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Induced Fit and the Entropy of Structural Adaptation in the Complexation of CAP and λ-Repressor with Cognate DNA Sequences

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ABSTRACT Molecular dynamics (MD) simulations of 5 ns on protein-DNA complexes of catabolite-activator protein (CAP), λ-repressor, and their corresponding uncomplexed protein and DNA, are reported. These cases represent two extremes of DNA bending, with CAP DNA bent severely and the λ-operator nearly straight when complexed with protein. The calculations were performed using the *AMBER* suite of programs and the *parm94* force field, validated for these studies by good agreement with experimental nuclear magnetic resonance data on DNA. An explicit computational model of structural adaptation and computation of the quasiharmonic entropy of association were obtained from the MD. The results indicate that, with respect to canonical B-form DNA, the extreme bending of the DNA in the complex with CAP is ~60% protein-induced and 40% intrinsic to the sequence-dependent structure of the free oligomer. The DNA in the complex is an energetically strained form, and the MD results are consistent with a conformational-capture mechanism. The calculated quasiharmonic entropy change accounts for the entropy difference between the two cases. The calculated entropy was decomposed into contributions from protein adaptation, DNA adaptation, and protein-DNA structural correlations. The origin of the entropy difference between CAP and λ-repressor complexation arises more from the additional protein adaptation in the case of λ, than to DNA bending and entropy contribution from DNA bending. The entropy arising from protein DNA cross-correlations, a contribution not previously discussed, is surprisingly large.

INTRODUCTION

Understanding the nature of binding affinities, and the specificities of the complexes between regulatory proteins and control regions of genomic DNA in terms of molecular structure and interactions, is essential for the total understanding of gene expression at the molecular level. Considerable thermodynamic binding data is available and a number of x-ray co-crystal structures of regulatory protein-DNA complexes have been obtained. Binding constants of regulatory protein-DNA interactions are typically in the range of 10^9–10^{12} M^{-1}, and the ratio of specific/nonspecific binding is in the range of 10^3–10^7. The basic structural motifs involved in protein-DNA binding such as helix-turn-helix, leucine zipper, etc., have been elucidated (Jones et al., 1999). However, the results also indicate that a given structural motif is utilized in significantly different ways in different complexes, and that there are few regularities in the data that could lead to a quantitative account of affinities and specificities. As a further complication, protein-DNA recognition does not occur via simple lock and key mechanism, and considerable structural adaptation may be involved in the protein, the DNA, or both, upon complexation.

The contribution of structural adaptation to binding thermodynamics is typically difficult, if not impossible, to isolate directly in an experiment. However, the importance of this contribution has been inferred in comparison studies (Spolar and Record, 1994) and from theoretical estimates (Duan et al., 1996). Investigation of structural adaptation requires not only the structure of the complex, but also the corresponding uncomplexed forms of protein and DNA free in solution. These structures, particularly for the DNA component, are not usually available. With the present generation of computing power and advances in simulation methodology, it has become possible to obtain accurate computational models of a protein DNA complex and corresponding uncomplexed protein and DNA in solution using molecular dynamics (MD) simulation. The structural adaptation process can be studied directly in an MD on the uncomplexed protein or DNA beginning at the structure observed in the complex. In addition, the entropy contribution to protein-DNA complexation originating in the changes in vibrational and configurational quasiharmonic degrees of freedom (key components in the thermodynamics of binding) can be calculated from the MD trajectories for the complex and the protein and DNA components.

We focus in this study on two protein DNA complexes: the catabolite-activator protein (CAP) DNA complex, in which the DNA makes a right turn (Schultz et al., 1991)—i.e., the DNA sequence is bent by nearly 90°—and the λ-repressor/operator (Beamer and Pabo, 1992), in which the DNA remains relatively straight. A comparative study of these two systems is well suited to the investigation of structural adaptation and induced fit. There is the added issue of balance between enthalpy and entropy that leads both these complexes to form with similar binding free-energy

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changes whereby the binding of CAP to cognate DNA is entropy-controlled, and the λ-repressor/operator binding is enthalpy-controlled (Jen-Jacobson et al., 2000). An explanation proposed for this result is that CAP requires additional enthalpy for bending the DNA in order to arrive at a viable binding free energy for a regulatory process; CAP DNA then binds with an increased flexibility and an increase in entropy. However, the origin of the increased flexibility on CAP DNA complexation is not obvious, since there is typically a rigidification of structure on complex formation.

MD simulations were performed on the protein DNA complex and uncomplexed forms of protein and DNA, all in solution including counterions and water explicitly, and each beginning with the corresponding structures in the crystallographic complexes. The quasiharmonic entropy for each species was calculated using Schlitter's method (Schlitter, 1993) adapted for macromolecules and examined critically in light of the idea of increased flexibility on CAP DNA complex formation. The results of this study contribute to an improved understanding of the relationship of structure to functional thermodynamics, and exemplify how subtle but significant questions about protein DNA recognition at the molecular level can be addressed with a combination of experimental data and computational modeling of complex systems using MD simulation.

BACKGROUND

The study of protein DNA complexation at the molecular level has been advanced dramatically in recent years with reports of now over 200 protein DNA crystal structures (Berman et al., 2002). However, understanding the thermodynamics of protein DNA binding in terms of molecular structures obtained from crystallography and increasingly, nuclear magnetic resonance (NMR) spectroscopy remains a formidable challenge due to the multiplicity of effects involved. The two systems under consideration in this study, the prokaryotic regulatory CAP complexed with the DNA 30-mer, d(GCGAAAAGTGTGACATATGTCACACTTTTCG), and the λ-repressor complexed with the 20-mer operator, d(AATACCACTGGCGGTGATAT), provide a case study of the difficulties involved and also what can be accomplished on the basis of experimental structural data complemented with computational models of dynamical structures of complexed and free forms of protein and DNA using MD simulation.

