PROTEINS: Structure, Function, and Genetics 2:283–289 (1987)

Electrostatic Calculations and Model-Building Suggest That DNA Bound to CAP Is Sharply Bent

J. Warwicker, B.P. Engelman, and T.A. Steitz
Department of Molecular Biophysics and Biochemistry, and the Howard Hughes Medical Institute, Yale University, New Haven, Connecticut 06511

ABSTRACT **Two observations suggest that DNA, upon binding to *E. coli* catabolite gene activator protein (CAP), is sharply bent by a total angle of at least 100–150 degrees: (1) The electrostatic potential field of CAP shows regions of positive potential that form a ramp on 3 sides of the protein. (2) The DNA binding site size as determined by DNA ethylation interference with binding, (Majors: "*Control of the* E. coli *Lac Operon at the Molecular Level.*" Ph.D. Thesis, Harvard University, Cambridge, 1977) and by relative affinities of DNA fragments of various lengths (Liu-Johnson et al.: *Cell* 47:995–1005, 1986) requires severe bending of the DNA to maintain its favorable electrostatic contact with the protein.**

Key words: bent DNA, electrostatics, base-pair rolls, finite difference calculations, gene activation, DNA-protein complex

The catabolite gene activator protein (CAP) of *E. coli*, when complexed with cyclic AMP (cAMP), binds in a DNA sequence-specific manner in the vicinity of a number of *E. coli* promoters and stimulates the transcription of these operons by RNA polymerase. Since the position of the CAP binding site in various operons ranges from about −40 to −100, the search for a common mechanism for stimulation of polymerase initiation provides an interesting challenge.[1,2]

It has been concluded that the binding of CAP to its DNA binding site induces a DNA bend from the analysis of the mobility of CAP-DNA complexes in polyacrylamide gel electrophoresis.[3–5] The same conclusion has been reached from electrodichroism measurements of the rotational relaxation times of CAP-DNA complexes.[6] Neither the nature of the bend nor its role (if any) in transcription activation has been documented.

The crystal structure of CAP from *E. coli* complexed with cAMP has been solved[7,8] and its coordinates have been refined at 2.5-Å resolution (Steitz and Weber, submitted to J. Mol. Biol.). Each subunit of the dimer consists of a large N-terminal domain that binds cAMP and a smaller C-terminal domain that contains the helix-turn-helix motif found in prokaryotic regulatory proteins.[9,10] The subunit structure in the CAP crystal is asymmetric.[7,8] One subunit has a relatively large cleft between the two domains (the "open" subunit) while the other does not (the "closed" subunit).

A model for CAP bound to right-handed B-DNA has previously been presented[1,11,12] based on the CAP-cAMP structure, electrostatic calculations, and the results of chemical and enzymatic protection experiments.[13,14] This model has investigated possible sequence-specific interactions between CAP and the 18-b.p. range of the consensus sequence of the CAP binding site.[1] Since a model of CAP interacting with straight B-DNA fails to account for the site size of at least 26 b.p. indicated by ethylation interference of CAP binding,[13] a continuous deformation of the DNA with a 70-Å radius of curvature was introduced. This increased the binding site size to about 20 b.p.[12] Here we present a model for CAP complexed with severely bent DNA that increases the electrostatic complementarity between the DNA and a new calculation of the protein's electrostatic potential field and that accommodates a DNA binding site size of 26–30 b.p.[13,15]

MATERIALS AND METHODS

Electrostatic Calculations

The electrostatic calculations performed on the CAP-DNA system involve 2 parts. The fields used for graphics display and docking are calculated by using the finite difference method.[16,17] Dielectric values of 3 for protein and 80 for solvent are incorporated together with an ionic strength of 0.1 M for the field evaluation. Since the finite difference method does not have sufficient accuracy at short range for energy calculations, these are performed by using an empirical method.[18] An effective dielectric value of 50 and a Debye-Huckel counter-ion screening term at 0.1 M ionic strength were used for the empirical energy evaluations. All electrostatic calculations were made at pH 7.0. This overall approach applied to 2 DNA-binding proteins, the Klenow fragment[18] and the Trp repressor (P.B. Sigler, private communication), has shown that a striking complementarity exists between the protein electrostatic fields and the negative charges on the phosphate backbones of the modelled DNA in each case.

Received May 19, 1987; accepted August 10, 1987.

