INTERACTION OF cAMP RECEPTOR PROTEIN WITH THE *ompA* GENE, A GENE FOR A MAJOR OUTER MEMBRANE PROTEIN OF *ESCHERICHIA COLI*

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1. Introduction

We have determined the entire DNA sequence of the *ompA* gene, a gene for a major outer membrane protein of *Escherichia coli* [1]. The promoter region of this gene was found to be remarkably homologous with the *lac* and *gal* P_2 promoters [2]. Particularly from the fact that both the *ompA* and *gal* P_2 promoters have the same octanucleotide sequence, T–C–A–C–A–C–T–T in their RNA polymerase recognition site (–35 region), it became relevant to find out if the cAMP receptor protein (CRP) binds to this region of the *ompA* gene to influence the cell-free transcription of the *ompA* gene [2].

Here we present the effects of CRP and cAMP on the cell-free transcription of the *ompA* gene and studies aimed at determining the CRP binding site on the *ompA* promoter. The results obtained indicate that CRP has a negative effect on the transcription of the *ompA* gene. We have also rechecked the gels of the DNA sequence of the *ompA* gene at 7 different positions where the DNA sequence we determined [1] differed from that in [3], including the sequence at the putative transcription termination site, where there is an extremely stable stem-and-loop structure in our sequence but not in theirs.

2. Materials and methods

The isolation of DNA fragments from the *ompA* gene, in vitro transcription of the DNA fragments, and the determination of DNA sequence have been described [1,2]. The deoxyribonuclease I (DNase I) protection experiment was done as in [4]. CRP was kindly provided by J. Krakow.

3. Results

3.1. *Effects of CRP on in vitro transcription of the* ompA *gene*

The in vitro transcription of the *ompA* gene was done in the presence of 22.5% glycerol as described using the *Hin*fI/*Hae*II fragment (position 1–425; fig.2 in [2]) which encompasses the *ompA* promoter and the 5′-portion of the *ompA* mRNA. A DNA fragment which contained the entire lipoprotein *(lpp)* gene (2.8 kilobases *Hae*III fragment; [5]) was also added in the reaction mixture as an internal control. The *lpp* gene expression is constitutive and not under cAMP control. As shown in fig.1A (1), both the *lpp* mRNA (322 bases) and the *ompA* transcript (313 bases) were produced in the absence of CRP and cAMP. However, when both CRP and cAMP were added to the reaction mixture, the production of the *ompA* transcript was completely inhibited, whereas the production of the *lpp* mRNA was hardly affected by CRP and cAMP (fig.1A (2)).

The inhibitory effect of CRP was observed even without the addition of cAMP (fig.1B (2)) although the addition of cAMP enhanced the inhibitory effect of CRP (fig.1B (3)). When the amount of CRP added was reduced to 1/10, which was equivalent to ~20 CRP molecules/DNA fragment, the in vitro transcription was not completely inhibited even in the presence of cAMP (fig.1B (4)). cAMP alone did not show any inhibitory effect on the in vitro transcription of the *ompA* gene (fig.1B (5)).

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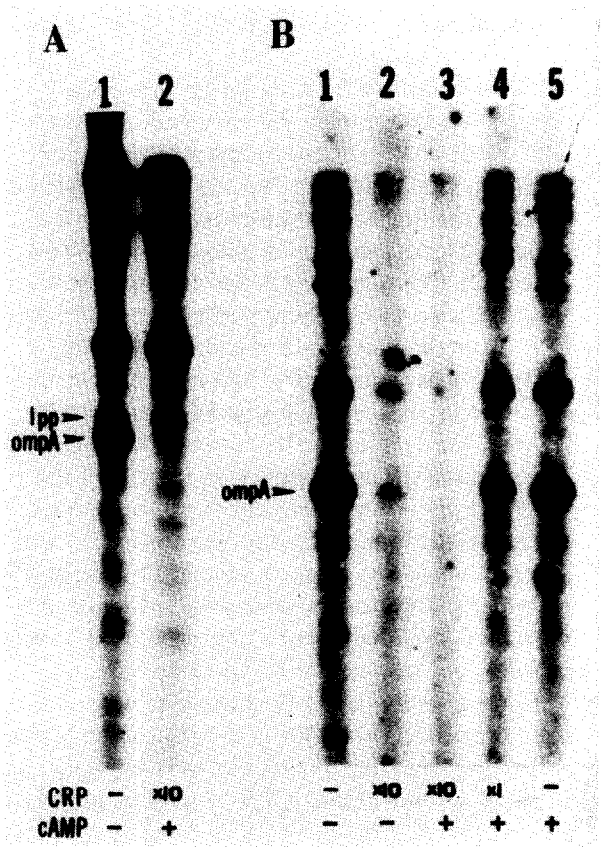


