A model for the non-specific binding of catabolite gene activator protein to DNA

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Received 10 July 1984; Revised and Accepted 18 October 1984

ABSTRACT

The binding of *E. coli* catabolite gene activator protein (CAP) to non-specific sequences of DNA has been modelled as an electrostatic interaction between four basic side chains of the CAP dimer and the charged phosphates of DNA. Calculation of the electrostatic contribution to the binding free energy at various separations of the two molecules shows that complex formation is favored when CAP and DNA are separated by as much as 12 Å. Thus, the long range electrostatic interactions may provide the initial energy for complex formation and also the correct relative orientation of CAP and DNA. The non-specific complex does not involve the penetration of amino acid side chains into the major grooves of DNA and permits 'sliding' of the protein along DNA, which would enhance the rate of association of CAP with the specific site as has been proposed previously for *lac* repressor. We propose that, as it 'slides', CAP is moving in and out of the major grooves in order to sample the DNA sequence. Recognition of the specific DNA site is achieved by a complementarity in structure and hydrogen bonding between amino acids and the edges of base pairs exposed in the major grooves of DNA.

INTRODUCTION

Transcription in *Escherichia coli* is regulated by repressor and activator proteins, among them the catabolite gene activator protein (CAP) (1, 2, 3) and *lac* repressor(4). CAP regulates transcription from many promoters including the lactose (5), galactose (6) and arabinose (7) operons. Cyclic AMP is an allosteric activator of CAP that increases the affinity of CAP for the specific DNA sites. In the absence of cAMP, CAP binds to non-specific DNA sequences about six orders of magnitude less tightly than to its specific site in the *lac* operon (8,9). Similarly, the affinity of *lac* repressor for operator DNA is about 7 orders of magnitude greater than the affinity for non-specific DNA sequences (10).

The presence of non-specific sequences of DNA appears to facilitate the search of *lac* repressor for the operator (11,12). *Lac* repressor (13) and the EcoRI endonuclease (14) locate their recognition site faster than

expected for a diffusion limited collision of macromolecules. These proteins appear to bind to the specific sites by a two-step process that involves binding first to non-specific sequences of DNA and then translocation to the high affinity specific site (15). It has been proposed that the protein moves along the DNA molecule by a type of one-dimensional random walk or 'sliding' process until the specific high affinity site is located (16,17,18). In this paper, we provide structural models of how non-specific complexes and 'sliding' might facilitate recognition of a specific DNA sequence by CAP.

The crystal structure of a CAP dimer complexed with cAMP has been determined at 2.9 Å resolution (19,20). The two F alpha helices are implicated in the binding of CAP to DNA and are predicted to lie in successive major grooves of B-form DNA in the complex (21,22). A two-helix structure similar to the consecutive alpha helices E and F in CAP is seen in the known crystal structures of cI and cro repressor from lambda phage (23,24). Sequence homologies have suggested the same structure occurs in lac repressor and many other gene regulatory proteins (25-27). Recently it has been shown by two dimensional NMR (28) that lac repressor headpiece folds into two alpha helices in approximately the predicted positions. Therefore CAP and lac repressor appear to share a common structure of two consecutive alpha helices that is involved in binding to and recognition of the specific DNA sites.

The atomic coordinates of the CAP structure have enabled us to calculate the electrostatic potential energy surfaces of the CAP dimer (29). This was valuable in building a model of the complex between CAP and the specific site in the lac operon (21). The specific site appears to be recognized by a set of complementary hydrogen bond interactions that are formed between amino acid side chains of the CAP F helices and the edges of base pairs that are exposed in the major grooves of DNA. The model complex that we have described is in good agreement with the experimental data for specific binding of CAP to the lac site.

