A negative electrostatic determinant mediates the association between the *Escherichia coli trp* repressor and its operator DNA

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Abstract

The electrostatic potential surfaces were characterized for *trp* repressor models that bind to DNA with sequence specificity, without specificity, and not at all. Comparisons among the surfaces were used to isolate protein surface features likely to be important in DNA binding. Models that differ in protein conformation and tryptophan-analogue binding consistently showed positive potential associated with the protein surfaces that interact with the DNA major groove. However, negative potential is associated with the *trp* repressor surface that contacts the DNA minor groove. This negative potential is significantly neutralized in the protein conformation that is bound to DNA. Positive potential is also associated with the tryptophan binding-site surface, a consequence of the tryptophan- or tryptophan analogue-induced allosteric change. This protein region is complementary to the strongest negative potential associated with the DNA phosphate backbone and is also present in the isolated protein structure from the protein–DNA complex. The effects of charge–change mutation, pH dependence, and salt dependence on the electrostatic potential surfaces were also examined with regard to their effects on protein–DNA binding constants. A consistent model is formed that defines a role for long-range electrostatics early in the protein–DNA association process and complements previous structural, molecular association, and mutagenesis studies.

Keywords: electrostatic potential; helix–turn–helix; pH; protein–DNA binding; protein mutants; pseudorepressor; super-repressor

The *Escherichia coli trp* repressor is a ligand-mediated DNA regulatory protein comprised of 2 identical subunits (Kinemage 1). Sequence-specific *trp* repressor binding to its operator DNA precludes RNA polymerase binding to the overlapping promoter region (Rose et al., 1973; Shimizu et al., 1973; Rose & Yanofsky, 1974; Squires et al., 1975), which inhibits transcription of the *aroH* (Brown, 1968; Pittard et al., 1969), *trpABCDE* (Morse & Yanofsky, 1969), and *trpR* (Gunsalus & Yanofsky, 1980) genes responsible for intracellular tryptophan biosynthesis (Yanofsky, 1971). The aporepressor binds 2 molecules of L-tryptophan prior to sequence-specific association to operator DNA. In the absence

of tryptophan, the protein binds without sequence specificity to DNA. Because tryptophan is the “switch” that distinguishes between sequence-specific and nonspecific protein–DNA recognition, its structural and energetic effects on the protein structure may provide valuable insight to understanding the mechanics of protein–DNA association.

The tryptophan-induced conformational change alters the shape of the protein–DNA contact surface, which changes the local steric and electrostatic interactions. Although global shape complementarity is necessary for sequence-specific DNA binding, it alone is not sufficient for either sequence-specific or non-specific DNA binding. Some tryptophan analogues bind more tightly to the aporepressor than does tryptophan, induce the conformational change, and yet these form protein–ligand complexes or *pseudorepressors* that cannot bind to DNA (Marmorstein et al., 1987; Lawson & Sigler, 1988; Marmorstein & Sigler, 1989). For example, the tryptophan analogue, indole-3-propionate (IPA), lacks the positively charged amino group found on tryptophan and binds to the protein using a different binding mode than tryptophan (Lawson & Sigler, 1988; Borden et al., 1991). Lawson and co-workers proposed that the IPA binding mode

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Abbreviations: Repressor variants are labeled according to the altered amino acid: E13K, glutamate replaced by lysine at position 13; E18K, glutamate replaced by lysine at position 18; D46N, aspartate replaced by asparagine at position 46; E49K, glutamate replaced by lysine at position 49; A77V, alanine replaced by valine at position 77; IPA, indole-3-propionate.

would interfere with pseudorepressor-DNA binding due to steric and electrostatic clashes between the IPA carboxylate and a DNA backbone phosphate. Local steric and electrostatic complementarity surrounding the tryptophan binding site are important recognition factors, particularly because the tryptophan ligand contacts DNA in the protein-DNA complex (Otwinoski et al., 1988).