CAP (also known as *cyclic adenosine monophosphate receptor protein*, i.e., cAMP, *catabolite gene-activator protein*; *catabolite-activator protein*; *catabolite-repressor protein*; and *cAMP-responsive element binding protein*) is one of the most extensively studied regulatory proteins. CAP has been implicated in both upregulation and downregulation of the expression of hundreds of target genes in bacterial cells. CAP acts in response to the level of cAMP, an indicator of carbon source availability that converts CAP from a nonspecific DNA binder to a specific one (Busby and Ebright, 1999). The CAP protein is a 45-kDa symmetric homodimer, with each dimer consisting of a 139-residue N-terminal domain for binding cAMP and subunit interactions for dimerization, and a 70-residue C-terminal domain containing the helix-turn-helix motif for binding DNA. Upon cAMP uptake, CAP undergoes a conformational change that optimally spaces the recognition helices for interaction with the major groove of DNA, conferring characteristic specificity for binding to its target DNA sequences.

There are presently nine relevant crystallographic studies of CAP DNA complexes (Benoff et al., 2002; Chen et al., 2001a,b; Lawson et al., 2004; Parkinson et al., 1996; Passner and Steitz, 1997; Schultz et al., 1991). In the original CAP-DNA structure by Steitz and co-workers, residues of the recognition helix interact both with phosphates and the major groove side of the bases (Schultz et al., 1991). The 90° bend in the DNA in the CAP-DNA crystal structure originates from the two ~45° bends at the second TG step of each of the TGTGA recognition sequences (Schultz et al., 1991). The highly bent structure for CAP-DNA was anticipated in the analysis of gel electrophoresis experiments (Liu-Johnson et al., 1986) and electrostatic calculations (Warwicker et al., 1987). Subsequent crystal structures (Passner and Steitz, 1997) and solution state topological (Lutter et al., 1996) and spectroscopic studies (Kapanidis et al., 2001) on CAP-DNA show some variation in the overall bend angle, but the DNA is highly bent in all cases. Important questions, from the perspective of molecular biophysics, are how much of the DNA bending in the complex is protein-induced? And how much is intrinsic to sequence-dependent structure of the uncomplexed DNA?

The λ-repressor/operator complex is implicated in the choice between the lysogenic and lytic phases in the development cycle of bacteriophage λ and is a participant in the prototype case of a genetic switch (Ptashne, 1986). The crystallography of this and other protein-DNA complexes with the helix-turn-helix recognition motif has been reviewed by Harrison and Aggarwal (1990). In the crystal structure of the λ-repressor/operator complex, the N-terminal domain of the repressor protein is bound to the OL1 operator (Beamer and Pabo, 1992). Here the DNA sequence is essentially straight and B-form. The major groove of the operator DNA makes direct contacts with the so-called *recognition helix* of the helix-turn-helix motif in the λ-repressor, and the basic amino-acid sequence (STKKKP) at the N-terminal end of the protein forms a flexible arm that makes contacts into the minor groove of the DNA at the center of the operator site. Eliason et al. (1985) and Clarke et al. (1991) have shown that deleting or mutating these six residues of the arm greatly affect binding affinity of the repressor protein for the operator site.

Previous MD studies of the λ-repressor/operator complex are due to Kombo et al. (2002, 2001). The thermodynamic study of protein-DNA complexes has a very extensive

literature. Protein DNA binding involves a diverse range of noncovalent associations and solvent contributions. An enumeration and description of the various components contributing to protein DNA binding has been provided by Jayaram et al. (1999, 2002) among others (Jen-Jacobson, 1997; Reyes and Kollman, 2000). Assuming additivity (Dill, 1997), the free energy of binding can be expressed as a sum of direct enthalpic and entropic interactions between the protein and DNA, and solvent-mediated contributions including the hydrophobic effect and the polyelectrolyte effect (Carra and Privalov, 1997; Jen-Jacobson, 1997; Merabet and Ackers, 1995; Saecker and Record, 2002; Spolar and Record, 1994; von Hippel, 1994). Theoretical analysis reveals that the relatively modest free energy of binding required for the on/off rates for regulatory protein-DNA complexes to function as molecular switches arises as the result of positive and negative contributions of much larger magnitude from various enthalpic and entropic components (Jayaram et al., 1999, 2002). This phenomena is referred to in the earlier literature as *hidden thermodynamics*, with respect to observed results (Gao et al., 1989; Lachenmann et al., 2002). Moreover, entropy is a collective property of the system, which makes it difficult to clearly discern the effect of various components to the net change in free energy, although such an understanding is significant for developing insights into the complex thermodynamics involved in molecular recognition processes (Boresch and Karplus, 1995; Brady and Sharp, 1997; Mark and van Gunsteren, 1994).

Specific contributions relevant to this study are as follows: Spolar and Record (1994) discovered that negative heat capacity and positive entropy changes are thermodynamic signatures of protein DNA complexation in which considerable structural adaptation is involved, and they advanced the case that it is important to consider induced fit in the protein DNA recognition processes at the molecular level. Subsequently, Jen-Jacobson et al. (2000) examined 10 cases of protein DNA complexation for which both thermodynamic (standard free energies, enthalpies, and entropies) and crystal structures of the corresponding complexes were available. Processes in which the protein DNA complexes with relatively undistorted, essentially straight DNA turned out to be enthalpy-driven, whereas the formation of complexes in which the DNA was distorted due to ligand induced bending were entropy-driven. An idea pursued in the interpretation of results is that recognition processes that strongly distort the DNA have net endothermic enthalpies of complexation, which must be counterbalanced by an increase in entropy to obtain the free energy of complexation optimum for recognition.