Address reprint requests to Dr. Thomas A. Steitz, Department of Molecular Biophysics and Biochemistry, Yale University, 260 Whitney Avenue, New Haven, CT 06511.

DNA Manipulation

The DNA model-building was based upon right-handed B-DNA coordinates[19] generated with FORTRAN computer code written by D.B. McKay. This code was incorporated into a FORTRAN program that further manipulates the DNA.

A 7-Å resolution structure of the nucleosome core indicates that the DNA makes sharp bends at specific sites.[20] One way of generating a sharp bend in DNA is through a rotation about the "roll" or "twist" axis[21] that runs across a base-pair, (and is perpendicular to the helix and dyad axes). The roll appears to be favoured over the other possible deviations from regular B-DNA structure in the generation of sharp DNA bends.[22] The crystal structure of kinked DNA with the EcoRI endonuclease[23] contains one site with a roll angle of about 23 degrees, (labeled a "neo-2" kink).

The FORTRAN program applies a roll between 2 b.p. as a rotation about the average of the roll axes of the 2 b.p. The program first generates the DNA and moves it into the appropriate orientation relative to the protein and then performs a specified set of rolls, moving out from the dyad axis. A check of the stereochemical overlap between the transformed DNA and the protein is maintained. Steric overlap between DNA segments surrounding a roll site is alleviated with a small translation of the transformed segment (about 0.75 Å). Although we have chosen to use only base-pair rolling, we do not intend to exclude the possibility that other rearrangements (such as "tilt" about an axis in the base-pair plane perpendicular to the roll axis) could give the sharp bends that are made.

A 44 b.p. fragment of DNA was used. This length was sufficient to provide all the possible intimate CAP-DNA interactions for the various models studied. It also facilitated comparison with the gel electrophoresis data.[15]

Graphics Display

The DNA kinked by base-pair rolls was modelled into the electrostatic field displayed on an Evans and Sutherland PS 300 graphics system with the program FRODO.[24]

RESULTS AND DISCUSSION

Positive Electrostatic Potential Field

The electrostatic potential field of the CAP dimer at pH 7.0 (Fig. 1) shows extensive positive potential over the protein regions flanking the proposed specific DNA binding sites of the F-helices and extending down the sides of the protein. The overall form of this field does not differ substantially from that calculated previously.[11,12] However, the finite difference method accentuates the potential envelopes in solvent regions due to the presence of the low dielectric protein region in the calculations. The "track" or "ramp" of positive potential runs along three sides of the protein and is the basis for the current model-building study. We assume that the path of the electrostatically negative DNA bound to the protein will follow the track of the positive potential. The positive potential arises from several patches of basic amino acids grouped on both the C-terminal and N-terminal domains. Straight or smoothly curved DNA cannot be made to interact with the positive potential that exists on the sides of the protein. However, DNA that is sharply bent can interact with various regions of the flanking positive potential depending upon the bend locations. Further, the asymmetry of the CAP structure in the crystal gives rise to a considerable asymmetry in the flanking electrostatic potential regions. In the "open" subunit (on the left-hand side of Fig. 1) the separation between different patches of basic groups is greater than it is in the "closed" subunit (on the right-hand side of Fig. 1).

Bent DNA-CAP Models

The central region of the specific DNA binding site has been fitted so that the approximate dyad symmetries of the DNA sequence and the CAP dimer superimpose. In addition the central 16 b.p. of the DNA has been positioned so as to maintain the sequence-specific protein contacts used in the previous CAP-DNA model.[12] The major objective of the present analysis has been to bend the DNA sharply to create kinked DNAs that are complementary to the positive electrostatic potential on the sides of CAP. This has been achieved by rolling around the base-pair axis connecting the sugars.

We present 2 complexes (models A and B) that represent extremes of an ensemble of possible ways to bend DNA in such a manner. In Figure 2 we show the location of the roll sites for these 2 models on the DNA of the lac operon together with the ethylation interference results of Majors.[13] Figure 3 shows model A in which the DNA has been fitted to positive potential on both the C-terminal and N-terminal domains. In Figure 4, model B shows a DNA molecule which forms close contacts exclusively with the C-terminal domain. Both models are able to account for the ethylation interference data.[13] Model A has three rolls (see Fig. 2), each of 30 degrees, on either side of the dyad. The base-pair positions of the 3 rolls with respect to the dyad axis are between b.p. 5 and 6, 7 and 8, and 10 and 11. Model B has 2 rolls (see Fig. 2) on either side of the dyad axis between b.p. 5 and 6 and 9 and 10. In this case each roll is 30 degrees on the "closed" subunit side and 35 degrees on the "open" subunit side. These different roll angles are required to maintain a similar fit on the asymmetric CAP structure. That the differences are required for model B which follows the C-terminal domain more closely than does model A is a manifestation of the lack of dyad symmetry between the C-terminal domains of the crystal structure. The 2 models share the first (5, 6) roll, which, at 5 b.p. away from the dyad axis, causes the

