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Some Energetic and Kinetic Aspects of Protein-DNA Interactions : A Theoretical Study on the λ Repressor-Operator Complex

B. Jayaram,
Department of Chemistry,
Indian Institute of Technology,
Hauz Khas, New Delhi-110016, India.

Abstract

An analysis of the energetics of λ repressor-operator complex, with a potential function developed exclusively for evaluating protein-DNA interactions, reveals the predominant role of van der Waals forces in complexation. The nature of recognition in this system appears primarily to be a lock and key fit of helix-3 and its flanking residues of the protein in the major groove of DNA, augmented by hydrogen bonds. The contribution of the direct code (side chain-base interactions) is only one fourth of the total interaction energy. The protein fold and the DNA conformation appear to be equally critical to recognition from an energetic point of view. Brownian dynamics simulations were performed monitoring the diffusion of several models of the repressor protein towards the OL1 operator. The computed rate constants were all in the range of 10^9-10^{10} $M^{-1}s^{-1}$. The calculated encounter probabilities suggest that it is more facile for the protein to diffuse to the DNA in a non-specific manner followed by a sliding of the protein to the operator site than it is to approach the operator site directly via a three dimensional search.

Introduction

Developing an atomic level understanding of the elements of recognition in protein-DNA systems, via structural, thermodynamic and kinetic investigations, both experimental and computational, is at the frontiers of current day biomolecular research. Several elegant single crystal X-ray and NMR studies on protein-DNA complexes reported in the last decade have enriched our knowledge considerably on the diversity of recognition motifs in these systems [1-7]. No simple code for protein-DNA recognition has emerged, however. Efforts aimed at complementing the structural view by providing a thermodynamic and dynamic perspective via computational means have just begun. A force field which can rapidly and accurately predict the energies and forces of interaction between protein and DNA, considering the solvent and salt effects, is required to be integrated into mole-

cular simulations and to examine the energetics of the available structures. Based on the collective experience accumulated via theoretical studies on the nucleic acid systems [8-18], we have recently put together a force field for evaluating the energetics of protein-DNA complexation. This has further been implemented in a Brownian dynamics algorithm to unravel some mechanistic issues of relevance to the kinetics of protein-DNA complexation. In the following, a detailed atomic level analysis of the energetics of λ repressor-operator complexation together with some kinetic aspects, is presented.

Background

The system bacteriophage λ offers a well characterized example of a genetic switch [19-21]. Simply stated, the switch works as follows. When the repressor protein binds to the operator, the switch is thrown into the off position and the host cell perpetuates in a state called lysogeny but if the cro protein binds in the operator region in preference to the repressor, the switch is turned on leading to the lysis of the host cell. Six operator sites have been identified on the λ genome : OL1, OL2 and OL3, called the left operators and OR1, OR2 and OR3 termed the right operators. Both λ cI repressor protein and λ cro protein act as transcriptional regulators and bind as dimers to the cognate operator sites on the genome with the repressor showing selectivity for OL1 and OR1 operators and cro protein showing preference for OR3 operator. The bound repressor protein physically blocks the access of RNA polymerase to the promoter region corresponding to the early lytic genes, but giving access to the promoter region for its (repressor's) own gene. When cro protein is bound to the operator, it blocks the access of the polymerase to the promoter of the repressor gene. Thus lysis-lysogeny decision depends upon the ability of the polymerase to bind to one of the two promoters, which in turn is controlled by the preferential binding of the repressor and cro

