Volume 11 Number 8 1983 Nucleic Acids Research

CAP binding to B and Z forms of DNA

Michael G.Fried, Hen-Ming Wu and Donald M.Crothers

Departments of Molecular Biophysics and Biochemistry and Chemistry, Yale University, P.O. Box 6666, New Haven, CT 06511, USA

Received 18 November 1982; Revised and Accepted 21 March 1983

ABSTRACT

We have examined the interaction between the cyclic AMP receptor protein (CAP) and a small DNA fragment containing its specific recognition sequence by circular dichroism spectroscopy. The binding of CAP to this fragment induces a B to "C-like" change in the CD spectrum, which is different from that observed for non-specific binding. A one-to-one (CAP dimer to DNA) binding stoichiometry was deduced from spectroscopic titration data, as was a non-specific binding site size of 17 bp/dimer. In addition, we have compared the non-specific binding affinity of CAP for the B and Z forms of synthetic DNA copolymers. A slight preference for the B form was found. These results do not support the recent specific suggestion that CAP binds to a left-handed form of DNA (1), but indicate more generally that an optically detectable conformational change takes place in DNA on binding CAP.

INTRODUCTION

The cAMP receptor protein of Escherichia coli (CAP) is a dimeric protein of 45,000 MW which modulates transcription of catabolite sensitive operons in response to changes in intracellular cAMP concentration (2-4). Stimulation of transcription is apparently achieved through the binding of cAMP-CAP to specific sites within promoter regions (5-7), although the detailed mechanism remains unknown. Two general models for the function of CAP have been advanced (8). The first argues that direct contact between RNA polymerase and CAP stabilizes the polymerase-promoter complex. The second model requires that CAP induce a conformational change in the promoter, possibly aiding polymerase in formation of the "open" complex required for initiation of transcription.

Recently a more detailed mechanism for transcriptional activation by CAP has been proposed by McKay and Steitz (1). In a study of the crystal structure of the CAP-cAMP complex they found two domains of the CAP dimer oriented appropriately to interact with the major groove of a left-handed form of B DNA. Based on this observation they suggested that the binding

of CAP to its recognition sequence causes a right-handed to left-handed conformational transition in the DNA. Such a transition could stimulate transcription by destabilizing the promoter, or by providing new features for recognition by RNA polymerase.

In order to assess the effects DNA structure on CAP binding, we have measured the relative binding affinity of CAP for left-handed Z form DNA with respect to the right-handed B form. In addition we have performed a circular dichroism study of CAP binding to a 16 bp synthetic DNA molecule, containing the CAP recognition sequence. Our results do not support the specific proposal of McKay and Steitz that CAP binding induces a right to left-handed change in the conformation of the lac promoter, but do, however, indicate that detectable structural changes take place upon binding.

MATERIALS AND METHODS

Reagents. Acrylamide and N,N-methylene bisacrylamide were purchased from BioRad. Bovine serum albumin and cAMP were from Sigma. T_4 polynucleotide kinase was from P-L laboratories, and ^{32}P- ATP from New England Nuclear. Micrococcal and S1 nucleases were from Worthington.

Proteins. Lac repressor, purified according to a published method (9) was the gift of M. Leahy. CAP was purified from E. coli K-12 (10) and was judged to be greater than 95% pure by SDS polyacrylamide gel electrophoresis. The preparation used in this study was approximately 90% active in cAMP-dependent binding to the lac promoter (18).

DNA samples. High molecular weight E. coli chromosomal DNA was the gift of H. Eshaghpour. This was dialyzed into 10 mM Tris (pH 7.4 at 4° C), 1 mM EDTA, and sheared for 1 h at 4° C in a Virtis blender operating at 950 rpm. The sheared DNA was fractionated by sedimentation in 5-20% sucrose gradients. Peak fractions were pooled: the weight-average molecular weight was approximately 3×10^6 by agarose gel electrophoresis.

Calf thymus DNA (Sigma) was phenol extracted to remove protein, and dialyzed into 10 mM Tris (pH 7.4 at 4° C), 1 mM EDTA. Shearing and fractionation was as described for the E. coli DNA sample. The weight average molecular weight was 3.5×10^6 by agarose gel electrophoresis.

A synthetic 16 bp DNA fragment containing the lac promoter CAP binding site sequence was the generous gift of K. Itakura (43). The purity and monomeric state of this fragment were verified by gel electro-

phoresis under native (11) and denaturing (12) conditions.

Poly(dG-m^5dC) was prepared as described by Behe and Felsenfeld (13), except that poly(dG-dC) (Sigma) was used as the template. Following synthesis, much of the template was removed by repeated digestion with the restriction endonuclease Hha I (New England BioLabs), which cleaves the sequence G-C-G-C, but not G-m^5C-G-m^5C. From the Mg^{++} dependence of the B-Z transition (13), We estimate that the product contained at least 70% m^5C. Fragments of poly(dG-dC) and poly(dG-m^5dC) were prepared by partial digestion with micrococcal nuclease. Digestion products were treated with S1 nuclease to remove any single-stranded regions, followed by preparative electrophoresis on a 5% polyacrylamide gel, using a Hae III digest of pBR 322 as size standards. Fractions approximately 170 bp in length were excised and eluted from the gel, and end-labeled with ^{32}P according to Maxam and Gilbert (12).