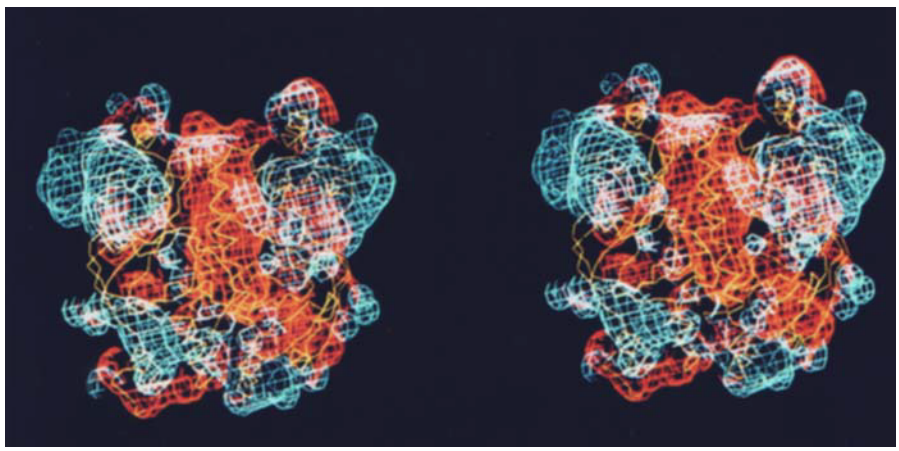


Fig. 1. A stereodiagram of the electrostatic potential field of CAP at pH 7.0. The pseudodimer axis runs vertically with the C-terminal domains at the top. The potential is plotted at −50 mV (red) and at +50 mV (blue) levels. Only those parts of the potential surface outside of the protein are displayed. The alpha-carbon backbone of CAP is drawn in yellow. The F-helices that are proposed to fit into successive B-DNA major grooves have both negative and positive potential regions. The protein flanking the F-helices is substantially positive over the C-terminal domain, and over parts of the N-terminal domain.

