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Location of DNA-binding segment of a positive regulator, OmpR, involved in activation of the *ompF* and *ompC* genes of *Escherichia coli*

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OmpR protein is a positive regulator involved in activation of the *ompF* and *ompC* genes which encode the major outer membrane proteins OmpF and OmpC, respectively. OmpR protein is considered to have a two-domain structure. In the present study we isolated a C-terminal fragment of the OmpR molecule, which bound to a specific promoter sequence of the *ompF* gene. We conclude that the C-terminal portion of the OmpR protein contains a DNA-binding site.

Protein, OmpR; Activator protein; DNA recognition; (*E. coli* outer membrane)

1. INTRODUCTION

Expression of the *ompF* and *ompC* genes, which encode the *Escherichia coli* outer membrane proteins OmpF and OmpC, respectively, is affected in a reciprocal manner by the osmolarity of the growth medium [1]. Several lines of genetic and biochemical evidence support the view that a regulatory factor, OmpR, acts as a *trans*-acting activator that interacts with a *cis*-acting upstream sequence of the *ompF* and *ompC* promoters [2–4]. Among bacterial gene-activator proteins such as the cyclic AMP receptor protein (CRP) and the lambda repressor, OmpR protein is unique from the following point of view. It is well known that most bacterial site-specific DNA-binding proteins have a helix-turn-helix motif in their secondary structures and this motif plays a crucial role in DNA recognition [5]. OmpR protein, however, does not contain a readily recognizable helix-turn-helix motif [6], suggesting that the mode of DNA recognition of OmpR protein is different from that of most other bacterial site-specific DNA-binding proteins.

We have previously suggested that the C-terminal portion of OmpR protein is responsible for its binding to the *ompF* and *ompC* promoter DNAs [7]. In this study, we purified a C-terminal fragment of OmpR protein, and by using this fragment, we have directly demonstrated that the C-terminal portion of OmpR protein contains a DNA-binding site.

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Abbreviations: SDS, sodium dodecyl sulfate; bp, base pairs; NMR, nuclear magnetic resonance

2. MATERIALS AND METHODS

2.1. *Materials*

Restriction endonucleases, Klenow fragment of DNA polymerase I and DNase I were obtained from Takara Shuzo Co. Ltd. [α-^{32}P]CTP (3000 Ci/mmol) was purchased from Amersham International. All other reagents were of reagent grade.

2.2. *Purification of OmpR protein and its C-terminal fragment*

OmpR protein was purified from *E. coli* JA221 carrying

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plasmid pFN108, which overproduces OmpR protein [3], according to the method described previously with a slight modification [7]. Purified OmpR protein was stored in 50 mM Tris-HCl (pH 7.8) containing 200 mM NaCl at 4°C. During storage, it underwent spontaneous degradation, giving a smaller polypeptide fragment. The time required for optimal generation of the smaller polypeptide fragment was about one month. This fragment was purified by using a Pharmacia Mono-Q column.

2.3. *DNA-binding assay*

The binding of OmpR protein to DNA was assayed essentially as described [3], by using a 463-bp *Eco*RI-*Hin*dIII fragment encompassing the *ompF* promoter region and its upstream sequence [7].

2.4. *DNase I footprinting*

The DNase I footprinting was carried out as described [7].

2.5. *Amino acid sequencing*

The amino acid sequencing was performed with an automated amino acid sequencer (Applied Biosystems Model-477A).

2.6. *Polyacrylamide gel electrophoresis*

An SDS-polyacrylamide gel (15% acrylamide) system was used [8].