Formation of CAP-DNA complexes. DNA concentrations were determined spectrophotometrically (ε_{260} = 1.3 x 10^4 M^{-1} cm^{-1} per bp) or by titration with a standardized lac repressor preparation under quantitative binding conditions (11). Protein concentrations were determined according to Sedmak and Grossberg (14), or spectrophotometrically, using ε_{280} = 3.5 x 10^4 M^{-1} cm^{-1} per CAP dimer (15). Differences between non-spectrophotometric and spectrophotometric determinations were generally less than 5%.

For CD analysis, samples of protein and DNA were dialyzed into 10 mM Tris (pH 8.0 at 21° C), 1 mM EDTA. To form complexes, protein was slowly added to the DNA solution with continuous gentle mixing. Cyclic AMP was then added to obtain the desired concentration, and spectroscopy performed within 5 min. We found that slow addition of CAP to the DNA solutions, in the absence of cAMP, was essential to prevent the formation of aggregates. Even so, turbidity was observed in some samples within 1 hr of mixing. Samples with high CAP concentrations were particularly susceptible. In most cases the problem was minimized by taking CD spectra immediately after addition of cAMP. For questionable samples several spectra were taken over a 20 min period. If significant differences were observed as a function of time, the data were not used.

For analysis by gel electrophoresis, binding reactions were carried out in 10 mM Tris (pH 8.0 at 21° C), 1 mM EDTA, 10% glycerol, 0.1 mg/ml BSA, with or without the addition of 4 mM $MgCl_2$, as indicated. Reaction mixtures contained 8.25 x 10^{-8} M poly(dG-dC) or poly(dG-m^5dC) (based on 170 bp/molecule) and variable amounts of CAP. After equilibration for 30

min at 21° C, solutions were applied directly to polyacrylamide gels, and electrophoresis begun immediately.

Spectroscopy. Circular dichroism measurements were carried out at 20 ± 1° C with a Cary 60 spectropolarimeter, with CD attachment. Each CD spectrum was taken at least twice, and the baseline was redetermined after every second scan. Absorption spectra were recorded with a Cary 219 spectrophotometer.

Protein distribution analysis by gel electrophoresis. Polyacrylamide gels were prepared as described (11), and equilibrated with 10 mM Tris (pH 8.0 at 21° C), 1 mM EDTA, plus or minus 4 mM $MgCl_2$, by electrophoresis for 1 hr at 15 V cm^{-1}. Samples were applied, and electrophoresis continued for 1.5 hr under the same conditions. Autoradiograms were obtained by exposing the gels to Dupont Cronex film at -20° C. Care was taken to ensure that film exposure was within the linear range of dose response. Developed film was scanned with a Joyce-Loebl microdensitometer. Peak areas were determined by planimetry.

RESULTS

Electrophoretic mobilities of B and Z forms of poly(dG-m^5dC). In a low salt buffer double stranded poly(dG-m^5dC) undergoes the transition from B to Z form at Mg^{++} concentrations in the millimolar range (13). For the related polymer poly(dG-dC) this transition occurs at about 0.7 M (16). Figure 1A shows the inversion of the circular dichroism spectrum characteristic of the B-Z transition of poly(dG-m^5dC). For the sample used in these studies, dissolved in electrophoresis buffer (10 mM Tris (pH 7.4 at 20° C), 1 mM EDTA), addition of $MgCl_2$ to a final concentration of 1.7 mM was sufficient to complete the B-Z transition.

The effect of added $MgCl_2$ on the gel mobilities of poly(dG-m^5dC) and poly(dG-dC) is shown in Figure 1B. In the absence of Mg^{++} both polymers migrate as single bands of comparable mobility (band β). In the presence of 4 mM $MgCl_2$ about 70% of the methylated sample runs as a band of diminished mobility (band α). The remainder co-migrates with the single band of the non-methylated sample.

The fraction of the poly(dG-m^5dC) appearing as a new band in the presence of Mg^{++} corresponds well to the extent of C-methylation of the sample, a relationship also found for the B-Z transition of this polymer in solution (13). In addition, the observed change occurs over the range of Mg^{++} concentrations appropriate for the change from B to Z: no other

