

DNA sequence of the promoter region of the *ompC* gene and the amino acid sequence of the signal peptide of pro-OmpC protein of *Escherichia coli*

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The osmoregulated *ompC* gene of *Escherichia coli* was cloned and the DNA sequence of a fragment encompassing the promoter region and a portion of the coding region was determined. There were no obvious homologies in the DNA sequences of the promoter regions of the *ompC* and *ompF* genes, in contrast to those of the coding regions of the two genes, both of which code for the matrix porins (major outer membrane proteins) and form passive diffusion pores. The amino acid sequence of the signal peptide of pro-OmpC protein was also deduced from the DNA sequence

<i>Pro-OmpC protein</i>	<i>Signal peptide sequence</i> (<i>Escherichia coli</i>)	<i>ompC gene</i> <i>Matrix porin</i>	<i>Promoter region sequence</i>
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1. INTRODUCTION

The matrix proteins (major outer membrane proteins) of *Escherichia coli*, are known to consist of OmpF and OmpC proteins. Both proteins form passive diffusion pores across the outer membrane, which are essential for the up-take of nutrients such as sugars and amino acids (review [1]).

These two proteins are coded for by two independent structural genes, the *ompF* gene and the *ompC* gene, which map at 21 and 47 min, respectively, on the *E. coli* chromosome (review [2]). The expression of these genes is controlled by the osmolarity of the culture medium; the *ompF* gene is preferentially expressed in low osmolarity, while the *ompC* gene is expressed under conditions of high osmolarity [3]. This osmoregulation of the *ompF* and *ompC* genes has been shown to be controlled by another unlinked gene, called the *ompB* operon, which maps at 74 min on the *E. coli* chromosome [2]. The DNA sequence of the entire *ompB* operon has been recently determined and its gene products have been identified [4,5].

To fully understand the molecular mechanism of the osmoregulation of the *ompF* and *ompC* genes,

it is essential to isolate these genes, and to determine the structures of their promoters, with which the *ompB* gene products are considered to interact. The DNA sequence of the *ompF* promoter region has been determined in [6].

Here, we report the cloning of the entire *ompC* gene and the DNA sequence of its promoter region. The amino acid sequence of the signal peptide of the pro-OmpC protein has been deduced from the DNA sequence. These features will be compared with those of the *ompF* gene.

2. MATERIALS AND METHODS

Restriction endonucleases were obtained from Bethesda Res. Lab. and New England BioLabs. T4 DNA ligase was from Bethesda Res. Lab. DNA polymerase I Klenow fragment was from New England Nuclear. [α - 32 P]dNTPs (2000–3000 Ci/mmol) were purchased from Amersham International.

2.1. Bacterial strains and bacteriophages

The following *E. coli* strains and phages were used: *E. coli* T19 *recA* (F^- *tsx-354 ompB*) [7],

E. coli W620 *recA* ($F^{-}\lambda^{-}thi-1$ *pyrD36 gltA6 galK30 strAD9 supE44 relA1?*), *E. coli* W620-78 *ompC::Mu* which was derived from W620 by integrating phage Mu into the *ompC* gene [7], phage λ p10-25 (*ompC-lacZ*⁺ 10-25 *lacY*⁺ *lacA*⁺; a gift from T.J. Silhavy [8]), and phage Tu1b (from J. Foulds).

2.2. Preparation of DNA and restriction endonuclease digestion

Plasmid DNA, chromosomal DNA, and phage DNA were prepared as in [7]. Electrophoresis of DNA fragments [9] and restriction enzyme digestions [10] were carried out as described.

2.3. DNA sequence determination

Restriction fragments were labeled at their 3'-ends using [α -³²P]dNTPs and DNA polymerase I Klenow fragment [11]. Singly end-labeled DNA fragments were obtained either by digestion with a second restriction enzyme or electrophoretic strand

separation [12]. DNA sequences were determined as in [12,13].

2.4. Other methods

Conditions for ligation and transformation of plasmids were as in [10]. DNA used for Southern blot hybridization [14] was nick translated as in [15].

