Poisson-Boltzmann analysis of the λ repressor-operator interaction

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ABSTRACT A theoretical study of the ion atmosphere contribution to the binding free energy of the λ repressor-operator complex is presented. The finite-difference form of the Poisson-Boltzmann equation was solved to calculate the electrostatic interaction energy of the amino-terminal domain of the λ repressor with a 9 or 45 base pair oligonucleotide. Calculations were performed at various distances between repressor and operator as well as at different salt concentrations to determine ion atmosphere contributions to the total electrostatic interaction. Details in the distribution of charges on DNA and protein atoms had a strong influence on the calculated total interaction energies. In contrast, the calculated salt contributions are relatively insensitive to changes in the details of the charge distribution. The results indicate that the ion atmosphere contribution favors association at all protein-DNA distances studied. The theoretical number of ions released upon repressor-operator binding appears to be in reasonable agreement with experimental data.

1. INTRODUCTION

An understanding of the thermodynamic basis of the formation of protein-DNA complexes is of primary importance in determining the role of DNA-binding proteins in biological processes, like the control of gene expression. The formation of a protein-DNA complex is influenced by direct interactions between both molecules as well as indirect contributions, such as release of water molecules and ions bound to protein and DNA. In contrast to simple electrolytes, DNA is surrounded by a cloud of condensed counterions that cannot be removed by dilution (1, 2). The condensation of counterions is a consequence of the high linear charge density on DNA and forms the basis for the electrostatic stability of DNA in solution (1–3). However, the creation of an ion atmosphere is an entropically costly process. Therefore, replacing part of the ion cloud surrounding DNA by a protein is considered to make a significant entropic contribution to the binding to DNA (1). This contribution decreases with increasing salt concentration since the entropy change upon releasing bound counterions depends on the ratio of bulk salt concentration to the concentration in the condensed ion atmosphere. In the framework of the counterion condensation theory, the counterion diffusion potential RTln (c_b/c_{loc}) was introduced by Manning (1) as a thermodynamic driving force for ligand-DNA association (c_b is the bulk salt concentration and c_{loc} is the salt concentration in the condensed cloud). This model predicts a linear relationship between the protein-DNA binding constant and ln (c_b), which is indeed observed for protein-DNA complexes (4). A plot of the logarithm of experimentally determined association constants versus logarithm of the ion concentration gives a straight line with negative slope M. The slope of the curve is interpreted as a measure of the number of ionic contacts between DNA and protein or equivalently proportional to the number of ions replaced upon binding (3).

The counterion condensation theory, which is based on a simplified description of DNA as a line of negative charges, is remarkably successful in describing the basic behavior of ions around DNA. A more sophisticated approach, including the shape of the molecule and a realistic atomic charge distribution, is necessary to study the electrostatics of protein-DNA recognition in detail. Recently, the ion contribution to the association of the λ repressor with its operator was studied by Monte Carlo (MC) simulation, which included explicit sodium and chloride ions surrounding the complex (5). This study concluded that the ion atmosphere contribution favors association only at small distances (<15 Å) and opposes association at larger distances between repressor and DNA. Herein we present a comparative study using finite-difference solutions of the Poisson-Boltzmann equation (FDPB) to calculate electrostatic interaction energies as well as ionic contributions to the λ repressor-operator interaction. In comparison with MC-studies, the FDPB method has the advantage of treating the complex in atomic detail with partial charges on all atoms and including the effect of a dielectric boundary between complex and solvent. Ions are not treated explicitly but are distributed according to Boltzmann weighting of the electrostatic potential. Ion–ion correlations are therefore neglected in the Poisson-Boltzmann (PB) approach. It has, however, proven to be successful in predicting hydration energies of small organic molecules (6, 7) and also has been used to calculate electrostatic potentials and ion distributions around B-DNA (8) and DNA-binding proteins (9).

The λ repressor-operator complex is one of the most extensively studied protein-DNA complexes. The three-dimensional structure of the amino-terminal domain of the λ repressor in complex with the O_L1 operator is known to high resolution (10). The binding affinity as a function of salt concentration was determined experimentally for a number of operator sequences recognized

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by the λ repressor (11, 12) and can be compared with theoretical predictions. In this study, we are particularly interested in calculating the salt contribution at different distances between repressor and operator and in the sensitivity of the results to the details of the charge distribution as well as to the length of the operator-containing DNA.

