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Primary structure of the *ompF* gene that codes for a major outer membrane protein of *Escherichia coli* K-12

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ABSTRACT

The nucleotide sequence of the ompF gene coding for a major outer membrane protein of Escherichia coli K-12 has been determined and the amino acid sequence of the OmpF protein was deduced from it. The OmpF protein contains 340 amino acid residues, and is produced from a precursor having 22 extra amino acid residues, the signal peptide, at the amino terminus. The expected secondary structure of the OmpF protein had a high β-sheet content with a low α-helix content. The promoter region and the transcription termination region of the ompF gene had a significantly high AT content, while the AT content of the coding region was about the same as the average AT content of the E. coli chromosome. Following the termination codon, a typical ρ-independent transcription termination signal was observed. The codon usage in the ompF gene was highly nonrandom; the codons preferably utilized are those recognized by the most abundant species of isoaccepting tRNAs or those, among synonymouns codons recognized by the same tRNA, that can interact more properly with the anticodon.

INTRODUCTION

The outer membrane of Escherichia coli K-12 usually contains four major proteins, the OmpF protein, the OmpC protein, the OmpA protein and Braun's lipoprotein, and these are the most abundant proteins in the cell (1). These proteins were first synthesized in a precursor form having a signal peptide at the NH_2-terminus (2, 3, 4). Two of these proteins, OmpF and OmpC, resemble each other with respect to the apparent molecular weight (5), total amino acid composition and NH_2-terminal sequence (6), strong association with the peptidoglycan layer (7), extremely high contents of β-structured polypeptide (5), trimeric structures (8), and pore functions for small hydrophilic molecules (9). Four genetic loci (ompF, ompC, ompR and envZ) are known to be involved in the synthesis of these two proteins. The ompF and ompC loci represent structural genes for the corresponding proteins, respectively (6, 10, 11), and are suggested to be derived from a single ancestral gene (6). On the other hand, gene products of ompR and envZ are shown to positively

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regulate the synthesis of these proteins (12). The ompR-envZ region was formerly called ompB.

Despite the similarities described above, the biosynthesis of the two proteins is regulated differentially. The envZ mutation results in the lack of the OmpF protein, while mutations in the ompR gene cause either the lack of OmpC alone or both proteins (12). In addition, the synthesis of the two proteins is affected in opposite directions by high concentrations of substances like sucrose and NaCl in culture media (13, 14). Upon addition of these substances to culture media, protein synthesis is immediately switched from OmpF to OmpC. It is suggested that recognition by cells of the osmotic difference between the outside and inside of the outer membrane is involved in this switching phenomenon (14).

In order to study the molecular mechanism controlling the synthesis of these outer membrane proteins, we isolated a specialized transducing lambda phage that carries the ompF gene of E. coli K-12 (15). In a previous study (4), we determined the DNA sequence that covers the NH_2-terminal region of OmpF and deduced the amino acid sequence of the signal peptide. Here we present the entire DNA sequence of the ompF gene.

MATERIALS AND METHODS

Enzymes and Chemicals.

Restriction endonucleases Rsa I, Pst I, Alu I, Taq I and Pvu II were obtained from New England Biolabs. Other restriction endonucleases were from Takara Shuzo Co. Bacterial alkaline phosphatase was from Worthington Biochemical Corp., bacteriophage T4 polynucleotide kinase from P.L.Biochemicals, and T4 ligase from Takara Shuzo Co. [γ-^{32}P]ATP (9,000 Ci/mmol) was prepared from carrier-free [^{32}P]orthophosphoric acid (Amersham Intl.) and ADP (Sigma Chemicals) by the method of Johnson and Walseth (16).

Bacterial Strains and Bacteriophages.

The following strains derived from E. coli K-12 and phages were used: KY2562 (thi tsx malA ompB101) (17); HO202mal$^+$ (F$^-$ thi rel asnS^{ts}, a mal$^+$ derivative of HO202)(4); λ ompF1 (a specialized transducing λ phage carrying the asnS-ompF region of the E. coli chromosome) (15); and λ cI857Sam7.

