Mutant cAMP Receptor Protein Binds to DNA without DNA Bending

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Cyclic AMP receptor protein (CRP) complexed with cAMP binds to DNA and induces sharp DNA bending around ~90 degree. Previous publication [5], however, reported that mutant CRP:cGMP complex showed high migration rate relative to mutant CRP:cAMP complex on native polyacrylamide gel. To confirm DNA structural change in the presence of CRP and cyclic nucleotide, molar cyclization factor (j_M) [13] was measured with 6 constructed DNA fragments. Nonlinear regression analysis of j_M data indicated that mutant CRP did not induce DNA bending in the presence of cGMP but bent DNA in the presence of cAMP without any helical twist change in DNA.

Key words – Cyclic AMP receptor protein, molar cyclization factor(j_M), DNA structural change.

The cyclic AMP receptor protein (CRP) controls transcription of many genes either positively or negatively in response to carbon sources as nutrient in *Escherichia coli* [1,9,12] CRP exists as homodimer. Each subunit contains cAMP binding pocket (N-terminal domain) and DNA binding motif (C-terminal domain). By binding of cAMP, CRP is converted into active conformation and its complex bound to the target site induces sharp bending in DNA [6].

Mutant 91 CRP, which was substituted serine for alanine 144 in helix D, showed the characteristic DNA binding property. 91 CRP did not bind to its binding site at *lac*P, although it was sensitive to protease in the absence of cyclic nucleotide similar to the conformation of wild-type CRP:cAMP complex [7]. In the presence of RNA polymerase, however, 91 CRP bound to DNA and activated transcription of *lac*P without either cAMP or cGMP [5]. In addition, 91 CRP activated the transcription of *lac*P not only in the presence of cAMP but also in the presence of cGMP [7]. It was reported by gel mobility shift assay [3] that 91 CRP:cGMP complex bound to *lac*P showed high migration relative to CRP:cAMP complex on native polyacrylamide gel. This result suggests that 91 CRP retains different protein conformation and binding property to compare wild-type CRP.

This study is designed to elucidate how 91 CRP affects DNA structure in the presence of either cAMP or cGMP, although FRET (fluorescence resonance energy transfer) experiments [8,11] and molar cyclization factor (j_M) measurement [4] reported that wild-type CRP induces symmetric DNA bending without helical twist change in the presence of cAMP.

As mentioned by Gang [4] for details, Several *XbaI* linkers were ligated to end-filled *HindIII* site of pBR322*lac*, which obtained lactose control region from pKL201 [7] resulted in the following three plasmids; pBR322*lac*(0) pBR322*lac*(2), and pBR322*lac*(4). Numbers in each plasmid indicate the additive numbers of base pairs based on pBR322*lac*(0), which has no added or deleted bases to its original sequence. Plasmid pBR322*lac*(6) was produced by Klenow treatment of *XbaI* digested pBR322*lac*(2). Mung bean nuclease, however, did not generate plasmids pBR322*lac*(-2) and pBR322*lac*(-4) due to several missed bases. Instead, pBR322*lac*(-3) and pBR322*lac*(-6) were obtained. The purified EcoRI-digested DNA fragments were named as followed; *lac*(-6), *lac*(-3), *lac*(0), *lac*(2), *lac*(4), and *lac*(6) as shown in Fig. 1.

To measure j_M values [13], time-dependent ligation reactions (see Fig. 2) were conducted by adding 4μl of ligase (0.04 Weiss units of T4 DNA ligase) in 25 mM Tris-HCl (pH 7.8), 10mM $MgCl_2$, 2 mM dithiothreitol, and 0.4 mM ATP. Eight microliters of reaction mixture was withdrawn at 0, 30, 60, 120, 150, and 180 sec. Reactions were terminated by adding an equal volume of 150 mM EDTA. Each reaction was applied on 5% nondenaturing polyacrylamide gel.

j_M values [13] are determined from simple and convenient ligation assay experiments. j_M is defined as $4MoR_{MC/LD}(0)$ where Mo is the initial concentration of linear DNA monomer and $R_{MC/LD}(0)$ can be determined by extrapolation of $R_{MC/LD}(t)$ graph to zero reaction time.