Fig.1. Effects of CRP and cAMP on in vitro transcription of the *ompA* gene. In vitro transcription was carried out in 20 μl of the reaction mixture with [α-^{32}P]UTP as in [2]. The reaction was carried out in 22.5% glycerol with 0.04 pmol *Hin*fI/*Hae*II fragment (position 1–425; fig.2 in [1,2]). (A) The reaction mixture also contained 0.02 pmol 2.8 kilobases *Hae*III fragment containing the *lpp* gene [5]. (1) no CRP and no cAMP; (2) 0.52 μg CRP and 0.5 mM cAMP were added to the reaction mixture. (B): (1) no CRP and no cAMP; (2) 0.52 μg CRP; (3) 0.52 μg CRP and 0.5 mM cAMP; (4) 0.052 μg CRP and 0.5 mM cAMP; (5) no CRP and 0.5 mM cAMP were added to the reaction mixture. RNA products were analyzed by a 5% polyacrylamide gel in 7 M urea. Arrows with *lpp* and *ompA* represent the positions of the *lpp* gene transcript (322 bases) and the *ompA* gene transcript (313 bases), respectively.

3.2. *CRP binding site*

To determine the CRP binding site, a DNase I protection experiment was carried out in the presence of CRP. The same *Hin*fI/*Hae*II fragment that was used in in vitro transcription experiments described above was used to obtain the *Hin*fI/*Msp*I fragment (position 1–178; fig.2 in [1,2]) which encompasses the promoter region as well as the 5′-end region of the *ompA* mRNA of 66 bases. Position 1 of this fragment was labeled with ^{32}P at its 5′-end [1]. The ^{32}P-labeled fragment was digested with DNase I in the absence and the presence of CRP. The location of the CRP binding site was then determined by electrophoresis of the DNase I digestion products, and cleavage positions were determined by parallel electrophoresis of the same ^{32}P-end labeled DNA fragment which was cleaved at C and T positions as in [6]. Fig.2 shows that positions 80–87 were clearly affected by the addition of CRP and cAMP: T at 80, C at 81 and T at 87 were protected, whereas cleavage at C at 83 was enhanced; T at 71, and G at 72 seem to be weakly protected, and cleavage at A at 102 was weakly enhanced.

3.3. *Re-examination of DNA sequence of the* ompA *gene*

There are 7 discrepancies between the DNA sequence of the *ompA* gene determined in [1] and that in [3]. Autoradiograms of DNA sequencing gels covering these regions are shown in fig.3A–G:

(A) In [3], A-124 is G and there is an extra C between C-123 and A-124. As shown in fig.3A, the position at 124 is clearly A. However, there is an extra blank space between C-123 and A-124, which cannot be identified by this gel. If this position is occupied by a methylated C, C–C*–A–G–G should be cleaved by *Bst*NI. We found that the *Hin*fI/*Hae*II fragment was cleaved by *Bst*NI at this position (not shown). Thus, in our original sequence of the *ompA* gene [1], one C was missing between position 123 and 124. However, the cleavage by *Bst*NI at this position confirmed A-124.

(B) The base at 173 is A not G in our original sequence. The opposite strand is shown in fig.3B where 173 is T rather than C. The sequence, GATC (172–175) was confirmed by cleavage with *Sau*3A at this position (not shown).

