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The role of DNA bending in Cro protein–DNA interactions

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Abstract

Binding energy of DNA–Cro protein complexes is analyzed in terms of DNA elasticity, using a sequence-dependent anisotropic bendability (SDAB) model of DNA, developed recently [M.M. Gromiha, M.G. Munteanu, A. Gabrielian and S. Pongor, J. Biol. Phys. 22 (1996) 227–243.]. The protein is considered to bind aspecifically to DNA that reduces the freedom of movement in the DNA molecule. In cognate DNA, the Cro protein moves on to form specific interactions and bends DNA. A comparison of the experimental data [Y. Takeda, A. Sarai and V.M. Rivera, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 439–443.] with the calculated DNA stiffness data shows that ΔG of the complex formation increases with the stiffness of the ligand when the interactions are nonspecific ones, while an opposite trend is observed for specific binding. Both of these trends are in agreement with our approach using the SDAB model. A decomposition of the energy terms suggests that binding energy in the nonspecific case is used mainly to compensate the free energy changes due to entropy lost by DNA, while the energy of specific interactions provide enough energy both to bend the DNA molecule and to change the conformation of the Cro protein upon ligand binding. © 1997 Elsevier Science B.V.

Keywords: Bending energy; Cro protein; DNA; Elastic entropy; Elastic stiffness; Free energy change; Operator sequences

1. Introduction

Protein–DNA interactions play a key role in most vital processes such as gene regulation, DNA replication and packaging. The remarkable sequence specificity that makes it possible for proteins to recognize exact locations within a quasi-infinite DNA chain is of considerable theoretical and practical interest. Many features of both macromolecules can be described at an atomic level by means of molecular mechanics and dynamics based on Newtonian mechanics. On one hand, as the specificity is believed to depend on intermolecular hydrogen bonds as well as ionic and van der Waals contacts, atomic detail seems in fact necessary. On the other hand, considerable work has been done on modelling DNA as an elastic rod of uniform rigidity (for review, see [1–3]). These 'low resolution models' were very successful in describing macroscopic properties of long DNA molecules but they lack sequence-dependent features

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necessary to study local protein recognition phenomena. Recently, we developed DNA bendability parameters from protein/DNA interaction data [4] and incorporated them into an elastic DNA-bending model [5] in which the rigidity of DNA is sequence-dependent and anisotropic (see Fig. 1a). In practical terms, DNA is considered as a cylindrical rod of 20 Å diameter, consisting of 3.4 Å thick disks representing base pairs. The rigidity of this disk is taken from a trinucleotide table (Table 1), i.e., a disk representing an AT base pair between a 5′ GC and a 3′ AT can be found at GAC. The rigidity of the disk is considered anisotropic in the sense that it can bend more easily towards the major groove and 10 times less in opposite and perpendicular directions. Only bending is considered, stretching/compression and torsional elasticity is neglected. Finite element analysis of this model showed that sequence motifs corresponding to curved DNA will show asymmetric bendability in one direction so they will behave as curved, due to thermal fluctuations. It was also found that sequence-dependent bendability (or stiffness) correlates well with protein-induced bends seen in X-ray crystallography [4]. Also, a correlation was seen between the stability of the complex of the Cro/cognate DNA complexes and the rigidity of the cognate molecule [5]. In this paper, we should like to determine what specific role sequence-dependent rigidity may play in a specific protein/DNA interaction, using the Cro/DNA recognition as an example.