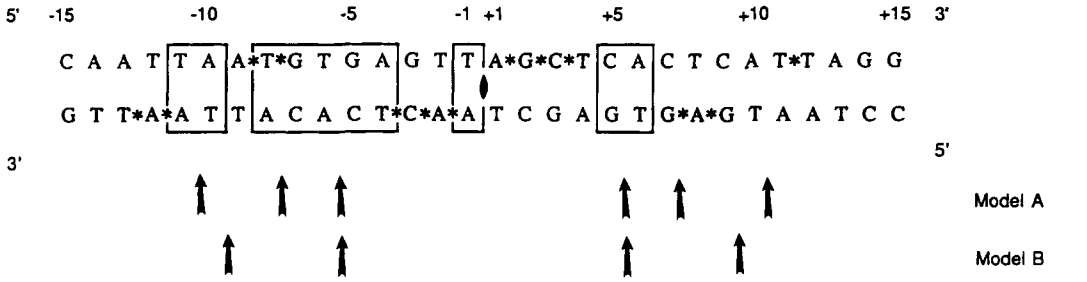


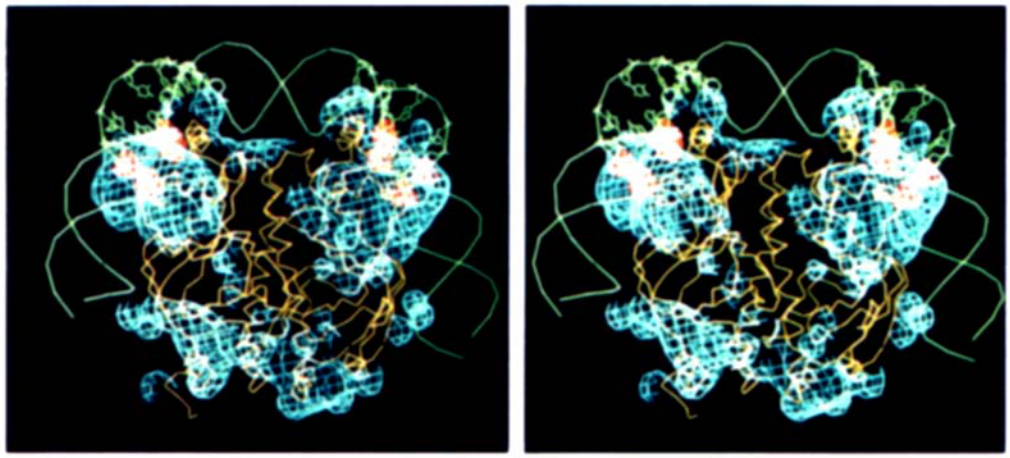
Fig. 2. The lac operon sequence is shown for 15 b.p. on either side of the pseudodyad axis (marked by the dyad symbol). Transcription start is to the right of the figure. Base-pairs that are highly conserved among CAP-sensitive operons are boxed. The phosphates whose ethylation interferes with CAP binding[13] are marked by asterisks. The sites of rolling for model A (3 on either side of the dyad) and model B (2 on either side of the dyad) are indicated by arrows.

DNA to bend toward the protein while maintaining the plane of the DNA. In model A the second roll, still within the consensus sequence region, serves to bend the DNA toward protein and to carry it somewhat out of plane in a direction which allows it to interact with the N-terminal domain further away from the dyad. The third roll of model A again bends the DNA toward protein and slightly compensates the out-of-plane movement of the second roll. The result for model A is a substantially bent DNA molecule which remains roughly in plane and interacts closely with CAP over most of its 44-b.p. length. The overall bend angle is about 150 degrees. In model B the second and final roll bends the DNA toward the C-terminal domain but away from the N-terminal domain so that close interactions with CAP exist over only 30 b.p. of the DNA. The second roll also bends the DNA substantially out of plane into the form of a left-handed supercoil. Model B has an overall bend angle of about 90 degrees. A DNA conformation intermediate between that of model A and model B on the ramp of positive potential could attain a bending angle greater even than the 150 degrees of model A. It should be noted that this modelling, which has DNA wrapping around the protein, is in conflict with the suggestion from electron microscopy that DNA is bent away from the protein by an angle of 30–45 degrees.[25]

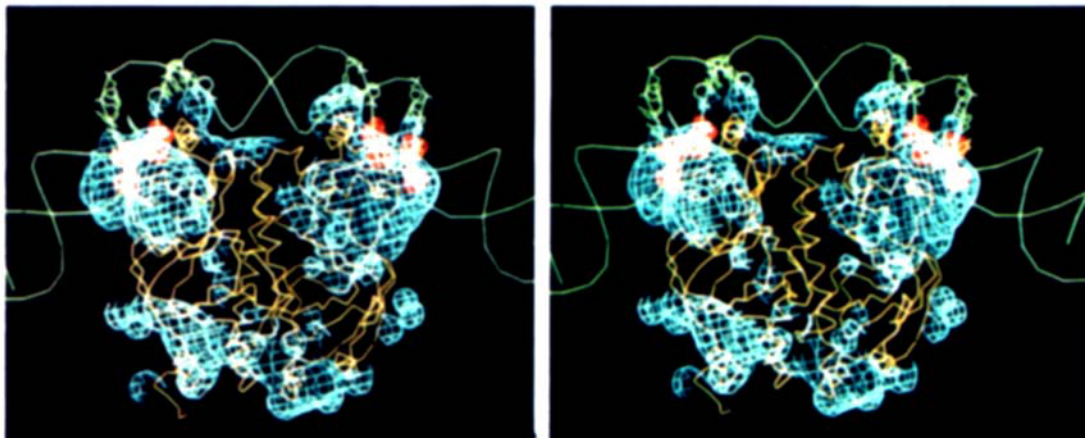
The first roll, common to both models, and the second roll of model A lie within the consensus CAP binding sequence and are rolls into the major groove. The first roll that is common to both models and the second roll of model A both lie at G/T junctions that are in a conserved sequence.[1,2] The third roll of model A and the second roll of model B lie within predominantly poly A/T stretches on either side of the pseudodyad. These 2 rolls are consistent with the poly A/T preference for rolling into the minor groove, deduced from studies of rotational positioning of DNA around the nucleosome.[22] The second and third rolls of model A are within regions of conserved sequence that cannot be model-built into specific base-to-side-chain interactions.[12]