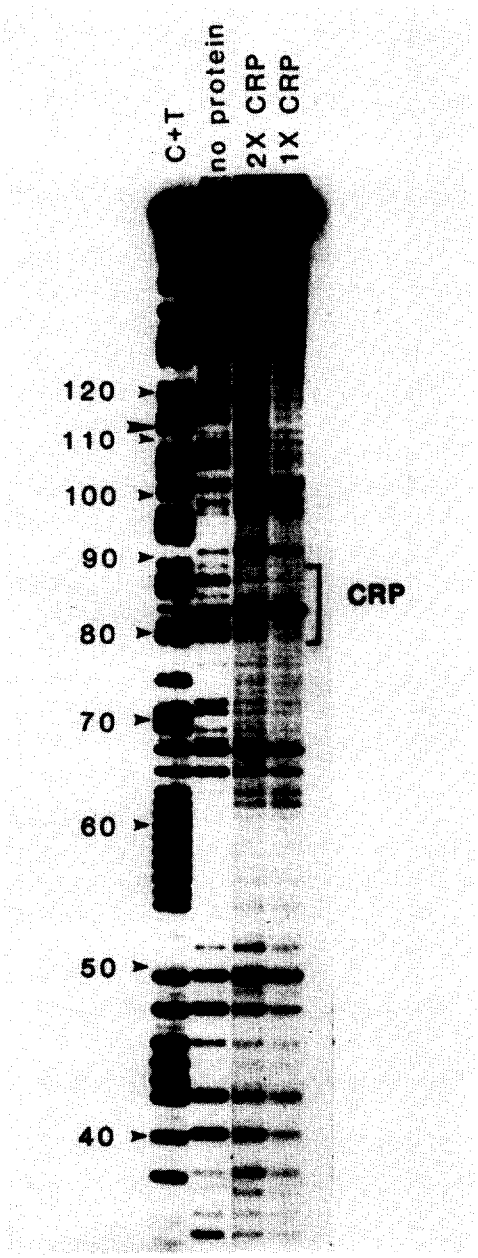
(C) The position at 375 is C not T (fig.3C).

(D) The position at 747 is C not G (fig.3D).

(E) The position at 837 is A not G. The opposite strand is shown in fig.3E where 837 is T rather than C.

(F) The position at 994 is C instead of T (fig.3F).

(G) In [3] there seems to be a deletion of 3 Cs from 1314–1316 and the base position at 1329 is T. In our sequence, there is a row of 4 Cs from 1314–1317 and the position at 1329 is G not T. There is a stretch of 8 Ts rather than 9 Ts at this position (fig.3G (1)). These can also be confirmed by the sequencing gel of the opposite strand; the position at 1329 is C not A and there is a stretch of 8 As (fig.3G (2)).



4. Discussion

4.1. *Interaction of CRP with the* ompA *promoter*

As pointed out earlier, there is a striking homology in the DNA sequence between the *gal* P2 and *ompA* promoters ([2] and fig.4). This was further substantiated by the finding that the *ompA* promoter was stimulated by the addition of glycerol in a cell-free system [2]. This homology in the RNA polymerase recognition site (–35 region) seems to be involved in CRP binding and inhibition of transcription [2].

Here, we have shown that CRP and cAMP inhibit in vitro transcription of the *ompA* gene but not the *lpp* gene. Furthermore, CRP was found to bind at –35 region of the *ompA* gene as in the case of the *gal* P2 [7]. Fig.4 summarizes the results of DNase I protection experiments for the *ompA* promoter and they are compared with those obtained for the *gal* P2 promoter [7]. One can again observe similarities in the patterns of DNase I protection as well as cleavage enhancement between the two promoters.

However, it is not clearly understood why no significant effects of cAMP on the production of the *ompA* protein have been observed in vivo ([8,9]; R. M., M. I., unpublished). It is possible that there is an unknown positive factor which competes with the inhibitory effect of CRP on the *ompA* gene.