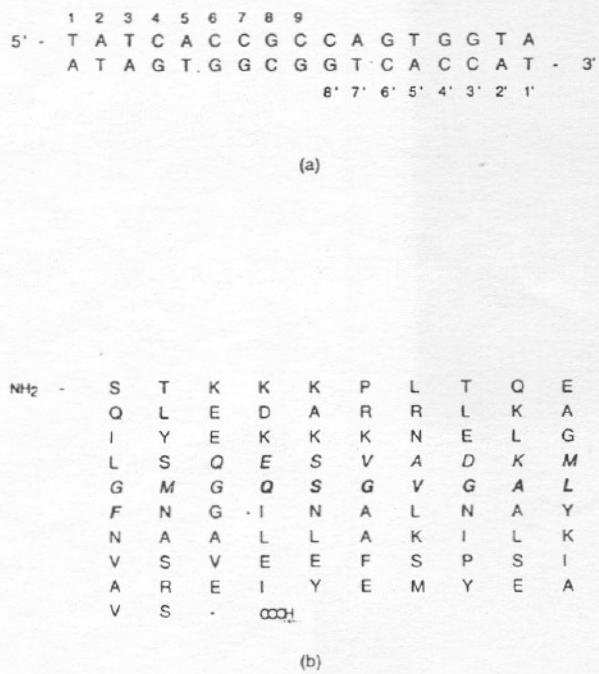

Figure 1:
(a). Base sequence of the λ OL1 operator (17 bp) taken from Jordan and Pabo [xx]. Left half of the operator (bases 1 to 9) is the consensus half (CHS) and the right half the non-consensus half (NCHS).
(b). Amino acid sequence of the N-terminal fragment of the λ cI repressor protein monomer. N-terminal arm consists of residues (1-8), helix-1 (9-23), helix-2 (33-39), helix-3 (44-51), helix-4 (61-69) and helix-5 (79-92).

proteins to the operators.

Structural studies on the repressor and cro proteins separately and in complex with DNA revealed several features of interest to recognition [22-27]. Both repressor and cro proteins contain a super-secondary structural unit called the hth (helix-turn-helix) motif in which the second helix, designated as the recognition helix, binds to the DNA in the major groove. Several interactions critical to recognition have been identifed in the crystal structures of the N-terminal fragment of the repressor complexed with OL1 operator [23,25]. The base sequence of the OL1 operator and the amino acid sequence of the N-terminal arm of a monomer of the repressor are shown in Figure 1. An extensive network of hydrogen bond interactions between the protein and the sugar-phosphate backbone and between the side chains and functional groups of the bases exposed in the major groove have been noted. In addition, some hydrophobic contacts are also implicated as significant to specificity. The picture is less clear with regard to those residues which contribute to specificity through van der Waals interactions.

Sequence analysis of the lambda genome (48,502 bp long; GenBank. Locus GB: LAMCG, ACCESSION J02459 M17233) undertaken with a view to identifying features which are common to all the six operator sites differentiating them from the remaining sites on the genome, has culminated in a proposal on symmetry driven extended consensus set {T1 A2 C4 A5 C6 G8 G4' T2'} [Jayaram and Beveridge, 1994, unpublished]. The base pairs identified in the above set are implicated in the structural studies as making important contacts with the repressor protein.

Computer modeling and theoretical studies based on solution or crystal structures of protein-DNA complexes can help delineate the nature and strength of intermolecular interactions, providing an energetic view point of biomolecular recognition and furthering an atomic level comprehension of specificity in binding. Such studies, in particular, can quantify the relative contributions of all protein subunits in unambiguous terms and enable a classification of the amino acid residues as important or unimportant for recognition complementing the atomic level proximity information provided by the structural studies. The results, although sensitive to the force fields employed, are becoming increasingly reliable with a constantly growing knowledge of the parametric dependence of the thermodynamic predictions and the availability of improved potential energy functions. These and other considerations have led us to assemble a force field for evaluating the energetics of DNA-ligand systems exclusively. The details of our attempts to provide an energy based rationale for the complexation of the λ repressor protein with the OL1 operator are given in the following.

Another point of interest in the context of protein-DNA interactions is the mechanism of association [28-31]. von Hippel and coworkers [29-31] interpreted the magnitude of the measured rate constants ($\sim 10^{10}$ $M^{-1}s^{-1}$) as providing a compelling evidence for a facilitated diffusion (translocation) of the protein towards the operator (active site). Brownian dynamics simulations can account for the rate constants of diffusion controlled reactions [32-36]. It was thus interesting to integrate our force field into a Brownian dynamics algorithm to monitor the rate and mechanism of association of the protein and the DNA.

Conclusions as emerging from our simulation work are also presented in the following.