The models presented here cannot be definitive in regard to the precise sites and degrees of rolling that actually occur in the CAP-DNA complex. Indeed, it is possible that some degree of base-pair tilt and/or other DNA distortions such as slide contribute to produce sharply bent DNA bound to CAP. However, the

3



4



Figs. 3, 4. Models A (Fig. 3) and B (Fig. 4) are shown with CAP in the same orientation as in Figure 1. The phosphate backbone of B-DNA is shown along with those base-pairs that surround roll sites. The blue potential contour at +50 mV is also displayed. Red stippled phosphates are the ethylation interference[13] markers. The ethylation interference data are displayed only for the DNA which has been rolled away from linear, and the single asymmetry between the data on the 2 strands has been removed by including 2 phosphates in the 12–13 b.p. from the dyad region, since we are not attempting to define a specific relationship between the CAP crystal asymmetry and any asymmetry in the data.

models presented here contain DNA fragments with the fewest base-pair roll sites that are able to fit the different regions of the flanking CAP-positive electrostatic potential, thus allowing us to examine the energetic and functional consequences of sharply bent DNA bound to CAP.

Electrostatic and Bending Energies

For both models the calculated electrostatic energy of stabilization that is achieved by bending the DNA is of the same order or larger than the expected energy cost of bending the DNA. The difference in calculated electrostatic energy of interaction between model A and straight DNA interacting with CAP is −19.8 kcal/mole while the same difference for model B is −17.3 kcal/mole (at 300°K). Since the difference in calculated stabilization of these 2 models is not large with respect to the anticipated errors in the calculation, this value cannot be used to discriminate between the 2 models. We also expect that the large number of different bent DNA conformations that lie between models A and B (as a result of different choices of roll locations and angles) will have similar electrostatic interaction energies. However, the calculated electrostatic energy of binding resulting from sharp bending of the DNA is of the same order or larger than the expected free energy cost of bending the DNA, depending on the assumptions made. Using the Bloomfield et al. equation,[26] and assuming a 135-degree overall bend spread over 30 b.p., the free energy cost of bending is estimated to be 8–9 kcal/mole.[15] For more sharply bent DNA over smaller base-pair ranges, as proposed here, the energy cost would be higher and more difficult to estimate correctly. The large electrostatic free energy of stabilization of bent DNA could account for the significantly greater observed free energy of binding of a 30-b.p. DNA fragment as compared with an 18-b.p. DNA fragment (about 5.8 kcal/mole).[15]

Although these electrostatic calculations alone cannot identify a unique bent DNA conformation, other (non-electrostatic) interactions between CAP and DNA may stabilize a particular bent conformation, and some kinks in the DNA may cost less energy