Tryptophan and its analogues may also affect the long-range electrostatic interactions involved in protein-DNA association. Because *trp* repressor and DNA both carry a relatively large net negative charge, the net interaction between the 2 molecules should be repulsive rather than attractive. The *trp* repressor, which carries a -6 net charge at neutral pH, does interact with DNA, indicating that the protein charge distribution is critical for DNA binding. Experimentally, when the overall protein charge is made more negative on the protein-DNA binding surface, as occurs with the pseudorepressors, the protein loses affinity for DNA. In contrast, the E13K, E18K, D46N, and E49K mutants, or *super-repressors*, increase the net positive charge on the protein-DNA binding surface by 2-4 units and enhance DNA binding affinities by factors of 2-10 in vitro relative to the wild-type *trp* repressor (Hurlburt & Yanofsky, 1990).

The electrostatic potential at a point on the surface of a molecule is the energy a unit charge experiences at that particular site. The electrostatic forces on the unit charge are a function of the molecular charge distribution and are very large for highly charged biomolecules. Consequently, macromolecules may interact through a distance before their complementary surfaces contact, which may influence how they associate. In this current work, the electrostatic potential surfaces of the *trp* repressor crystal structures and model-built super-repressor mutants are used to describe how changes in the protein surface potentials may affect *trp* repressor-DNA association. Protein orientations used in the figures, structural unit definitions, and positions of mutation are described in Figure 1.

Results

The trp repressor electrostatic potential surface suggests how the protein binds to DNA

The electrostatic potential surface of 1 *trp* repressor structure (1wrp), solved using a crystal obtained by co-crystallizing aporepressor and tryptophan, shows how the negatively charged protein is able to interact with the polyanion. The strongest positive potential is associated with the solvent-accessible D-helix and the N-terminal tip and midsection of the E-helix in the helix-turn-helix units (Fig. 2A,B), which are inserted into the B-DNA major groove (Otwinoski et al., 1988). Positive potential is also associated with the *trp* repressor tryptophan binding site and the N-terminal residues. The protein is likely to be oriented with the helix-turn-helix structural units facing DNA before association because only negative potential is observed on the surface opposite the DNA binding face (Fig. 2B,C).

Negative potential extends across the DNA binding surface (Fig. 2A). This is anti-complementary to the negative potential associated with the DNA minor groove and may cause significant repulsion between protein and DNA during association. However, because the helix-turn-helix units extend several angstroms beyond the minor groove contact region (Fig. 2B), the positive potential surface will encounter DNA before this neg-

ative potential surface. Because the strength of the electrostatic forces is inversely proportional to the distance separating the 2 molecules, the repulsive potential will be weaker early in the association process. Solvent screening from water trapped between the 2 macromolecules may further weaken the repulsion between macromolecules. Thus, the extension of the DNA reading heads beyond the minor groove contact region and the attraction between DNA reading heads and DNA favor complex formation despite the negative potential on the DNA contact surface.

The DNA binding surface features are a consequence of the DNA reading head conformation

A second *trp* repressor structure (2wrp), solved from protein crystals obtained from infusing tryptophan into the aporepressor crystal, differs from the previously described structure in the DNA reading head conformation (Lawson et al., 1988). The structural flexibility of these units may be important in the search for the sequence-specific operator sequences from many nonspecific DNA sites (Lawson et al., 1988). The electrostatic potential surfaces for these protein structures differ only on the DNA contact surface (Fig. 3A). The positive potential at the E-helix midsection is enhanced for 2wrp relative to 1wrp. Positive potential is also enhanced at the center of the 2wrp DNA binding surface, as indicated by the positive potential increase on the tryptophan binding site and neutralization of 2 small negative potential regions. These differences are associated with a decreased separation distance between the DNA minor groove and major groove contact regions for the 2wrp structure relative to 1wrp (Figs. 2B, 3B). The DNA reading head conformation appears to be coupled to the strength and the location of negative and positive protein potentials on the DNA binding surface. As a result, the DNA electrostatic forces may be able to induce a reading head conformation or limit conformational sampling during association.

