
The nucleotide sequence coding for major outer membrane protein OmpA of *Shigella dysenteriae*

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ABSTRACT

The nucleotide sequence of the ompA gene from Shigella dysenteriae has been determined and the amino acid sequence of the pro-OmpA protein predicted. Sequence comparison between the ompA genes of S.dysenteriae and Escherichia coli showed that features such as mRNA secondary structure and codon usage, as well as polypeptide function, have been conserved during evolution. The pro-OmpA protein of S.dysenteriae consists of 351 residues, as opposed to the 346 of the E.coli protein and also shows several amino acid changes. These changes have been used to interpret differences in the biological activity of the two proteins.

INTRODUCTION

The outer membrane of most Gram-negative bacteria contains a major, heat-modifiable polypeptide, the OmpA protein (1). In Escherichia coli this transmembranous protein (2) confers stability on the outer membrane (3,4) and mediates in F-dependent conjugation (5-7). It also serves as the receptor for several phages (7,8) and is involved in the uptake of colicin L (9). We are currently studying the evolution of the OmpA protein amongst the Enterobacteriaceae by cloning the corresponding genes (10) and determining their nucleotide sequences. It is hoped that this approach, together with the characterisation of the respective proteins, will permit identification of the functional domains of the polypeptide. In this paper we present the nucleotide sequence of the ompA gene from Shigella dysenteriae and compare the predicted pro-OmpA protein with that from E.coli.

MATERIALS AND METHODS

Bacteria, Phages and Plasmids

The E. coli K12 strains 5K (11) and SNO1 (12) were used for phage and plasmid production, respectively. The λ ompA Sh phages contain the ompA gene from Shigella dysenteriae cloned into the EcoRI vector, λ NM816 (13). Plasmids used as vectors in subcloning experiments were pBR322 (14), pACYC184 (15) and pHSG415 (16).

Preparation of DNA

The preparation of phage DNA and the isolation of restriction fragments were recently described (10,17). High-copy number plasmids were purified from chloramphenicol-treated cultures by a cleared lysate procedure (18) and two rounds of CsCl gradient centrifugation in a TV 865 rotor. Low-copy number plasmids were precipitated from cleared lysates with polyethylene glycol (19), resuspended in TES buffer (50 mM Tris, 5 mM EDTA, 50 mM NaCl, pH 8.0) then similarly centrifuged.

DNA:DNA Hybridization

DNA fragments were transferred from agarose gels to nitrocellulose filters as described by Southern (2) and hybridised with 32 P-labelled probes according to Wahl et al. (21). In some instances, colonies harbouring recombinant plasmids containing parts of the ompA gene were identified by the colony hybridisation procedure of Grunstein and Hogness (22).

Mini-lysate Screening

Plasmids of interest were further characterised by restriction analysis of DNA produced by the mini-lysate method of Birnboim and Doly (23). This worked well with high-copy number plasmids but proved very erratic with low-copy number vectors, such as pHSG415. Consequently, the following procedure was developed. A cleared lysate (24) was produced from a 10 ml overnight culture, mixed with an equal volume of a solution containing 1 M NaCl and 20% (^W/v) polyethylene glycol 6000 and placed on ice for 1 h. The

plasmid DNA was precipitated by centrifuging for 5 min in an Eppendorf centrifuge and usually sufficed for 2 analyses.

DNA Sequence Analysis

The DNA sequencing procedure of Maxam and Gilbert (25) was employed but with the modifications of Smith and Calvo (26).

Sources of Materials

Restriction enzymes were obtained from New England Biolabs, Bethesda Research Laboratories and Boehringer, Mannheim. Calf-intestinal alkaline phosphatase, polynucleotide kinase and DNA polymerase I (Klenow fragment) were supplied by Boehringer, Mannheim. Nitrocellulose filters were purchased from Sartorius and all isotopes from Amersham, Buchler GmbH.