Preparation of λompF1 DNA and Subcloning into pBR322.

The methods used for the propagation of the λompF1 were those described by Schrenk and Weisberg (18). Strain HO202mal$^+$ grown in L-broth at 30°C was used as the host strain and λcI857Sam7 as the helper phage. The λompF1 DNA was extracted with phenol as described (19) and recovered by ethanol pre-

cipitation. After digestion with Sal I, the 14-kb DNA fragment shown in Fig. 1 was prepared using agarose gel and ligated with cloning vector pBR322 as described (20). Ligation mixtures were used directly for transformation using the procedure of Dagert and Ehrlich (21).

Purification of Plasmid DNA.

Strain KY2562 harboring a pBR322-derived plasmid was grown until A_{660}= 1.3 at 37°C in L-broth supplemented with glucose (1 g/l) and ampicillin (20 mg/l). Chloramphenicol (100 mg/l) was then added and the cells were harvested 18 h later. Purification of plasmid DNA was carried out as described by Matsubara (22).

Gel Electrophoresis of DNA Fragments.

Gel electrophoresis was carried out for both analytical and preparative purposes. Polyacrylamide gel(5%) was used for the separation of DNA fragments smaller than 1,500 bp and 0.8% agarose gel was used for fragments larger than 1,500 bp. The buffer for electrophoresis contained 50 mM Tris-borate (pH 8.3) and 1 mM EDTA. DNA fragments were eluted from the polyacrylamide gel by the crush and soak technique (23) and from the agarose gel by the freeze-squeeze technique (24), extracted with phenol and precipitated with ethanol.

Restriction Endonuclease Mapping and DNA Sequencing.

A restriction enzyme cleavage map was constructed using both the end-labeling method (25) and the double digestion method (26). All DNA sequencing methods were according to Maxam and Gilbert (23). Single end-labeled fragments obtained after the chemical cleavages were analyzed by means of a thin sequencing gel system (0.04 x 20 x 40cm) with 20% and 10% polyacrylamide in 7 M urea.

RESULTS

Cloning of the ompF Gene on Plasmid Vector pBR322.

We constructed various hybrid plasmids carrying the ompF region of λ ompF1 as shown in Fig. 1. The 14-kb Sal I DNA fragment from λ ompF1 was inserted into the Sal I site of pBR322. The ligated mixture was used to transform KY2562 selecting for ampicillin resistant and tetracycline sensitive. Two types of 18.3-kb plasmids having the 14-kb insert at the Sal I site of pBR322 with opposite orientation to each other were found in the resulting transformants. Representative plasmids were designated as pLF2 and pLF3, respectively. pLF10 was further derived from pLF2 by digestion with Pvu II followed by religation. pLF11 was derived from pLF10 by Eco RI digestion. Similary, pLF4 was derived from pLF3 by Hin dIII digestion, and pLF9 from