Tryptophan contributes to the DNA binding surface potentials through the allosteric change

Sequence-specific *trp* repressor binding to a 20-base pair operator DNA consensus fragment is distinguished from nonspecific aporepressor binding by a 6,500-fold difference in dissociation constant (Marmorstein et al., 1991). The tryptophan-induced conformational change has been implicated as the cause for the enhanced binding affinity of the *trp* repressor relative to the aporepressor (Schevitz et al., 1985; Zhang et al., 1987). In addition to changing the surface complementarity between protein and DNA, the conformational change shifts the positive protein potential from the E-helix C-terminus in the aporepressor (Fig. 4A,B) to the E-helix N-terminus, which interacts with the DNA major groove in the *trp* repressor-DNA complex (Kinemage 1; Otwinoski et al., 1988). In addition, positive potential is enhanced on the *trp* repressor DNA binding surface regions surrounding the tryptophan binding site, relative to the aporepressor. Because this region interacts with the negatively charged phosphate backbone in the protein-DNA complex (Otwinoski et al., 1988), the *trp* repressor DNA binding surface is more complementary to DNA than is the aporepressor surface. Because the "aporepressor," modeled by removing the tryptophan from the *trp* repressor structure, exhibits minor changes in the region surrounding the trypt-

Fig. 1. Key to the protein orientations relative to DNA, definitions of structural units, and sites of HIS protonation and super-repressor mutations for the *trp* repressor. The front view (**A**) displays the protein surface that contacts DNA. A 90° and 180° counterclockwise rotation about the z-axis produce side (**B**) and rear (**C**) views, respectively. The structural units for *trp* repressor in front (**D**) and side (**E**) views, displaying the α carbon backbone (green), helix-turn-helix DNA reading heads (blue), bound tryptophan CPK surface (yellow), and the N-termini (red). Helix D of the helix D-turn-helix E DNA reading head is the shorter of the 2 helices. HIS 16 and the charge-change super-repressor mutations, which alter the electrostatic potential on the DNA binding surface (**F**): HIS 16 (gray), E13K (magenta), E18K (blue), D46N (cyan), and E49K (red). The N-terminal MET of the *trp* repressor gene product is posttranslationally cleaved (Gunsalus & Yanofsky, 1980) and was not included during protein model building.

Fig. 2. The electrostatic potential of the isolated *trp* repressor (PDB entry 1wrp) from the trigonal crystal form (Schevitz et al., 1985) at 0.145 M in front (A), side (B), and back (C) views. The C α backbone of the protein is shown in a ribbon diagram in green and the tryptophan ligand is displayed with a CPK surface in yellow. The solid contours correspond to ± 2 kT/e, and the line contours correspond to ± 1 kT/e. The negative potential less than -1 kT/e is colored red and the positive potential greater than $+1$ kT/e is colored blue. The regions between -1 kT/e and $+1$ kT/e are considered to be neutral.

Fig. 3. The electrostatic potentials of the orthorhombic crystal form (Lawson et al., 1988) of *trp* repressor (PDB entry 2wrp) at 0.145 M in front (A) and side (B) views. Comparisons to Figure 2 show the effects of crystal packing and helix-turn-helix conformation on the protein potentials. The coloring scheme and contour levels are described in the Figure 2 caption.

tophan binding site relative to the *trp* repressor model (data not shown), the conformational change, and not the position of the tryptophan charges on the DNA binding surface, gives rise to the electrostatic potential differences between *trp* repressor and aporepressor.

The positive potential shift may be important for distinguishing sequence-specific protein-DNA binding from nonspecific binding because it is the predominant electrostatic feature that differs between the aporepressor and *trp* repressor. Support for this may be gained from the A77V *trp* repressor mutant. The A77V mutation, which occurs in the turn of the helix-turn-helix binding motif (Kinemage 1), induces the conformational change (Luisi & Sigler, 1990) that enables the protein to bind with sequence specificity in the absence of bound tryptophan. If the conformational transition is induced to the same extent by the valine substitution as with tryptophan, the A77V mutant should have an electrostatic potential surface that is very similar to *trp*