RESULTS AND DISCUSSION

Location and Subcloning of the Gene

The ompA gene of S.dysenteriae was initially cloned (10) on a 9.5 Kb EcoRI fragment into the replacement vector, λ NM 816 (13). Four different isolates, λ ompA-Sh 1-4, were characterised by restriction endonuclease analysis and each was found to contain the same fragment in the same orientation. Consequently, only one isolate, λ ompA-Sh3, was studied further. A more detailed restriction map of the insert carried by this phage was produced and is presented in Fig. 1A. This map is in good agreement with that determined in a preliminary DNA:DNA hybridization study in which 32 P-labelled probes prepared from the E.coli ompA gene were used to detect homologies in restriction digests of chromosomal DNA from S.dysenteriae (unpublished data).

A more precise location of the gene was obtained by hybridising restriction fragments of λ ompA-Sh3 DNA with probes prepared from known regions of the E.coli ompA gene. Two different probes specific for the 5' and 3' ends of the gene, respectively, were used. It was thereby established that the ompA gene was located on a 4.6 Kb HindIII-EcoRI fragment and contained an internal BamHI site. Furthermore,

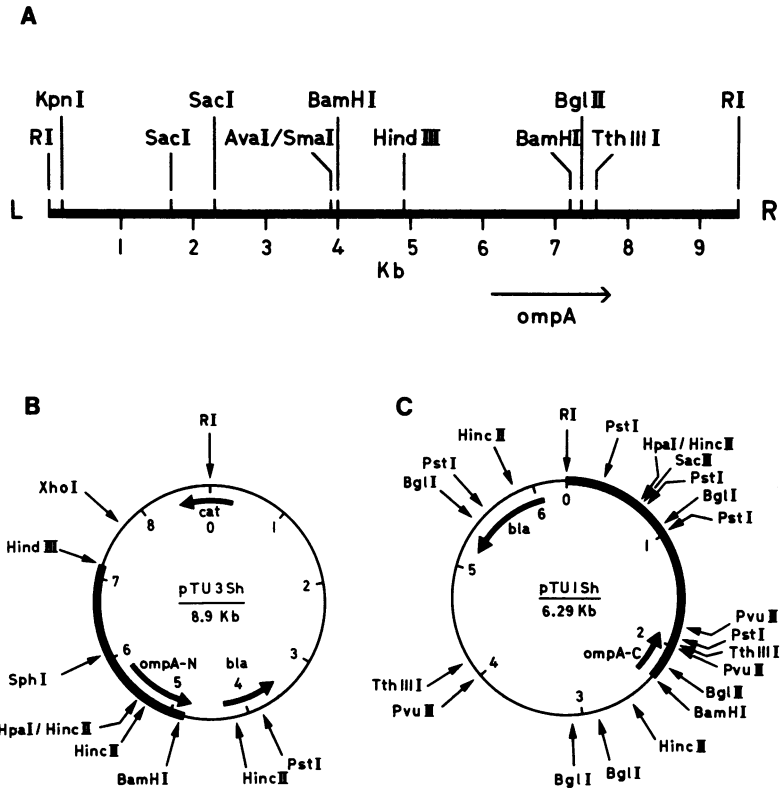


Figure 1. **A.** Restriction map of the RI fragment carried by λ ompA-Sh3 showing the location of the *ompA* gene and its direction of transcription. L and R correspond to the left and right arms of the vector, respectively. **B.**, **C.** Restriction maps of subclones pTU3Sh and pTU1Sh, the thinner lines correspond to the pHSG415 and pBR322 parts, respectively.

it could be inferred that transcription proceeded from left to right as shown in Fig. 1A.

To facilitate the production of DNA for sequencing we attempted to subclone appropriate restriction fragments (BamHI, BamHI-EcoRI, BamHI-HindIII, EcoRI-HindIII) into the *tet* gene of pBR322. Tetracycline-sensitive transformants were then screened for plasmids containing *ompA* sequences by colony hybridisation. From those clones exhibiting a positive signal mini-lysates were produced and the plasmid DNA analysed by digestion with the appropriate restriction

enzymes. It was found that only restriction fragments bearing the 3'-end of the gene (Fig. 1C) could be cloned into pBR322. This is consistent with the findings of Bremer et al. (27) for the ompA gene of E.coli. The failure to recover clones carrying large parts from the 5'-end of the ompA gene is almost certainly due to lethal over-expression of the residual gene fragment when cloned in a high-copy number vector. This problem was overcome by cloning a 2.3 Kb HindIII-BamHI fragment bearing the promoter and about two thirds of the coding sequence into the low-copy number vector, pHSG415 (16). This plasmid, pTU3Sh (Fig. 1B), is stable and the truncated ompA gene which it carries produces a functional protein. Analysis of pTU3Sh with HincII revealed two sites, about 100 bp apart, within the insert. This enabled the transfer of a 1.08 Kb HincII fragment (470 bp of ompA, 610 bp from the vector) to pACYC184 thus creating pTU4Sh. Plasmids pTU1, 3 and 4 Sh were used as the sources of restriction fragments for sequence determination.