Fig.2. DNase I protection of the *ompA* promoter in the absence and presence of CRP and cAMP. The *Hin*fI/*Msp*I fragment (position 1–178) labeled with ^{32}P at the *Hin*fI site (position 1 in [1]) was used for this experiment. DNase I protection was done as in [4]; each 22.5 μl reaction mixture contained 66 ng ^{32}P-labeled DNA fragment in 20 mM Tris–HCl (pH 8.0), 3 mM $MgCl_2$, 0.1 mM DDT and 0.5 mM cAMP. After 10 min preincubation at 37°C, 0.9 μl of no protein, 2 × CRP (4.7 μg), and 1 × CRP (2.3 μg) solution (50 mM potassium phosphate (pH 7), 0.1 mM EDTA, 1.0 M NaCl) was added in 3 independent reaction mixtures, respectively. The mixtures were incubated for 10 more min at 37°C and for 1 min at 20°C. DNase I solution (0.12 μg/ml; Millipore Corp.) (2 μl) was added. After a 30 s incubation at 20°C, the reaction was stopped by adding stop buffer. The DNA was prepared and separated by electrophoresis. (1) C + T sequencing size control; (2) DNase digestion with no CRP addition; (3,4) DNase digestion with 4.7 μg and 2.3 μg CRP, respectively. Numbers with small arrows correspond to those published (fig.2 in [1,2]). A big arrow indicates the transcription initiation site.