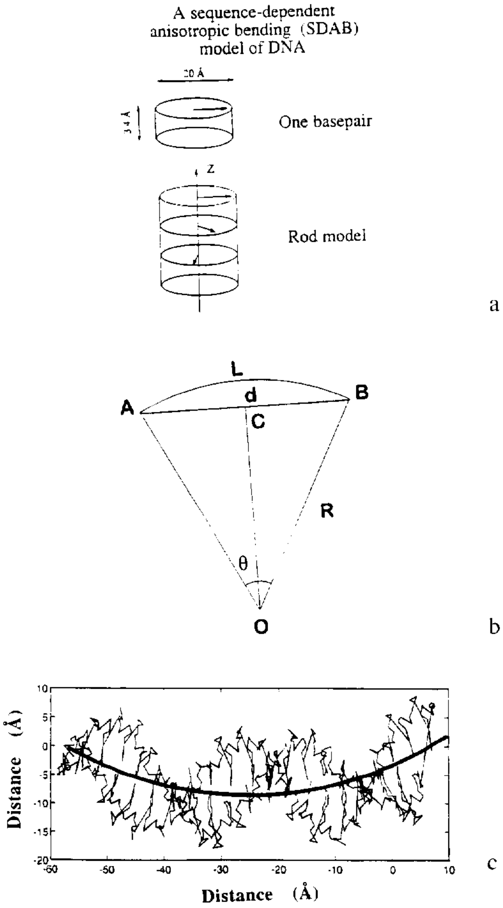

Fig. 1. (a) Schematic diagram of the sequence-dependent anisotropic bending (SDAB) model of DNA. (b) Parameters used to compute the bending angle from the 3D structure (L, arc length; d, end to end distance; θ, bending angle; and R, radius of curvature. (c) Model diagram to compute the bending angle by fitting a circular arch to the DNA trajectory. X-ray coordinates are taken from PDB3CRO.ENT.

Cro protein represents a DNA-binding protein with a helix-turn-helix structure as a specific DNA recognition motif. The conformation of Cro–OR3 complex was deduced from NMR spectroscopy by Lee et al. [6], and the crystallographic structure of the complex was reported by Brennen et al. [7]. Cro is well known to bend DNA upon specific binding [8–12] and several investigations have been carried out on its interaction with cognate and non-cognate DNA molecules. Takeda et al. [13] analyzed the interaction of Cro with specific and nonspecific sites on DNA and suggested that Cro/nonspecific DNA interaction is predominantly electrostatic and the Cro/specific DNA interaction includes hydrogen bonds and hydrophobic interaction as well. Kim et al. [14] analyzed the Cro–DNA interaction with filter binding assays by measuring the dissociation constants and suggested that Cro has the highest affinity to the consensus operator. The conformational changes due to sequence specific interaction of operator DNA with Cro repressor was studied by Lee et al. [15]. Torigoe et al. [16] introduced some base substitutions in the operator and studied the effects on their conformations and thermodynamic parameters for the dissociation of the complex. Baleja et al., [17] compared the binding strengths and the conformation of Cro–DNA complexes and showed that base pair differences between the two halves of the OR3 operator affects the binding of the Cro protein.

Table 1
DNA stiffness (Young's modulus) scale for the trinucleotides in DNA [5]

Trinucleotide	Young's modulus, E (10^8 N/m^2)	Trinucleotide	Young's modulus, E (10^8 N/m^2)
AAA/TTT	7.176	CAG/CTG	1.668
AAC/GTT	6.272	CCA/TGG	6.813
AAG/CTT	4.736	CCC/GGG	3.868
AAT/ATT	7.237	CCG/CGG	5.440
ACA/TGT	3.810	CGA/TCG	3.810
ACC/GGT	4.156	CGC/GCG	4.678
ACG/CGT	4.156	CTA/TAG	2.673
ACT/AGT	6.033	CTC/GAG	3.353
AGA/TCT	3.410	GAA/TTC	4.214
AGC/GCT	3.524	GAC/GTC	3.925
AGG/CCT	4.445	GCA/TGC	2.842
ATA/TAT	1.613	GCC/GGC	2.448
ATC/GAT	5.087	GGA/TCC	3.581
ATG/CAT	2.169	GTA/TAC	3.467
CAA/TTG	3.581	TAA/TTA	2.955
CAC/GTG	3.239	TCA/TGA	1.447