2. METHODS

2.1 Calculation of electrostatic energies

The electrostatic interaction energy of the λ repressor and operator DNA was calculated by solving the finite-difference form of the PB equation implemented in the University of Houston Brownian dynamics program (13). The finite-difference scheme to solve the PB equation allows the calculation of the electrostatic energy of molecules of arbitrary shape. The interior of a molecule is treated as a region of low dielectric constant with charges assigned to the center of each atom. Surrounding water is treated as a high dielectric continuum, and the counterions and coions are distributed according to a Boltzmann weighting of the electrostatic potential in regions outside the molecule. Initially, a cubic grid is set up, and the molecule is mapped onto the center of the grid. The charge on each atom is distributed over the eight neighboring grid points according to a trilinear weighting function that preserves monopole and dipole moments of the charge distribution (13, 14). All grid points interior/exterior to the molecule were assigned a low/high dielectric constant ($\epsilon = 2.0/\epsilon = 78.0$). Grid points near the boundary between the interior and exterior were assigned intermediate values (15). The potential at the boundary of the grid was calculated analytically by treating each charged atom as a Debye-Hückel sphere. As an alternative, boundary potentials for smaller grids could be calculated from large grid calculations using the focusing technique (16). The size of the ions was accounted for by adding a Stern layer (2.5 Å, the radius of hydrated sodium ions) to the molecular surface of the molecules.

Details of the finite-difference method to calculate the electrostatic potential according to the linearized PB (LPB) or nonlinear PB (NLPB) equation are given in (17, 18). For the LPB equation, the electrostatic energy of the system is given by the sum over the fixed charges times the electrostatic potential (13):

$$E = \sum_{i=1}^{n} \frac{q_i \phi_i}{2} \qquad (1)$$

In case of the NLPB equation, the electrostatic energy can be calculated from

$$E = \int \left(\frac{q^f \phi}{2} - \frac{q^m \phi}{2} - \Delta\Pi \right) dv \qquad (2)$$

where q^f and q^m are the distribution of fixed and mobile (ionic) charges (19). The term $\Delta\Pi$ denotes the osmotic pressure contribution to the energy due to the nonuniform distribution of ions in the electric field. The integral in Eq. 2 is over all grid points after solving the finite-difference form of the PB equation (in contrast to Eq. 1, where the sum is taken only over those grid points with fixed charges). Electrostatic interaction energies between two molecules can be obtained by calculating the difference between the electrostatic energy of two molecules in a complex and the electrostatic energy of each isolated molecule. This corresponds to the change in energy of bringing the two molecules from infinite distance to the complexed state.

2.2 Model

Coordinates of the λ repressor-operator complex were taken from the Brookhaven Protein Data Bank as deposited by Jordan and Pabo (10). All calculations were performed using one λ repressor monomer complexed with DNA consisting of either 9 base pair (bp) (model 1, DNA-sequence: 5′-TATCACCGC) or 45 bp (model 2, DNA-sequence: 5′-A_{15}ATATCACCGCCAGTGT$_{15}$). The DNA-sequence for model 1 corresponds to the consensus half site of the O_L1 operator (20). In the case of model 2, the DNA operator sequence was extended to yield 45 bp by using the molecular modeling program QUANTA (21). To calculate electrostatic energies between repressor and operator at different distances, it was necessary to choose a sufficiently large grid. On the other hand, the spacing between grid points should be as small as possible to obtain accurate electrostatic energies (i.e., insensitive to changes in the grid spacing or the position of the complex in the grid). For model 1, we found a grid spacing of 0.75 Å and a 100^3 grid sufficient to calculate interaction energies that did not vary by >0.5 kcal/mol on moving the complex by about half a grid spacing or changing the grid spacing by about ±0.1 Å. However, we were particularly interested in the salt dependence of the interaction energies. Differences in interaction energies at different ion concentrations showed even smaller variations on changes in the grid parameters (<0.2 kcal · mol^{-1}). For model 2 (the λ repressor complexed with a 45-bp DNA), the structure was centered on a 80 × 170 × 80 grid with a spacing of 1.1 Å. The y-direction (170 grid points) was aligned with the helical axis of the DNA. The electrostatic potential calculated using this grid served to set up the boundaries for a 100^3 grid with a spacing of 0.75 Å containing the operator sequence and the λ repressor.