One of the possible implications of this observed positive entropy change is that complexation accompanied by bending is achieved by more disorder in the system than in cases where no DNA bending is involved. However, this is not easily susceptible to direct experimental measurement, although efforts have been made to study this behavior in NMR experiments (Bruschweiler, 2003; Dyson and Wright, 2002; Kay, 1998; Stone, 2001; Wand, 2001). Advances in computational modeling make it possible to test this hypothesis, based on inspections of MD trajectories of the protein DNA complexes and the corresponding uncomplexed protein and DNA, and to quantitate matters further via the calculation of the quasiharmonic component to the entropy of binding—a key quantity that directly reflects the structural adaptation of protein and DNA. In general, it is of interest to inquire whether the entropy difference between protein DNA complexes originates in an increased disorder in the complex or in an increased order in the uncomplexed DNA and/or protein, testing the idea that the observed difference in thermodynamics is dominated by the DNA component of the problem.

Molecular dynamics (MD) simulations have the advantage of providing the evolution of structure with time and can serve to bridge the gap between dynamical structure and functional thermodynamics. All-atom MD simulations has been established as a useful tool to understand the internal motions and resultant conformational changes in a range of molecular systems of biological interest (Hansson et al., 2002; Karplus and McCammon, 2002). On the nanosecond-timescale of simulations currently being performed on large biomolecules, MD simulation captures the vibrational motions, punctuated by transitions between multiple local minima and changes in configurational orientations (Berendsen and Hayward, 2000). The general background for the MD simulations on DNA and studies related to DNA hydration, the ion atmosphere of DNA, DNA bending, and protein-DNA complexes, has been reviewed recently (Beveridge et al., 2004b, and the references therein). Independent perspectives are available in review articles by Cheatham and Kollman (2000), Giudice and Lavery (2002), Norberg and Nilsson (2002), Orozco et al. (2003), and most recently by Cheatham (2004).

The oligonucleotide d(CGCGAATTCGCG) has served as prototype for studies of the ability of nucleic-acid force fields to reproduce experimental structural results for a specific oligonucleotide in a realistic representation of the solvent environment. These results have been described in a series of articles reporting MD trajectories based on the *AMBER* suite of programs and ranging now from 5 to 60 ns (Ponomarev et al., 2004; Young et al., 1997a,b). Calculated time-correlation functions and relaxation times for the internal structural parameters of the DNA are found to be in the range of ~500 ps; and thus, sampling at the level of 10× the relaxation times of properties of interest (Haile, 1992), 5-ns MD trajectories should be sufficient for the purposes of this study. Arthanari et al. (2003) have reported a detailed comparison of calculated and observed results on DNA in solution, comparing MD results on the d(CGCGAATTCGCG) duplex at in vivo ionic strength with both two-dimensional NOESY spectra and RDC structures from NMR spectroscopy. Further studies (McConnell and Beveridge, 2001)

indicate that MD provides an accurate description and plausible model of the dynamical structure of A-tracts in DNA oligonucleotides. In a key NMR structure determination of d(GGCAAAAAACGG) (MacDonald et al., 2001) in which the results differs from that found in the crystal (Nelson et al., 1987), MD beginning at NMR structure, canonical B-form, or the crystal structure supports the NMR solution structure, and provides an indication that the crystal structure is subject to packing effects (Dixit et al., 2004). The contributions of MD simulation to the problem of DNA bending and curvature have been recently reviewed (Beveridge et al., 2004a). The MD results support a model with essentially straight A-tracts and local deformations at pyrimidine-purine YpR basepair steps in both intrinsic curvature and ligand-induced DNA bending.

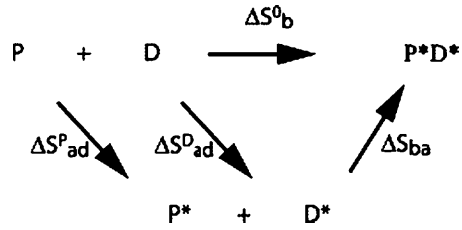
There are several instances of MD simulations on protein-DNA and protein-RNA complexes reported in the literature from this laboratory (Kombo et al., 2001; Pitici et al., 2002) and elsewhere (for a recent review, see Zakrzewska and Lavery, 1999). A singular feature of the results to date is the extent to which the MD of the complex exhibits a reduced range of dynamical motion. The atoms in the complex oscillate with a fairly narrow window of thermal dispersion around the starting structure, which in all studies to date is a crystal form. A primary focus in this project is on the entropy of association in protein DNA complexation, which in an additive model consists of contributions from changes in translational, rotational, vibrational, and conformational motions as well as release of ions and water on complexation. The vibrational and conformational entropies are typically computed together as the quasiharmonic (QH) contribution, which is obtained from the determinant of the cross-correlation matrix of atomic motions for a molecule (Karplus and Kushick, 1981). This quantity has been previously considered in MD studies of the Eco RI endonuclease protein DNA complex by Duan et al. (1996), and the cross-correlation matrix, per se, has served as a basis for the study of correlated domain motions in protein structures (Harte et al., 1990; Hunenberger et al., 1995) and allosteric effects in the SH2 and SH3 domains responsible for the activation of Src kinases (Young et al., 2001).

METHODS AND CALCULATIONS

Our MD simulations of the CAP-DNA system are based on the protein DNA complex structure solved at 2.5 Å resolution by Parkinson et al. (1996). Independent simulations were carried out on the complex and unbound form of the protein and DNA structures derived from the structure of the complex. The nick in the original CAP-DNA complex co-crystal due to missing phosphate groups in the backbone of each of the two DNA strands was patched by modeling to form continuous single strands. The two cAMP molecules bound to CAP in the original crystal structure (Parkinson et al., 1996), one bound to each monomer of CAP, were maintained in the simulation. MD simulations on the λ-repressor-OL1 operator complex were started from the co-crystal structure solved at 1.8 Å resolution by Beamer and Pabo (1992). The missing N-terminal arm of the λ-repressor protein for the non-consensus half-site of this complex was modeled on the basis of the details of the arm in the consensus half-site that has been resolved in the crystallographic study.