It has also been shown that DNA bending is induced by Cro at the OR3 operator site [12,18] and Cro prefers to bind OR3 more than OR1 [19,20]. Evertsz et al. [21] performed Raman spectroscopic studies on DNA–Cro binding sites and suggested that DNA undergoes significant structural changes upon binding. This was supported by Gursky et al. [22] who noted that the binding of Cro with OR3 attributes to DNA-dependent structural changes in the protein. Takeda et al. [23,24] measured quantitatively the binding affinities of the Cro repressor to the chemically synthesized operators and mutants of OR1 operators and other thermodynamic parameters, ΔH, ΔS, ΔG and ΔCp, for Cro protein–DNA association process. In spite of all these investigations, the role of elastic stiffness in Cro–DNA interactions has not yet been explored.

The aim of the present work is to understand the relationship between elastic stiffness and free energy change (ΔG) due to binding of Cro to cognate or non-cognate sequences, using thermodynamic data on the interaction of Cro with 14 cognate and non-cognate DNA molecules, determined by Takeda et al. [23]. Here, we use the simplified sequence-dependent anisotropic bendability (SDAB) model of DNA we recently developed [5] in which only bending is considered. We suggest a new approach to describe Cro/DNA interactions and estimate the contribution of bending energy to protein/DNA recognition.

2. Methods

2.1. Computation of bending energy

DNA can be considered as an elastic rod of radius, r, [5,25,26]. The bending energy of this elastic rod of length, L, subjected to pure bending can be given by the classical formula:

$$\Delta G = BL/2R^2 \tag{1}$$

where R is the radius of curvature and B is the bending rigidity given as $B = EI$, E is the Young's modulus, a parameter characteristic of the material of the rod whose value for DNA is measured to be 3.4×10^8 N/m^2 [27]. I is the moment of inertia and for a rod of circular cross section, I is given as $I = \pi r^4/4$, where r is the radius.

The radius of curvature, R, is related with the bending angle, θ, with the formula [5,28]:

$$R = L/\theta \tag{2}$$

and the bending energy is given by:

$$\Delta G = EI\theta^2/2L \tag{3}$$

The average Young's modulus is calculated with the formula [5]:

$$\frac{1}{E} = \frac{1}{n} \Sigma \frac{1}{E_i} \tag{4}$$

where n is the number of nucleotides. E_i is the Young's modulus values for the trinucleotide units.

We have multiplied the bending energy with the Avogadro number to get the values related to one mole of the material.

2.2. Computation of the average stiffness for various DNA molecules

We have computed the average stiffness values for all the six operators, three operator mutants and five non-operator sequences given in Takeda et al. [23], according to Eq. (4). We used the stiffness scale for trinucleotide units we developed [5] to compute the average stiffness value. The Young's modulus (stiffness) scale is given in Table 1. The computed average stiffness values (Young's modulus) are presented in Table 2.

2.3. Computation of elastic entropy

Marko and Siggia [29] stated that the thermal fluctuations are responsible for elastic entropy. The direct relationship between bending angle due to thermal fluctuations and bending stiffness [1,5] leads us to compute the elastic entropy, ΔS, with the formula:

$$\Delta S = nR \ln \left[\frac{\langle \theta^2_{\mathrm{bound}} \rangle^{1/2}}{\langle \theta^2_{\mathrm{free}} \rangle^{1/2}} \right] = nR \ln \left[\frac{E_{\mathrm{free}}}{E_{\mathrm{bound}}} \right] \tag{5}$$

where E_{free} is the average Young's modulus from Eq. (4) and n is the number of degrees of freedom. E_{bound} is the Young's modulus of the bound (quasi-immobilized) DNA.

2.4. Computation of bending angle

We have used the crystal structure of Cro–OR3 complex, available in Protein Data Bank, to compute the bending angle. We have computed the end to end distance (d) of the OR3 operator using the crystal coordinates. We set up an equation connecting bending angle (θ), arc length (L) and d (Fig. 1b) as:

$$\frac{\sin(\theta/2)}{\theta/2} = \frac{d}{L} \tag{6}$$

We use this equation to compute the bending angle, which is 76.4° for the Cro–OR3 complex.

