OmpC protein present in the Hex$^+$ mutants (Fig. 1). Only RAM359 (R74 to S, R37 to H) had wild-type levels of OmpC. The other Hex$^+$ mutants had slightly reduced levels of OmpC in the outer membrane. The Hex$^+$ mutants were tested for sensitivity to antibiotics and SDS (Table 2). These mutants showed a slight increase in their sensitivity to β-lactams and chloramphenicol when

compared with the Dex$^+$ mutants carrying the appropriate single substitutions (Table 2). There was no apparent change in sensitivity to rifampin, erythromycin, or SDS (Table 2).

As expected, the Hex$^+$ mutants had faster growth rates on the larger maltodextrin sugars than the Dex$^+$ mutants carrying the appropriate single mutations (Fig. 2). Although these mutants were selected for their ability to grow on maltohexose with solid medium, they had slow growth rates on maltohexose in liquid. This difference may reflect the fact that on the solid media, colonies were not scored until after 96 h of incubation, while the liquid growth tests were done for only 6 h.

As a further test of outer membrane permeability, we assayed [^{14}C]maltose uptake (Fig. 3). As expected, the uptake rates of the Hex$^+$ mutants were faster than those of the corresponding Dex$^+$ mutants which carry single substitutions. The rates observed for the Hex$^+$ mutants were always greater than the summations of the rates conferred by the appropriate individual substitutions.

DISCUSSION

In this paper, we report studies on *ompC* mutations which affect pore properties of the OmpC porin. The point mutations identify four residues (R37, R74, D105, and R124) of OmpC which alter pore function. Alterations at these residues result in increased sensitivity to β-lactams (ampicillin, benzylpenicillin, and cephalothin) and chloramphenicol, which are thought to enter the cell through the OmpF and OmpC porins (21). These mutations do not alter sensitivity to hydrophobic antibiotics, such as rifampin and erythromycin, which cross the outer membrane in a porin-independent manner (21). The mutations confer an extended growth range which includes large defined maltodextrins and increase the rate of [^{14}C]maltose uptake. Thus, we conclude that these mutations result in a larger porin channel.

We have developed a genetic technique, which utilizes the positive (Dex$^+$) phenotype of the *ompC*(Dex) mutation, to clone the mutant *ompC* gene from the chromosome onto a low-copy-number plasmid. This involves detection of larger Dex$^+$ colonies in a diploid strain carrying an *ompC*(Dex) allele in the chromosome over the wild-type *ompC* allele on the plasmid. In the diploid strains, the *ompC*(Dex) allele is codominant and results in an intermediate Dex$^+$ phenotype (i.e., small colonies on maltodextrin medium). The recombination event between chromosome and plasmid, which transfers the *ompC*(Dex) allele onto the plasmid, results in larger colonies, thus allowing us to use in vivo cloning to obtain the desired clones. This general technique is simple and efficient and should be applicable to other similar systems which can utilize a selectable phenotype.

We have characterized several spontaneous and NTG-derived mutations in the *ompC* gene which include transversions, transitions, insertions, and deletions. All the point mutations altered charged residues, and in three cases (R37, R74, and R124) this residue was an arginine. One possible explanation for the phenotypes conferred by the amino acid changes is that they alter electrostatic interactions. Consistent with this notion, we found that the R37-to-H substitution resulted in only a modest change in pore function. One explanation for this is that at physiological pH, the histidine residue can be protonated and therefore might be expected to compensate, at least in part, for the loss of the positively charged arginine residue. In contrast, the R37-to-C substitution significantly affected pore function, as might be expected, with a complete loss of the ionic pairing ability. At position R74, the three substitutions (R74 to S, R74 to C, and R74 to G) had moderate effects on pore function. Although these amino acids are chemically different, they are all uncharged. Since the R74-to-C change resulted in a less dramatic phenotype than does the R37-to-C change, it appears that the types of perturbations caused by these subtitutions are not identical. One possibility is that R74 is at or near the exterior surface of the protein, while the R37 residue is present in the pore channel. The homologous residues (R82 and R42) in OmpF have been proposed to be present on the outside and within the transmembrane regions of the peptide, respectively (23, 25).

At this stage, it is not possible to suggest whether the substitutions exert their effects by altering intramolecular interactions (i.e., within a monomer) or intermolecular interactions, (i.e., between the monomers of a trimer). In addition, we cannot eliminate the possibility that the various substitutions exert their effects by replacement of the bulky arginine and aspartic acid residues with smaller, less bulky residues. We believe that this explanation is unlikely, since we did not obtain mutations affecting other bulky amino acids present in the region immediately adjacent to the substituted charged residues.

The V-A insert between R74 and V75 supports the idea that the R74 residue is at an exterior face of the peptide. The fact that the insertion did not dramatically disrupt overall protein conformation, as was shown by the retention of phage sensitivity, protease insensitivity (data not shown), and pore function, suggests that this region is not buried within the protein. At an exterior face, the addition of two amino acids might be more easily tolerated. We believe that the SDS sensitivity conferred by the V-A insert results from an altered interaction with other outer membrane components, further supporting our contention that these residues are located at an exterior face of the peptide.

The deletion mutation causes a significant increase in sensitivity to both hydrophilic (β-lactams and chloramphenicol) and hydrophobic antibiotics (erythromycin and rifampin) as well as to detergents. It is striking that the loss of eight amino acids does not result in the loss of overall protein conformation; this suggests that this region of the protein contains no critical information for membrane insertion or correct folding. It seems unlikely that the deletion destroys a transmembrane segment. We suggest that the deletion removes a region of the peptide at least partly exposed at the protein surface and that this may result in defective interactions with other outer membrane components. This may account in part for the dramatic increase in sensitivity to both hydrophilic and hydrophobic antibiotics. Similar deletions in the OmpF porin have similar phenotypes (S. A. Benson, J. L. Occi, and B. A. Sampson, J. Biol. Chem., in press).