Two sets of charge distributions were examined. For the first set, charges of −1 were assigned to the phosphates on DNA and carboxyl groups of Glu and Asp residues in the protein. Lys and Arg residues were assigned a charge of +1 on the amino or guanidinium groups. The amino and carboxyl termini of the protein molecule carried charges of +1 and −1. Atomic radii were taken from the OPLS force field parameter set (22, 23). The second charge model used a more detailed distribution of charges on all polar atoms (including polar hydrogens) on DNA and protein using the OPLS force field.

2.3 Calculations at different distances

The complex was oriented on the grid such that the protein could be separated from the DNA in the x-direction of the grid without causing atomic overlap between the molecules at any distance. Note that this is possible for the monomeric complex but not for the λ repressor dimer (as it is deposited in the Brookhaven Protein Data Bank) because part of the protein wraps around DNA. The distance between protein and DNA was varied between 0 Å (corresponding to the coordinates as deposited in the Data Bank) and 27 Å. The maximum distance was limited by the condition that there should be a space of ≥5–10 Å between the grid boundary and protein or DNA molecules. Otherwise, the potential at the boundary cannot be accurately approximated by the Debye-Hückel equation. At each distance, the electrostatic interaction energy between protein and DNA was calculated. To obtain salt contributions at different distances, the interaction energy was calculated for two different salt concentrations. We choose 22 mM as low ion concentration to compare our calculations with MC simulations. This value corresponds to the zero added salt condition Jayaram et al. (5) used to keep electroneutrality during their MC simulation. The high salt concentration was 122 mM (corresponding to the highest salt concentration in the MC study). The reference state for all calculations was the complexed state (R = 0 Å). Therefore, the interaction energy difference (between low and high ion concentration) at each given repressor-operator distance was finally subtracted from the corresponding value at zero distance.

3. RESULTS

The electrostatic interaction energy of the λ repressor and operator was calculated for several distances in the range of 0–27 Å at two salt concentrations (22 and 122

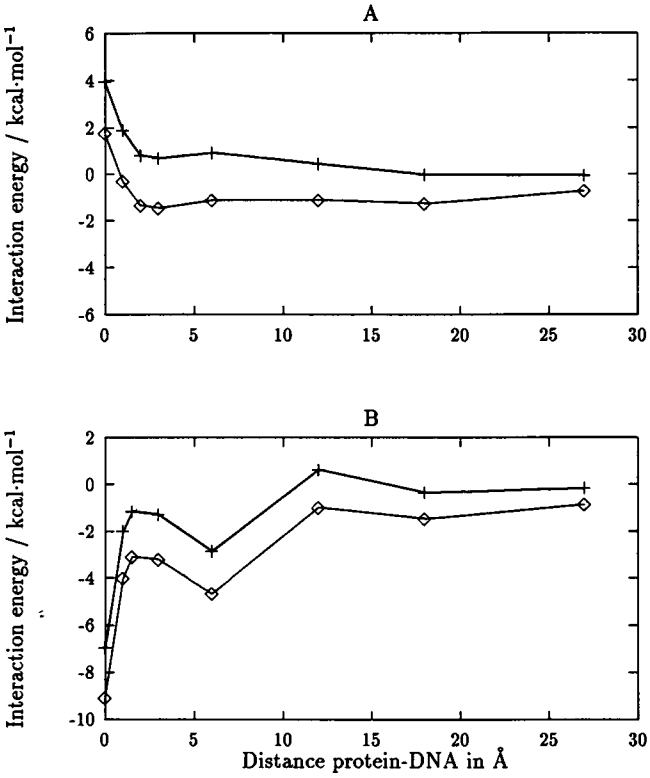

FIGURE 1 Calculated electrostatic interaction between λ repressor and operator as a function of distance (Å) for two salt concentrations (22 and 122 mM). The interaction energy at any given distance is the electrostatic energy of the protein-DNA complex minus electrostatic energy of the isolated components. (*A*) Simplified charge model; (*B*) detailed charge model (see text). The upper (+) and lower (◇) curves correspond to the high (122 mM) and low (22 mM) ion concentration, respectively.

mM). Throughout this study, electrostatic interaction energies were obtained by solving the NLPB equation. However, in the range of salt concentrations studied, the results did not differ significantly from electrostatic interaction energies obtained using the LPB equation. The difference was <0.3 kcal/mol.