3. RESULTS

3.1. *Isolation of a polypeptide fragment derived from the C-terminal portion of OmpR protein*

During the course of characterization of purified OmpR protein (molecular weight 27353), we noticed that it undergoes characteristic degradation during storage, giving a smaller fragment with an apparent molecular weight of 16000. To study the structure and function of OmpR protein, we purified this fragment. Fig.1A shows a SDS-polyacrylamide gel profile of the isolated fragment with a molecular weight of 16000 (lane 3). A minor polypeptide with a molecular weight of 14500 was also seen. This polypeptide, however, turned out to be a product formed from the major polypeptide fragment by further degradation, since it was observed that the polypeptide fragment with a molecular weight of 16000 gradually disappeared and the polypeptide fragment with a molecular weight of 14500 concomitantly accumulated during further storage (not shown). The N-terminal sequence of the fragment was determined to be NH_2-Asn-Glu-Leu-Pro-Gly. This sequence was found in the central portion of the amino acid sequence of intact OmpR protein which was deduced

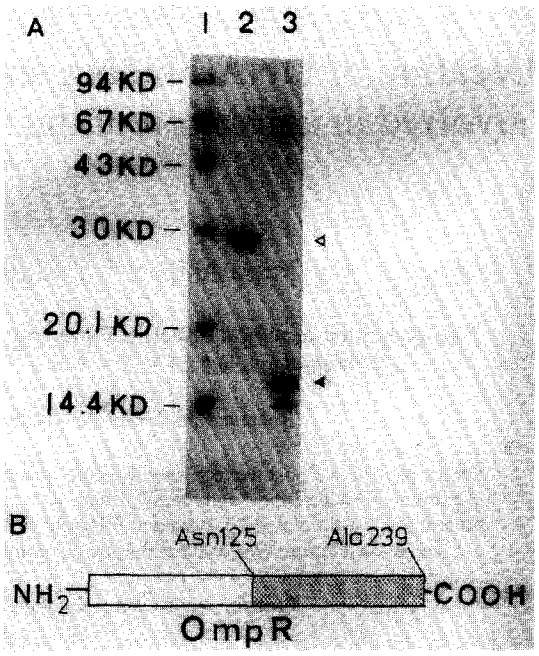

Fig.1. SDS-polyacrylamide gel electrophoresis of purified OmpR protein and its C-terminal fragment and a schematic representation of OmpR protein. (A) Proteins were subjected to SDS-polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue; lane 1, molecular weight standards; lane 2, purified OmpR protein (5 μg); lane 3, purified C-terminal fragment (3 μg). (B) Structure of the OmpR protein is schematically presented. The shaded region denotes the C-terminal fragment characterized in this study. The positions of the relevant amino acids are indicated.

from the nucleotide sequence of the *ompR* gene [6]. The first amino acid residue Asn corresponds to Asn-125 of the intact protein (fig.1B). Although the C-terminal residue of the fragment could not be determined, its apparent molecular weight (16000) estimated by SDS-polyacrylamide gel electrophoresis agrees well with that calculated on the basis of the amino acid sequence for the segment extending from Asn-125 to the C-terminal Ala-239. We therefore conclude that the purified fragment contains most, if not all, of the C-terminal portion of the OmpR protein. It should be emphasized that the degradative change of purified OmpR protein was reproducible, and that we were able to isolate the same fragment at least twice from OmpR protein preparations purified independently.

3.2. *DNA-binding ability of the C-terminal fragment of OmpR protein*

We have previously proposed that the C-terminal portion of OmpR protein is responsible for its binding to the *ompF* and *ompC* promoter DNAs [7]. To test this, the DNA-binding ability of the isolated C-terminal fragment was compared in vitro with that of intact OmpR protein. A 463-bp *Eco*RI-*Hin*dIII fragment encompassing the *ompF* promoter and its upstream sequence (fig.2A) was labeled with [α-^{32}P]CTP at its *Hin*dIII site. After incubation of this DNA fragment with intact OmpR protein or the fragment, the mixtures were subjected to non-denaturing polyacrylamide gel electrophoresis. It is expected that the protein binding to the DNA fragment would decrease the mobility of the DNA fragment. As shown in fig.2B, the C-terminal fragment as well as intact OmpR protein were capable of binding to the *ompF* promoter DNA, suggesting that the C-