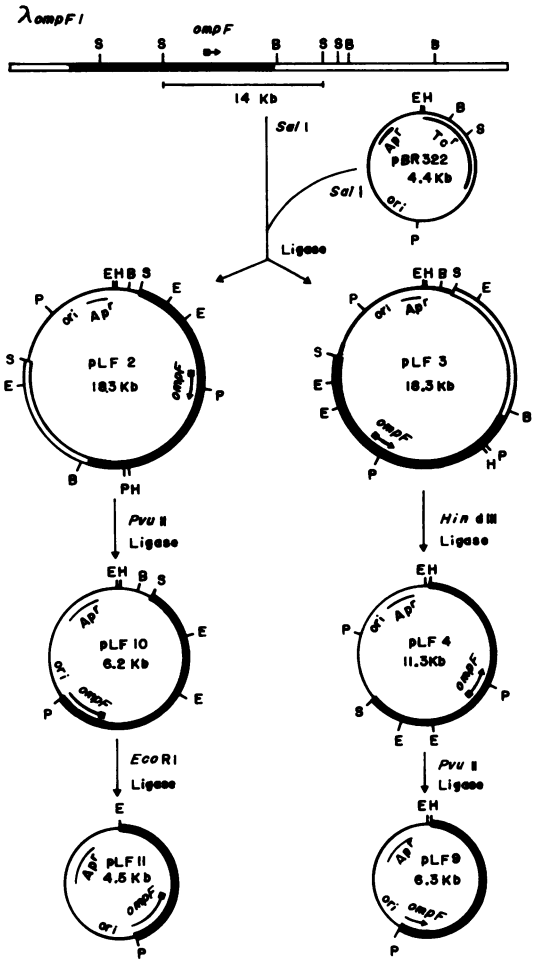

Figure 1. Construction of hybrid plasmids carrying the ompF gene. The heavy solid lines represent E. coli chromosomal DNA and the open segments represent λ phage DNA. pBR322 DNA is shown as thin lines. The position of the ompF gene is indicated by a line with ■ (initiation site) and/or ▶ (termination site). S, Sal I; B, Bam HI; E, Eco RI; H, Hin dIII; P, Pvu II; Ap^r, ampicilin resistant; Tc^r, tetracycline resistant; ori, origin of replication. In the λompF1 DNA, only Bam HI and Sal I sites are shown.

pLF4 by Pvu II digestion. The 3.7-kb Sal I-Pvu II fragment involved in pLF10 was called Fragment 2 in a previous paper (4).

Restriction Endonuclease Mapping and DNA Sequencing.

Figure 2 shows a restriction endonuclease cleavage map around the ompF gene. It was incorrectly reported in a previous paper that the region derived

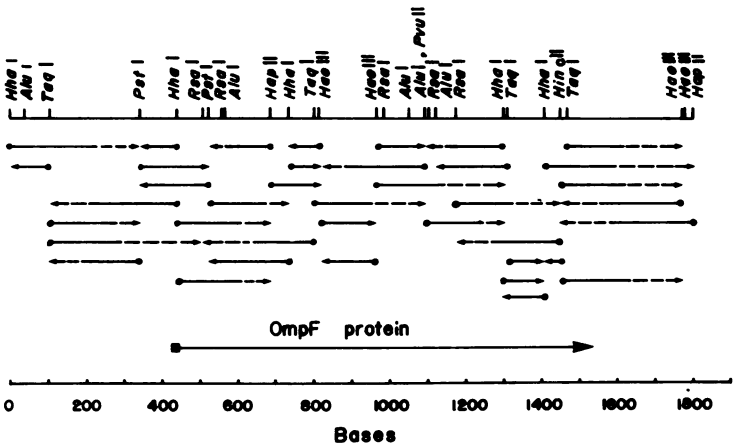

Figure 2. Restriction endonuclease cleavage sites and sequence strategy around the ompF gene. The singly labeled fragments are indicated by arrows. (●) The position of the ^{32}P-label at 5'-end. Broken regions of the arrows indicate that the sequence of these regions were not determined in these experiments. The coding region of the ompF gene is also indicated with ■ (initiation site) and ▶(termination site).

from the E. coli chromosome shown in Fig. 1 has one Bam HI site(15). However, no Bam HI site was found to exist in this region. The nucleotide sequence shown in Fig. 3 also confirmed this fact. We showed previously (4) that the OmpF signal peptide is coded for by the 180-bp Pst I DNA fragment and the direction of transcription is from left to right in Fig. 2. In order to determine the entire ompF gene we sequenced the entire 1.8-kb region shown in Fig. 2. The restriction endonuclease fragments used for the sequence analyses are also shown in Fig. 2.