Fig. 1 shows the results of the calculations using the NLPB equation for the two sets of charges (see Methods). The calculated interaction energies differ for the two charge sets at distances of <12 Å. Note that only the second more realistic charge set includes short range dipole–dipole interactions as well as electrostatic contributions to hydrogen bonds. This probably accounts for the negative interaction energy of the two molecules at short distances observed using the second set of charges. At distances beyond 15 Å, the interaction energies for both charge models became similar because the interaction is increasingly determined by the overall charge of the molecules.

Although the direction chosen to move the protein away from the DNA allows for separation without atomic overlap, certain atoms of the flexible NH_2-terminal λ "arm" can approach DNA atoms closely. The λ arm consists of the first six amino acids of the protein and forms the part of the repressor that wraps around DNA upon binding. To test whether the maximum in electrostatic interaction energy for the detailed charge model at a protein-DNA distance around 1–4 Å is influenced by this effect, the calculations were repeated with the λ arm slightly rotated to ensure a minimum distance of 3 Å between λ arm and DNA at any distance between the two molecules. It is obvious from Fig. 2 That the interaction energy maximum disappears in this case and the resulting curve is smoother. The electrostatic interaction at zero distance is larger. This indicates that wrapping the λ arm around DNA contributes to the stability of the complex. It is interesting that the calculations predict a minimum of the electrostatic energy at a distance of 4–6 Å between open form of the repressor and DNA. This could indicate that the open form is related to a repressor structure that binds nonspecifically to DNA and prefers to form a more loosely bound complex with DNA.

Fig. 3 shows the change in ion atmosphere contribution to the free energy of interaction as a function of the distance between repressor (with the arm in the wrapped state) and operator. A negative value at a given distance *R* signifies that this energy is released (at low ion concentration compared with high ion concentration) upon bringing protein and DNA to the complexed state ($R = 0$ Å). The calculated ion atmosphere contribution for the λ repressor-operator interaction was generally negative for all given distances, implying that it favors association. The results were similar for both charge models, deviating slightly in magnitude at small repressor-operator distances. At very large distances between protein and DNA, the interaction energy should become zero, implying that the ion atmosphere contribution, as it is defined in Methods, should reflect the difference at the reference state. In other words, the interaction energy difference between calculations at 22 and 122 mM salt

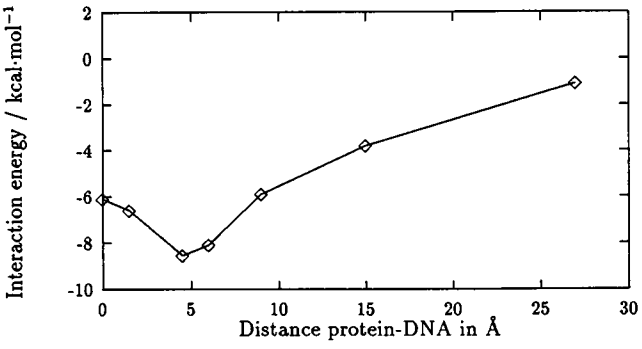

FIGURE 2 Distance dependence of the calculated electrostatic interaction energy between an "open" form of the λ repressor and operator. The open form differs from the λ repressor crystal structure by a slight rotation of the λ "arm" away from the DNA. With the repressor in this form, a minimum distance between λ arm and DNA of 3 Å is ensured in the course of protein-DNA separation. The detailed charge model and a salt concentration of 22 mM were used.