All simulations were carried out using the *AMBER* (Case et al., 1997) suite of programs and the *parm94* force field developed by Cornell et al. (1995). The atomic charges for the cAMP molecule presented in Fig. 1 were derived using the RESP fitting procedure (Cieplak et al., 1995), compatible with the rest of the *AMBER* force field. The system was solvated in a box of TIP3P (Jorgensen et al., 1983) water molecules extending to a minimum distance of 12 Å from all DNA and protein atoms. Na^+ and Cl^- ions were randomly placed in the simulation box to provide an ionic strength of ~120 mM. Minimization and subsequent molecular dynamics of the protein-DNA complex, ions, and water were performed using the protocol described by Kombo et al. (2001). A 9 Å cutoff was employed along with the particle-mesh Ewald method (Darden et al., 1993; Essmann et al., 1995) to treat long-range electrostatics. Constant pressure of 1 atm and temperature of 300 K was maintained using the Berendsen coupling scheme (Berendsen et al., 1984) and the SHAKE procedure (Ryckaert et al., 1977) was used to hold all the covalent bonds involving hydrogen atoms rigid. The 2-fs time-step was used for the simulation and the results presented here are based on trajectories 4-ns long for the λ-repressor-operator and 5 ns for the CAP-DNA system. Fig. 2 shows the convergence profile of the calculated quasi-harmonic entropy for the protein, DNA, and their complexes. The estimated entropy is >99% converged in the simulation lengths employed here. Principal component analysis of the displacement in the essential dynamical modes indicates that the available trajectory is fairly well converged.

The entropy calculations carried out are referenced to the binding thermocycle (Scheme 1),



where *P* is protein, *D* is DNA, and *P*D** is the protein-DNA complex. The subscripts *ad* and *ba* refer to adaptation and binding of adapted forms, respectively. The asterisk in *P*D** denotes that both protein and DNA in the complex are in structurally altered forms compared to the uncomplexed

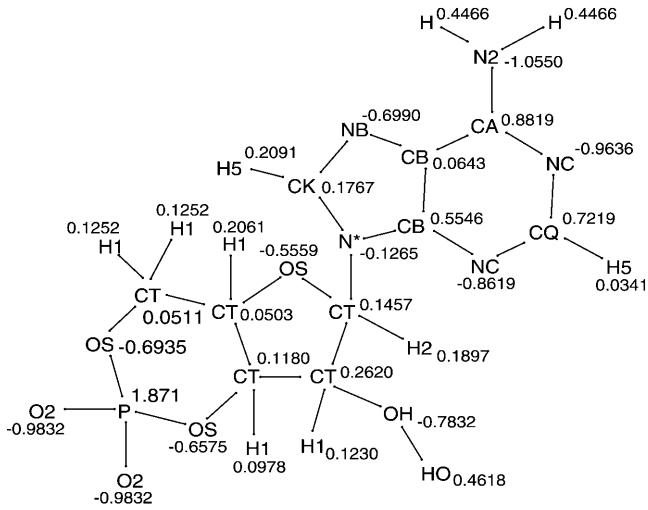


FIGURE 1 *AMBER parm94*-compatible atom types and the partial atomic charges derived using the RESP procedure for the cyclic AMP molecule.

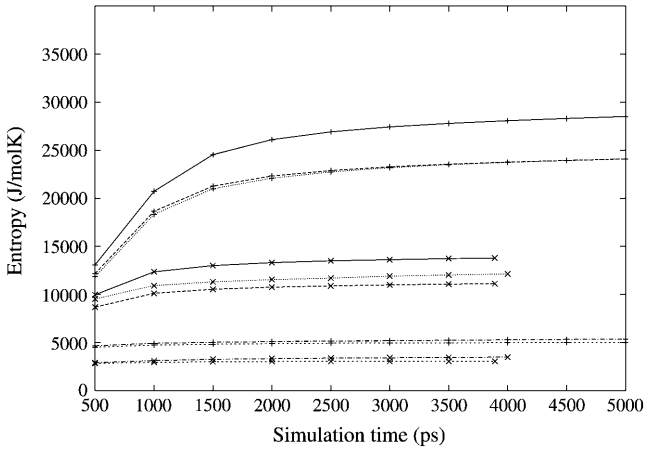


FIGURE 2 Convergence profile of the estimated configurational entropy based on the quasiharmonic analysis of molecular dynamics trajectories. The data for the CAP-DNA (5 ns) and λ-repressor-operator system (4 ns) is marked with plus and cross symbols, respectively. The plot presents the convergence of the configurational entropy of the complex, the protein, and DNA in the trajectory of the complex and the configurational entropy of unbound forms of the constituent protein and DNA.

protein and DNA as a consequence of induced fit. All species are presumed to be in solution phase. Thus the configurational entropy calculations presented here are based on simulations of the protein and DNA in the free and bound forms, and is computationally less demanding in comparison to the more rigorous free energy and entropy calculations (Beveridge and DiCapua, 1989; Peter et al., 2004).