The nucleotide sequence of 1807 bp covering the entire region shown in Fig. 2 was determined (Fig. 3). The determination was carried out for both strands of most of the DNA. In addition, all restriction sites were overlapped by sequence determinations of different DNA fragments. Therefore, no sequence information was missed as a result of the loss of a very small restriction fragment. Following the sequence that covers the promoter, signal peptide and NH_2-terminal regions of the OmpF protein (4), there was an open reading frame sufficiently long to encode the OmpF protein. This is the only long open reading frame that exists in this region. This reading frame is terminated by the two contiguous termination codons (nucleotides 1542-1547), thus making a protein of a molecular weight of 37,082 (excluding the signal

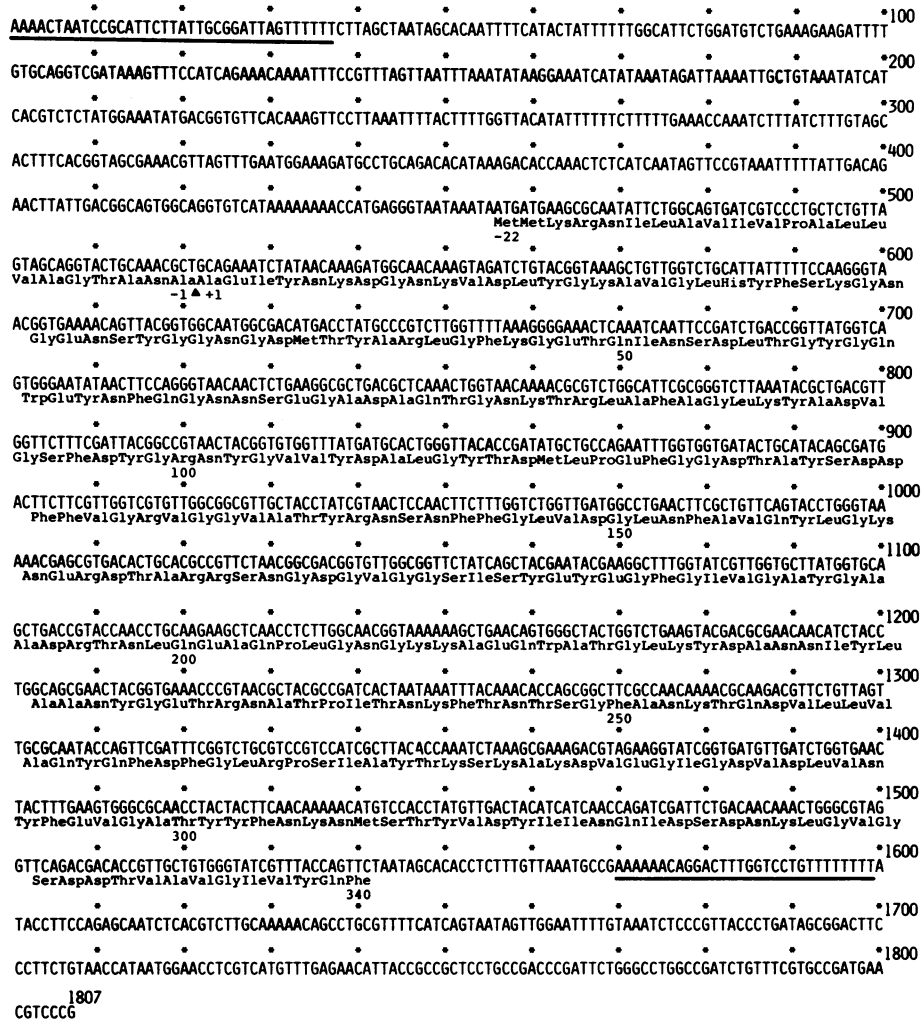

Figure 3. The 1807 base pairs DNA sequence encompassing the ompF gene. The amino acid sequence of the pro-OmpF protein deduced from the DNA sequence is also shown. The cleavage site of the signal peptide is shown by a triangle. The amino acid residues are numbered from the amino terminus of the OmpF protein. Possible transcription termination signals are underlined.

peptide). This molecular weight is in good agreement with that estimated from the gel electrophoretic behavior of the OmpF protein (MW= 38,000) (5). In Fig. 3 is also shown the amino acid sequence deduced from the nucleotide sequence. The amino acid composition derived from this sequence is also in

excellent agreement with our earlier estimates based on amino acid analysis of acid hydrolyzates of the whole OmpF protein (6). In addition, the deduced sequence is almost the same as that of a corresponding protein of E. coli B determined by Chen et al. (27). Following the termination codons, there is a possible ρ-independent transcription termination signal which is characterized by an oligo(T) sequence and a stable stem-and-loop structure (nucleotides 1570-1599)(28). This will be discussed later. From these results we conclude that the DNA sequenced here represents the entire ompF gene.