Theory and Methodology

(a). Energetics

The interaction energy between the protein and the DNA is expressed as a sum of three terms - electrostatic (E_{el}), van der Waals (E_{vdw}) and a desolvation term (E_{hpb}) for the non-polar groups.

$$E_{pro\text{-}DNA} = \sum E_{el} + E_{vdw} + E_{hpb} \qquad 1$$

The summation runs over all the atoms of protein and DNA. The electrostatic contribution is evaluated as

$$E_{el} = \frac{q_i\, q_j}{D(r)\, r_{ij}} \qquad 2$$

where q_i are the partial atomic charges taken from the OPLS force field [37,38] and r_{ij} is the distance between the interacting particles. D(r) is a distance dependent dielectric function [16,21,39] given by

$$D(r) = D - [\,(\frac{D - D_i}{2})\,(\alpha^2 + 2\,\alpha + 2)\,e^{-\alpha}\,] \qquad 3$$

where D=78, D_i=4, α=sr and s=0.395. D(r) is a sigmoidal function and s has been calibrated previously on the base pairing energies reported by Newmark and Cantor [40] and further supported by finite difference Poisson-Boltzmann calculations [41]. The van der Waals term consists of a (12,6) Lennard-Jones potential function between the interacting atoms.

$$E_{vdw} = \frac{C_{12}^{ij}}{r_{ij}^{12}} - \frac{C_6^{ij}}{r_{ij}^{6}} \qquad 4$$

The C_6 and C_{12} parameters are evaluated from the van der Waals diameters (σ) and well depths (ε) as follows.

$$C_6^{ij} = \sqrt{C_6^i\, C_6^j}\;; \quad C_{12}^{ij} = \sqrt{C_{12}^i\, C_{12}^j}$$
$$C_6^i = 4\,\varepsilon_i\,\sigma_i^6\;; \quad C_{12}^j = 4\,\varepsilon_i\,\sigma_i^{12} \qquad 5$$

In view of the significance attached to the hydrophobic interactions involving the thymine methyl groups in the structural studies, it was deemed fit to estimate their contribution via an additional term (as adopted by Friedman and coworkers [42,43] in their Gurney parameter approach and as in the hydration shell model [44-46] of Scheraga and coworkers).

$$E_{hpb} = f_{ij} \times \frac{V_{excl}}{V_w} \quad \text{if } r_{ij} \geq (R_{Hi} + R_{Hj}) \; ; \; 0 \text{ otherwise} \qquad 6$$

Here f_{ij} are the free energy parameters [45] and V_{excl} is the volume of exclusion when the hydration sphere of a nonpolar atom on the protein encroaches upon the hydration sphere of a nonpolar atom on the DNA.

$$V_{excl} = \frac{r_{ij}^3}{12} - \frac{(R_{Hi}^2 + R_{Hj}^2)^2}{4\, r_{ij}} + \frac{2}{3}(R_{Hi}^3 + R_{Hj}^3) - \frac{r_{ij}}{2}(R_{Hi}^2 + R_{Hj}^2) \qquad 7$$

R_{Hi} and R_{Hj} are the hydration sphere radii of the atoms involved. These are evaluated as $R_{Hi}=a\,\sigma_i/2$ with a=0.769, chosen to give -1 kcal/mol for the overlap of the hydration spheres of two methyl groups [47]. $V_w=(4/3)r_w^3$ and r_w=1.575Å, the radius of a water molecule as represented by TIP4P water [48]. Thus equation (1) captures the electrostatic and van der Waals contributions to the interaction energy between protein and DNA along with the desolvation energy of the nonpolar groups with an implicit water model. The energy analysis of the crystal structure attempted here, is based on the above potential energy function.

(b). Kinetics

Brownian dynamics simulations were performed to monitor the kinetics of complexation. The Langevin equations for a system of N Brownons are [32,33]

$$m_i a_i = -\sum_i \zeta_{ij} v_j + F_i + \sum_i \alpha_{ij} f_j \qquad 8$$

where m_i is the mass of the i th particle, r_i its position, v_i its velocity and a_i its acceleration. The first term on the right hand side represents frictional force with ζ_{ij} giving the friction tensor. The second term F_i handles the systematic force on the i th particle arising due to interactions with all other particles in the system. This is evaluated from the specified potential function. The third term represents a randomly fluctuating force upon the particle due to the surrounding solvent. f_j obeys a Gaussian distribution. The propagation equation in configuration space is then given as

$$r(t + \Delta t) = r(t) + \frac{\Delta t}{kT} \sum_{i,j} D_i(t)\, F_{ij}(t) + R(D, \Delta t) \qquad 9$$

The time step Δt, must be greater than the momentum relaxation time (mD/kT) and yet small enough that ∇D and F are constant during this interval. D is determined as

where a is the hydrodynamic radius, η the coefficient of viscosity of the solvent. R(D, Δt) is a random displacement with a Gaussian distribution. R is calculated by generating normal random deviates

[{xi} : <xi>=0, <xixj> = 2 dij D Dt]

The procedure adopted above has been reported previously in the literature [33,35]. Also, our Brownian dynamics program has been tested and ensured to reproduce the simulation and analytical results given by Ermak and McCammon [33].