Assuming additivity, the entropy of association can be partitioned into five terms,

$$\Delta S^0_{\text{assoc}} = \Delta S^0_{\text{H}\phi} + \Delta S^0_{\text{ions}} + \Delta S^0_{\text{rt}} + \Delta S^0_{\text{vib}} + \Delta S^0_{\text{conf}}, \quad (1)$$

where $\Delta S^0_{\text{assoc}}$ is the standard entropy of binding, and the terms$\Delta S^0_{\text{H}\phi}$ and ΔS^0_{ions} capture the increase in entropy from water and ion reorganization, i.e., the hydrophobic and polyelectrolyte effects, respectively; ΔS^0_{rt} is the entropy decrease due to the reduction in translational and rotational degrees of freedom on complex formation; ΔS^0_{vib} is the entropy due to the population of new low frequency vibrational modes created in the complex; and ΔS^0_{conf} is the configurational entropy change arising from structural changes in the protein and the DNA on complex formation. The contributions from solvent reorganization, $\Delta S^0_{\text{H}\phi}$ and ΔS^0_{ions}, are typically positive since solvent and ion release favor complexation, whereas ΔS^0_{rt} is negative and opposes complexation.

The crucial difference when structural adaptation is involved comes in terms ΔS^0_{vib} and ΔS^0_{conf}, which are computed together from MD trajectories as the quasiharmonic entropy ΔS^0_{QH} (Karplus et al., 1987), $\Delta S^0_{\text{QH}} = \Delta S^0_{\text{vib}} + \Delta S^0_{\text{conf}}$.

It follows from the postulated thermocycle that

$$\Delta S^0_{\text{QH}}(P + D \rightarrow P^*D^*) = \Delta S^0_{\text{QH}}(P^*D^*) - \Delta S^0_{\text{QH}}(P) - \Delta S^0_{\text{QH}}(D). \quad (2)$$

The quantity ΔS^0_{QH} can be resolved as

$$\Delta S^0_{\text{QH}}(P + D \rightarrow P^*D^*) = \Delta S^0_{\text{QH}}(P \rightarrow P^*) + \Delta S^0_{\text{QH}}(D \rightarrow D^*) + \Delta S^0_{\text{QH}}(P^* + D^* \rightarrow P^*D^*), \quad (3)$$

where $\Delta S^0_{\text{QH}}(P \rightarrow P^*) = S(P^*) - S(P)$ and $\Delta S^0_{\text{QH}}(D \rightarrow D^*) = S(D^*) - S(D)$.

The absolute entropies $S(P)$ and $S(D)$ are obtained from the MD trajectories of the uncomplexed P and D. The quantities $S(P^*)$ and $S(D^*)$ can be obtained from the trajectories of the protein and DNA in the complex. Thus the quasiharmonic entropy change can be resolved into contributions from protein adaptation, DNA adaptation, and protein DNA intermolecular correlations and provides us a means to approach one of the main questions posed in this study.

The calculation of the quasiharmonic entropies from MD trajectories are carried out using the heuristic approach developed by Schlitter (1993), which is based on the entropy equation of a one-dimensional quantum mechanical harmonic oscillator. Extending the equation to a system with many degrees of freedom, the entropy definition based on covariance matrix of atomic positional fluctuations of the Cartesian coordinates of all the particles in the system is given as

$$S = \frac{1}{2} k_{\text{B}} \ln \det \left[1 + \frac{k_{\text{B}} T e^2}{\hbar^2} M\sigma \right], \quad (4)$$

where k_{B} is the Boltzmann constant, $\hbar = h/2\pi$, h is Planck's constant, T is the temperature, e is the Euler value, and M is a mass matrix of rank $3N$ in which the diagonal elements hold the masses, and off-diagonal elements have value zero. The value σ is the covariance matrix of the atomic positional fluctuations with elements

$$\sigma = \langle (x_{\text{i}} - \langle x_{\text{i}} \rangle)(x_{\text{j}} - \langle x_{\text{j}} \rangle) \rangle. \quad (5)$$

The approximation yields an upper limit of the classical entropy, and is useful since it overcomes numerical instabilities that could occur due to singularity of the covariance matrix in a Cartesian coordinate system. Recently, Schafer et al. (2000, 2002) have verified the applicability of this procedure for peptides and small protein-folding problems. A further refinement of the quasiharmonic approach to entropy calculation has been provided in the work of Andricioaei and Karplus (2001).

Before calculating the configurational entropy, the structures in the trajectory are spatially superposed to a common reference structure to exclude all rotational and translational motions exhibited by the molecule in the MD trajectory. The covariance matrix calculated from the positional fluctuations of all the degrees of freedom in individual snapshots in the trajectory provides the lead to the entropy. The application of this covariance matrix calculation for all the atoms of a large system such as the CAP-DNA complex, which has 8382 atoms (25,146 degrees of freedom) is a memory-intensive computational task. Instead, we analyze the fluctuations of the residue center of mass as a reduced representation of the system. This approximation also avoids the estimation of effects from the motions of atoms within a residue, which may be expected to be on a timescale different from the important large-scale structural changes that would more greatly contribute to the entropy.

RESULTS AND DISCUSSION

Overlays of the time-resolved structures from the MD trajectories of the protein-DNA complexes and the corresponding unbound forms of the protein and DNA are presented for CAP-DNA and λ-repressor-operator complexes in Figs. 3 and 4, respectively. In each case the MD structures have been fit to a reference form to minimize the root mean-square deviations. The dispersion of structures in the various images reflects the range of the dynamics observed in the simulations of the protein, DNA, and complex in solution. Visual examination of the structures of the unbound and bound form leads to some ideas about the dynamics of the system. In both cases there appears to be a net decrease in the ordering of the DNA on binding. With respect to the protein, the CAP appears more disordered in

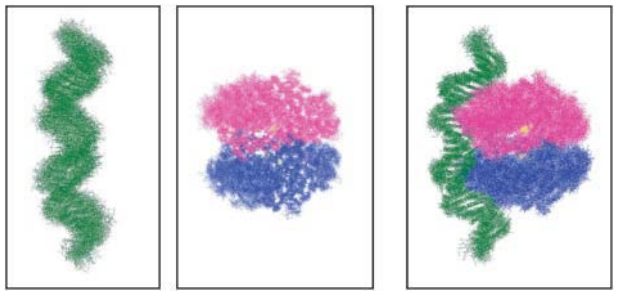


FIGURE 3 Overlay of superimposed structures from the MD simulation of the unbound form of the CAP binding site (*left*), CAP (*middle*), and the CAP-DNA complex (*right*), respectively. Figures prepared using MOLMOL (Koradi et al., 1996).

the complex (Fig. 3), whereas the λ-repressor protein (Fig. 4) appears to be more ordered in the complex. However, these ideas, based on inspection, need to be backed up by calculations (see below).