We have constructed a model of CAP bound to non-specific DNA that is consistent with the more extensive data on the binding of lac repressor to DNA. In this non-specific complex, CAP and DNA are further apart than in the specific complex and less contact occurs between the two molecules in the major grooves of DNA so that there is no physical barrier to the relative motion of the molecules. The electrostatic interaction between CAP and DNA plays a major role in formation of the complex and is consist-

ent with the type of facilitated translocation along the DNA that has been proposed for lac repressor.

METHODS

Electrostatic Potential Surface

The electrostatic potential surfaces of the CAP dimer were calculated by the method described in Matthew and Richards (30). This method is a modification of the Tanford-Kirkwood theory (31) and scales the local dielectric constant of the charged group according to the solvent accessible area (32). The charged sites were taken from the crystallographic coordinates of the titratable amino acid side chains of the CAP dimer and the phosphates of the two bound molecules of cAMP. The electrostatic work factors (33) between pairs of charges were computed for a sphere of equivalent volume to the protein. The electrostatic potential surfaces were also calculated for a 24-base pair molecule of B-DNA with 20 Na⁺ counterions of variable occupancy as described in Matthew and Richards (34) and for a longer 44-base pair DNA molecule with 40 Na⁺ counterions.

Modelling a CAP-DNA Complex

A 24-base pair molecule of B-DNA was placed with two successive major grooves facing the two parallel F alpha helices of the CAP dimer so that the pseudo two-fold axis of the DNA was coincident with the approximate two-fold axis of the CAP small domains. Then the DNA was rotated to optimize the overlap of the phosphate backbone with the positive electrostatic potential surfaces of CAP (29,21). In the non-specific complex the DNA is moved further from the CAP molecule and a lysine and arginine side chain from each F helix were moved to within 3 Å of two phosphate oxygens of DNA so that a total of 4 ion pairs were formed.

Electrostatic Stability of the Non-Specific Complex

The electrostatic free energy for the formation of this non-specific complex was calculated by subtracting the electrostatic energy of the two separate molecules from that of the complex. The electrostatic stability of the complex was also calculated as a function of ionic strength and of distance between the two molecules. When the CAP and DNA were separated the ion pair interactions were reformed where possible by adjusting the ends of the basic side chains to about 3 Å of the phosphates in order to simulate the minimum energy association of the two molecules. The electrostatic free energy of the complex was also calculated as CAP was moved along the helix axis of a 44 base pair DNA molecule in order to simulate a one-dimensional sliding.

Figure 1 A stereo picture of the electrostatic potential energy surfaces of the CAP dimer with cAMP are shown together with the alpha carbon backbone of CAP. The positive (dashed lines) and negative (dotted lines) equipotential surfaces are contoured at a level of $2kT$ and the calculation was performed at pH 7.0 and an ionic strength of 0.01.

RESULTS AND DISCUSSION

Electrostatic Potential Energy Surfaces of CAP

The electrostatic potential surfaces of the CAP dimer are asymmetric as shown in Figure 1. The positive charge density is located on the sides of the two small DNA-binding domains while the negative charge is distributed centrally in this view and along the large amino-terminal domains. The positive charge distribution also extends along and parallel to each of the protruding F alpha helices of CAP. This region of positive charge is expected to interact with the negatively charged DNA molecule. The maximum of the negative electrostatic charge distribution of B-DNA follows the helical path of the charged phosphates; however, at a lower potential energy level of $2kT$ the charge distribution of DNA approximates a cylinder (34).

Model Complex of CAP and Non-specific DNA

In the complex between CAP and a non-specific sequence of DNA shown in Figure 2 the primary interaction is electrostatic. The two regions of positive potential in CAP lie close to the phosphates of DNA. Four ion pairs are formed between a lysine and arginine from each F helix and the charged phosphates. These basic side chains are long and it is possible to form ionic interactions when the DNA is at a distance from the protein. The total contribution of four ionic interactions between CAP and DNA in this non-specific complex is in agreement with the reported values (35,36,37) of from 4 to 7 ion pairs obtained from the variation of the binding constant as a function of ionic strength. The electrostatic contribution to the

formation of this non-specific complex is calculated to be -8.0 kcal/Mole at pH 7.0 and 0.01 ionic strength. This can be compared with the calculated electrostatic free energy of the specific complex between CAP and bent DNA of -13.4 kcal/Mole (21) which corresponds to a difference of 5.4 kcal/Mole.