Calculations and Results

(a). Energetics

The coordinates for the N-terminal fragment of the λ repressor in complex with OL1 operator were taken from the Brookhaven protein data bank [49] as deposited by Pabo and coworkers [23,25]. The missing N-terminal arm of one of the monomers was constructed and minimized. Hydrogen atoms were added to the crystal structure and their geometry optimized [50]. The interaction energy between the protein and the DNA is calculated

Table I

Calculated interaction energy between protein and DNA (in kcal/mol) partitioned into contributions from (A) protein subunits and (B) DNA subunits.

	Ele.	vdW	hpb	Total
(A)				
N-ter. arms	-122.28	-67.45	-8.84	-199.17
h2th3	-7.97	-75.88	-7.43	-91.29
Other res.	-18.99	-15.48	-1.61	-36.07
Total	-149.83	-158.81	-17.88	-326.53
th3t	-23.68	-92.08	-8.75	-124.51
(B)				
Base	-38.26	-83.60	-5.54	-127.30
Sugar	+60.04	-49.64	-12.44	-2.04
Phosphate	-171.61	-25.58	0.0	-197.18
Total	-149.83	-158.82	-17.88	-326.52

using equation (1) and shown in Table I after partitioning on a subunit basis. Some notable features in Table I are the significant electrostatic contributions of the N-terminal arms and the phosphate backbone and the van der Waals contributions of the h_2th_3 motif (the th_3t has an even larger contribution) and the bases to the total energy. A tight fit of the recognition helices (residues: Q44 to F51) and their adjacent residues in the major groove of the DNA appears to be one of the tenets of recognition in this system. The energy analysis also led to a recovery of all the hydrogen bonds identified in the crystal structure as significant. An interesting feature of the energy analysis is a quantification of the contribution of the direct code (side chain-base interactions which was found to be about one fourth of the total interaction energy).

To establish a proper reference state, the desolvation energy of polar atoms must also be included in the energetics. Implicit water description tends to underestimate the energetics of solvation/desolvation relative to the electrostatic and van der Waals interactions computed on an atomic basis explicitly. To be consistent with the parameter set employed in the force field, a layer of explicit (TIP4P) waters was generated around the protein, the DNA and the complex, their orientation and location minimized and their interaction energy with the solute calculated. The sum of the interaction energies of the isolated protein and the DNA with waters is subtracted from the interaction energy of the complex with waters to obtain the desolvation energy. (The waters were generated randomly and using the SOAK option of INSIGHTII of BIOSYM [50]. The calculated desolvation energies with these two different methods for obtaining starting structures of water molecules, agree to within 0.6 kcal/mol of the complex. Such an agreement may be fortuitous. Some protocol issues such as the number of layers of water molecules, the status of condensed counterions during minimization, are under investigation.) The total interaction energies comprising electrostatic and van der Waals contributions as in equation (1), together with the desolvation energy estimated with explicit waters is reported in Table II. The desolvation term is positive and exceeds in magnitude both the electrostatic and the van der Waals terms individually. The entropic contribution originating in the release of water molecules from the surface of the protein and the DNA upon complexation is missing in these estimates although it would not be expected to dominate the energetics of highly polar solutes. It may be recalled that the energies quoted here refer to single point energies and no statistical mechanical averaging is involved. The major objective here is to arrive at a methodology to analyze the energetics of crystal structures to draw meaningful inferences on the issue of specificity in protein-DNA systems. Table II simply reiterates the point that any discussion on specificity must take into consideration, the favorable and significant contribution of the van der Waals forces to complexation.

Table II

Calculated interaction energy between the protein and the DNA (in kcal/mol).