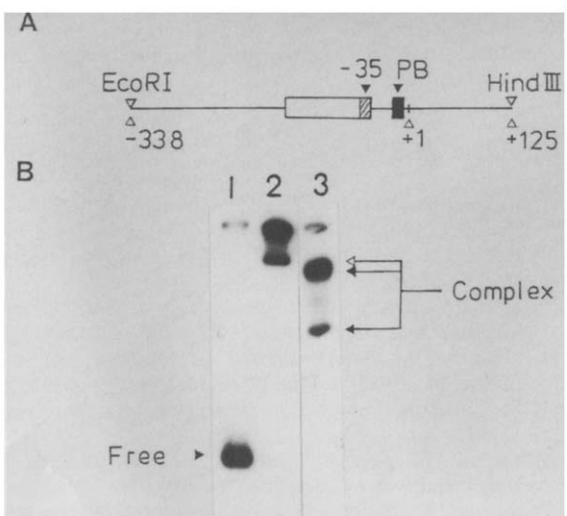

Fig.2. Binding of the C-terminal fragment of OmpR protein to the *ompF* promoter DNA. (A) The 463-bp *Eco*RI-*Hin*dIII fragment encompassing the *ompF* promoter region is schematically presented. The position of the *ompF* transcription start site is shown as +1. The open rectangle denotes the OmpR binding region which is located upstream of the −35 region (−35) and the Pribnow box (PB) of the *ompF* promoter. (B) The *Eco*RI-*Hin*dIII fragment was isolated and labeled with [α-^{32}P]CTP at its *Hin*dIII site. The endo-labeled fragment (about 0.5 pmol) was incubated either with the intact OmpR protein (lane 2, about 15 pmol) or the C-terminal fragment (lane 3, about 150 pmol). After the incubation, the samples were subjected to non-denaturing polyacrylamide (6%) gel electrophoresis and then analyzed by autoradiography.

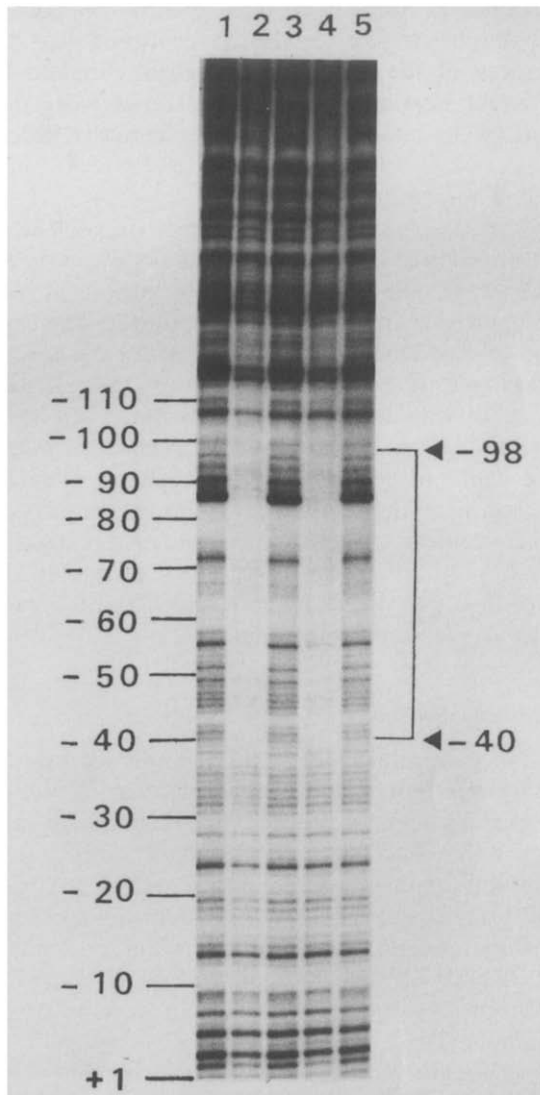

Fig.3. DNase I footprinting of the *ompF* promoter region with the C-terminal fragment of the OmpR protein. The endo-labeled *Eco*RI-*Hin*dIII fragment (see fig.2A) was incubated either with the intact OmpR protein (lane 2, 120 pmol) or the C-terminal fragment (lane 4, 880 pmol) under exactly the same conditions described in the legend to fig.2. Lanes 1, 3 and 5 represent control experiments without addition of the OmpR protein. After 60 s treatment with DNase I, the samples were subjected to polyacrylamide (8%) sequencing gel electrophoresis and then analyzed by autoradiography. The numbers on the left-hand side of the gel correspond to those of the nucleotide sequence of the *ompF* promoter; +1 represents the *ompF* transcription start site. The precise positions of nucleotides were determined by comparison with a parallel run of the product of the Maxam-Gilbert 'G' cleavage reaction (not shown). The regions protected by the OmpR protein and the C-terminal fragment were indicated by a bracket on the right-hand side of the gel.