The observed and calculated variation of the stability of this complex with ionic strength are in modest agreement and decrease slightly with increasing ionic strength (Figure 3). The experimental values of Saxe and Rezvin (35) and Takahashi et al (36) for the comparative binding of CAP to non-specific DNA show a somewhat greater ionic strength dependence than that of the calculated binding energy. The calculated values are closer to those measured for the case of CAP binding to DNA in the presence of cAMP which may be a more appropriate comparison since CAP has been crystallized as a complex with cAMP. Probably, the conformation of the protein differs in the absence of cAMP.

The electrostatic contribution of non-specific binding has also been calculated as the CAP and DNA molecules are separated from the position of a model specific complex (Figure 4). The two molecules have been moved apart in steps of one to $2 \text{ \AA}$ and each distance the long flexible lysine and arginine side chains are adjusted into positions close to the phosphates of DNA. This procedure results in a binding energy that is close to a minimum energy association at each separation distance. Alternatively, if the four basic side chains are maintained at the position that they occupy in the closest non-specific complex (which is $3.6 \text{ \AA}$ from the position of the molecules in the specific complex), there is a loss of about 3 kcal/Mole at $7.6 \text{ \AA}$ separation and about 0.5 kcal/Mole at $11.6 \text{ \AA}$ distance for low ionic strength. Therefore, the distance dependence of the electrostatic stabilization of the complex falls off more rapidly when the mobile side chains are not adjusted towards the charged phosphates.

The electrostatic interaction favors the association of the two molecules up to a separation of about $12 \text{ \AA}$ at which point electrostatic binding energy falls below 2 kT or 1.2 kcal/Mole at a physiological ionic strength. Therefore the long range electrostatic attraction may provide the initial energy for formation of the complex between CAP and DNA and also orient the protein relative to the DNA. This complex is electrostatically stable at a protein - DNA separation where there are no specific hydrogen bonds or other binding contacts of a directional character between CAP and DNA. Thus, the movement of the protein along the DNA does not require the making and breaking of specific bonds.

Figure 2 Space filling representations of 3 model complexes of CAP and DNA looking down the major grooves at an angle of 30° to the DNA helix axis. a) The specific complex of CAP and DNA that is bent with a $70 \text{ \AA}$ radius of curvature. The 2 F alpha helices penetrate into the major grooves of DNA and form hydrogen bonds with the base pairs. b) A non-specific complex at $3.6 \text{ \AA}$ separation distance (see Figure 4), where there is little or no penetration of protein atoms into the major grooves. Only lysine 188 and arginine 185 from each F helix are sufficiently close to make ionic interactions with the phosphates of DNA. c) A non-specific complex at $7.6 \text{ \AA}$ separation distance. The DNA is represented as straight for these non-specific complexes, however there is little information on whether the DNA is straight or bent as shown for the specific complex.

Specific and Non-specific Interaction: Analogy with Lac Repressor

The non-specific complex of CAP and DNA has been constructed to be consistent with the extensive data on the non-specific binding of lac repressor protein to DNA. Lac repressor has a significant affinity (10^{-5} to 10^{-8} M , depending on the DNA) for non-operator sequences of DNA (38). It has been estimated from the ionic strength dependence of binding that the non-specific complex of lac repressor and DNA involves about 10-12 ionic interactions (39,40) whereas approximately 8 ion pairs are formed in the lac repressor-operator complex (41). Thus ionic interactions are more im-

Figure 3 The variation in electrostatic stability of the non-specific CAP-DNA complex at 3.6 Å separation (Figure 2b) with ionic strength. The line connects the calculated values for pH 7.0. The experimental observations are: for CAP and double stranded DNA, (Δ) from Saxe and Rezvin (35); (∇) from Takahashi et al. (36) and ($\square$) CAP, cAMP and double stranded DNA (36).