Determination of the DNA Sequence

To facilitate the DNA sequencing fine structure restriction maps of the appropriate regions were made by the partial digest method of Smith and Birnstiel (28). These were used as the basis of the sequencing strategy which is shown in Fig. 2. The composite DNA sequence obtained is presented in Fig. 3.

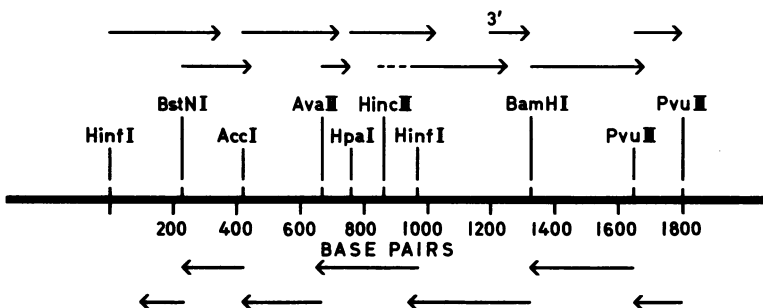


Figure 2. The strategy used to sequence the ompA gene showing the restriction sites employed. The arrows are proportional to the sequence obtained and the 3' denotes that this sequence was obtained after 3'-end labelling.

1 GAGTCAA*GGTTCGCGCTT*ACGCACGGCAAT*ACAGCTGTGGTATCGGTTGGT*GGCAGATGATT*GCATGAAGAAGACATGCTGAAC*TTGTTGATG
GluSerMetValArgAlaLeuArgThrGlyAsnTyrSerValValIleGlyTrpLeuAlaAspAspLeuThrGluGluGluHisAlaGluLeuValAspAla

101 CGCAAA*TAAGGTAA*CGCTATG*GGGTTA*TTATCGCTCGGTAAGCGCTTCC*CTCAGCAGCAGAGACAAC*TTCCGGGCTAA*AAATTCAC*TTAA*TTT
AlaAsnGluGlyAsnAlaMetGlyPheIleMetArgPheValSerAlaSerSerHisAlaThrArgGlnLeuSerGlyLeuLysIleHisSerAsnLeu

IleHis
A A A A C C

201 GTATC*ATAAGTA*AAATTTAGG*ATAATCCTGGA*ACTTT*TTTGTGCGCCAGCCAATGCTTTCAGTGGT*GACTA*ATTTCCTTGTGAGGC*TTGCTGAA
TyrHis***

301 GCGGTTCCGCGATTTCTTCTGTA*AAATGCGCTGCACAAAAAGATTAACGTACCTTATACA*GACTTTTTTCATATGCC*TCAGCGAGGTTCACACT

C C A A -35

401 TGTAAGTTTCACTACGTGTGTAGACTTTACATCGCTAGGGGTCTCGGCATAAGCCGAAGATATCGCTAGAGTTAATATTGAGCAGATCCCCGGTGA

-10

501 GGATTTAACC*GTGTATCTCGTGGAGATA*TTCAATGGCGTATTTTGAGTATAACGAGCCCAAAAAATGA*AAAGACAGCATCGCGATTACAGTGGCA
MetLysLysThrAlaIleAlaIleValAla

Ala
G

601 CTGGCTGGTTTCGCTACCGTAGCCAGGCGCC*TCGCAAA*GATAACACCTGGTACACTGGTCTAACTGGCTGGTCCAGTACCATTGA*CTGGTTCA
LeuAlaGlyPheAlaThrValAlaGlnAlaAlaPheLysAspAsnThrTrpTyrThrGlyAlaLysLeuGlyTrpSerGlnTyrHisAspThrGlyPheIle