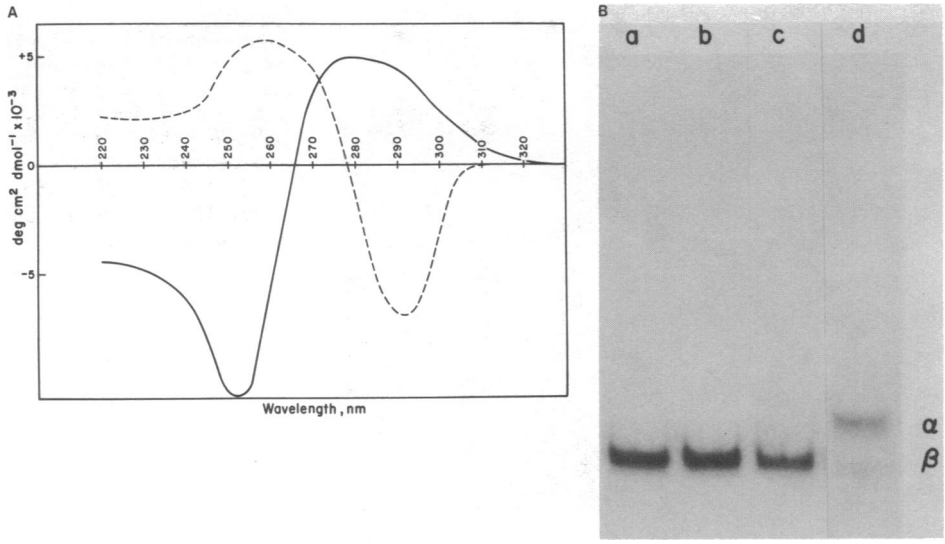

FIGURE 1A. CD spectrum of poly (dG-m^{5}dC) in the presence (-----) and absence (———) of 2 mM $MgCl_2$. Buffer: 10 mM Tris (pH 7.4 at 21° C), 1 mM EDTA. Spectra obtained at 21 ± 1° C.
FIGURE 1B. Electrophoresis of poly (dG-dC) (lanes a and c) and poly (dG-m^{5}dC) (lanes b and d) in 5% polyacrylamide gels. Buffer, lanes a and b: 10 mM Tris (pH 7.4 at 21° C), 1 mM EDTA; lanes c and d: 10 mM Tris (pH 7.4 at 21° C), 1 mM EDTA, 4 mM $MgCl_2$.

structural change has been found for poly(dG-m^{5}dC) under these conditions. Finally, hydrodynamic measurements indicate that the rise per residue of Z DNA is about 9% greater than B DNA (17). The increased length to charge ratio of Z DNA should diminish its electrophoretic mobility relative to the B form. Taken together, these observations argue strongly that the new electrophoretic species formed in the presence of Mg^{++} is Z DNA. The residual 30% of the sample unable to make the transition most probably consists of partially methylated hybrid molecules resistant to the Hha I digestion, or possibly some residual poly (dG-dC) primer.

It was of interest to compare the binding affinities of CAP for the B and Z forms of DNA found in this poly(dG-m^{5}dC) sample at 4 mM $MgCl_2$. The differences in gel mobility of the B and Z forms allowed us to use a DNA binding assay based on polyacrylamide gel electrophoresis (11). Such an experiment is shown in Figure 2A. The only observable complexes are of such high molecular weight that they hardly enter the 5% polyacryl-

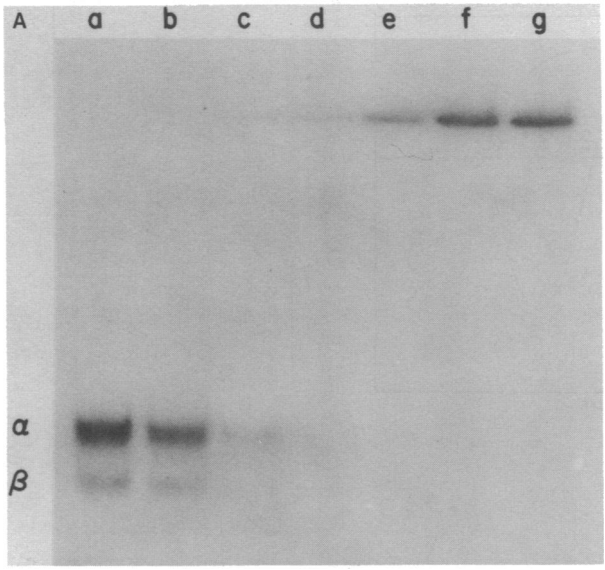

FIGURE 2A. Electrophoretic analysis of CAP: poly (dG-m^{5}dC) complexes formed in the presence of 4 mM $MgCl_2$. CAP:DNA ratios for lanes a-g respectively are: 0, 0.32, 0.63, 0.95, 1.59, 5.29, 10.57 moles of CAP per DNA molecule.

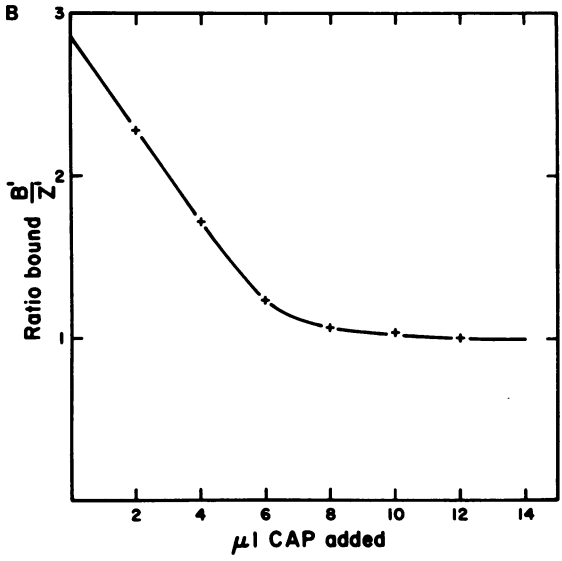

FIGURE 2B. Plot of B bound/Z bound versus volume of CAP added. Data of Figure 2A.

amide gel. Under these gel conditions 1:1 and 2:1 CAP complexes with a 203 bp restriction fragment run several cm into the gel (18). The appearance of complexes with CAP-DNA ratios apparently greater than one without prior formation of lower complexes, is indicative of a cooperative binding process. Similar cooperativity has been shown for non-

specific CAP binding to both single stranded and duplex DNAs (19, 20).