DISCUSSION

We previously determined the DNA sequence for the promoter-signal sequence region of the ompF gene and presented the amino acid sequence of the signal peptide (4). Here we present the DNA sequence of 1807 bp that encompassing the entire ompF gene. Its unique features are discussed in the following sections.

Structure of the ompF gene.

The DNA sequence shown in Fig. 3 together with previous results (4, 29) indicates that the OmpF protein is first synthesized as a precursor form that consists of 362 amino acid residues starting with initiation codon ATG (nucleotides 456-458) and terminating with termination codon TAA (nucleotides 1542-1544). The precursor is converted to the OmpF protein having 340 amino acid residues by release of the NH_2-terminal signal peptide (22 amino acid residues). It is interesting that the ompF gene has both the initiation codons (nucleotides 456-461) and the termination codons (nucleotides 1542-1547) in tandem fashion. No potential open reading frame that could code for other proteins can be found in this locus.

The coding region of the ompF gene is preceded by candidates for the Shine-Dalgarno sequence, Pribnow box and the RNA polymerase recognition site as discussed in a previous paper(4). The initiation site of the transcription is being studied in this laboratory in relation to the mechanism of the ompF expression that is regulated by osmolarity of the culture media. The DNA sequence also shows the possible ρ-independent transcription termination signal (nucleotides 1570-1599) that can form an extremely stable stem-and-loop structure with an oligo(T) sequence at the end (28). A similar structure has been found at the corresponding region of the lpp and ompA genes, the genes for other major outer membrane proteins (30, 31, 32). A similar stem-and-loop structure also is located preceding the ompF gene(nucleotides 1-37).

The asnS gene is located right upstream of the ompF gene (N. Mutoh, Y. Koga, K. Inokuchi and S. Mizushima, manuscript in preparation). Therefore, this region may represent the transcription termination signal of the asnS gene.

Figure 4 shows the distribution of AT base pairs around the ompF gene. It is apparent that the first 455 base pairs that cover the promoter region of the ompF gene have a significantly high AT content (69%) as in the corresponding region of the major lipoprotein gene, lpp (80%) (33), in comparison with the average AT content (49%) of the E. coli chromosomal DNA (34). Many A or T clusters (9-15 contiguous nucleotides) are found in this region as in the case of the lpp gene. On the other hand, the AT content of the promoter region of the ompA gene is not so high (56%)(31). The high AT content in the promoter region possibly contributes to destabilization of the double helical structure of the DNA and facilitates RNA polymerase-mediated strand-unwinding (35, 36). The AT content decreased to 52% in the coding region (nucleotides 456-1541), and again increased to 67% in the region probably representing the 3'-terminus of the mRNA (nucleotides 1542-1599).

Codon Usage.

The codon usage in the ompF gene is highly nonrandom (Table 1). A similar feature was also found in genes for other major outer membrane proteins (30, 31, 37), ribosomal proteins (for example 38, 39) and others (40, 41) that are efficiently synthesized in E. coli cells. The codon usage in ompA, lpp and lamB genes are also shown in Table 1 for comparison. It was suggested that the codons preferentially utilized in genes conducting efficient protein synthesis are those recognized by the most abundant species of isoaccepting tRNAs (42, 43). It was further suggested for these genes that a preferentially utilized codon among synonymous codons that are recognized by the same tRNA has a preference in a codon-anticodon interaction (42,