Electrostatic	-149.83
van der Waals	-158.82
Total	-308.65
Desolvation	+263.05
Net interaction energy	-45.60

(b). Kinetics

A series of Brownian dynamics simulations were performed on simplified models for both

protein and DNA to focus on the following issues: (i) the role of intermolecular forces in protein-DNA association, (ii) the relative rates of specific and nonspecific association and (iii) the relative probabilities of a three dimensional search for the operator site versus search in a space of reduced dimensionality. The DNA was modeled as a cylinder of radius 10Å and height 57.8 Å, with a charge of -34 distributed along its axis to mimic a 17 base pair operator. The protein was modeled as a sphere and as a dumb-bell carrying a net charge of +2 corresponding to the repressor protein. Each trajectory was initiated at a distance of 65Å from the axis of the cylinder and truncated 200Å away or when the sphere contacted the cylinder. The time step was taken to be 0.01 ps after several trial runs with varying time steps were conducted and the calculated probabilities were ensured to remain fairly stable. The rate constant k was estimated via the following relation [35].

$$k = 4\pi DbP/\{1-(1-P)\gamma\}$$

where P is the probability of a hit and $\gamma = (b/\rho)$, b and ρ are the starting and truncation radii respectively.

A number of cases were considered as shown in Table III. Case I deals with the diffusion of an uncharged sphere of radius 18Å (computed by considering the surface area of the dimeric repressor protein and equating it to that of a sphere) to an uncharged cylinder. Case II models the diffusion of a sphere towards a cylinder in a potential well of the form [51]

$$E = A \ln r_{cs} + (B/r_{cs}^9)$$

Here r_{cs} is the distance of the center of the sphere from the axis of the cylinder. A and B were determined by calibrating the respective terms to get the same energies as obtained for the electrostatic and van der Waals terms using equation (1). Case III introduces a slightly better description for the protein (dumb-bell, a dimer of two spheres of radius 12.8Å instead of a single sphere) and the remaining as in Case I. Case IV considers the dumb-bell model for the protein with an external potential as in Case II except that the

Table III

Calculated encounter probabilities and association rate constants with different models for the repressor-operator complex.

	Probability	Rate constants (109 M-1 s-1)	Remarks
Case I	0.074	0.709	Simple diffusion of sphere
Case II	0.938	6.413	Diffusion of sphere with systematic
Case III	0.072	0.691	Simple diffusion of dumb-bell
Case IV (nonspecific 3D-diffusion)	0.905	6.258	Diffusion of dumb-bell with systematic forces
Case V (Specific 3D-diffusion)	0.154	1.423	Diffusion of dumb-bell with reactive patch
Case-VI (Specific 1D-diffusion)	0.585	13.359	Sliding of dumb-bell with reactive patch

energies are the sum of energies of the individual monomers. A and B for each monomer were recalibrated. Periodic boundary conditions were employed along the axial direction for cases I to IV. To bring specificity into picture and to model the operator (target) site on the DNA, a reactive patch was defined on both monomers by computing the spatial extent of the hth motifs from the cartesian coordinates of the protein and defining the same on the surface of both monomers. This is considered in Case V. The DNA was divided into two parts and each part was reactive to the extent of 180 degrees. The cylindrical DNA was extended to 200Å along the axis in each direction from the center with the middle 57.8Å containing the reactive patch. The simulation was carried out using rotations about the center of mass of the protein dimer due to torques in addition to translations. A trajectory was said to be successful if the reactive patches of both the monomers contacted the corresponding reactive areas on the DNA simultaneously. Case VI deals with a one dimensional sliding model. The simulation was started with the (dumb-bell) model dimeric protein 65Å away from the reactive patch and moved on the surface of the cylinder by computing the axial displacement and getting the final position by considering the fact that 3.4Å change axially corresponds to an angular variation of 36 degrees for canonical B-DNA. In fact the axial net attractive force if any was due to van der Waals forces only, as there was no axial component to the electrostatic force in the model studied. The average number of time steps in a trajectory was of the order of 10^6 and over 1000 separate trajectories were simulated in each case on a 486DX2 and SG Indigo workstation.