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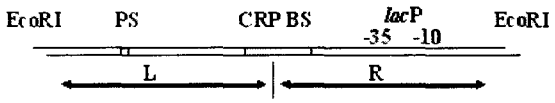

lac	L (bp)	PS	R (bp)
-6	111	CA	113
-3	114	CAGCA	113
0	117	CTCTAGAG	113
2	119	CTCTAGAGCA	113
4	121	TGCTCTAGAGCA	113
6	123	CTCTAGCTAGAGCA	113

Fig. 1. Organization of *EcoRI* DNA fragments. CRP BS (CRP binding site), PS (phasing set), -35 and - 10 regions in *lac* operon are highlighted. L and R represent left and right arms based on the location of CRP BS, respectively. 6 consecutive DNA fragments differ in base sequences of PS. The numbers of bp in L and R are indicated the length of DNA fragments from CRP BS to left (L) or right (R) end of *EcoRI* fragments. Reprinted from *Biochem. Biophys. Res. Commun.*, 322, Gang J., Measurement of DNA helical change for the binding of cyclic AMP receptor protein to *lac* DNA, 994, 2004, with permission from Elsevier.

$R_{MC/LD}(t)$ is the ratio of bands intensities of monomeric circle (MC) to linear dimer (LD) at each reaction time. Band intensity was measured directly by cutting off bands from gel and reading them with scintillation counter. Monomeric circles were highly increased relative to linear dimers and linear trimers in the presence of 91 CRP + cAMP in Fig. 2. This result is consistent with the previous publications [2,4,10], which showed the highly increased monomeric circle by CRP-induced bending or synthetic sequence-dependent curvature. j_M values in this study could not be compared to data reported in previous publications, because we used different length of DNA fragment around 230 bp. Recent publication [4] reported that j_M was 3.1 x 10^{-9} for DNA alone with *lac*(2) DNA fragment. This result is consistent with j_M data measured with same *lac*(2) DNA fragment (3.4 x 10^{-9}) in this study. It indicates that this method is consistently reproducible under different sets of experiments.

In Fig. 3, each set of j_M data was fitted by nonlinear regression analysis with Origin 6.0 (Microcal Software, Inc.). The equation used for this fitting is as follows

$y = Asin[P\ I*(x-xc)/w] + B,$

which A, xc, and w mean amplitude, phase, and half of

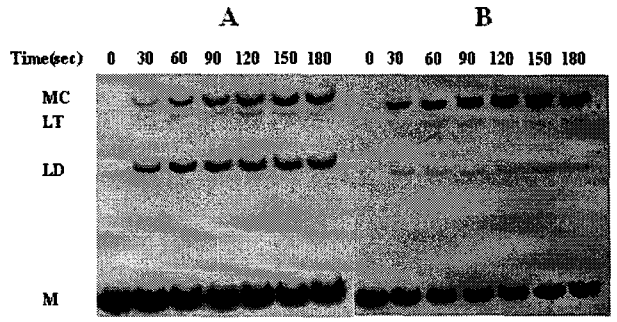

Fig. 2. Autoradiograph showing time-dependent ligation of *lac(0) EcoRI* DNA fragment at either DNA only(A) or 91 CRP + cAMP (B). The concentrations of DNA, 91 CRP and cAMP were 7 nM, 100 nM, and 25 μM , respectively, in 60 μl of reaction volume. Bands on gel images show monomer (M), linear dimmer (LD), linear trimer (LT), and monomeric circle (MC) molecules.

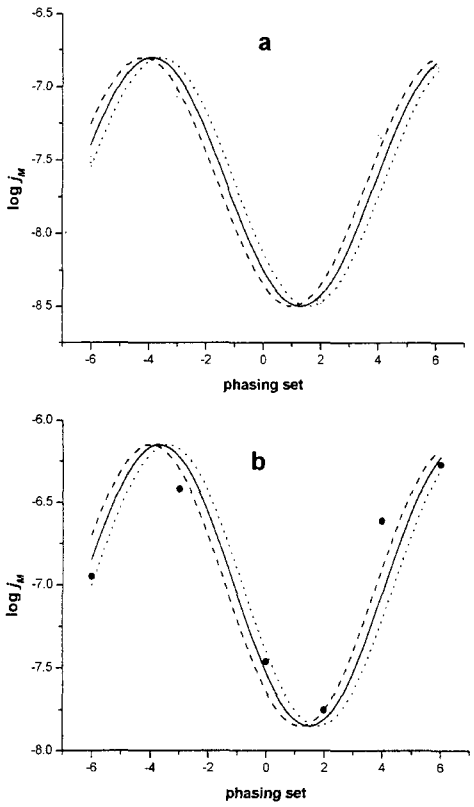

Fig. 3. Analysis of plots of log j_M against phasing set by nonlinear regression analysis with Origin 6.0 (Microcal Software, Inc.). The method used in the analysis was mentioned in Results and discussion. In Fig. 3-a, set of j_M values was obtained in the absence of 91 CRP. Graph was fitted at xc values of - 6.8 (dash line), -6.5 (straight line), and - 6.2 (dot line). Another set of j_M values were fitted at xc of -6.6 (dash line), -6.3 (straight line), and -6.0 (dot line) in 91 CRP + cAMP in Fig. 3-b. Values of xc at -6.5 and -6.3 were best fitted to j_M data of graph a and b, respectively.

periodicity (helical repeat) in wave function, respectively. PI is a constant (3.14) and B represents y shift in the plot. All the parameters are determined from each set of j_M values by changing each variable for the optimal fitting.