Fig. 4. The electrostatic potential of the orthorhombic crystal form (Zhang et al., 1987) of the aporepressor (PDB entry 3wrp) in front (**A**) and side (**B**) views, and the IPA pseudorepressor (Lawson & Sigler, 1988) in front (**C**) and side (**D**) views. Comparison to the *trp* repressor shows how tryptophan enhances the positive potential over the tryptophan binding site, and the allosteric change focuses the electrostatic potential associated with the E-helix onto the DNA binding surface. The coloring scheme and contour levels are described in the Figure 2 caption.

repressor, even though it lacks the tryptophan charges in the tryptophan binding site. This remains to be tested.

The effects of tryptophan analogue charge on the DNA binding surface potentials

IPA, which induces a protein conformational change similar to that produced by tryptophan (Lawson & Sigler, 1988), significantly enhances negative potential on the DNA binding surface and diminishes the positive potential associated with the DNA reading heads relative to the *trp* repressor structures (Fig. 4C,D). Because the interaction between the DNA binding surface and DNA relies on the proper balance of attractive and repulsive forces, this may be sufficient for the loss of protein–DNA interaction observed with this pseudorepressor. However, IPA also neutralizes positive potential at the tryptophan binding site on the DNA binding surface. Even if the attractive long-range forces could overcome the repulsion on the DNA binding surface

at the minor groove contact region, there would be no attraction between protein and DNA at the tryptophan binding site where the phosphate backbone contacts the protein. As a result, the long-range electrostatic forces may disfavor pseudorepressor–DNA binding at a distance before the surfaces come into contact.

Protein–tryptophan analogue and protein–analogue–DNA binding studies have suggested that the negative charge on the tryptophan zwitterion facilitates ligand binding to the protein and the positive charge is required for subsequent high-affinity DNA binding (Marmorstein et al., 1987; Marmorstein & Sigler, 1989). The electrostatic potential surfaces for *trp* repressor are consistent with these experimental studies. Tryptophan analogues with a negative charge only, like IPA, will be more attracted to the positive tryptophan binding site than ligands that have a positive charge, leading to enhanced protein binding affinities. Although positively charged ligands may be more difficult to bind than tryptophan because the site is associated with positive potential, once bound, they may enhance protein–DNA

Fig. 5. The electrostatic potential for the 1wrp model-built E13K (A), E18K (B), D46N (C), and E49K (D) super-repressors, showing the changes in DNA binding surfaces of the protein upon charge modification. The E49K mutant was also model-built from the 3wrp structure, which contains no bound tryptophan (E). The effect of protonating HIS 16 on wild-type (1wrp) electrostatic potentials (F) is compared to the effect of super-repressor mutations in the text. The positive potential that appears at the protein center when the surrounding negative potential is neutralized is located in the protein matrix, rather than on the solvent-accessible surface, and therefore is not important in the context of this work.

binding by increasing the positive potential on the tryptophan binding site.

A positive correlation between position of super-repressor mutation and DNA affinity

DNA binding affinity increases relative to the wild-type protein as the super-repressor mutation position moves closer to the molecule center (Fig. 1F; Kinemage 1): the E13K mutant binds twice as strongly as wild type; the E18K mutation binds 4 times as strongly as wild type; and both the D46N and E49K mutant *trp* repressors bind 10 times as strongly as wild type (Hurlburt & Yanofsky, 1990). The electrostatic potential surfaces of the super-repressors model built from both the *trp* repressor (Fig. 5A,D) and aporepressor (data not shown) structures show that these charge-change mutations strengthen the positive potential between the A-helices and the helix-turn-helix structural units, enhance positive potential over the tryptophan binding site, and neutralize the negative potential on the DNA binding surface relative to the wild-type model. These effects are enhanced as the position of mutation moves toward the protein center. The most dramatic effect on binding would be expected for the E49K mutant aporepressor because mutation completely removes negative potential from the DNA binding surface (Fig. 5E). The E49K mutant aporepressor actually binds 100 times more strongly to operator DNA than the wild-type aporepressor, exhibiting the strongest DNA affinity for mutants lacking bound tryptophan (Marmorstein et al., 1991).