A comparison of the results (Table III) for Case I with Case II or Case III with Case IV clearly indicates the role intermolecular forces - notably the radial component of electrostatics - in the kinetics of complexation. Electrostatics assists nonspecific association in the λ system. {An all atom model of hth motif (net charge -1) was found to be extremely sluggish in its approach towards DNA than an all atom model of a monomer (net charge +1) - results not shown}. Also, it appears that the level of detail (sphere versus dumb-bell) does not seem to affect the kinetics significantly so long as the forces were made to correspond to the same all atom model. Finally, the joint probability of a nonspecific association followed by a one dimensional diffusion to the active site (a product of the encounter probabilities for Cases IV and VI) was computed to be 0.529. This is much larger than the probability associated with a three dimensional search for the active site (case V).

Discussion

The energetic analysis attempted in this study based on a protein-DNA force field, reveals several interesting features pertinent to recognition supplementing the structural knowledge. Seeman et al. proposed a mechanism very early on [52] for protein-DNA recognition based on the sequence specific hydrogen bonding donor and acceptor atoms in the major groove of DNA (called the direct code). Further studies led to the recognition of the role of structure in recognition (indirect code) [53-58]. Crystal studies enabled a verification of the existence of hydrogen bonds between a number of proteins and their cognate sequences of DNA but no code could be established. The rules of recognition remain unclear as yet. Presumably both direct and indirect codes contribute to specificity in some unknown ways. Proximity information alone as provided by the structural studies is obviously insufficient to judge the role of direct versus indirect codes. This provides the rationale for our studies on the energetic aspects of protein-DNA recognition.

The magnitude of the van der Waals contribution (which may loosely be categorized as judging the extent of steric fit/shape complementarity) of the recognition helix and its flanking residues with the bases in the major groove of DNA is significant and this is a hitherto unknown feature. An equally interesting aspect of the energetics is the contribution of the direct code (base-side chain interactions which includes electrostatic, van der Waals and hydrophobic contributions) which is only one fourth. The remaining three fourths is made up by the interactions of protein backbone with nucleic acid bases, nucleic acid backbone with protein side chains and also protein backbone with sugar phosphate backbone. Perhaps, it is this high level of noise which has impeded progress in deciphering such a code. It is equally probable that the protein fold (protein backbone interactions) and DNA conformation (DNA backbone interactions) are also an integral part of the recognition code.

An energetic analysis of the complexes of the repressor protein with all the six operators would probably give a better insight into the common factors for specific and non-specific association and highlight the features responsible for specific association. Preliminary studies with the consensus half sites of all the six operators did not yield any clue to selectivity [S. Dixit, M.Sc. Thesis, IIT, Delhi, 1995]. Further studies with both consensus and non-consensus half sites are in progress.

Rates exceeding 10^9 $M^{-1}s^{-1}$ are typical of diffusion controlled reactions [60]. Berg, Winter and von Hippel [29-31], based on a theoretical analysis of the measured association rate constants ($10^{10}M^{-1}s^{-1}$) for the lac repressor-operator binding, proposed a two step process which incorporates a facilitated diffusion of the protein to the target site. The results of our Brownian dynamics simulations on the λ system conform to their model.

Lastly, although several simplifying (reasonable) assumptions were made in arriving at the force field and in the simulation set up, the conclusions appear to be significant enough and seem to hold promise for a better understanding of the protein-DNA recognition at a molecular level. A systematic improvement of the force field and the models involved is on hand. Also, a cross sectional analysis of the protein-DNA complexes where structural data is available, is also in progress.

Conclusions

A computational approach to deciphering the elements and mechanism of recognition in protein-DNA systems has been described. Overall, while confirming the hydrogen bond interactions noted in the X-ray studies and highlighting the residue-wise relative contributions to the binding energy, the theoretical study here underscores van der Waals interactions of the turn-helix3-turn (tht) as an essential determinant in DNA binding. Lock and key hypothesis [61], proposed in 1894 by Emil Fischer, puts forth structural complementarity as a hall mark of biomolecular recognition and this appears to hold true even after hundred years, albeit in a modified form.

Investigations on the kinetics of association favor a two step mechanism for the recognition of the target site, in further support of the model proposed by von Hippel and cowork-

ers. A non-specific association of the protein and the DNA, followed by a facilitated diffusion of the protein to the operator region, in a reduced dimensional configuration space, is favored over a three dimensional diffusion of the protein to the operator site.

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