3. RESULTS

Originally we attempted to determine the DNA sequence of the *ompC* promoter region and the region corresponding to the 5'-end of the *ompC* mRNA by directly sequencing a DNA fragment derived from λ p10-25 DNA carrying an *ompC-lacZ* fusion [8]. However, this attempt was unsuccessful since it was found that this fusion phage contained a fragment of Mu DNA between the *ompC* gene and the *lacZ* gene as shown in fig.1A. The DNA sequence of the *PvuII-HpaI* fragment obtained

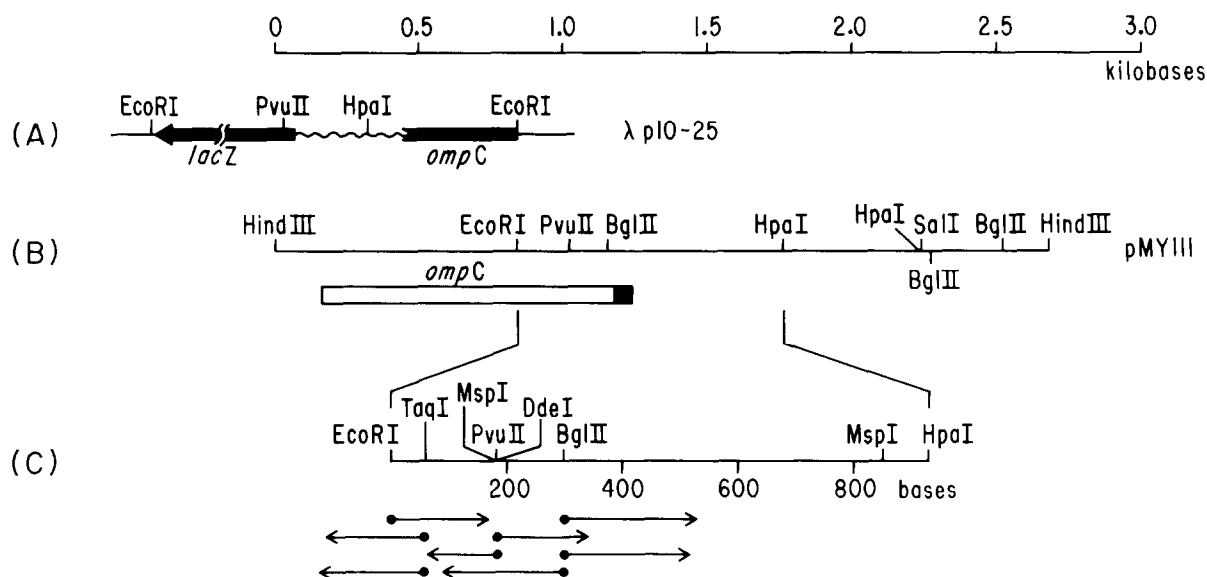
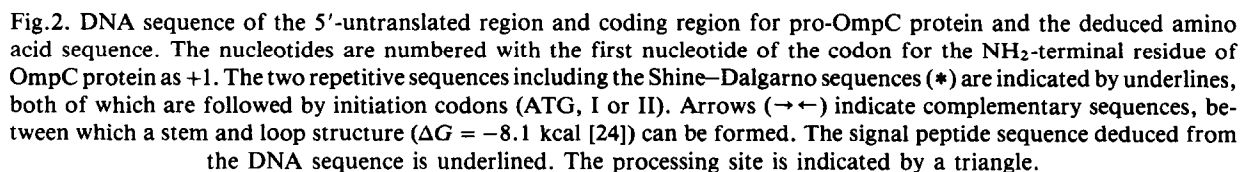


Fig.1. Cloning of the *ompC* gene and the sequencing strategy. (A) The 3.7-kb *EcoRI* fragment in λ p10-25 carrying an *ompC-lacZ* gene fusion. The *lacZ* gene ($\leftarrow$) was assigned in the 2.9-kb *EcoRI-PvuII* region on the basis of DNA sequencing results. The region indicated by a wavy line (~~~~) was found to be derived from Mu phage DNA (see text). (B) The 2.7-kb *HindIII* fragment from chromosomal DNA, which was cloned into pBR322 (the plasmid was designated as pMY111). The *EcoRI* site corresponds to the *EcoRI* site of the *HpaI-EcoRI* fragment in λ p10-25. The approximate coding region for the *ompC* gene ($\square$) is shown under the restriction map. The closed box ($\blacksquare$) indicates the coding region of the signal peptide sequence. (C) Sequencing strategy: ($\rightarrow$) extent of sequences determination using 3'-end ($\bullet$) labeled fragments.