-10 -10 -1 +1 10 20

701 TCGACAACAATGGCCGACCCATGAA*AAACACTGGCGCTGGTCTTTTGGTGGTACCAGGTTAACCCGTATGTGGCTTTGAAATGGTTACGACTG
AspAsnAsnGlyProThrHisGluAsnGlnLeuGlyAlaGlyAlaPheGlyGlyTyrGlnValAsnProTyrValGlyPheGluMetGlyTyrAspTrp

Asn
A
30 40 50

801 GTTAGTGTATGCCGTACA*AAAGCCAGCGTTG*AAACGGTGCATCA*AAAGCTCAGGGCGTTCAGTTGACCGCTAAACTGGTTACCA*ACTCACGACAC
LeuGlyArgPheCProTyrLysGlySerValGluAsnGlyAlaTyrLysAlaGlnGlyValGlnLeuThrAlaLysLeuGlyTyrProIleThrAspAsp

60 70 80 90

901 CTGGCAGTGTACACTCGTCTGGTGGTATGGT*TTGGCTGCAGACACCAAAAGCTCACAACAATGTCAGCAGG*TAATCGAGAAA*ACCAGCATCCGGCG
LeuAspValTyrThrArgLeuGlyGlyMetValTrpArgAlaAspThrLysAlaHisAsnAsnValThrGlyGluSerGlyLysAsnHisAspThrGlyVal

A C* C * A * T * Ser AsnValTyrGly
TCC AACGTATTGGT C

1001 TTTCTCCGGTATTCCGAGGTGGTGTGAATGGGCCATCAC*CTCGAAATCGCTACCCGCTTGAATACAGTGGACCACAACATCCGGTACGCACACAC
SerProValPheAlaGlyValGluTrpAlaIleThrProGluIleAlaThrArgLeuGluTyrGlnTrpThrAsnAsnIleGlyAspAlaHisThr

*C T C Tyr
G*AC G

1101 CATCGGCATCTGCCGACACCGCTCTGAGCTTGGGTTTCTCTACCGTTTCGGTCCAGGGCAAGCAGCTCCAGTAGTTGCTCCGGCTCCAGCTCCG
IleGlyThrArgProAspAsnGlyLeuLeuSerLeuGlyValSerTyrArgPheGlyGlnGlyGlyAlaAlaPheValAlaAlaPheAlaProAlaPro

Met
A C
160 170 180 190

1201 GCACCGAAGTACAGACCAAGCACTTCACTCTGAA*GTCGACGCTCTGTTCAACTCAACAAAGCAACCCCTGAA*ACCGAGGATCAAGCTGCTCGATC
AlaProGluValGlnThrLysHisPheThrLeuLysSerAspValLeuPheAsnPheAsnLysAlaThrLeuLysPProGluGlyGlnAlaAlaLeuAspGln

200 210 220

1301 AGCTGTACACCGCACTGACCACTGGATCGAAAGCGGTTCCGTAGTGTCTCTGGGTACACCGACCGCATCGGTTGACGCTTACAACCGGGTCT
LeuTyrSerGlnLeuSerAsnLeuAspProLysAspGlySerValValValLeuGlyTyrThrAspArgIleGlySerAspAlaTyrAsnGlnGlyLeu

230 240 250

1401 GTCCGAGCGCCGTCTCAGTCTGTGTGTGATTACCTGATCTCCA*AAAGTATCCCGCAGACAAGATCTCCGCACTGGTATGGCGAATCAACCCGGTT
SerGluArgArgAlaGlnSerValValAspTyrLeuIleSerLysGlyIleProAlaAspLysIleSerAlaArgGlyMetGlyGluSerAsnProVal

260 270 280 290

1501 ACTGGCAACACCTGTGACAACGTGAACAGCGGTCTGC*ACTGATCGACTGCC*GGCTCCGGTGCATCGCTGGT*AGAGATCGAAAGTAAAGCGCATTCAAGACG
ThrGlyAsnThrCysAspAsnValLysGlnArgAlaAlaLeuIleAspCysLeuAlaPheAspArgArgValGluIleGluValLysGlyIleLysAspVal