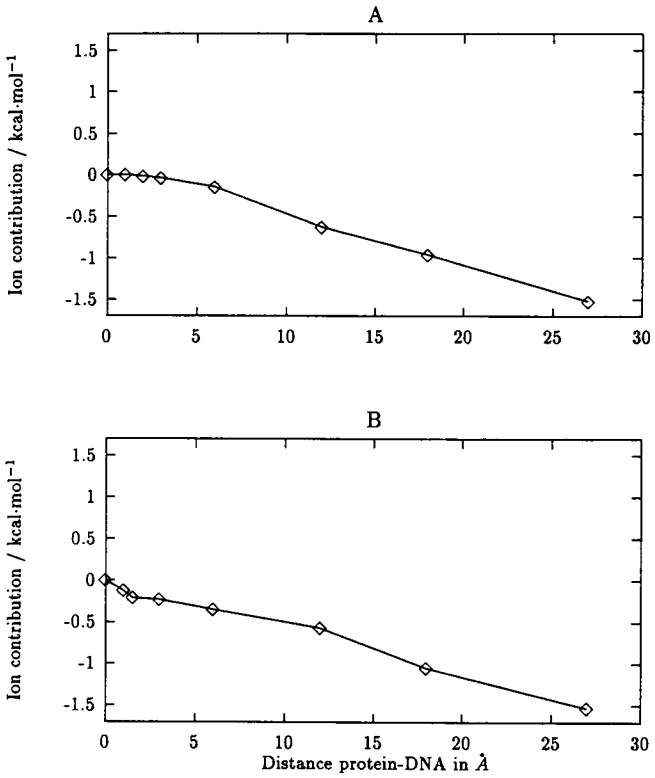

FIGURE 3 Calculated ion atmosphere contribution to the repressor-operator interaction at different distances (Å). The ion atmosphere contribution at a given distance R is the difference in interaction energy at low salt concentration versus high ion concentration of bringing protein and DNA to the complexed state (R = 0 Å). That is, the quantity shown is $[E_{22}(R = 0) - E_{22}(R)] - [E_{122}(R = 0) - E_{122}(R)]$, where $E_{22}(R)/E_{122}(R)$ is the electrostatic interaction at 22/122 mM and distance R. (A) Simplified set of charges; (B) detailed charge model.

and at R = 0 Å corresponds to the (relative) ion atmosphere contribution of bringing protein and DNA from infinite distance to the complexed state.

The electrostatic interaction energy between λ repressor and operator was calculated for several different ion concentrations (at R = 0 Å). Fig. 4 shows a plot of these interaction energies as a function of the logarithm of the ion concentration. For the simplified as well as the detailed charge model, a straight line was obtained. In case of using the detailed charge distribution, the slope M was −1.7, and for the simplified charge model, M = −2.4. As outlined in the Introduction, the slope of the plot in Fig. 4 (multiplied by −1) is a measure of the number of ions released during the protein association process. According to Record et al. (3), $-M$ is equal to the number of released ions, N, times their screening activity Ψ. For long chains, Ψ = 0.88, which is the sum of condensation (0.76) and diffusive screening (0.12) (3).

However, this screening parameter is certainly different for a small piece of DNA compared with a long chain, which complicates the estimation of the number of released ions. Record and Lohman (24) developed a semiempirical extension of the polyelectrolyte theory of DNA to treat oligonucleotides. They introduced an end-effect parameter reflecting the reduced association of counterions with the terminal regions of the oligonucleotide. The overall screening factor is then given by:

$$\Psi = 0.88 - 2.53/L,$$

where L is the number of base pairs. This translates to a screening factor Ψ = 0.6 in case of 9 bp, resulting in 4.0 released ions per λ repressor monomer for the simple charge set and 2.9 for the detailed charge set. However, the full validity of Eq. 3 was only proven for oligonucleotides containing 18 or more base pairs (24).

Therefore, the salt dependence of the protein–DNA interaction was also calculated for the λ repressor binding at the center of a longer piece of DNA (45 bp). In this case, end-effects play only a minor role in counterion condensation, and the polyelectrolyte limit is applicable (the screening parameter Ψ is close to 0.88 [24]). Again, a 100^3 box with 0.75 Å grid spacing surrounding the repressor-operator complex was used to calculate the interaction energy at zero distance. Since this grid is too small to embed the whole 45 bp of DNA, the focusing technique was used to calculate electrostatic potentials at