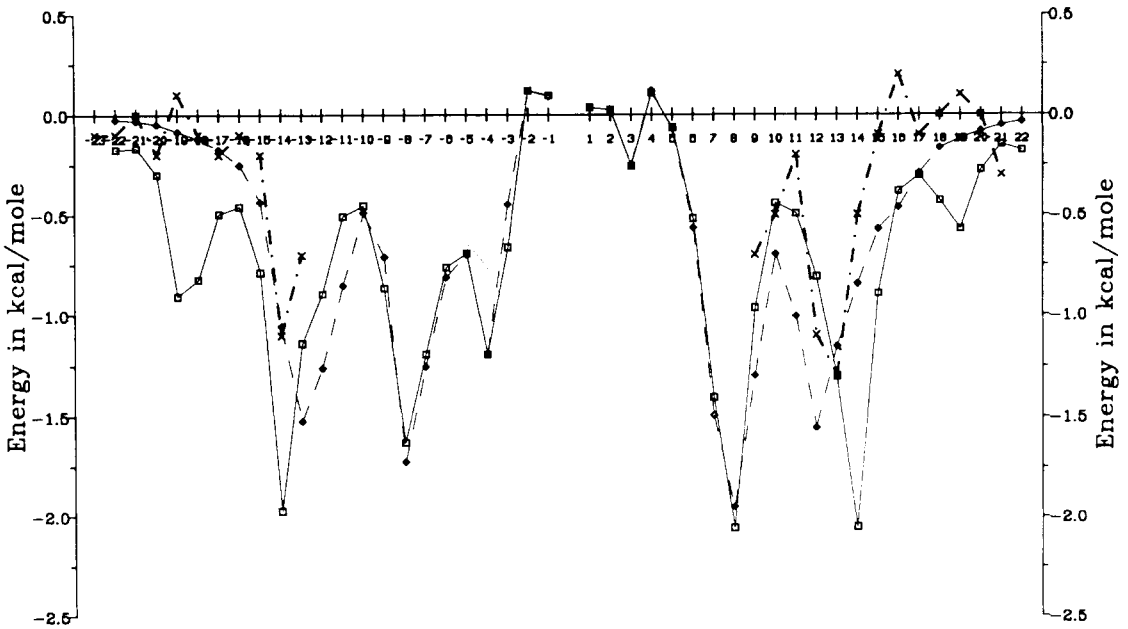


Fig. 5. Incremental free energies of binding in kcal/mole from Liu-Johnson et al.[15] are plotted with calculations of the incremental electrostatic energies for models A and B. The centre of the x-axis marks the psuedodyad symmetry, so that base-pair numbering gives the distance from the pseudodyad axis. The transcription start is located to the right of the x-axis, (toward the higher base-pair numbers). Crosses joined by a dot-dash line show the experimental data of Liu-Johnson et al.[15] while squares joined by a solid line give model A, and diamonds joined by a dashed line give model B.

than others. However, we cannot exclude the possibility that the lack of calculated electrostatic preference for a particular bent DNA conformation implies a flexibility of bent DNA conformation that is a real property of the system.

Comparison of Calculated and Experimental Binding Energies

Figure 5 displays the calculated electrostatic binding energy contributed by the addition of individual base pairs for both models A and B compared with the experimental free energies determined by a gel electrophoresis method.[15] The asymmetry of the calculated energies for both A and B data arises from the inexact dyad of the crystal structure. The direction of the model A and B data relative to the defined direction of the experimental data is arbitrary, since we are not attempting to assign asymmetric experimental data to the CAP crystal structure asymmetry. The energies on this plot are not equivalent. The free energy of binding is being compared to an electrostatic model energy that excludes contributions to the free energy of interaction from other sources, notably the conformational entropy of the DNA, and the loss of solvation energy upon binding. In addition, the model energy increments are derived from the 44-b.p. conformation, while presumably the experimental DNA fragments of various lengths can adopt different conformations dependent upon their lengths. This figure will be used to look at the correlation of interaction energy as a function of distance from the dyad axis between the models and the experimental data.

Both models agree qualitatively with the experimental data that exist from about 9 to 16 b.p. distant from the dyad axis. In particular the experimental downstream maximum of the free energy 11 b.p. from the dyad axis and the experimental minimum at around 13 b.p. from the dyad axis on both sides have counter-parts at +/− 1 b.p. in models A and B. The region around 13 b.p. that shows the most favorable interaction in both the experimental data and the electrostatic calculations also corresponds to the ethylation interference data[13] (Figs. 3, 4).

Liu-Johnson et al.[15] have suggested that those base-pair positions for which the incremental free energy is near zero in their experimental analysis correspond to the positions at which the DNA is being bent. They presumed that the energy cost of bending balances the incremental interaction energy at these base-pair positions. However, the electrostatic calculations presented in Figure 5 show another possible explanation. The incremental binding free energy for adding a base-pair can approach zero simply as a result of a large distance of the DNA phosphates from the protein. Thus, phosphate positions of b.p. 10 and 11, where the experimental increment in the free energy of binding is small, are distant from the DNA and show a small electrostatic stabilization. Other kinds of non-electrostatic interactions of these nucleotide base pairs with the protein also appear not possible at b.p. 10 and 11.

Model A shows a relatively large CAP-DNA interaction around b.p. 19, where the DNA backbone again comes into close contact with basic residues on the

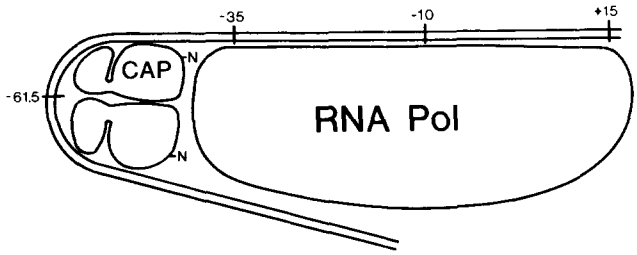


Fig. 6. A schematic drawing of the relative orientation of CAP and RNA polymerase on the lac promoter if the DNA is bent on CAP as in model A.