The structural adaptation of the protein and DNA components can be studied directly with MD by following the time course of simulations of the free protein and DNA components, beginning with the structures obtained from crystallography of the complexes and allowing the structures to relax to the forms free in solution predicted by MD. The structural adaptation of the DNA bound to CAP with reference to the unbound form of DNA is represented by the bundle of helix axes for the ensemble of DNA structures in the MD simulation (Fig. 5), calculated by the program *Curves* (Lavery and Sklenar, 1996). The direction and extent of helix axis curvature in the DNA bound to CAP is shown, overlaid with the axis curvature observed in the simulation of the unbound form of DNA. The DNA bound to CAP forms a tight bundle, demonstrating considerable immobilization of the structures in comparison to the unbound form. The helical axis of the DNA in bound form clearly exhibits curvature originating near the two TpG kink points, similar to that observed in the original crystal structure. Interestingly, MD simulation of the unbound form of DNA also shows significant bending at the TpG positions, implying pre-organization of the structure for the ligand-induced bending by CAP. The calculated curvature in the uncomplexed form is in the range of 25–40° compared to 60–80° for the complex, indicating that ~40% of the curvature in the structure of the complex is intrinsic, and that 60% is induced by the protein.

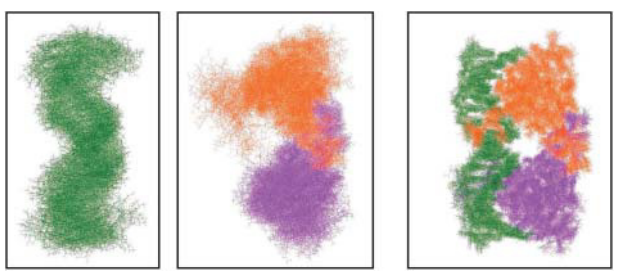


FIGURE 4 Overlay of superimposed structures from the MD simulation of the unbound OL1 operator DNA (*left*), λ-repressor protein (*middle*), and the λ-repressor-operator complex (*right*), respectively.

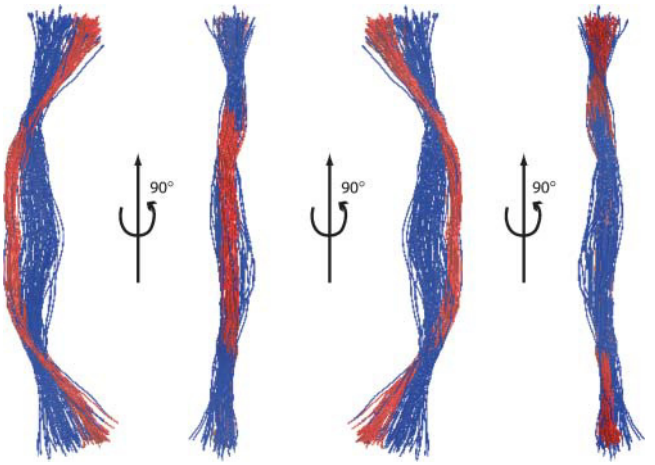


FIGURE 5 Direction of DNA axis curvature as observed in the MD simulation of DNA bound to CAP (*red*) and the free form of DNA (*blue*). The image presents four different views of the same set of structures.

The DNA axis in the co-crystal structure with CAP (Schultz et al., 1991) is not planar, but exhibits an effective dihedral angle close to 35° due to out-of-plane bending between the two ends of the DNA, as shown in Fig. 6. The dihedral angle observed during the simulation of the free and protein-bound DNA is shown in Fig. 7. Simulations of the free DNA in solution exhibit large flopping motions of this axial dihedral angle, averaging ~0° with the fluctuations ranging from +80 to −80°. On the other hand, the DNA in the complex exhibits significant localization of dihedral twist with the average ~38° in quantitative agreement with the original co-crystal structure. The observed fluctuations in the bound form are in the range of 80–0°, significantly reduced in comparison to the trajectory of the unbound DNA. Since the free DNA relaxes to a significantly different structural form in MD beginning with the DNA structure in the complex, this indicates that the structure of the DNA in the complex is an unstable, energetically strained form and not a stable or metastable substate. However, the dynamic range of the free DNA at its outer limit encompasses the structure of the protein-bound form, indicating that conformational capture phenomena (Leulliot and Varani, 2001) may be involved—in which the protein selects out, and stabilizes, complex-adapted conformable structures of the nucleic acid.