300 310 320

1601 TTTGTAACTAGCCGAGGCTAGGTTCTGCTTGGTAGAAA*ACCCCGACTCGGGGCTTTTTTTCGCTTTAGTAAATGAACTGACTGTGTCAGT
ValThrGlnProGlnAla***

330

1701 ATTCTTACCAGCAATGCCTCGACATCC*TCGTCAGAAAAGACATTTTATTCGCGTATTC*TCITTTGTTTTCCGCACTTTCAGTCACTG

Analysis of the DNA Sequence

On examination of the sequence two reading-frames were found. These correspond to the pro-OmpA protein and the COOH-terminal part of an unidentified polypeptide (29). The overall sequence is about 97 % homologous with the corresponding region of the E.coli chromosome (29,30) which is to be expected in view of the close degree of relatedness between the two bacteria (31). Many of the differences occur in the non-coding regions or in the wobble position of the codon and most (66%) are accounted for by transitions.

The promoter sequence is identical to that of the E.coli gene (32, Cole et al., in preparation) and the region corresponding to the 5'-untranslated end of the mRNA shows only one change (Fig. 3). Furthermore, the sequence encoding the signal peptide and the first 90 residues of the OmpA protein is also highly conserved. Many base differences can be found in the next stretch which encodes residues 90-170 and the most remarkable of these occurs between positions 951-981 (Fig. 3). This 30 bp sequence is not found in the E.coli gene and the corresponding region, consisting of 15 bp, bears slight resemblance. Consequently, the reading frame of the S.dysenteriae ompA gene contains 5 codons more than that of E.coli. Towards the 3'-end of the gene the most extensive region of homology (450 bp) with the E.coli sequence can be seen although there are some differences around the transcriptional terminator (positions 1639-1667; Fig. 4).

Secondary Structure of the mRNA

The coding sequences of the ompA genes from S.dysenteriae and E.coli (29,30) exhibit extensive dyad symmetries and the corresponding mRNAs thus have the potential to form stable secondary structures. This probably accounts for their very long functional half-lives (33).

Figure 3. Nucleotide sequence of the ompA gene from S.dysenteriae. Nucleotides which differ in the E.coli sequence (29,30) are indicated. Positions at which bases have been deleted (-) or inserted (∇) in the E.coli sequence are also shown.

It is interesting to note that in the region where the *S.dysenteriae* sequence differs extensively from the *E.coli* sequence dyad symmetry may also be found. The stable stem and loop structure that can be formed is shown in Fig. 4 and compared with the corresponding structure from the *E.coli ompA* mRNA. This finding suggests that the evolution of the *ompA* gene amongst the Enterobacteriaceae may have been subject to the additional constraint of conserving mRNA secondary structure as well as that of polypeptide function.

Codon Usage

The *S.dysenteriae ompA* gene shows 19 codon changes to the *E.coli* gene but only 5 of these result in an amino acid change. The distribution of the codons used in the

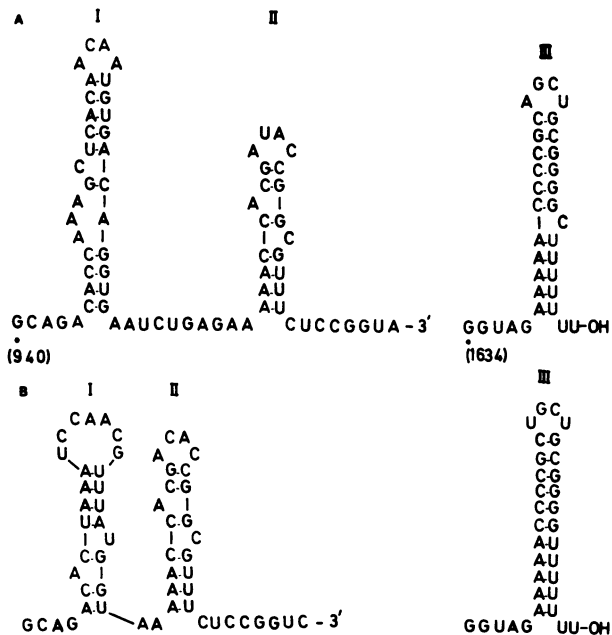


Figure 4. Potential secondary structures of the regions where the *ompA* mRNA from *S.dysenteriae* (A) and *E.coli* (B) show major differences (I) and of the transcriptional terminators (III). The ΔG values for the loops calculated according to (38) are: A -5.4 (I), -6.8 (II), -20.4 (III) Kcal. B -1.6 (I), -6.8 (II), -23.4 (III) Kcal.