N-terminal domain. Model B has no such contact, and its interactions fall off sharply in this region. In this outer region, neither of the models shows a clear correlation with the experimental data on its upstream or downstream side.

Liu-Johnson et al.[15] have reported that the binding energy and the bending of the lac operon DNA to CAP is greater upstream than downstream. This difference could result from the asymmetry of the DNA sequence alone, since the tighter binding half contains more consensus bases. Additionally the CAP protein asymmetry itself, if it persists in solution, could contribute to asymmetry of binding and bending. Thus, the conformation of a sharply bent DNA may be dependent upon both the DNA sequence and the protein conformation. It is possible that asymmetry in the CAP-DNA system extends to include the conformation of DNA in the region 7–20 b.p. from the pseudodyad axis, making the bending properties upstream and downstream different.

DNA Bending and RNA Polymerase Activation

Although there is no experimental evidence on whether or how DNA bending is involved in the activation of transcription by RNA polymerase, bending as in model A would certainly affect the relative orientation of CAP and the polymerase (Fig. 6). Data from the Gilbert laboratory[27] show that in the lac operon CAP and RNA polymerase bind on the same side of the DNA helix and adjacent to one another. Bending the DNA as in model A would then place the polymerase adjacent to the cAMP binding domain. Any protein-protein interaction that occurs between CAP and polymerase would most likely involve CAP side-chains on its "back" side. How an analogous interaction could occur between polymerase and the CAP molecule that binds 10 b.p. further upstream in the mal T operon remains an interesting and challenging question.

CONCLUSIONS

This article shows that it is possible to maintain a specific DNA binding region to CAP while also fitting more distant parts of the DNA into regions of positive electrostatic potential that form a ramp on the sides of the CAP protein. Such modeling increases the CAP binding site size on the DNA, is able to account for the ethylation interference data of Majors,[13] and also gives a good qualitative fit to experimental free energy of binding data, where this is available, over a 30-b.p. region around the pseudodyad.[15] The sharp bends that are necessary to give this agreement with the experimental data and the electrostatics have been produced by base-pair rolling, which appears to be the favoured mechanism for producing sharp bends in DNA.[22]

The CAP protein has been co-crystallized with a 31 base-pair fragment of DNA (S. Schultz, G. Shields, T.A. Steitz, unpublished). These crystals diffract to at least 4-Å resolution. Comparison of the models prsented here with a CAP-DNA complex structure will be interesting. In particular it will be possible to assess the usefulness of electrostatic modeling as a tool in predicting protein-DNA conformation.

ACKNOWLEDGMENTS

We wish to thank F.M. Richards for discussion and support and J. Mouning and P. Eatherton for technical assistance. S. Schultz and G. Shields have also participated in valuable discussion. We thank D. Crothers and H.-N. Lui-Johnson for providing us with their experimental data prior to its publication. This research has been supported by NSF grant PCM-8316666, USPHS grant GM 22778, and the Howard Hughes Medical Institute.