The case of λ has been discussed previously (Kombo et al., 2000), in which it was pointed out that the N-terminal arm of the repressor undergoes significant conformational change and rigidification upon complexation. The extent of this is evident from a comparison of the free and bound forms of the MD structures for λ-repressor in Fig. 4. The quasiharmonic entropy calculations allow us to quantify the extent to which

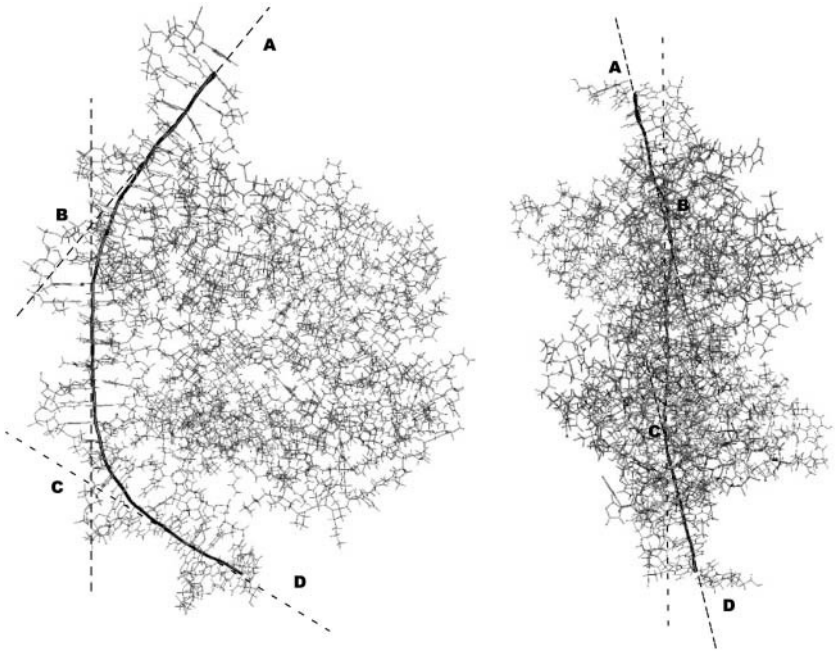


FIGURE 6 The original crystallographic structure of the CAP-DNA complex (1CGP) with the helix axis shown with a dark solid line. The ~35° out-of-plane bend in the helical axis is presented by the dihedral angle between the sections *A*,*B*,*C* and *B*,*C*,*D* of the DNA.

these structural changes in the MD models of both CAP and λ-systems translate into the quantitative thermodynamics. The convergence profiles of the entropy calculations are shown in Fig. 2 and the calculated quasiharmonic entropy values are listed in Table 1. The resulting values are $\Delta S_{QH} = -1140$ J/mol K for the CAP-DNA complex and $\Delta S_{QH} = -1749$ J/mol K for the λ-repressor-operator complex. These quantities are both negative, which indicates that a net ordering of the structure in the complex dominates the quasiharmonic entropy change in the case of both CAP and λ. However, the ΔS_{QH} for the λ-repressor-operator complex is considerably more negative than for CAP, supporting the interpretation of Jen-Jacobson et al. (2000) that the CAP complex is ''sloppier'' than the λ-repressor-operator complex.

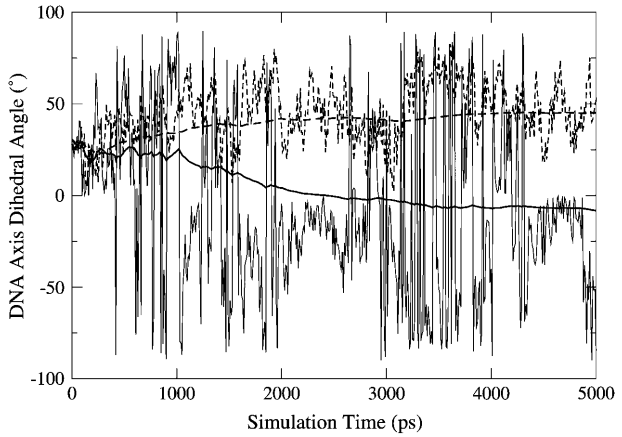


FIGURE 7 Time series of the out-of-plane bend in the DNA helical axis in the 5-ns MD simulation of DNA bound to CAP (*dotted line*) and the unbound form (*solid line*). The running average of the out-of-plane bend dihedral angle is shown for the two cases as thick lines.

We now turn to the relative contribution of protein and DNA to the quasiharmonic entropy, and investigate the nature of the difference between ΔS_{QH} for CAP and λ-complexation. The relevant quantities are $\Delta S^0_{QH}(P+D \rightarrow P^*D^*)$, $\Delta S^0_{QH}(P \rightarrow P^*)$, $\Delta S^0_{QH}(D \rightarrow D^*)$, and $\Delta S^0_{QH}(P^*+D^* \rightarrow P^*D^*)$ as defined above, and the calculated values are listed in Table 1. The contribution to the quasiharmonic entropy change for CAP protein adaptation is positive, an increase in the disorder that is consistent with the visual examination of the dynamics of the free and bound forms of CAP noted earlier in this article. The contribution from DNA adaptation is net-negative, i.e., increased order in the adapted form relative to the unbound form, also consistent with visual inspection of the dynamical structures from MD in Fig. 3. The net contribution from correlated motions that arise when the structurally adapted forms of CAP and cognate DNA are bound is likewise net-negative, as any form of protein-DNA correlation would have a net-ordering effect on the system. The magnitude of this term, with a $-T\Delta S$ of 57 kcal/mol, is surprisingly large. This contribution does not appear to be recognized previously in the protein DNA literature. The MD results indicate that ΔS_{QH} is not dominated by DNA, but arises due to a balance of terms of significant magnitude. For λ-repressor-operator complex the contributions from the protein, DNA, and the complexation are all negative. This reflects the idea that the λ-repressor protein exhibits net-ordering on complexation, a likely consequence of the immobilization of the N-terminal arm at the protein-DNA interface. The $\Delta S(D \rightarrow D^*)$ for the DNA complexed to λ-repressor protein is slightly lower than the cognate DNA in the CAP-DNA complex. The $\Delta S_{QH}(P^*$

TABLE 1 Calculated configurational entropy values (in units of J/mol K)

		Complex	Protein		DNA		ΔS_{QH}	
		S_{QH}^{P*D**}	S_{QH}^{P*} †	S_{QH}^{P}	S_{QH}^{D*}	S_{QH}^{D}	$\Delta S_{QH}^{(P*D*-P*-D*)}$	$\Delta S_{QH}^{(P*D*-P-D)}$
CAP	No. of residues	460	398		62			
	S	27,869	23,631	23,464	5032	5545	−794	−1140
	S/residue	60.6	59.4	59.0	81.2	89.4	−1.73	−2.5
λ cI	No. of residues	222	184		38			
	S	13,736	11,077	12,030	3045	3455	−386	−1749
	S/residue	61.9	60.2	65.4	80.1	90.9	−1.74	−7.9

*Configurational entropy from the MD trajectory of protein-DNA complex.
†Configurational entropy of protein or DNA from the trajectories of their unbound form.