translation of the *S.dysenteriae* pro-OmpA protein is presented in Table 1. Of the 61 sense codons, 16 are not used and 7 are used only once. In contrast, 16 codons are employed ten or more times and these account for 251 of the 351 codons found in the mRNA. Most of the codons used by *ompA* are recognised by the major iso-accepting species of tRNAs (34) and such biased codon usage is believed to be a mechanism of ensuring efficient translation (35).

Some Features of the *S.dysenteriae* pro-OmpA Protein

The pro-OmpA protein predicted by the DNA sequence consists of 351 residues, 21 of which form the signal pep-

Table 1

Codons used in the *S.dysenteriae ompA* mRNA.
The corresponding values for *E.coli* are shown on the right.

	U		C		A		G	
U	UUU Phe	2: 2	UCU Ser	5: 4	UAU Tyr	1: 2	UGU Cys	1: 1
	UUC Phe	7: 7	UCC Ser	7: 8	UAC Tyr	14:15	UGC Cys	1: 1
	UUA Leu	1: 1	UCA Ser	0: 0	UAA Och	1: 1	UGA Opa	0: 0
	UUG Leu	2: 0	UCG Ser	0: 0	UAG Amb	0: 0	UGG Trp	6: 5
C	CUU Leu	0: 0	CCU Pro	1: 1	CAU His	2: 2	CGU Arg	10:10
	CUC Leu	0: 0	CCC Pro	0: 0	CAC His	4: 3	CGC Arg	3: 3
	CUA Leu	0: 0	CCA Pro	3: 3	CAA Gln	1: 2	CGA Arg	0: 0
	CUG Leu	21:22	CCG Pro	15:15	CAG Gln	16:15	CGG Arg	0: 0
A	AUU Ile	1: 1	ACU Thr	9:10	AAU Asn	2: 1	AGU Ser	0: 0
	AUC Ile	14:15	ACC Thr	13:12	AAC Asn	17:18	AGC Ser	4: 4
	AUA Ile	0: 0	ACA Thr	3: 1	AAA Lys	15:15	AGA Arg	0: 0
	AUG Met	5: 6	ACG Thr	0: 0	AAG Lys	4: 4	AGG Arg	0: 0
G	GUU Val	17:17	GCU Ala	21:22	GAU Asp	6: 5	GGU Gly	25:24
	GUC Val	0: 1	GCC Ala	3: 1	GAC Asp	17:17	GGC Gly	13:14
	GUA Val	7: 7	GCA Ala	11:11	GAA Glu	12:10	GGA Gly	0: 0
	GUG Val	4: 2	GCG Ala	2: 3	GAG Glu	3: 3	GGG Gly	0: 0

tide, and has a molecular weight of 37,729. Excluding the sequence between residues 108-117, there are five differences between the S.dysenteriae and E.coli proteins (29,30, 36). Four of these represent conservative substitutions and these are at positions -13 (Thr:Ala; S.d.:E.c.), 93 (Val:Ile), 134 (Trp:Tyr) and 166 (Leu:Met). The fifth difference at residue 25 (Asp:Asn) is probably the most significant biologically.

We have established that the S.dysenteriae OmpA protein (10,37) can replace the E.coli protein in conjugation and as the receptor for phage Ox2. It does not, however, enable colicin L to be taken up or permit successful infection of phage K3 although, it does inactivate this phage. A receptor site for K3 is known to be centred around residue 70 of the E.coli protein (Henning et al., in preparation). Since this region of the S.dysenteriae protein is identical it appears that another part of the protein is required to trigger the infection process. This could be situated around residue 25, or residues 108-117, since this is where the two proteins differ most significantly. Furthermore, one or other of these differences must account for the defect in colicin L uptake. It is hoped that a more precise understanding of these processes will be obtained when the sequences of other OmpA proteins and of defined mutations are available.

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