A more general procedure is to approximate the

Table 2
Computed Young's modulus for Cro operators, mutants and non-operators

No.	Code	Sequences	E (10^8 N/m^2)	ΔG (kcal/mole)
Operators				
1	OR1	TACCTCTGGCGGTGATA	3.45	−15.4
2	OR2	CAACACGCACGGTGTTA	4.07	−14.1
3	OR3	TATCCCTTGCGGTGATA	3.45	−16.1
4	OL1	TACCACTGGCGGTGATA	3.74	−15.5
5	OL2	TATCTCTGGCGGTGTTG	3.90	−14.9
6	OL3	AACCATCTGCGGTGATA	4.01	−14.4
Operator mutants				
7	OM1	TTTACCTCTGGCGGTGATAAT	4.30	−13.33
8	OM2	TTTACCTCTGGCGGTATTAAT	4.48	-13.11
9	OM3	TTTACCTCTGGCGGAGTTAAT	4.61	−13.26
Non-operators				
10	N1	TAAAACACCTCACGAGTTAAT	4.49	−13.40
11	N2	TAAATCACTCCCGGGTATATT	4.22	−12.90
12	N3	TATATCAGTGGCAGTGTGAAT	3.60	−10.97
13	N4	TAGATCACCGCAGCGGTTGCT	3.88	−11.76
14	N5	TTCAGCACCGCTGATGCTGCT	3.40	−9.97

curvature of DNA by that of a perfect circular arch that fits the best the atomic coordinates of the DNA molecule, in terms of the root-mean-square distance. The calculation consists of two steps: (i) first, the optimal plane is determined by minimizing the sum of the square distances between the atomic coordinates and the plane; (ii) second, all the atoms are projected on this plane, and the circle best fitting the points on the plane is determined using a nonlinear optimization based on the Newton–Raphson method. Finally, the radius, the length of the circular arch and the corresponding angle are computed (Fig. 1c). For the Cro–DNA complex, the bending angle is computed to be 78.5°. If the DNA sequence is too short, and therefore the points are too dispersed, the possibility exists that the iterative method does not converge. A computer code written in MATLAB was used by the authors to examine a number of DNA sequences. More details about the algorithm are available upon request to MGM (e-mail: mmmm@starnets.ro).

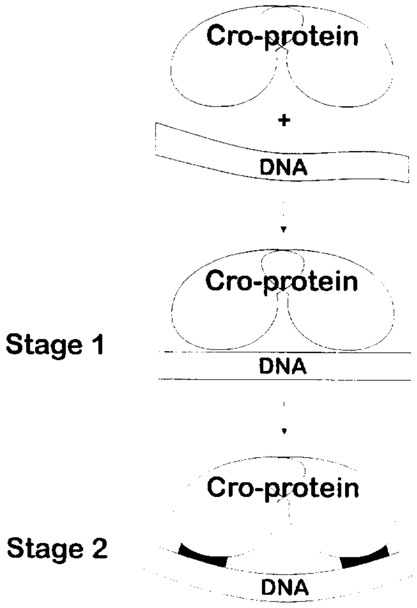

Fig. 3. A simple model of Cro–DNA interactions.

3. Results and discussions

3.1. Correlation of average stiffness with binding to Cro (ΔG)

The ΔG (free energy due to binding of the Cro repressor to operators and non-operators) values of Takeda et al. [23] were first plotted against the calculated stiffness values (Table 2). Fig. 2 shows that cognate (operator) and non-cognate (non-operator) DNA follow two adverse, quasi-linear relationships. In the operator sequences, ΔG is higher for stiffer molecules ($R = 0.95$), i.e., the stiffer the molecule, the weaker the binding. In non-operator sequences, ΔG is lower for stiffer sequences ($R = 0.99$), i.e., the stiffer the sequence, the stronger the binding.