Fig. 3-a shows plot of log j_M against phasing set in the absence of CRP. Parameters for w, A, and B were optimally fixed except xc at 5.2, 0.75, and - 7.0, respectively. Value of xc needs to be carefully analyzed to figure out the phase change of plot by binding of protein. Phase change (xc difference) means the helical twist (or end alignment) change of DNA by bound CRP. Fig. 3-a shows that xc = -6.5 represents the best fit to j_M data of DNA only. In Fig. 3-b, j_Ms were increased largely in the presence of 91 CRP and cAMP relative to those of DNA alone. In this case, xc = -6.3 shows the best fit to j_M data. Although there is 0.2 deviation in xc values between Fig. 3-a and b, it does not mean that there is DNA helical twist change by 91 CRP:cAMP complex.

In *lac*(6) DNA fragment, it contains 240 bp including the overhanging bases. Thus, number of helical turn (DNA length in bp / helical repeat) is 23.0. It means that both ends of *lac*(6) DNA fragment are in good alignment for ligation reaction.

Using xc = -6.3, we plotted four sets of log j_M against phasing set of *lac*(-6) through *lac*(6) in Fig. 4. Fig. 4-c and d show that DNA is not bent in the presence of either 91 CRP alone (graph c) or 91 CRP + cGMP (graph d) because B values(y shift) are not increased largely at graphs c (B= -7.70) and d (B = -7.75) relative to graph a (B=-7.0) for 91 CRP + cAMP. It demonstrates that 91 CRP:cGMP make complexes with DNA without DNA bending and helical change.

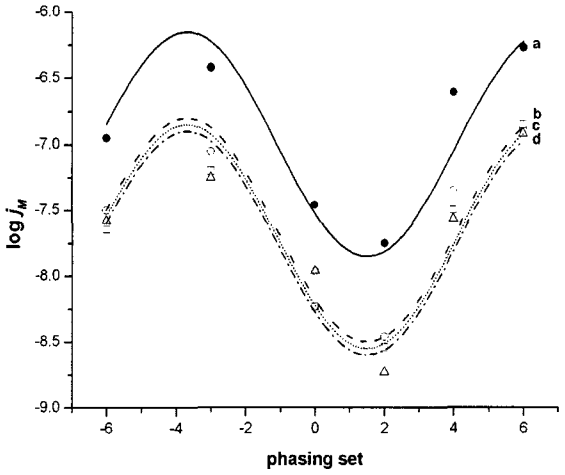

Fig. 4. Plot of log j_M against phasing set of *lac*(-6) ~ *lac*(6) DNA fragments. Graph a, b, c, and d represent the best fit to each set of j_M values at B= -7.0 for 91 CRP + cAMP (filled circle, straight line), -7.65 for no CRP (open circle, dash line), - 7.7 for 91 CRP only (open square, dot line), and -7.75 for 91 CRP + cGMP (open triangle, dash dot line), respectively.

In summary, even though 91 CRP showed different protein conformation and binding property, 91 CRP:cGMP did not induce DNA bending and 91 CRP:cAMP induced DNA bending without a helical twist change as wild-type CRP [4,8,11].

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초록 : DNA 벤딩(휨) 없이 돌연변이 cAMP 수용체 단백질의 결합

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cAMP와 복합체를 형성한 cAMP 수용성 단백질은 DNA와 결합하여 ~90도 정도의 예리한 DNA bending을 유도한다. 그러나 이전의 논문[5]에 의하면 돌연변이 CRP:cGMP 복합체는 돌연변이 CRP:cAMP 복합체보다 아크릴아미드 겔에서 상대적으로 빠른 이동속도를 보였다. CRP와 cyclic nucleotide 존재하에서 DNA의 구조 변화를 알아보기 위하여 6가지 준비된 DNA 조각들을 사용하여 몰 고리화 인자(molar cyclization factor)[13]를 측정하였다. 이들 자료를 사용하여 nonlinear regression analysis를 통하여 cGMP 존재하에서 돌연변이 CRP는 DNA bending을 형성하지 않으나 cAMP 존재하에서 나선 꼬임과 같은 DNA 구조 변화없이 DNA bending을 형성한다.