When a DNA binding protein such as CAP is added to a solution containing more than one type of DNA, the protein partitions between the available binding sites according to the relative binding constants of each. By analogy to equation 9 of reference 11, it can be shown that

$$\lim_{[CAP] \to 0} \frac{P(B)}{P(Z)} = \frac{K_B}{K_Z}$$

where P(B) and P(Z) are the probabilities of CAP binding to B form and Z form DNAs; K_B and K_Z are the respective equilibrium constants for cooperative formation of the high molecular weight complexes observed. Thus we can determine the relative binding constants for the two DNA forms from the titration data by extrapolating to zero CAP added the ratio of B bound to Z bound, as shown in Figure 2B. The limiting value of K_B/K_Z is slightly more than 2.8, indicating only a very slight preference for B form DNA.

Strictly speaking, the small observed preference of CAP in this experiment is probably for non-methylated or under-methylated B-DNA over fully-methylated Z-DNA. To determine what effect methylation has on the nonspecific binding of CAP to B-DNA, we compared the strength of binding to methylated and unmethylated DNA. For the reaction

$$nC + D \rightleftharpoons DC_n, \qquad K = \frac{[DCn]}{[D][C]^n}$$

in which C is CAP, D is DNA, and DC_n is the complex of n CAP molecules with DNA. Taking the $\log_{10}$ of both sides of the equation and rearranging yields

$$\log_{10} \frac{[DCn]}{[D]} = n \log_{10} [C] + \log_{10} K$$

The binding constant K can be determined graphically from a plot of $\log_{10}$ [DCn]/[D] versus $\log_{10}$ [C]: when $\log_{10}$[DCn]/[D] = 0, then $-n \log_{10} [C] = \log_{10} K$. Data from titrations of B-form poly(dG-dC) and B-form poly(dG-m^5dC) are plotted in this fashion in Figure 3.

The free CAP concentration at equilibrium [C], was calculated using the relation $[C] = [C]_{tot} - n[DCn]$, in which $[C]_{tot}$ is the total concentration of CAP in the mixture. We took the value of n to be 10, based on a DNA fragment size of 170 bp and an assumed non-specific binding site size of 17 bp/dimer. Data justifying this assumption are presented later in this paper. Because of the efficient binding of CAP under these conditions, values of n[DCn] and $[C]_{tot}$ are of similar size. Therefore,

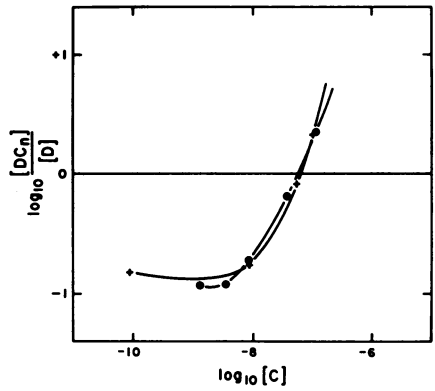

FIGURE 3. Analysis of CAP binding to B-form poly (dG-dC) (+) and B-form poly (dG-m^5dC) (●). DNA fragment concentrations were fixed at 2.0×10^{-8} M, CAP concentrations were varied from 6.0×10^{-9} to 2.0×10^{-7} M. Binding buffer was 10 mM Tris (pH 8.0 at 21° C), 1 mM EDTA. Complexes were resolved from free DNA by electrophoresis in 10 mM Tris (pH 8.0 at 21° C), 1 mM EDTA in 5% polyacrylamide gels.

estimates of [C], and hence K are probably somewhat imprecise. With this reservation, the common X-intercept of both DNA binding curves indicates that there is little difference in the binding of CAP to these polymers to attribute to methylation.