*The effect of pH on *trp* repressor–DNA binding*

Histidine (HIS) 16 has a pK_a of 6.22 (Hyde et al., 1989) and lies on the DNA contact surface between the E13K and E18K super-repressor mutation positions (Fig. 1F). HIS protonation increases the protein charge to -4 and significantly increases the positive potential between the helix-turn-helix regions and the N-termini (Fig. 5F) relative to that for the E13K mutant (Fig. 5A). However, like the E13K mutant, the negative potential is not affected. The position of this charge change, and the fact that the E13K mutant exhibits a 2-fold increase, whereas the E18K mutant exhibits a 4-fold increase in binding affinity relative to wild-type *trp* repressor, suggests that if the protein binds to operator DNA with HIS 16 protonated, then a 2–4-fold enhancement in binding constant relative to the unprotonated protein species would be expected based on an extrapolation of the position effects observed with the super-repressors. This was observed experimentally. The dissociation constant in the *trp* repressor–operator complex, calculated using the gel retardation assay (Carey et al., 1991) at pH 6.0, was approximately 4-fold larger than that determined from the alkaline phosphatase protection assay (Marmorstein et al., 1991) at pH 7.4 and filter binding assay (Klig & Yanofsky, 1988) at pH 7.5. Marmorstein et al. (1991) noted that the pH difference could give rise to this discrepancy in dissociation constants, and our calculations support this.

*The DNA-induced *trp* repressor conformational change and changes in the protein potentials*

The electrostatic potential surface was calculated for the protein isolated from the protein–DNA complex to evaluate electrostatic potential changes that occur in the later stages of DNA

association. *The DNA was not present in these calculations.* The qualitative features of the electrostatic potentials are similar to those of the isolated *trp* repressor structures, although the negative potential on the DNA binding surface is neutralized substantially (Fig. 6A,B). Positive potential is associated with the tryptophan binding site, indicating that this feature, which distinguishes aporepressor from repressor, is important for DNA binding.

Discussion

Salt sensitivity is expected for the association of oppositely charged surfaces and is typical for protein–DNA association. Early reports concluded that electrostatic interactions were not important for *trp* repressor–operator association based on the insensitivity to salt concentration compared to other DNA binding proteins (Lane et al., 1986; Chandler & Lane, 1988). The dis-

Fig. 6. The electrostatic potentials of *trp* repressor from the protein–DNA complex (Otwinski et al., 1988) in front (A) and side (B) views. These potentials were calculated without the DNA present. Identical results were obtained with both molecules in the asymmetric unit of the 2.5-Å resolution protein–DNA complex structure. The coloring scheme and contour levels are described in the Figure 2 caption.

crepancy arising from our proposal that electrostatic interactions are important for this association and the apparent insensitivity to salt concentration can be resolved by considering the nature of the *trp* repressor DNA binding surface. If the negative potential on the DNA binding surface is responsible for the salt insensitivity, then "neutralizing" it by mutation would yield a protein with increased sensitivity to salt during DNA association. This is experimentally observed for the E49K mutant aporepressor, which exhibits salt-sensitive binding, but not observed for the wild-type aporepressor (Klig & Yanofsky, 1988; Marmorstein et al., 1991). Comparisons with the λ repressor and *cro* repressor electrostatic potential surfaces also suggest that the negative potential on the DNA binding surface is a feature particular to *trp* repressor (Guenot, 1993). This is expected because neither the λ nor *cro* repressor binds to DNA with an insensitivity to salt concentration.