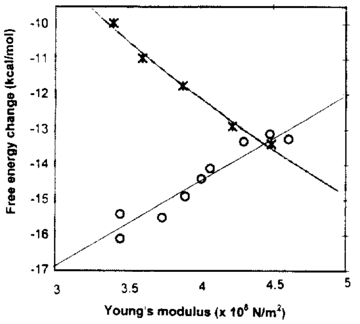

Fig. 2. Relationship between the average stiffness (Young's modulus) of DNA and the free energy change (ΔG) of cognate (○) and non-cognate (*) DNA sequences. Eq. (10) was fitted to the non-cognate data ($R = 0.99$) and a straight line was fitted to the cognate data ($R = 0.95$)

3.2. Construction of a simple model for Cro / DNA interactions

The following approach is suggested to describe specific Cro–DNA interactions in terms of sequence-dependent bending (Fig. 3).

Before binding, protein and DNA are free to move in the solution. At this stage, DNA undergoes thermal fluctuations whose entropy contribution can be calculated using Eq. (5).

Stage 1: The protein binds to DNA in an aspecific manner, i.e., via interactions independent of the sequence of DNA. These interactions are considered to be the same for interactions with operator and non-operator DNA. The freedom of movement of DNA is lost by these interactions and the enthalpy gained is used for compensating the free energy changes due to the entropy lost.

Stage 2: In the case of operator sequences, the protein binds to the specific recognition site and bends DNA. The enthalpy of binding is used in part to cover the energy required for bending DNA. DNA is considered to be bent to the same shape as in the Cro/DNA complex, i.e., the angle of curvature is 78.5°.

This model incorporates a number of simplifications. First, the interaction between the protein and the DNA backbone is considered roughly the same for specific and nonspecific interactions. This means that the difference between the two kinds of interactions is taken to originate solely from the energy gained from the hydrogen bonds between the protein and the DNA bases during specific binding. This energy is then spent on two purposes: (i) to cause a conformational change in DNA, which is described here in terms of elastic bending, and (ii) to change the conformation of the protein.

Note that the E values are between 3.2 and 4.5. In this range, the relationship between E and $\ln E$ is almost linear. Therefore, ΔG can vary practically in a linear manner as a function of E and of $\ln E$ in this range (Fig. 2).

The free energy of binding can be specified in the following way for the two kinds of binding.

Nonspecific:

$$\Delta G_{\text{nonspecific}} = \Delta H_{\text{nonspecific}} - T\Delta S_{\text{nonspecific}} \tag{7}$$

Specific:

$$\Delta G_{\text{specific}} = \Delta H_{\text{specific}} - T\Delta S_{\text{specific}} + \Delta G_{\text{bend}} \tag{8}$$

To find out the role of elastic stiffness in specific and nonspecific protein–DNA interactions, it is worth decomposing the free energy terms into sub-terms related and unrelated to elasticity.

$$\Delta G_{\text{total}} = \Delta G_{\text{elastic}} + \Delta G_{\text{nonelastic}}. \tag{9}$$

3.3. Comparing the model with experimental data

The ΔG of nonspecific binding can be calculated in the form:

$$\Delta G_{\text{nonspecific}} = K_1 - nRT \ln E_{\text{free}}. \tag{10}$$

The value of E_{free} is the average bending rigidity of non-operator DNA and is obtained from Eq. (4). The value of n, i.e., the number of degrees of freedom affected, can be estimated as follows: (i) bending degrees of freedom: Cro binds to a segment of 14 base pairs within DNA each of which has one degree of freedom; (ii) translational and rotational degrees of freedom: before complex formation, both Cro and the DNA have three translational and three rotational degrees of freedom, respectively. These 12 degrees of freedom will diminish to six on complex formation. Taken together, the number of degrees of freedom affected are 20. Substituting $n = 20$ into Eq. (10), we have quite a good agreement with the experimental data (Fig. 4). In fact, the agreement is illustrated by the fact that determining n from fitting Eq. (10) to the data, we get $n = 20.58$, the value of K_1 is 4.89. Since, $nR \ln [\langle \theta^2_{\text{bound}} \rangle^{1/2}]$, according to Eq. (5), one can say that the root-mean-square fluctuation of bending in the bound state is 2.2° (in the free state, it is 5.7°). Taken together, it appears that the elastic vibrations of DNA account for the majority of the free energy changes occurring on nonspecific binding.