Figure 4 The variation in electrostatic stability of the model CAP-DNA complexes as the 2 molecules are separated. The zero separation corresponds to the position of the two molecules in the complex of CAP with straight DNA at the specific site. The calculations were performed at pH 7.0 and an ionic strength of 0.01 (open circles) and 0.10 (solid circles). The electrostatic interaction favors the formation of a complex up to about 12 Å separation where the electrostatic free energy falls below $2kT$ or 1.2 kcal/Mole.

portant for non-specific than for specific binding to DNA. The formation of a CAP complex with random sequences of DNA releases from 4 to 6 counterions (35,36) whereas the estimate for the specific complex is 4 counterions (37). It is expected that for both proteins there will be a large non-ionic component in the binding to specific DNA sequences. Winter and von Hippel (42) estimate an extra 9-12 kcal/Mole for lac repressor binding to operator as opposed to non-operator DNA and there is an added 5 kcal/Mole for the specific binding of CAP to the lac site compared with non-specific binding (37,9).

Lac repressor binding to non-operator sequences of DNA does not require the protein to penetrate into the major groove of the DNA since its binding is not reduced by steric blocking of the major grooves (38,43). In fact, lac repressor binds to analogs of poly dAT in which the major groove is filled by bulky substituents with affinities as high as 10^9 M^{-1} (43). Even the charge of the substituents has little influence on the non-specific binding. Leahy (44, 45) has shown that blocking the major grooves of DNA with bulky substituents of the Thymine methyl group abolishes specific binding of lac repressor to operator containing DNA but not its non-specific association. Thus, in the case of lac repressor, the specific complex with DNA involves penetration of the protein into the major grooves of the operator, while the non-specific interaction involves little or no penetration into the major grooves and more ion pairs. It has been predicted (25,26) that both lac repressor and CAP would form the specific complex with B-DNA by means of hydrogen bond interactions between amino acid side chains of an alpha helix and the edges of base pairs exposed in the major groove of DNA. Such a model has been built for the case of the CAP dimer interacting with the CAP site in the lac operon (21,22).

In the non-specific complex described here the relative orientation of CAP and DNA is the same as in the specific model, however, the two molecules are separated so that the protein side chains do not penetrate into the major grooves of DNA (Figure 2). In fact, the Arginine and Lysine side chains that form ion pairs with the DNA phosphate backbone in the non-specific complex penetrate into the major groove in the model of the specific complex and make hydrogen bonds with base pairs. The CAP dimer interacts with about 21 base pairs of DNA in a specific complex with B-DNA that has been bent smoothly with a 70 Å radius of curvature. Each subunit of the CAP dimer forms hydrogen bonds between 4 side chains of each F helix and 4 consecutive base pairs that form part of the conserved DNA sequence.

There are additional interactions occurring between CAP and the phosphates of DNA and these include a total of 5 ionic interactions between the CAP dimer and the DNA site.

Length and bending of the DNA affect the electrostatic contribution to the formation of the specific CAP-DNA complex but have little effect on the non-specific complexes described here. Whereas bending a 24 b.p. CAP site to a 70 Å radius of curvature improves the electrostatic stability of the specific complex by only -1 to -2 kcal/Mole, similar bending of a 44 b.p. fragment increases the stability by -3.8 kcal/Mole. The longer DNA gives a more accurate value in this case because of end effects when the two molecules are close together. Little variation (0.5 to 0.8 kcal/Mole) is seen in the electrostatic stability of CAP with straight DNA of different lengths. Thus, we expect that CAP will bend or kink DNA in the specific complex, but may not in the non-specific complex.