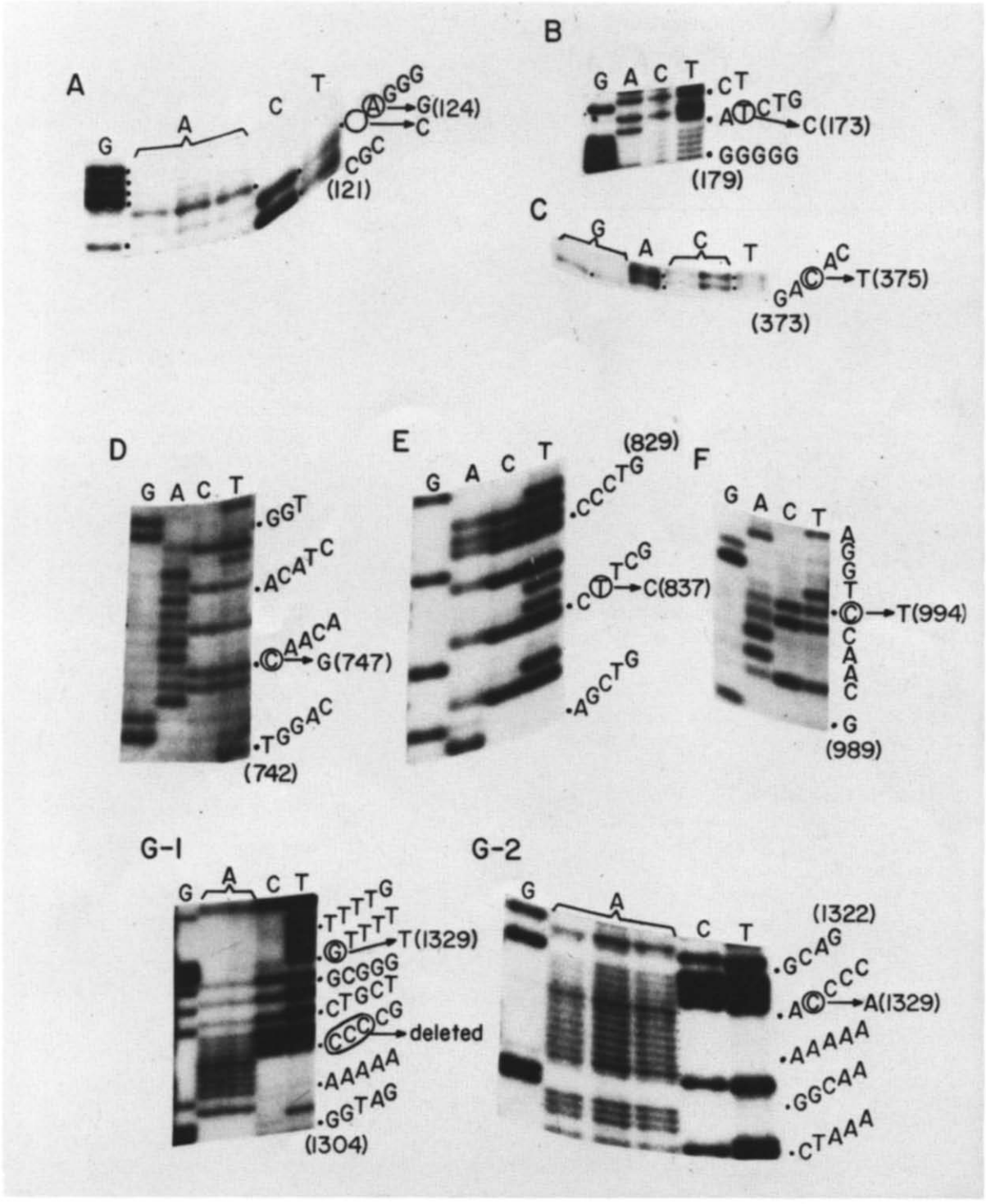


Fig.3. DNA sequencing gels of regions where the DNA sequence of the *ompA* gene determined in [1] differs from that in [3]. Numbers represent those previously described for the *ompA* gene (fig.2 in [1,2]). DNA sequences described at the right-hand sides are those determined by the gels shown. Circles indicate positions where sequences in [1] differ from those in [3]. Their sequences are shown by arrows. Note that in B, E and G-2 show sequences of the opposite strand.

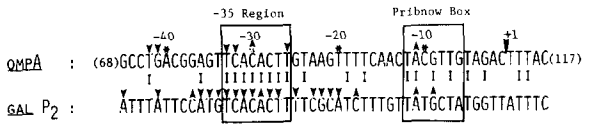


Fig.4. Summary of protection results, and comparison with the *gal* P2 promoter. The DNA sequence of the *ompA* promoter is from [1,2]. Small arrows pointing downward indicate protection against DNase digestion by cAMP–CRP at the 3′-side of the indicated base. Small arrows pointing upward indicate enhancement of digestion by DNase at the 3′-side of the indicated bases. The results for *gal* P2 were from [7]. A big arrow indicates the transcription initiation site. Numbers in parentheses indicate those described for the *ompA* gene [1,2]. Bars between 2 sequences show homologies between the 2 sequences.

4.2. *DNA sequence of the* ompA *gene*

We have confirmed the DNA sequence of the *ompA* gene determined in [1] except that 1 C was missing between the positions 123 and 124 (fig.2 in [1]). This C was not detected in a DNA sequencing gel possibly because it was methylated. Since this C is located in an early region of the 5′-untranslated region of the *ompA* mRNA, there is no effect on the coding region of the *ompA* gene.

Four other descripancies between our sequence and that in [3] are found in the coding region, although none of them change amino acid residues. However, all the codons found in these positions [3] sequence are either not used in our *ompA* sequence at all (UUG for leucine, and ACG for threonine [1]) or used less frequently (GAU for aspartic acid and GAG for glutamic acid [1]).

The difference between our sequence and [3] at the region of 1314–1337 is rather interesting. In our sequence, this region forms a potential extremely stable stem-and-loop structure, and show a remarkable homology with the 3′-end portion of the lipoprotein mRNA, and is considered to be a signal for transcription termination [1]. However, in [3], such a possibility does not exist. It is possible that mutations which reduce the production of the *ompA* protein may have occurred when the *ompA* gene was cloned in pSC101. It is interesting to note that a similar mutation (a deletion at the transcription termination site) was found in the *lpp* gene from *Serratia marcescens* cloned into Charon 14 phage vector [10].

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