Chromosomal DNA from *E. coli* W620 was digested with several restriction enzymes, and hybridized with the ³²P-labeled *HpaI*–*EcoRI* fragment. A 2.7-kb *HindIII*, a 4.7-kb *HpaI*, and a 2.0-kb *EcoRI* fragment were found to hybridize with the probe (not shown). The 2.7-kb *HindIII* fragment was then cloned into pBR322. *E. coli* T19 *recA*



ompB strain was used as a recipient for transformation to avoid a possible lethal effect due to an overproduction of OmpC protein, since the *ompC* gene is not expressed in an *ompB* mutant [8]. A clone (pMY111) carrying the 2.7-kb *Hind*III fragment was isolated by colony hybridization with the 32 P-labeled *Hpa*I–*Eco*RI fragment. When *E. coli* W620 *ompC*::Mu was transformed with the plasmid pMY111, an outer membrane protein with the same M_r -value as OmpC protein was produced in the transformed cells (not shown). Moreover, the cells became sensitive to phage Tu1b, which is known to use OmpC protein as its receptor [2]. These results indicate that pMY111 contains the entire *ompC* gene.

3.1. Identification of the *ompC* gene and its DNA sequence

To determine the position of the *ompC* gene in the 2.7-kb *Hind*III fragment, the restriction map of the fragment was determined as shown in fig.1B. An *Eco*RI site found in the fragment was considered to correspond to the *Eco*RI site of the *Hpa*I–*Eco*RI probe derived from λ p10-25. Therefore, we determined the DNA sequence around the *Eco*RI site. We found that sequence on the right

hand side of the *Eco*RI site was identical to that of the *Hpa*I–*Eco*RI probe (not shown). To find the coding region for the NH₂-terminal part of OmpC protein, we determined the DNA sequence to the left of the *Eco*RI site (fig.1C). The DNA sequence around the *Bgl*II site was found to code for the amino acid sequence:

Ala–Glu–Val–Tyr–Asn–Lys–Asp–Gly–
–Asn–Lys–Leu–Asp (fig.2)

which is exactly the same as the NH₂-terminal sequence of OmpC protein as determined in [16]. This result confirms that the cloned DNA fragment contains the *ompC* structural gene. Thus, the signal peptide sequence consisting of 21 amino acid residues was also deduced from the DNA sequence as shown in fig.2.

4. DISCUSSION

The NH₂-terminal amino acid sequence of OmpC protein of *E. coli* K-12 has been determined to be:

Ala–Glu–Val–Tyr–Asn–Lys–Asp–Gly–
–Asn–X–Leu–Asp [16]