Barring the unlikely possibility that addition of $MgCl_2$ dramatically increases the effect of C-methylation on CAP binding to DNA, these experiments indicate that the B to Z transformation has little effect on the non-specific DNA binding of CAP. One can also conclude as a corollary that non-specific binding of CAP has little influence on the conformational equilibrium between B and Z forms of DNA. The Mg^{2+} conentration added for CAP binding is more than twice the value required at the midpoint of the conversion to the Z form. According to the B-Z transition curves published by Pohl and Jovin (16), such an increase in log $[Mg^{2+}]$ should leave less than 1% residual B form. Hence if binding required reversion to B, binding to Z should be at least 100-fold weaker than to B. Since this is not the case, we conclude that the Z-form is bound by CAP without return to the B-form. However, we cannot rule out some other conformational change of the Z-form upon CAP binding. Previous studies have shown that CAP is able to bind nonspecifically to both single stranded and duplex forms of DNA (19, 20). The present results further demonstrate the great range of DNA structures that can be accommodated by

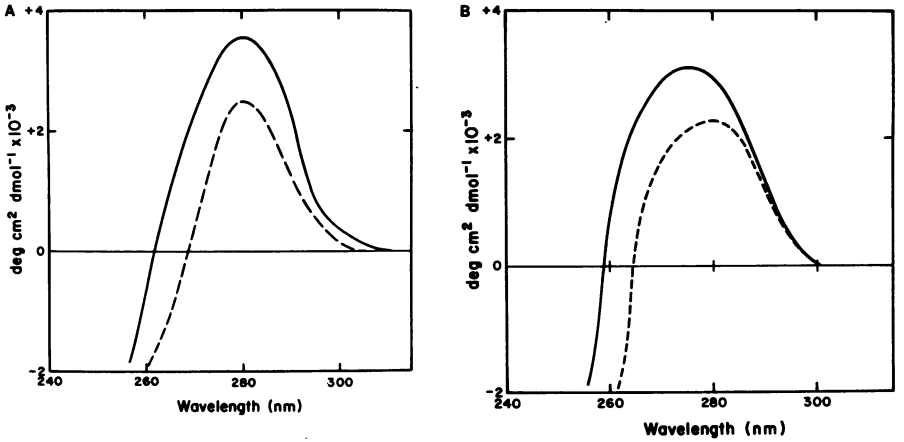

FIGURE 4A. Circular dichroism spectra of a 16 bp DNA fragment containing the CAP recognition sequence alone (———) or in the presence of an equimolar amount of CAP (-----). Binding buffer was 10 mM Tris (pH 8.0 at 21° C), 1 mM EDTA, 5 μM cAMP. The DNA concentration was 7.61 x 10^{-5} M bp. FIGURE 4B. Circular dichroism spectra of sheared E. coli DNA alone (———), or in the presence of CAP (-----) at a ratio of 20 bp of DNA per CAP dimer. Binding buffer was 10 mM Tris (pH 8.0 at 21° C), 1 mM EDTA, 5 μM cAMP. The DNA concentration was 7.8 x 10^{-5} M bp.

the nonspecific binding mode of CAP.

Circular dichroism. The sequence of the 16 bp fragment used in these studies is:

5' - T G T G A G T T A G C T C A C U
A C A C T C A A T C G A G T G A - 5'

The 3'-terminal residues are both ribonucleotides. Apart from this difference, the sequence is identical to that of residues -69 through -54 of the lac promoter, and contains the symmetry element T G T G N_8 C A C A recently shown to be a common feature of CAP binding sites in the lac, gal, pBR-p4, ara BAD, and ara C promoters (21). Figures 4A and 4B show the CD spectra (as ellipticities) of the 16-mer and of bulk E. coli chromosomal DNA. The strong similarity between spectra indicates that the 16-mer is present as a B-form duplex. The small differences in peak shape and amplitude are likely to reflect differences in base composition (22) and sequence (23, 24) between the samples.

Addition of CAP brings about a decrease in the amplitude of the CD signals of both samples, over the range from 310 nm to 240 nm (Figures 4A

and 4B). This decrease in ellipticity must be due to conformational changes in either CAP or in DNA, or in both. However, since the aromatic amino acids responsible for the ellipticity of CAP above 255 nm are found throughout the protein (25), and since the amplitude of the CD signal of CAP alone is less than 5% of that of the DNA in this range (20), radical reorganization of the protein would be required to produce a CD change of comparable magnitude to the one found here. For this reason, the observed decrease in the CD amplitude is most likely to be due to changes in DNA conformation.

In order to compare the effects of specific and nonspecific DNA binding on the CD spectrum under similar conditions of DNA occupancy, we determined the CAP to DNA ratio at which binding saturation occurs. For binding to high molecular weight DNA, this ratio yields an estimate of the number of base pairs occupied per CAP dimer (20). Subject to the assumption that a single CAP dimer excludes others from binding to the same region of DNA, this value is a measure of the length of the DNA binding site. Results are shown in Figure 5A. In this experiment a fixed amount of DNA was titrated with CAP, and the ellipticity followed at 280 nm. Due to the low ionic strength and relatively high protein and DNA concentrations employed in this experiment, binding is quantitative (18). At low protein to DNA ratios, CD changes are largely due to the interaction of CAP and DNA: once the DNA is saturated with CAP, any change must be due to the ellipticity of the free protein. Binding saturates at 0.058 dimers per base pair, or equivalently, 17.2 bp/dimer. This is significantly larger than the value of 13 bp/dimer found in a similar experiment by Saxe and Revzin (20), but is in substantial agreement with estimates made by other means (26-28).