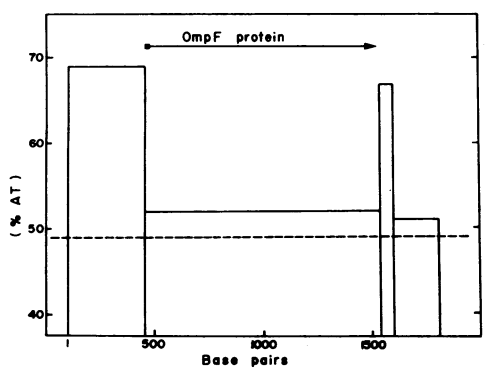

Figure 4. Distribution of AT base pairs around the ompF gene. The ompF gene region is divided into the following four sections: the region involving the promoter (1-455), the coding region (456-1541), the region probably representing the 3'-terminus of the mRNA (1542-1599), and the subsequent region (1600-1807). The average AT content of E. coli chromosomal DNA (49%) (34) is indicated by a broken line.

Table 1. Codon Usage in the Precursors of the Major Outer Membrane Proteins

First base	Second base: U					C					A					G					Third base
		(a)	(b)	(c)	(d)		(a)	(b)	(c)	(d)		(a)	(b)	(c)	(d)		(a)	(b)	(c)	(d)	
U	Phe	7	2	0	4	Ser	6	4	3	7	Tyr	9	2	0	7	Cys	0	1	0	2	U
		12	7	0	16		5	8	2	7		20	15	1	15		0	1	1	0	C
	Leu	2	1	0	4		1	0	0	1	Term	1	1	1	1	Term	-	-	-	-	A
		0	0	0	2		0	0	0	4		-	-	-	-	Trp	2	5	0	19	G
C	Leu	3	0	0	2	Pro	2	1	0	3	His	1	2	0	3	Arg	10	10	3	11	U
		0	0	0	3		0	0	0	0		0	3	0	3		2	3	1	6	C
		0	0	0	0		1	3	0	2	Gln	6	2	0	6		0	0	0	0	A
		18	22	9	11		2	15	0	4		7	15	5	16		0	0	0	0	G
A	Ile	1	1	0	7	Thr	7	10	4	7	Asn	4	1	0	4	Ser	1	0	0	2	U
		13	15	2	13		11	12	0	15		28	18	6	27		3	4	2	7	C
		0	0	0	0		1	1	0	2	Lys	16	15	6	12	Arg	0	0	0	0	A
	Met	5	6	3	16		3	0	0	1		3	4	1	8		0	0	0	0	G
G	Val	16	17	3	10	Ala	16	22	8	12	Asp	13	5	2	14	Gly	33	24	2	26	U
		1	1	0	7		2	1	0	10		14	17	6	19		15	14	1	22	C
		4	7	2	1		11	11	3	12	Glu	13	10	0	18		0	0	0	0	A
		5	2	1	6		5	3	1	6		1	3	0	3		1	0	0	1	G

(a) Precursor of the OmpF protein.
(b) Precursor of the OmpA protein(31,32).
(c) Precursor of the lipoprotein(30).
(d) Precursor of the LamB protein(37).

43). The nonrandom codon usage in the ompF , ompA and lpp genes meets these requirements: For example, preferential utilization of CUG for leucine, GGU/GGC for glycine and AUC/AUU for isoleucine must contribute to the efficient translation of the ompF gene through the recognition by the abundant species of isoaccepting tRNA, and preferential utilization of AAA over AAG for lysine, GAA over GAG for glutamic acid, AUC over AUU for isoleucine and AAC over AAU for asparagine must contribute to the efficient translation through the proper codon-anticodon interaction. Although the LamB protein becomes a major outer membrane protein when the synthesis is induced by maltose, the nonrandomness of the codon usage in the lamB gene is not as extreme as that in genes for major outer membrane proteins.

Protein Structure.