Sliding

Since the negative electrostatic charge potential of the DNA can be approximated by a cylinder and since the non-specific complex is stable at CAP-DNA separations up to 12 Å, it is possible for CAP to move freely along the DNA by a rapid one-dimensional diffusion or 'sliding'. In an attempt to approximate the size of the electrostatic energy barriers to 'sliding', the electrostatic energy of CAP moving along the DNA in a direction parallel to the DNA helix axis was calculated. This produced a variation in the electrostatic stability of the complex of about 2.5 kcal/Mole. A similar variation in electrostatic free energy was also calculated for a simulation of CAP moving in a spiral path around the ribose-phosphate backbone of B-DNA. This loss in electrostatic free energy is of the same order as that calculated when the four ion pairs are not made between the basic amino acids and the phosphates. This means that the variation of 3 kcal/Mole calculated for the 'sliding' of CAP along DNA is an extreme and unlikely case since it involves the loss of all four ionic interactions simultaneously. The basic amino acid side chains of CAP that form salt bridges to the DNA in this non-specific complex are long and flexible so that they can readily move from one phosphate site to an adjacent phosphate, although in these simulations the side chains were not moved independently of the whole protein. Presumably if CAP is to sample all contiguous base sequences during the search for the specific recognition site, the best route is a spiral path that follows the backbone of the DNA double helix. However, the approximate analysis presented here gives

similar energy barriers for a one-dimensional motion along the DNA helix axis and for a spiral path and therefore cannot distinguish between them.

Relation of Non-specific Complex to Sliding and Specific Sequence Recognition

How, then, does the 'sliding' of CAP along the DNA allow CAP to identify the correct sequence to which it binds specifically? The electrostatic attraction of DNA for CAP is like an elastic leash whose energy varies from 2 kcal/Mole at 12 Å separation to as much as 13 kcal/Mole in contact at the specific site (Figure 4). As CAP 'slides' down the DNA the electrostatic attraction will favor penetration of the two alpha helices into the major grooves and close contact with the DNA. However, as CAP moves closer to the DNA, the extent of complementarity between the hydrogen bond donors and acceptors of the side chains of the F helix and those donors and acceptors on the edges of the base pairs exposed in the major grooves becomes a dominant energy factor. If the pattern of donors and acceptors on the protein is not matched by those of DNA, the protein will be repulsed and will continue to 'slide'. Thus, CAP can 'sample' each sequence of base pairs as it 'slides' along the DNA. We suggest that the protein is 'bouncing' as well as 'sliding', in that it will move in and out of the groove, presumably at a rate that is faster than 'sliding'. When the correct base sequence is reached, the energy of hydrogen bonding and van der Waals contacts will add to the full 13 kcal/Mole of electrostatic energy rather than subtract from it.

CONCLUSIONS

The calculated electrostatic potential energy of the CAP molecule has an asymmetric distribution with the positive charge concentrated around the DNA-binding domains. This is similar to ribonuclease (30) which also has a region of positive electrostatic field close to the RNA site and cro repressor (47) from lambda phage which shows positive potential at the DNA binding site. Such an asymmetric charge distribution may be a common feature of proteins that interact with nucleic acids. The electrostatic contribution to the interaction of CAP and DNA is effective when the molecules are separated by up to 12 Å under physiological ionic strength. The complementary electrostatic fields of CAP and DNA can orient the protein relative to the DNA and favour the association of the two molecules. This is probably important for proteins that associate non-specifically with DNA and search for a specific sequence by a rapid facilitated trans-

location (14, 15). The protein molecule may form an electrostatic association at a distance and slide along the DNA until the specific site is recognized. During the diffusion of the protein along DNA the protein must sample the sequence of base pairs in a series of random associations and at the high affinity specific site there will be a minimum energy set of complementary hydrogen bonds formed between amino acid side chains and the base pairs of DNA.

ACKNOWLEDGMENTS

We thank Jim Matthew for advice on the electrostatic calculations. This work was supported by National Science Foundation grant PCM-8316666 and U. S. Public Health Service grant GM-22778.

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