A similar experiment was performed with the 16 bp fragment (Figure 5B). In this case, binding saturates at a 1:1 molar ratio (16 bp/dimer). Other binding experiments carried out with 203 bp *lac* promoter fragments have recently shown that one CAP dimer is bound per specific site (18), to be published). Thus the present result is not due to the small size of the synthetic fragment, or to the lack of DNA flanking the recognition sequence. It is possible that the 1:1 ratio reflects the functional stoichiometry of CAP *in vivo*.

Together, the 1:1 stoichiometry of the specific complex and the ratio of ~ 17 bp/dimer for non-specific interactions argue that a free CAP molecule can not bind and produce an optical change in a region of

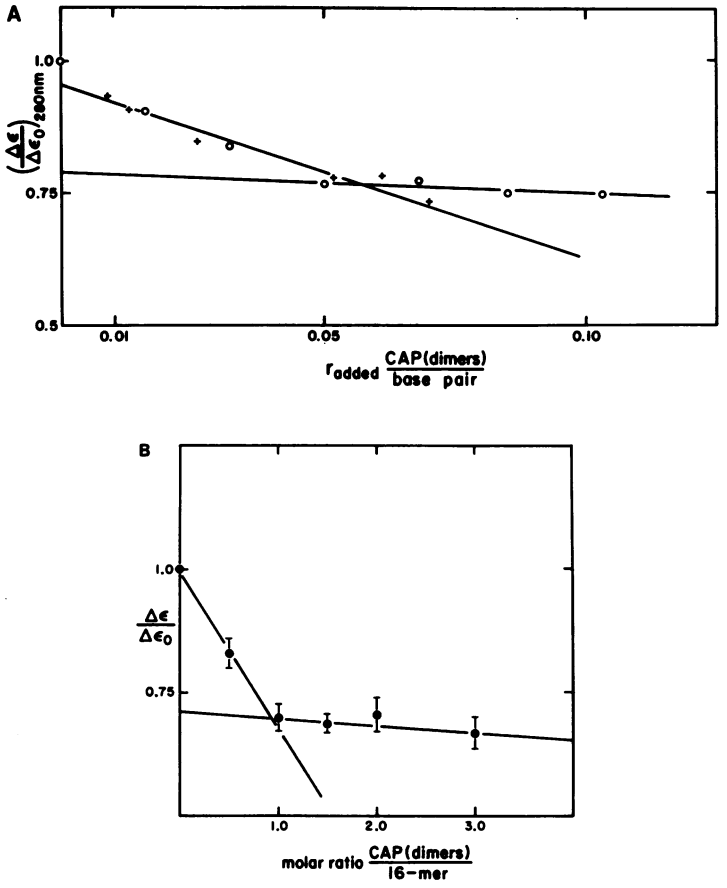

FIGURE 5A. Titration of native E. coli DNA (o) or calf thymus DNA (+) with CAP, monitored by circular dichroism. DNA concentrations were 5.2 x 10^{-5} M bp (E. coli) and 4.7 x 10^{-5} M bp (calf thymus). The binding buffer consisted of 10 mM Tris (pH 8.0 at 21° C), 1 mM EDTA, 5 µM cAMP. The lines drawn through the data points represent least squares fits of the data over the ranges 0.01 - 0.06 dimers CAP/bp and 0.05 - 0.10 dimers CAP/bp respectively.

FIGURE 5B. Titration of a 16 bp DNA fragment containing the CAP recognition sequence, monitored by circular dichroism. The DNA concentration was 7.61 x 10^{-5} M bp. The binding buffer was 10 mM Tris (pH 8.0 at 21° C), 1 mM EDTA, 5 µM cAMP.

DNA already in complex with CAP. Thus, the primary CAP binding site on the DNA must be approximately 17 bp long. This is the size of the site that one CAP dimer excludes from interactions with other dimers: the significantly larger region protected by CAP from nuclease digestion

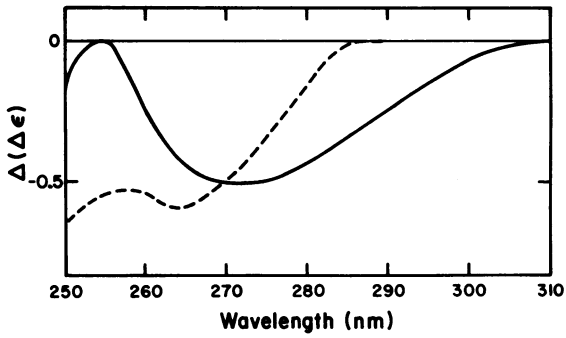

FIGURE 6. Circular dichroism difference spectra. An equimolar mixture of CAP with the 16-mer versus the 16-mer alone (———): a complex of CAP with E. coli DNA at 17.0 bp/dimer versus DNA_5 alone (-----). DNA concentrations were 7.61×10^{-5} M bp and 7.8×10^{-5} M bp for the 16-mer and E. coli DNA respectively. Binding buffer consisted of 10 mM Tris (pH 8.0 at 21° C), 1 mM EDTA, 5 μM cAMP.

(5,28) probably includes the distance from the CAP-occupied segment to the active site of the nuclease probe.