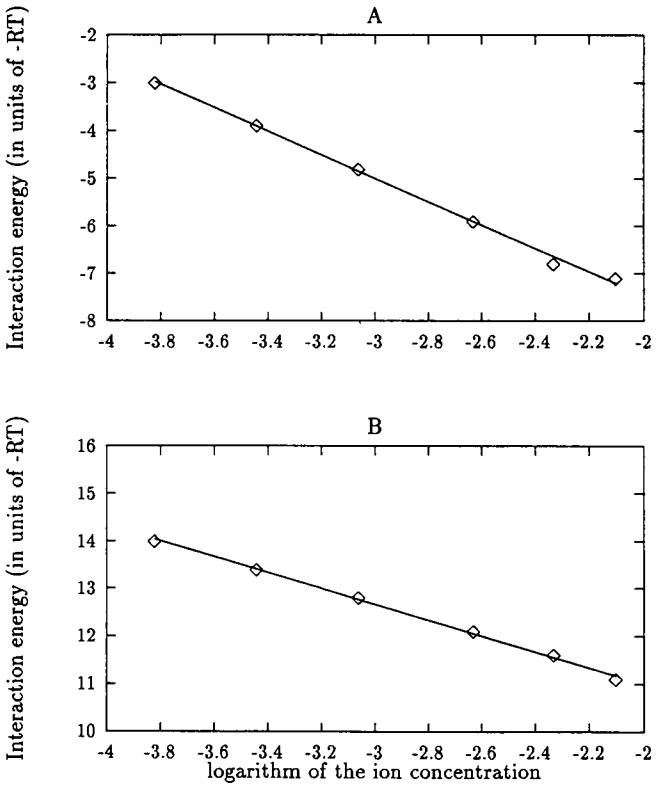

FIGURE 4 Dependence of the calculated interaction energies on the ion concentration. The electrostatic interaction energy of repressor and operator was calculated at various salt concentrations (in the complexed state with distance R = 0 Å). The interaction energy is given in units of $-RT$ ($E_I/(-RT)$, where I is the ion concentration) as a function of the logarithm of the ion concentration. (A) Simplified charge model; (B) detailed charge model.

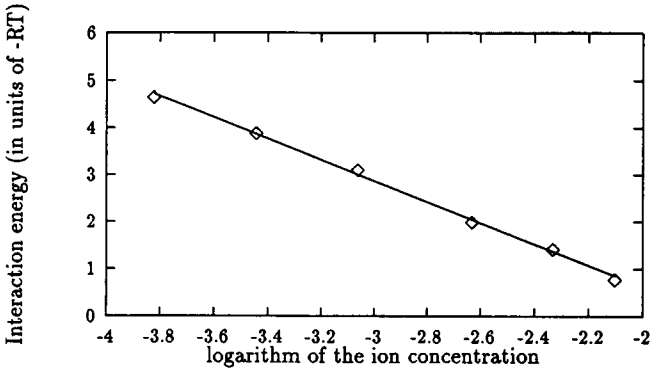

FIGURE 5 Calculated ion concentration dependence of the electrostatic interaction of the λ repressor in complex with the operator sequence in the center of a 45-bp oligonucleotide. The interaction energy was calculated at various salt concentrations and at zero distance between protein and DNA using the focusing technique as outlined in the text. For all calculations, the detailed charge set (see Methods) was used to assign atomic charges on DNA and protein. The calculated interaction energy is given in units of $-RT$ as a function of the logarithm of the salt concentration (see also Fig. 4).

the boundaries of the fine grid. The starting grid to calculate the potential at the boundaries comprised 80 grid points in both the x- and z-directions and 170 points in the y-direction with a grid spacing of 1.1 Å. The y-direction was aligned with the helical axis of the DNA. The result for a set of calculations at different ion concentrations is shown in Fig. 5. Again, the plot of interaction energy versus logarithm of the ion concentration resulted in a straight line. From the slope M we can obtain the number of released ions per bound repressor molecule to be 2.55 (Ψ taken to be 0.88). For the dimeric repressor-operator complex, this would imply the release of 5.1 ions upon binding. The experimentally determined number of released ions upon binding of the dimeric λ repressor was reported to be 4.8 in case of the O_R1 operator (11) and between 2 and 3 in the case of the O_L1 operator (10). Both O_R1 and O_L1 contain the same operator sequence we used in our calculations. It should be noted that a direct comparison of the slopes obtained experimentally and theoretically is also possible. Taking a screening factor of 0.88 into account allows a direct comparison of reported number of ions released with our calculations.