terminal portion of OmpR protein contains a DNA-binding site. It should be noted that the amount of the C-terminal fragment required for efficient binding was about ten times more than that of the intact OmpR protein in molar ratio.

3.3. *Footprinting analysis*

A critical question was whether or not the C-terminal fragment recognized the same specific sequence in the *ompF* promoter region as that recognized by intact OmpR protein [7]. The same end-labeled DNA fragment as used for the binding assay (see fig.2A) was subjected to DNase I footprinting with the C-terminal fragment (fig.3). It was found that the C-terminal fragment protected the same nucleotide sequence against DNase I digestion as that protected by intact OmpR protein. Namely the nucleotide sequence extending from −40 to −98 of the *ompF* promoter was specifically protected by both intact OmpR protein and its C-terminal fragment.

4. DISCUSSION

The C-terminal fragment of OmpR used in this study was spontaneously generated during storage of purified OmpR protein. The degradative reaction is reproducible and presumably results from contamination by cellular proteases. In any case, this fortuity enabled us to demonstrate that the C-terminal portion of OmpR protein contains a DNA-binding site. This conclusion is consistent with previous genetic studies on various *ompR* mutants [7].

Although the C-terminal fragment can specifically bind to the *ompF* promoter DNA, its binding efficiency appears to be lower than that of intact OmpR protein. We have previously suggested that OmpR protein has a two-domain structure, each domain exhibiting a different role in the gene regulation [9], and that more than one OmpR molecule bind to the *ompF* and *ompC* promoter DNAs [8,10]. The N-terminal portion of OmpR protein may play a role in stabilizing the OmpR–DNA interaction through an OmpR–OmpR interaction at its N-terminal portion.

Structural studies of purified OmpR protein with a variety of physicochemical techniques are now underway in our laboratories. NMR spectra of the C-terminal fragment isolated in this study were measured and compared with those of intact OmpR protein under various conditions. The results of NMR analysis suggest that the conformation of the C-terminal fragment, which we discussed above, is basically the same as that of the corresponding portion of the polypeptide chain in intact OmpR protein (Tate, S., et al., in preparation). Such physicochemical analyses of the C-terminal fragment and intact OmpR protein should shed light on the molecular mechanism underlying specific DNA recognition by the activator, OmpR.

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REFERENCES

[1] Kawaji, H., Mizuno, T. and Mizushima, S. (1979) J. Bacteriol. 140, 843–847.
[2] Hall, M.N. and Silhavy, T.J. (1981) Annu. Rev. Genet. 15, 91–142.
[3] Jo, Y.-L., Nara, F., Ichihara, S., Mizuno, T. and Mizushima, S. (1986) J. Biol. Chem. 261, 15252–15256.
[4] Norioka, S., Ramakrishman, G., Ikenaka, K. and Inouye, M. (1986) J. Biol. Chem. 261, 17113–17119.
[5] Pabo, C.D. and Sauer, R.T. (1984) Annu. Rev. Biochem. 53, 293–321.
[6] Nara, F., Matsuyama, S., Mizuno, T. and Mizushima, S. (1986) Mol. Gen. Genet. 202, 194–199.
[7] Mizuno, T., Kato, M., Jo, Y.-L. and Mizushima, S. (1988) J. Biol. Chem. 263, 1008–1012.
[8] Laemmli, U.K. (1970) Nature 227, 680–685.
[9] Nara, F., Mizuno, T. and Mizushima, S. (1986) Mol. Gen. Genet. 205, 51–55.
[10] Maeda, S., Ozawa, Y., Mizuno, T. and Mizushima, S. (1988) J. Mol. Biol. 202, 433–441.