Other comparisons among the *trp*, λ , and *cro* repressors provide interesting insight to *trp* repressor-DNA association (Guenot, 1993). All 3 repressors differ in magnitude and sign of the net protein charge, yet all 3 proteins bind to DNA with high affinity for their target operators. Of these proteins, only the *trp* repressor binds with an overall -6 charge, indicating that the distribution of positive potential on the DNA contact surface is critical for DNA binding. Positive potential associated with the helix-turn-helix structural units and the N-termini is common to all 3 proteins. Although nearly the entire electrostatic potential surface of *cro* repressor, a dimer with an overall $+18$ charge, is positive, negative potential is associated with the λ repressor surface opposite the DNA binding surface. Therefore, this negative potential is neither a general feature of the helix-turn-helix binding proteins nor specific to *trp* repressor. When these proteins are bound to their respective operator DNA, this potential surface retains the qualitative features observed for the isolated protein (Guenot, 1993). In vivo, the negative potential could camouflage the λ and *trp* repressor on the DNA so as not to weaken the DNA potential locally and interrupt other biological processes occurring nearby the operator sites. The negative potential on the *trp* and λ repressors may also ensure that the isolated proteins are properly oriented for interaction in the DNA electrostatic field.

Two theories describe the *trp* repressor-DNA complex as a nonspecific complex, because the contacts between protein and DNA are water-mediated rather than direct. One model assumes that the protein-DNA complex is nonspecific because the protein-DNA complex has the wrong DNA sequence (Staacke et al., 1990). The conclusions of this work have been challenged by 2 studies (Carey et al., 1991; Haran et al., 1992). A second model assumes that the correct DNA sequence is bound, but the protein-DNA complex is nonspecific because adding 2-methyl-2,4 pentanediol to the crystallization solution induced a state of supersaturation that could promote nonspecific binding (Brennan & Matthews, 1989; Carey et al., 1991). However, the structure of the *met* repressor-DNA complex has been reported (Somers & Phillips, 1992). This complex was also crystallized with 2-methyl-2,4 pentanediol, although Somers and Phillips observed direct contacts between the protein and DNA base pairs in the complex. Therefore, the crystallization conditions used for *trp* repressor will not necessarily lead to nonspecific protein-DNA binding, although nonspecific complexes may still form under these conditions. Whether the Otwinoski et al. (1988) model represents a sequence-specific or nonspecific protein-

DNA complex should not affect the conclusions of this study, which does not claim to distinguish between these binding modes. The electrostatic potential characterization of the protein-DNA complex is characteristic of whatever binding mode this complex represents. Structural support for the Otwinoski et al. (1988) proposal has recently been provided by Carey and co-workers, who have solved a protein-DNA complex crystal structure in which 2 *trp* repressor dimers bind in tandem to 1 DNA segment (Lawson & Carey, 1993). Similar protein-DNA contacts are made in the *trp* repressor-DNA complex structures.

It should be noted that the Lawson et al. (1988) and Otwinoski et al. (1988) protein-DNA complex structures were crystallized at different pH's. The protonated HIS form of *trp* repressor is likely binding to DNA at pH 4.8, whereas the Otwinoski et al. (1988) structure more likely characterizes the deprotonated HIS form of repressor at neutral pH. Whether HIS 16 is protonated or deprotonated during DNA association in vivo is not known, but its protonation state can affect the in vitro assays. Our electrostatic potential analysis indicates that HIS 16 protonation may affect protein-DNA binding in a manner similar to the charge-change super-repressor mutations. Because the results from different assays of biological activity may be dependent upon the HIS 16 protonation state, the effect of pH on *trp* repressor-operator binding should be better characterized.

Conclusions

Direct interactions between the DNA reading heads and the DNA major groove are expected to be important for *trp* repressor-operator recognition (Bass et al., 1988; Otwinoski et al., 1988). To date, changes in protein affinity for DNA arising from the super-repressor mutations (Klig & Yanofsky, 1988; Hurlburt & Yanofsky, 1990; Marmorstein et al., 1991) and tryptophan analogue binding (Lawson & Sigler, 1988; Marmorstein & Sigler, 1989) have been interpreted in terms of direct local interactions, which are or are not present in the crystal structure of the protein-DNA complex (Kinemage 1; Otwinoski et al., 1988). However, the protein and DNA surfaces must be in contact to form the salt bridges, hydrogen bonds, and dispersion interactions that stabilize the macromolecular complex. As a consequence, these sequence-specific elements play a very important role in the later phases of sequence-specific association.