4. SUMMARY AND DISCUSSION

The distance dependence and salt dependence of the electrostatic interaction between the λ repressor and operator were calculated using the PB equation. The electrostatic interaction energy at different distances depends strongly on the details of the charge distribution on DNA and protein. An attractive electrostatic interaction at any distance was only obtained with a detailed charge distribution, including polar hydrogens. The sensitivity of the calculated electrostatic interaction on the details of the charge distribution indicates that the PB approach also may be useful to study how the λ repressor discriminates between different DNA recognition sequences. Calculations on a repressor-operator complex with a slightly rotated λ arm showed that the process of approaching the DNA recognition sequence by the repressor in the open form is not only favored sterically but also electrostatically. In contrast to the distance dependence of the interaction energy, the salt contribution to the electrostatic interaction did not change significantly on changing the charge model. For two DNA molecules of different length, the predicted number of ions released upon repressor-operator binding agreed reasonably well with experimental data.

This is an important result, supporting the validity of the PB approach to calculate electrostatic interactions between biological macromolecules. Similar studies on other protein-DNA complexes should provide insight on whether salt contributions are, in general, predictable by the PB approach.

In addition, the calculated number of released ions showed only a slight dependence on the details of the charge distribution on the molecules. This finding may indicate that simplified charge models for DNA are valid for some applications and further justifies their success in predicting ion distributions around DNA and ion contributions to ligand binding (1, 2).

Jayaram et al. (5) observed an ion contribution to the λ repressor-operator interaction opposing complexation at distances beyond 12 Å using MC simulations, including explicit ions. In our calculations solving the PB equation, the ion contribution was negative at all distances between DNA and protein. It is important to note that all our calculations on the distance dependence of the salt contribution were done on the repressor interacting with a short piece of DNA without periodic boundary conditions in the direction of the helical axis of DNA. In contrast to our calculations, a cell model with a constant number of ions in the cell was used in the MC simulation. Although the ion distribution around DNA calculated by a PB approach is in reasonable agreement with MC-studies, including explicit ions (8, 25), the PB approach, as already noted in the Introduction, neglects ion–ion correlation effects. These might be reasons for the discrepancy. Additional calculations on the distance dependence of the salt contribution of the λ repressor interacting with longer chains of DNA are necessary to further investigate differences between these studies.

Besides the calculation of salt contributions to the λ repressor-operator interaction, this study showed that binding of a possible open form of the λ repressor has an optimum in electrostatic interaction not at zero distance, as in the "closed" form, but at a protein-DNA distance ~4–6 Å. Being aware of the above mentioned limitations of the PB approach, this result suggests the following possible mechanism of repressor-operator binding.

The repressor in the open form is loosely bound to DNA at a distance of ~5 Å. To reach the complexed

state, the repressor has to "climb" uphill in electrostatic energy (see Fig. 2). At zero distance, the λ arm can wrap around the operator and further stabilize the complex (the system would reach the electrostatic energy given in Fig. 1 *B* at $R = 0$ Å). In case of a sequence different from the recognition sequence, the necessary complementary hydrogen bonds between protein and DNA cannot be fully formed. This would imply that the energy at zero distance increases further so that the system has to overcome a higher barrier to reach the complexed state with the λ arm wrapped around the DNA. In addition, the complexed state is of course also destabilized by the "wrong" recognition sequence.

Although it is obvious (for steric reasons) that the λ repressor must approach the DNA target sequence in an open form, our particular choice for the open form is arbitrary. Therefore, it is necessary to confirm the scenario discussed above using other forms of "open" repressor structures.