The ΔG of specific binding can be calculated with the following formula:

$$\Delta G_{\text{specific}} = +\Delta G_{\text{entropic[Eq.(10)]}} + E_1 I \theta^2 / 2L. \tag{11}$$

E_1 can be determined from Eq. (4). θ is the value of bending that can be determined from fitting a circular arch to the DNA trajectory determined from the X-ray crystallography data as described in Section 2.

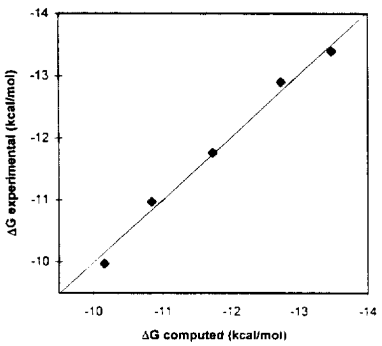

Fig. 4. Comparison of computed and experimental ΔG values in nonspecific DNA sequences.

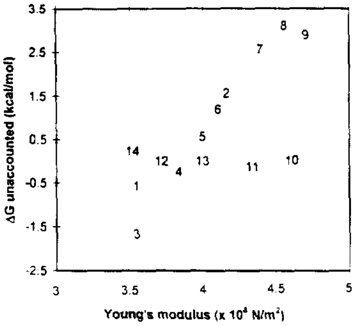

Fig. 5. Relationship between Young's modulus and unaccounted ΔG values in specific (1–9) and nonspecific (10–14) DNA sequences.

We can now plot equation [11] and compare calculated and observed values. The differences between the calculated and observed free energy change are plotted in Fig. 5. We can see that a portion of ΔG is not accounted for by the model. In other words, there is free energy left, and since our model does not contain the data for the protein conformation change, we can expect that the difference is in fact the energy requirement of this change. Qualitatively, the prediction is correct since we expect a greater conformational change if the DNA ligand is stiffer; moreover, it is also expected that the stiffer the DNA, the less stable is the complex. Quantitatively, the energy corresponds to the breaking of one hydrogen bond only. In fact, the conformational change of Cro was estimated to be relatively low [30].

We mention that one can, in principle, vary the angle, θ, to find an optimum fit between the measured and observed values. This would amount to ascribing all the energy change to DNA conformational change. In this case, however, we obtain a value of 141° for the angle of curvature, which is substantially higher then the one seen experimentally (80°). In other words, the contribution of protein conformational change cannot be disregarded.

Summarizing, we can conclude that a good correlation exists between the elastic stiffness of DNA cognates, calculated with a sequence-dependent anisotropic bendability (SDAB) model [5] and the experimentally determined binding affinity of the corresponding oligonucleotides to Cro protein [23]. However, cognate and non-cognate oligonucleotides show opposite trends. These can be interpreted in terms of a simplistic binding model in which Cro first nonspecifically binds to DNA, followed by a second, specific binding step in the case of cognate DNA which is accompanied with the bending of the DNA molecule. The goal of this work is not to develop a perfect model for Cro/DNA interactions but to find out if bending properties of DNA can explain some aspects of this interaction. The good correlation to experimental data suggest that this is indeed the case. Sequence dependent DNA bendability is an easily computable property so it can be incorporated into finite element calculations for modelling local DNA structures [26] such as a curvature [5]. It also has to be noted that DNA bendability and curvature propensity shows particular and differential distributions within various genomes [31,32] so this approach may be used to predict bendable and inherently curved sites within genomic DNA.

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