The maltohexose (Hex$^+$) selection resulted in the identification of the same charged residues as those identified in the Dex$^+$ selection and one additional charged residue, R124. It is interesting to note that in the Dex$^+$ selection, the R37-to-C alteration was more frequently obtained (in 4 of 18 strains) than were the D105-to-G (2 of 18) and R37-to-H (1 of 18) substitutions combined. However, in the Hex$^+$ selection, the R37-to-C substitution was not isolated. The R37-to-C substitution has a strong permeability phenotype, and it is possible that, in combination with other mutations, its effect is detrimental. This may account for the fact that when we started with a strain carrying the R37-to-C alteration, we obtained unstable Hex$^+$ mutants (data not shown). In addition, we found that the combination of two substitutions

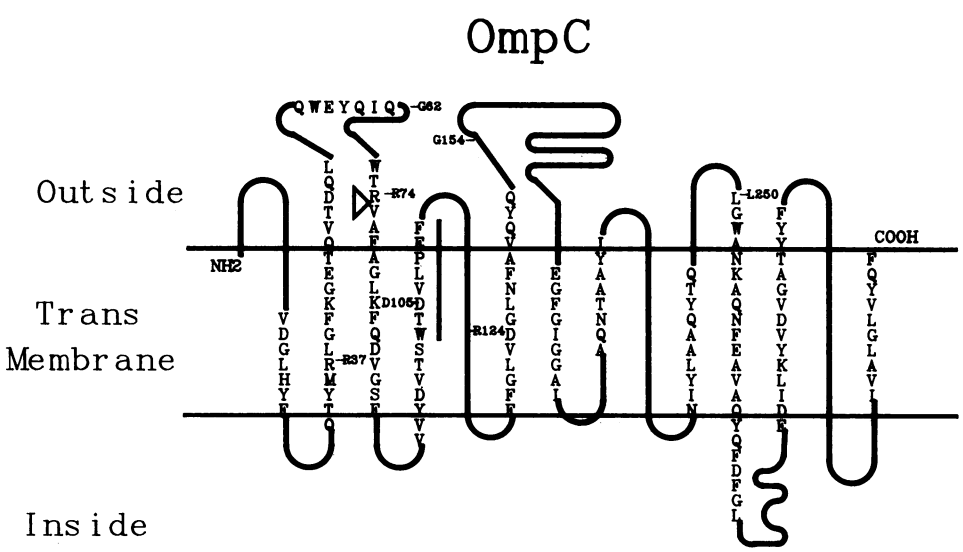

FIG. 4. A model of the topology of the OmpC protein. Regions which have a high probability of ordered structure as determined by the rules of Paul and Rosenbusch (23) are shown in single-letter amino acid codes. Regions that are likely to be turn regions or nonordered structures are shown as wide solid lines. The positions of the various point substitutions are indicated by single-letter amino acid codes. Mutations at residues R37, R74, D105, and R124 affect pore function, whereas alterations at G62, G154, and L250 affect phage receptor function of OmpC. The size and position of the deletion are shown by a short solid line. The position of the V-A insert is shown by the triangle. The topological arrangement in the membrane is hypothetical, as are the positions of the termini (see text for complete description).

which have relatively weak phenotypes (e.g., R37 to H and R74 to S) does not affect the amount of OmpC present in envelopes. Whereas combinations which involve at least one strong mutation resulted in reduced levels of OmpC, strain RAM363, which carries two relatively strong point mutations, had the least amount of the protein present (Fig. 1). The reduction in protein levels may reflect an impaired assembly, export, or stability of the peptide in the outer membrane.

Since the Hex^+ selection identifies the same set of residues as does the Dex^+ selection, this strongly suggests that we have identified important residues involved in pore function. Note that the Hex^+ selection identifies an additional residue (R124) not identified in the Dex^+ selection. The fact that we have obtained Dex^+ and Hex^+ point mutations at a limited number of charged residues does not necessarily mean that only these residues are involved in pore function. Alterations at other residues involved in pore function might be detrimental in that they block protein export, protein folding, or essential membrane interactions. Such mutations would not be obtained in selections which demand that the protein be exported and function as a pore.

Identification of the OmpC residues involved in pore function in combination with the membrane protein-folding rules of Paul and Rosenbush (23) allow us to construct a preliminary model of the OmpC protein (Fig. 4). In this model, residues R37, D105, and R124 are present in transmembrane segments. Since these are charged residues and as such are likely to be surface residues of the peptide (24), we suggest that they lie within the pore channel. We have placed the R74 residue outside of the plane of the membrane and close to a tentative turn segment on the basis of phenotypes conferred by the substitutions and insertions at this position (see above). However, we cannot rule out the possibility that this residue is present within the channel, in which case it might be within the plane of the membrane. The mutants suggest that the pore domain is located in the first one-third of the peptide. This is consistent with the analyses of the hybrid *ompC-phoE* genes (28) and *ompF*-(Dex) mutations (Benson et al., in press), which indicated that the first one-third of the PhoE and OmpF proteins are involved in pore function. The positions of the termini are hypothetical, and we know of no data that allow one to clearly place their positions. This model will serve as a guide for future experiments designed to further test this model.

It is clear that many more mutants need to be characterized in order to give a complete map of the various functional domains. The positive Dex^+ phenotype conferred by the point mutations will facilitate such approaches. Towards this end, we have obtained mutations in *ompC* which alter residues G62, G154, and L250 that affect bacteriophage receptor function (data not shown). In addition, the acquisition of unique cysteine residues at critical positions in the peptide opens up the possibility of the use of a number of chemical modification approaches to probe the structure of the protein.

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