Difference spectra for the binding of CAP to the 16-mer and to non-specific DNA are compared in Figure 6. Although these spectra were obtained at comparable binding ratios (1:1 for the 16-mer and 17 bp/dimer for non-specific DNA), both the amplitude and position of the local minima are different. In particular, the minimum of the CAP-16-mer difference spectrum is red-shifted by 6 nm with respect to the non-specific complex, and reduced in amplitude by about 15%.

DISCUSSION

Our results demonstrate that CAP binding to both specific and non-specific sequences produces DNA conformations that have altered circular dichroism when compared to free DNA. A wide variety of agents can cause reduction of the positive CD band in a manner similar to that of CAP. Some of these are: alcohols (29,39), salts (29,31,32), temperature shift (31,33), dehydration (34), and changes in linking number in covalently closed circular DNA molecules (35). Condensed or packaged forms of DNA, such as those occuring in chromatin or bacteriophage frequently show similar spectra (36, 37).

Reduction in the positive CD band has traditionally been correlated with a B to C-form conformational change (34). Recent evidence indicates

however, that DNA has a structure closely resembling the B form, with 10 bp per turn, under some conditions that produce the characteristic C-form CD spectrum (38). Unfortunately, the structural changes responsible for this "C-like" CD spectrum have not been identified to date. Thus it is impossible to say whether the CD changes induced by CAP binding reflect small or large changes in DNA conformation. Nonetheless, our results are not compatable with transition to the A form (34), the Z form (12), or melting of the duplex (33).

Differences in base composition between the E. coli DNA sample (51% G + C) and the 16-mer (44% G + C: We count the 3' terminal ribo-A and ribo-U as A and T respectively) may account for small variations between the DNA CD spectra (22), but cannot explain the relatively large difference in the spectral changes induced by CAP binding. In an analysis of the base composition dependence of the DNA CD spectrum, Gratzer et al demonstrated that above 275 nm the majority of the rotational strength of the positive CD band is contributed by signals from A-T components, while the positive band below 275 nm is dominated by G-C contributions (22). On this basis it is conceivable that the red shift of the difference minimum of the 16-mer relative to that of E. coli DNA reflects the interaction of CAP with one or more A-T sites in the 16-mer. Alternatively, cooperative binding to non-specific DNA may result in formation of regular higher-order structures, with intrinsic circular dichroism different from that produced by isolated binding interactions (such as with the 16-mer). Long range order has previously been observed in non-specific CAP-DNA complexes (27). A third possibility is that the 16-mer lacks DNA regions flanking the binding site which could provide a barrier to the propagation of conformational changes due to CAP binding. This is an advantage when one wishes to observe the CD spectrum of the binding site without interfering spectral contributions from other DNA regions, but remains a possible source of difference between CAP:16-mer and CAP: E. coli DNA complexes.

Recently Kolb and Buc have shown that the binding of CAP to closed circular plasmids containing lac or gal promoter sequences produces little change in the topological linking number of those DNA molecules (39). While in general their data do not support the proposal of McKay and Steitz that CAP binds to left-handed DNA sequences (1), their interpretation is complicated by the presence of a large amount of competing non-specific DNA, topologically coupled to the specific sites. In the

present study we have attempted to eliminate this complication by considering only the CAP recognition sequence. A right to left-handed transition, such as the one proposed, should result in net unwinding of the duplex: small decreases in duplex winding angle are correlated with increases in the positive CD band (40, 41), a trend opposite in sign to the one we observe. On the other hand, transition to the left-handed Z form, with accompanying large decrease in the winding angle, results in complete inversion of the CD spectrum, as shown in Figure 1A. The CD results do not support the notion that this type of process is occuring either: if anything, they suggest that CAP binding slightly increases the duplex winding angle. However, in spite of the CD evidence implying sequence dependent interaction between CAP and the 16-mer, it is possible that there may be some unknown deficiency in this DNA molecule which prevents its switching to the postulated left-handed form, or which prevents binding of CAP in its specificlly active form. Another possibility which we cannot exclude is that only a small fraction of the DNA molecules switch to a left-handed form.

Lastly, we note that CAP binds with marginal preference to B-DNA under conditions that allow the protein to partition between B and Z species. The efficient binding to single stranded and duplex DNA (20,14) as well as to Z-DNA, demonstrates the wide range of DNA structures accommodated by the non-specific binding mode. The absence on the gel of complexes of intermediate molecular weight is indicative of the cooperativity of these interactions.

Although these results do not support a left-handed binding model, they do imply that structural changes in the DNA are caused by CAP binding. It is curious that lac repressor binding to the lac operator causes a large increase in the positive CD band of the DNA (42), in contrast to the decrease induced by CAP binding at its site. In the future it may be possible to correlate the competing roles of these proteins with the different structural transitions they induce in DNA.

ACKNOWLEDGEMENTS

This work was supported by grant PCM 75-17879 from the National Science Foundation, and by grant GM-21966 from the National Institutes of Health.