$+ D^* \rightarrow P^*D^*$) is less negative than CAP, at least in part because the λ-repressor-operator system has a lower number of residues, and hence fewer degrees of freedom are involved. We have recalculated all the quantities on a per-residue basis (See Table 1) and find the trends to be similar, so the difference in the number of degrees of freedom does not introduce an artifact.

The results listed in Table 1 indicate that the differential entropy change, $\Delta\Delta S_{QH}$ ($\lambda \rightarrow$CAP) = +609 J/mol K, is consistent with the idea that the CAP complexation is more disordered than for λ. However, the $\Delta\Delta S_{QH}$ ($D \rightarrow D^*$) and $\Delta\Delta S_{QH}$ ($P^* + D^* \rightarrow P^*D^*$) are both negative, and oppose in sign the overall entropy difference $\Delta\Delta S_{QH}$ ($\lambda \rightarrow$ CAP). The key factor that this favors is the increase in relative ordering of the protein on complexation, a negative ΔS_{QH} ($P \rightarrow P^*$) of λ exceeding that of CAP—being 184% of the $\Delta\Delta S_{QH}$ ($\lambda \rightarrow$ CAP) accounts for all of the differential entropy compared with −17% from the differential DNA bending. Thus we find from the MD that CAP complexation is indeed a relatively less-ordering process than that of λ, but the nature of this differential entropy change lies predominantly in the contribution from protein rather than cognate DNA, and originates in the increased rigidification of the N-terminal arm region of the λ-repressor upon complexation. The large change in structure of the DNA induced by the protein does not translate into a major thermodynamic contribution, which exemplifies the difficulty with intuiting thermodynamic behavior from observed structures.

Concluding, we have combined our calculation of the quasiharmonic entropy change with estimates from the other terms contributing to the total entropy of CAP and λ-complexation with DNA. Here we utilize estimates of the entropy due to water release based on the calculated loss in solvent-accessible nonpolar surface area (Karplus, 1997) and the entropy due to ion release from Record et al. (1978). Yu et al. (2001) have reported that the translational and rotational motions make negligible contribution to the free energy of binding in aqueous solution at 1 M standard state. The results are collected in Table 2. The calculated entropy of complexation for CAP is −636 J/mol*deg and for λ is −1278 J/mol*deg. The observed trend is well reproduced. However, the calculated value for CAP is negative whereas the corresponding experimental value is positive. This is not surprising, due to the uncertainty in the estimates of the various components of the overall entropy change from diverse sources. The calculated overall entropy arises as a resultant of terms of considerable magnitude, whereas the uncertainties are positive-additive, a problem that affects all estimates of thermodynamic variables under the assumption of additive components.

SUMMARY AND CONCLUSIONS

MD simulations have been performed for CAP and λ-repressor systems treating the complexes and the corresponding uncomplexed forms. The quasiharmonic entropy change consists of a combination of vibrational and configurational changes on complexation and indicates that the complex of DNA with λ-repressor protein forms with considerably more ordering than that of CAP-DNA complex. These results are consistent with the trend implied from the experimental data, which shows CAP binding is favored by entropy change relative to the λ-repressor-operator, which is enthalpy-driven. Analysis of the differential $\Delta\Delta S(\lambda \rightarrow$ CAP) shows that the origin of the entropy difference lies in the contribution from structural adaptation of the protein component in CAP versus λ-repressor, and specifically the increased ordering in the λ-repressor due to the relative immobilization of the protein in the protein-DNA complex. This suggests that the origin of the entropy control in CAP-DNA complexation versus enthalpy control in

TABLE 2 Estimated net entropy during protein-DNA complexation

	ΔASA^{np}	ΔS_{HE}^0 J/molK	ΔS_{ions}^0* J/molK	ΔS_{QH} J/molK	ΔS_{assoc}^0 J/molK
CAP	1154	404	100 (8†)	−1140	−636
λ	1202	421	50 (3.7‡)	−1749	−1278

$\Delta S_{assoc}^0 = \Delta S_{HE}^0 + \Delta S_{ions}^0 + \Delta S_{QH}^0$, where $\Delta S_{HE}^0 = \Delta ASA^{np} \times 25 \times 4.18/300$.
*Numbers in parentheses are the experimentally reported number of ions released upon complexation.
†Ebright et al. (1989).
‡Koblan and Ackers (1991).

λ-repressor-operator complex comes about by increased ordering of the λ-repressor protein on complexation, not from the DNA components. The MD simulation and calculated ΔS_{QH} for CAP indicates that although both systems undergo some degree of rigidification upon complexation, it is a relatively more flexible complex than that of the λ-repressor-operator, but as a consequence of the relative extent of local folding of the protein coupled to binding, and not so much from DNA bending. It is notable that this interpretation is impossible to obtain from a straightforward visual examination of the protein DNA crystal structure or even the dynamical structure of bound and unbound constituents obtained from MD. The problem with visual interpretation of the results is that it is not possible to reliably intuit the quantitative magnitudes of the various contributions and thus the balance of terms which gives rise to the net entropy changes. This information can be obtained from MD simulations, which thus serve to complement the experimental structural data in the interpretation of results.

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