Events that take place in the early stages of association are likely to be directed by the long-range electrostatic forces between macromolecules. The objectives of this work were to describe the electrostatic potential features of *trp* repressor and to determine whether the differences in the protein electrostatic potential surfaces of related *trp* repressor models could be used to deduce the surface characteristics and their potential roles in protein-DNA binding. This was done by evaluating how differences in protein conformation, tryptophan and analogue binding, charge-change mutation, pH dependence, and salt dependence affected the protein electrostatic potential surface and then relating these to whether the altered protein binds to DNA with sequence specificity, without specificity, or not at all. Because the models we have examined cover all 3 of these possibilities with a range of binding affinities and the wealth of structural and mutational data, the *trp* repressor proved to be an excellent model system for this approach.

The electrostatic determinants that we propose to be important for *trp* repressor-DNA binding form a consistent model

that implicates a role for long-range electrostatics early in the association process and complements the interpretations of previous structural and mutational studies. The conformational change that occurs upon tryptophan binding positions positive potential on the DNA binding surface to complement the regions of highest negative DNA potential (i.e., the DNA-phosphate backbone). Negative potential on the DNA binding surface is enhanced along the nearby minor groove-base contact region, but upon binding DNA, much of this negative potential is neutralized. Comparisons among the charge-change super-repressor mutants suggest that the negative potential may be the origin of the salt insensitivity previously observed for wild-type *trp* repressor. These calculations also explain the assay-dependent discrepancy in sequence-specific DNA binding constants.

The role of the negative potential on the DNA binding surface of the protein is intriguing but not well understood. The electrostatic potentials may exhibit some long-range site selectivity in the early stages of protein-DNA binding. For example, DNA sequences unable to induce a protein conformation that would dissipate the negative potential may be unable to bind the protein with high affinity, or the negative potential on the DNA binding surface may direct the protein away from regions of strong negative DNA potential. Such selection processes would enable the protein to search for its sequence-specific site from a distance rather than sampling the entire contact surface, thereby reducing the amount of time required by the protein for finding its *in vivo* operator among the many nonspecific sites.

Methods

Atoms and residues that were not crystallographically resolved were modeled using the default INSIGHT templates. Because the model-built surface residues extend into the high dielectric medium in the Poisson-Boltzmann electrostatic model, different random side-chain conformers yielded very similar protein potentials. Electrostatic potentials were calculated using the nonlinear Poisson-Boltzmann equation, which extends the conventional Coulomb equation by explicitly incorporating ionic strength effects, and modeling the solvated macromolecule as a 2-dielectric system. The Poisson-Boltzmann equation was solved by a finite difference method on a grid (Warwicker & Watson, 1982) with a 1.25-Å/grid-point resolution in the DELPHI software package (Klapper et al., 1986; Gilson & Honig, 1987; Gilson et al., 1988), currently implemented in INSIGHT (BIOSYM version 2.0.1). The formalism for this approach has been described in 2 excellent reviews (Harvey, 1989; Sharp & Honig, 1990). Protein and DNA were assigned dielectric values of 4, and bulk solvent was assigned a dielectric value of 80. Physiological ionic strength (i.e., 0.145 M) was used for all calculations, although the dependence of the potentials on salt concentration was examined for selected models at 0.01 M, 0.4 M, and 1.0 M. The protein and DNA charges were taken from the all-atom force field currently implemented in AMBER 4.0 (Pearlman et al., 1991). These were derived from a least-squares fit of the *ab initio* electrostatic potential to each atomic center in the amino acid model compounds (Singh & Kollman, 1984). The van der Waals parameters were adopted from the work of Bryant et al. (1989). Calculations were run and displayed on Silicon Graphics Iris 4D and Indigo workstations.