REFERENCES

1. McKay, D.B. and Steitz, T.A. (1981) Nature 290, 744-749.
2. Emmer, M., deCrombrugghe, B., Pastan, I. and Perlman, R. (1970) Proc. Natl. Acad. Sci. USA 66, 480-487.
3. Magasanic, B. (1961) Cold. Spr. Harb. Symp. Quant. Biol. 26, 249-256.
4. Eron, L. and Block, R. (1970) Proc. Natl. Acad. Sci. USA 68, 1828-1832.
5. Schmitz, A. (1981) Nucl. Acids Res. 9, 277-292.
6. Taniguchi, T., O'Neill, M. and deCrombrugghe, B. (1979) Proc. Natl. Acad. Sci. USA 76, 5090-5094.
7. Ogden, S., Haggerty, D., Stoner, C.M., Kolodrubetz, D. and R. Schleif, (1980) Proc. Natl. Acad. Sci. USA 77, 3346-3350.
8. Gilbert, W. (1976) in RNA Polymerase, ed. Losick, R. and Chamberlin, M. Cold Spring Harbor Lab., Cold Spring Harbor, NY pp. 192-205.
9. Müller-Hill, B., Beyreuther, K. and Gilbert, W. (1971) Methods in Enzymology 21 (D), 484-501.
10. Fried, M.G. (1982) Ph.D. Dissertation, Yale University.
11. Fried, M. and Crothers, D.M. (1981)Nucl. Acids. Res. 9, 6505-6525.
12. Maxam, A. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560-564.
13. Behe, M. and Felsenfeld, G. (1981) Proc. Natl. Acad. Sci. USA 78, 1619-1623.
14. Sedmak, J. and Grossberg, S. (1977) Anal. Biochem. 79, 544-552.
15. Anderson, W.B., Schneider, A., Emmer, M. Perlman, R.L. and Pastan, I. (1971) J. Biol. Chem. 246, 5929-5937.
16. Pohl, F.M. and Jovin, T.M. (1972) J. Mol. Biol. 67, 375-396.
17. Wu, H.M., Dattagupta, N. and Crothers, D.M. (1981) Proc. Natl. Acad. Sci. USA 78, 6808-6811.
18. Fried, M.G. and Crothers, D.M. (1983) Nucl. Acids Res. 11, 141-158.
19. Garner, M.M. and Revzin, A. (1981) Biochemistry 20, 305-311.
20. Saxe, S. and Revzin, A. (1979) Biochemistry 18, 255-263.
21. Queen, C. and Rosenberg, M. (1981) Nucl. Acids. Res. 9, 3365-3377.
22. Gratzer, W., Hill, L.R. and Owen, R.J. (1970) Eur. J. Biochem. 15, 209-214.
23. Allen, F., Gray, D.M., Roberts, G.P. and Tinoco, T., Jr. (1972) Biopolymers 11, 853-879.
24. Gray, D.M., Hamilton, F.D. and Vaughan, M. (1979) Biopolymers 17, 85-106.
25. Aiba, H. personal communication.
26. Takahashi, M., Blazy, B. and Baudras, A. (1979) Nucl. Acids. Res. 7, 1699-1712.
27. Chang, J., Dubochet, J., Baudras, A., Blazy, B. and Takahashi, M. (1981) J. Mol. Biol. 150, 435-439.
28. Simpson, R. (1979) Nucl. Acids. Res. 8, 759-766.
29. Ivanov, A., Murkhenkova, L. Schyolkma, A.K. and Poletayev, A. (1973) Biopolymers 12, 89-110.
30. Girod, J., Johnson, W.C., Jr., Huntington, S.K. and Maestre, M.F. (1973) Biochemistry 12, 5092-5096.
31. Studdert, D., Patroni, M. and Davis, R.C. (1972) Biopolymers 11, 761-779.
32. Zimmer, C. and Luck, G. (1974) Biophys. Biochem. Acta 361, 11-32.
33. Gennis, R. and Cantor, C.R. (1972) J. Mol. Biol. 65, 381-399.
34. Tunis-Schneider, M.B. and Maestre, M.F. (1970) J. Mol. Biol. 52, 521-541.

35. Maestre, M.F. and Wang, J. (1971) Biopolymers 10, 1021-1030.
36. Shih, T.Y. and Fasman, G.D. (1970) J. Mol. Biol. 52, 125-129.
37. Maestre, M.F., Gray, D.M. and Cook, R.B. (1971) Biopolymers 10, 2537-2553.
38. Zimmerman, S.B. and Pheiffer, B.H. (1980) J. Mol. Biol. 142, 315-330.
39. Kolb, A. and Buc, H. (1982) Nucl. Acids Res. 10, 473-485.
40. Chan, A., Kilkuskie, R. and Hanlon, S. (1979) Biochemistry 18, 84-91.
41. Baase, W. and Johnson, W., Jr. (1979) Nucl. Acids. Res. 6, 797-814.
42. Culard, F. and Maurizot, J. (1981) Nucl. Acids Res. 9, 5175-5184.
43. Tan, Z.-K., Ikuta, S., Huang, T., Dugaiczyk, A. & Itakura, K. (1982) Cold Spring Harbor XLVII Symposium on Quantitative Biology, in press.