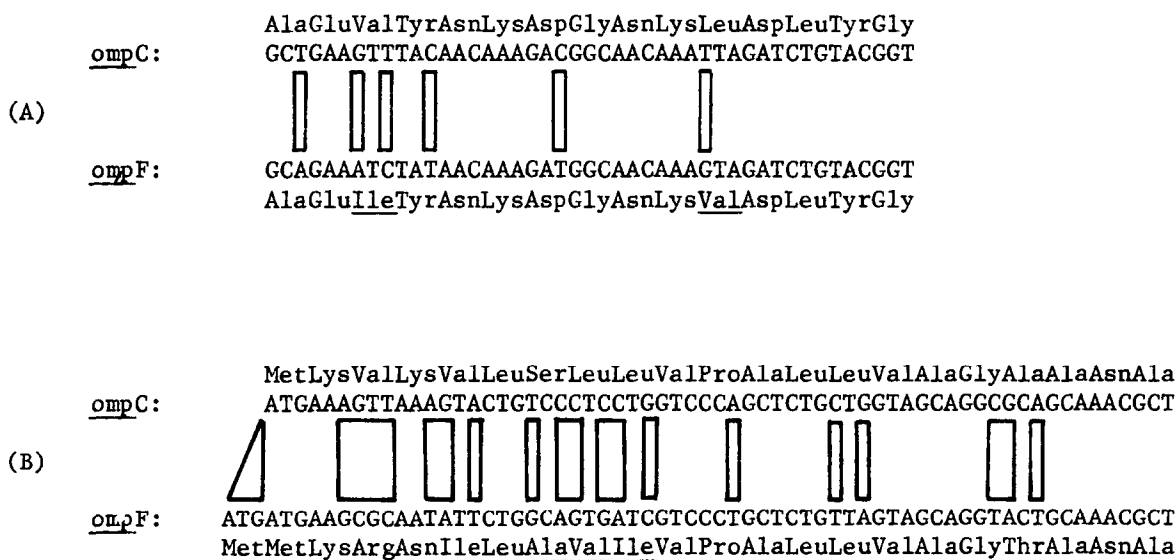


Fig.3. DNA sequence comparison between the *ompC* gene and the *ompF* gene: (A) DNA sequences encoding the first 15 amino acids of NH₂-terminal residues; (B) the signal peptides of OmpC and OmpF proteins are shown. The *ompF* gene sequence is cited from [6]. Non-homologous regions are connected with empty bars, amino acid differences are underlined.

Exactly the same sequence can be deduced from the DNA sequence (from positions +1 to +36 in fig.2). Since OmpC protein consists of about 340 amino acid residues which corresponds to 1020 base pairs, the 2.7-kb *Hind*III fragment should contain the entire *ompC* gene as shown in fig.1.

The DNA sequence also showed that OmpC protein is synthesized as a precursor with an extension at the NH₂-terminal end, consisting of 21 amino acid residues, which appears to have the well-known features of prokaryotic signal peptide sequences (review [18]). These characteristics are as follows:

- (i) The existence of basic amino acids near the NH₂-terminus;
- (ii) The existence of long hydrophobic residues at the central region;
- (iii) The existence of a glycine and a proline residue in the hydrophobic region;
- (iv) The existence of the cleavage site on the carboxyl terminal side of an alanine residue.

It is interesting to note that the cleavage site for pro-OmpC protein is Ala-Ala-Glu, which is identical with those for pro-OmpF [6] and pro-PhoE [19] proteins. All of these proteins are known to form diffusion pores across the outer membrane.

The DNA sequence coding for the first 15 amino acids of NH₂-terminal portion of OmpC protein reveals extensive homology with that of the *ompF* gene [6], only 6 out of 45 base pairs are substituted (fig.3). However, there are more drastic changes in the DNA sequences coding for the signal peptides of pro-OmpC and pro-OmpF proteins, including not only single base substitutions but also alterations of more than one base within a codon (fig.3).

More surprisingly, no such homology can be seen in the 5'-untranslated regions of the two proteins. In this region of pro-OmpC protein (fig.2), there are several unique features. The translation initiation codon (⁻⁶³ATG⁻⁶¹) is preceded by a Shine-Dalgarno sequence (⁻⁷⁶GAGG⁻⁷³) constituting the ribosome binding site [20]. The distance between these sequences is 9 base pairs. These facts are consistent with the general findings for prokaryotic translation initiation sites (review [21]). It should be pointed out that exactly the same sequence (⁻⁷⁶GAGGTTAAT⁻⁶⁷) which includes the Shine-Dalgarno sequence was found further upstream from positions -128 to -119, and that a stable stem and loop structure can be formed at this region. Moreover, this region is also followed

by another ⁻¹¹⁴ATG⁻¹¹². Protein synthesis beginning at this initiation codon would terminate just upstream of the putative ribosome binding site ⁻⁷⁶GAGG⁻⁷³. These features are summarized in fig.2. The 5'-untranslated region of another major outer membrane protein, the OmpA protein, has similar characteristics [22]. The exact role of such structures is unknown at present. Upstream of this region, there is a possible Pribnow-box (-10 region) ⁻¹⁵⁴gAgAATG⁻¹⁴⁸ (lower case letters denote nucleotides which do not share homology with the consensus sequence) and the RNA polymerase recognition site (-35 region) ⁻¹⁷⁹TGTTGgattaTT⁻¹⁶⁸, both of which have good homology with the consensus sequences [23].

The expression of the *ompC* and *ompF* genes are under complex regulation exerted mainly by the *ompB* operon. The *ompB* operon has been cloned, and its entire DNA sequence has been determined [4,5]. Since its gene products have been identified [4,5], it is now of great interest to examine how these gene products interact with the promoter regions of the *ompF* and *ompC* genes.

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