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REFERENCES

1. Manning, G. S. 1978. The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Q. Rev. Biophys.* 11:179–246.
2. Le Bret, M., and B. Zimm. 1984. Distribution of counterions around a cylindrical polyelectrolyte and Manning's condensation theory. *Biopolymers.* 23:287–312.
3. Record, M. T., C. F. Anderson, and T. M. Lohman. 1978. Thermodynamic analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the role of ion association or release, screening, and ion effects on water activity. *Q. Rev. Biophys.* 11:103–178.
4. Mossing, M. C., and M. T. Record. 1985. Thermodynamic origins of specificity in the *lac* repressor-operator interaction. *J. Mol. Biol.* 186:295–305.
5. Jayaram, B., F. M. DiCapua, and D. L. Beveridge. 1991. A theoretical study of polyelectrolyte effects in protein-DNA interactions: Monte Carlo free energy simulations on the ion atmosphere contribution to the thermodynamics of λ repressor-operator complex formation. *J. Am. Chem. Soc.* 113:5211–5215.
6. Jean-Charles, A., A. Nicholls, K. Sharp, B. Honig, A. Tempzyck, T. Hendrikson, and W. C. Still. 1991. Electrostatic contributions to solvation energies: comparison of free energy pertubation and continuum calculations. *J. Am. Chem. Soc.* 113:1454–1455.
7. Mohan, V., M. E. Davis, J. A. McCammon, and B. M. Pettitt. 1992. Continuum model calculations of solvation free energies: accurate evaluation of electrostatic contributions. *J. Phys. Chem.* 96:6428–6431.
8. Jarayam, B., K. A. Sharp, and B. Honig. 1989. The electrostatic potential of B-DNA. *Biopolymers.* 28:975–993.
9. Warwicker, J., D. Ollis, F. M. Richards, and T. A. Steitz. 1985. Electrostatic field of the large fragment of *Escherichia coli* DNA polymerase I. *J. Mol. Biol.* 186:645–649.
10. Jordan, S. R., and C. O. Pabo. 1988. Structure of the lambda complex at 2.5 Å resolution: details of the repressor-operator interactions. *Science (Wash. DC).* 242:893–899.
11. Nelson, H. C. M., and R. T. Sauer. 1985. Lambda repressor mutations that increase the affinity and specificity of operator binding. *Cell.* 42:549–558.
12. Senear, D. F., and R. Batey. 1991. Comparison of operator-specific and nonspecific DNA binding of the λ cI repressor: [KCl] and pH effects. *Biochemistry.* 30:6677–6688.
13. Davis, M. E., J. D. Madura, B. A. Luty, and J. A. McCammon. 1991. Electrostatics and diffusion of molecules in solution: simulations with the University of Houston Brownian dynamics program. *Comp. Phys. Comm.* 62:187–198.
14. Edmonds, D. T., N. K. Rogers, and M. J. E. Sternberg. 1984. Regular representation of irregular charge distributions: applications to the electrostatic potentials of globular proteins. *Mol. Phys.* 52:1487–1494.
15. Davis, M. E., and J. A. McCammon. 1991. Dielectric boundary smoothing in finite difference solutions of the Poisson equation: an approach to improve accuracy and convergence. *J. Comp. Chem.* 12:909–912.
16. Gilson, M. K., K. A. Sharp, and B. Honig. 1987. Calculating the electrostatic potential of molecules in solution: method and error assessment. *J. Comp. Chem.* 9:327–335.
17. Davis, M. E., and J. A. McCammon. 1989. Solving the finite difference linearized Poisson-Boltzmann equation: a comparison of relaxation and conjugate gradient methods. *J. Comp. Chem.* 10:386–391.
18. Luty, B. A., M. E. Davis, and J. A. McCammon. 1992. Solving the finite-difference non-linear Poisson-Boltzmann equation. *J. Comp. Chem.* In press.
19. Sharp, K. A., and B. Honig. 1990. Calculating total electrostatic energies with the nonlinear Poisson-Boltzmann equation. *J. Chem. Phys.* 94:7684–7692.
20. Clark, N. D., L. J. Beamer, H. R. Goldberg, C. Berkower, and C. O. Pabo. 1991. The DNA binding arm of λ repressor: critical contacts from a flexible region. *Science (Wash. DC).* 254:267–270.
21. QUANTA. Molecular modelling Package. Polygen Corporation, Waltham, MA.
22. Jorgensen, W. L., and J. Triado-Rives. 1988. The OPLS potential functions for proteins. Energy minimizations for crystals of cyclic peptides and crambin. *J. Am. Chem. Soc.* 110:1657–1666.
23. Pranata, J., S. G. Wierschke, and W. L. Jorgensen. 1991. OPLS potential functions for nucleotide bases. Relative association constants of hydrogen-bonded base pairs in chloroform. *J. Am. Chem. Soc.* 113:2810–2819.
24. Record, M. T., and T. M. Lohman. 1978. A semiempirical extension of polyelectrolyte theory to the treatment of oligoelectrolytes: application to oligonucleotide helix-coil transitions. *Biopolymers.* 17:159–166.
25. Jayaram, B., S. Swaminathan, D. L. Beveridge, K. Sharp, and B. Honig. 1990. Monte Carlo simulation studies on the structure of the counterion atmosphere of B-DNA. Variations on the primitive dielectric model. *Macromolecules.* 23:3156–3165.