E. coli K-12 possesses two matrix proteins, OmpF and OmpC, while the B strain has one such protein that migrates to the same position as the K-12 OmpF protein on polyacrylamide gel (44). The primary structure of the OmpF protein deduced from the DNA sequence (Fig. 3) is almost the same as that of the corresponding protein of the B strain reported by Chen et al. (27) except

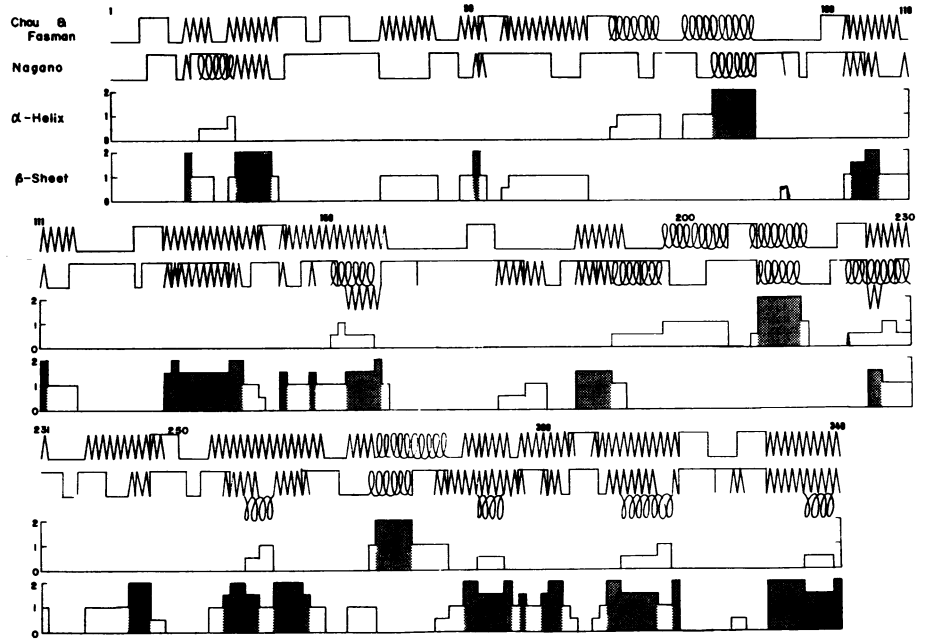

Figure 5. Expected secondary structure of the OmpF protein. Two expected structures predicted by the methods of Chou & Fasman (45) and that of Nagano (46) are shown. (llll) α-helix; (MM) β-sheet; (⌐¬) β-turn (Chou & Fasman) or β-turn and loop (Nagano); (___) coil. In cases of ambiguity in the prediction both conformational states are indicated. The histograms show the sum of the individual conformational states predicted by the two methods for α-helix and β-sheet. The values were calculated as follows: 1 was given to the region expected to be one of the relevant conformational states by one method, and 0.5 was given to the region in which two conformational states were expected by one method. The regions over 1.5 are shadowed.

for glutamine, glutamic acid and glutamine residues at positions 66, 117 and 262, respectively. These replacements of amino acid residues can be accounted for by a single base change in the gene. Therefore, we conclude that the two proteins are essentially the same.

The translated sequence of the OmpF protein was analyzed for the expected secondary structure by using the method of Chou and Fasman (45) and that of Nagano (46) (Fig. 5). Although the current state of the secondary structure prediction is not perfect and many contradictions are, indeed, found in the predicted structures with the two methods, it is clear that the possible β-sheet content is far greater than that of the α-helix. Figure 5 also shows histograms in which information obtained by the two methods is incorpo-

rated. The region strongly supported by the two methods to be β-sheet (larger than 1.5 in the histogram) is five times larger than the corresponding region for the α-helix. These results are consistent with our earlier studies with circular dichroism that the native OmpF protein is peculiar in that it has a very high content of β-sheet with a low content of α-helix (5). It should be taken into consideration,however, that the OmpF protein is a transmembrane protein and exists as a trimer, while most of the proteins used for the derivation of the prediction rules are globular hydrophilic proteins.

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ABBREVIATIONS

bp, base pair(s); kb, kilobases or kilobase pairs; EDTA, ethylene-diaminetetraacetate; Tris, Tris-(hydroxymethyl)-aminomethane; MW, molecular weight.

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