REFERENCES

1. Steitz, T.A., Weber, I.T.: Structure of catabolite gene activator protein. In: "Biological Macromolecules and Assemblies: Vol. 2—Nucleic Acids and Interactive Proteins." Jurnak, McPherson, eds. New York: John Wiley and Sons, 1985.
2. de Crombugghe, B., Busby, S., Buc, H.: Cyclic AMP receptor protein: Role in transcription activation. Science 224:831–838, 1984.
3. Fried, M.G., Crothers, D.M.: CAP and RNA polymerase interactions with the lac promoter: Binding stoichiometry and long range effects. Nucleic Acids Res. 11:141–158, 1983.
4. Kolb, A., Spassky, A., Chapon, C., Blazy, B., Buc, H.: On the different affinities of CRP at the lac, gal, and MalT promoter regions. Nucleic Acids Res. 11:7833–7852, 1983.
5. Wu, H.-M., Crothers, D.M.: The locus of sequence-directed and protein-induced DNA bending. Nature 308:509–513, 1984.
6. Porschke, D., Hillen, W., Takahashi, M.: The change of DNA structure by specific binding of the cAMP receptor protein from rotation diffusion and dichroism measurements. EMBO J. 3:2873–2878, 1984.
7. McKay, D.B., Steitz, T.A.: Structure of catabolite gene activator protein at 2.9 Å resolution suggests binding to left-handed B-DNA. Nature 290:774–749, 1981.
8. McKay, D.B., Weber, I.T., Steitz, T.A.: Strucutre of catabolite gene activator protein at 2.9 Å resolution: Incorporation of amino acid sequence and interactions with cAMP. J. Biol. Chem. 257:9518–9524, 1982.
9. Steitz, T.A., Ohlendorf, D.H., McKay, D.B., Anderson, W.F., Matthews, B.W.: Structural similarity in the DNA-binding domains of catabolite gene activator and Cro repressor proteins. Proc. Natl. Acad. Sci. USA 79:3097–3100, 1982.
10. Pabo, C.O., Sauer, R.T.: Protein-DNA recognition. Annu. Rev. Biochem. 53:293–321, 1984.
11. Steitz, T.A., Weber, I.T., Matthew, J.B.: Catabolite gene activator protein: Structure, homology with other proteins, and cyclic AMP and DNA binding. Cold Spring Harbor Symp. Quant. Biol. XLVII:419–426, 1983.

12. Weber, I.T., Steitz, T.A.: Model of specific complex between catabolite gene activator protein and B-DNA suggested by electrostatic complementarity. Proc. Natl. Acad. Sci. USA. 81:3973–3977, 1984.
13. Majors, J.E.: "Control of the *E. coli* Lac Operon at the Molecular Level." Ph.D. Thesis, Harvard University, Cambridge, Massachusetts, 1977.
14. Simpson, R.B.: Interaction of the cAMP receptor protein with the lac promoter. Nucleic Acids Res. 8:759–766, 1980.
15. Liu-Johnson, H.-N., Gartenberg, M.R., Crothers, D.M.: The DNA binding domain and bending angle of *E. coli* CAP protein. Cell 47:995–1005, 1986.
16. Warwicker, J., Watson, H.C.: Calculation of the electric potential in the active site cleft due to alpha-helix dipoles. J. Mol. Biol. 157:671–679, 1982.
17. Warwicker, J.: Continuum dielectric modelling of the protein-solvent system, and calculation of the long-range electrostatic field of the enzyme phosphoglycerate mutase. J. Theor. Biol. 121:199–210, 1986.
18. Warwicker, J., Ollis, D., Richards, F.M., Steitz, T.A.: Electrostatic field of the large fragment of Escherichia coli DNA polymerase I. J. Mol. Biol. 186:645–649, 1985.
19. Arnott, S., Hukins, D.W.L.: Optimised parameters for A-DNA and B-DNA. Biochem. Biophys. Res. Commun. 47:1504–1510, 1972.
20. Richmond, T.J., Finch, J.T., Rushton, B., Rhodes, D., Klug, A.: Structure of the nucleosome core particle at 7 Å resolution. Nature 311:532–537, 1984.
21. Arnott, S., Dover, S.D., Wonacott, A.J.: Least-squares refinement of the crystal and molecular structures of DNA and RNA from X-ray data and standard bond lengths and angles. Acta Cryst. B25:2192–2206, 1969.
22. Drew, H.R., Travers, A.A.: DNA bending and its relation to nucleosome positioning. J. Mol. Biol. 186:773–790, 1985.
23. Frederick, C.A., Grable, J., Melia, M., Samudzi, C., Jen-Jacobson, L., Wan.-C., Greene, P., Boyer, H.W., Rosenberg, J.M.: Kinked DNA in crystalline complex with EcoRI endonuclease. Nature 309:327–330, 1984.
24. Jones, T.A.: A graphics model building and refinement system for macromolecules. J. Appl. Crystallogr. 11:268–272, 1978.
25. Gronenborn, A.M., Nermut, M.V., Eason, P., Clore, G.M.: Visualization of cAMP receptor protein-induced DNA kinking by electron microscopy. J. Mol. Biol. 179:751–757, 1984.
26. Bloomfield, V.A., Crothers, D.M., Tinoco, I., Jr.: "Physical Chemistry of Nucleic Acids." New York: Harper and Row, 1974:164–166.
27. Siebenlist, U., Simpson, R.B., Gilbert, W.: *E. coli* RNA polymerase interacts homologously with two different promoters. Cell 20:269–281, 1980.