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References

- Bass S, Sorrells V, Youderian P. 1988. Mutant *trp* repressors with new DNA-binding specificities. *Science* 242:240-245.
- Borden KLB, Beckmann P, Lane AN. 1991. Determination of the orientations of tryptophan analogues bound to the *trp* repressor and the relationship to activation. *Eur J Biochem* 202:459-470.
- Brennan RG, Matthews BW. 1989. The helix-turn-helix DNA binding motif. *J Biol Chem* 264:1903-1906.
- Brown KD. 1968. Regulation of aromatic amino acid biosynthesis in *Escherichia coli* K12. *Genetics* 60:31-48.
- Bryant SH, Islam SA, Weaver DL. 1989. The surface area of monomeric proteins: Significance of power law behavior. *Proteins Struct Funct Genet* 6:418-423.
- Carey J, Lewis DEA, Lavoie TA, Yang J. 1991. How does *trp* repressor bind to its operator? *J Biol Chem* 266:24509-24513.
- Chandler LR, Lane AN. 1988. Interaction of the *trp* repressor from *Escherichia coli* with a constitutive *trp* operator. *Biochem J* 250:925-928.
- Gilson M, Honig B. 1987. Calculations of electrostatic potentials in an enzyme active site. *Nature* 330:84-86.
- Gilson MK, Sharp KA, Honig BH. 1988. Calculating the electrostatic potential of molecules in solution: Method and error assessment. *J Comput Chem* 9:327-335.
- Guenot J. 1993. Molecular dynamics and electrostatic modeling of proteins: Methodologies and applications for conformational sampling, structure prediction, structure refinement and macromolecular association [dissertation]. San Francisco, California: University of California.
- Gunsalus RP, Yanofsky C. 1980. Nucleotide sequence and expression of *Escherichia coli* *trpR*, the structural gene for the *trp* aporepressor. *Proc Natl Acad Sci USA* 77:7117-7121.
- Haran TE, Joachimiak A, Sigler PB. 1992. The DNA target of the *trp* repressor. *EMBO J* 11:3021-3030.
- Harvey SC. 1989. Treatment of electrostatic effects in macromolecular modeling. *Proteins Struct Funct Genet* 5:78-92.
- Hurlburt BK, Yanofsky C. 1990. Enhanced operator binding by *trp* super-repressors of *Escherichia coli*. *J Biol Chem* 265:7853-7858.
- Hyde EI, Ramesh V, Roberts GCK, Arrowsmith CH, Trest-Clemons L, Klaib B, Jardetzky O. 1989. NMR studies of the *Escherichia coli* *trp* aporepressor: Sequence-specific assignment of the aromatic proton resonances. *Eur J Biochem* 183:545-553.
- Klapper I, Hagstrom R, Fine R, Sharp K, Honig B. 1986. Focusing of electric fields in the active site of Cu-Zn superoxide dismutase. *Proteins Struct Funct Genet* 1:47-59.
- Klig LS, Yanofsky C. 1988. Increased binding of operator DNA by *trp* super-repressor EK49. *J Biol Chem* 263:243-246.
- Lane AN, Lefevre J, Jardetzky O. 1986. The effect of the *trp* repressor from *Escherichia coli* on the structure and dynamics of dA20dT20. *Biochim Biophys Acta* 867:45-56.
- Lawson CL, Carey J. 1993. Tandem binding in crystals of a *trp* repressor/operator half-site complex. *Nature* 366:178-182.
- Lawson CL, Sigler PB. 1988. The structure of *trp* pseudorepressor at 1.65 Å shows why indole propionate acts as a *trp* "inducer." *Nature* 333:869-871.
- Lawson CL, Zhang RG, Schevitz RW, Otwinoski Z, Joachimiak A, Sigler PB. 1988. Flexibility of the DNA-binding domains of *trp* repressor. *Proteins Struct Funct Genet* 3:18-31.
- Luisi BF, Sigler P. 1990. The stereochemistry and biochemistry of the *trp* repressor-operator complex. *Biochim Biophys Acta* 1048:113-126.
- Marmorstein RQ, Joachimiak A, Sprinzl M, Sigler PB. 1987. The structural basis for the interaction between L-tryptophan and the *Escherichia coli* *trp* aporepressor. *J Biol Chem* 262:4922-4927.
- Marmorstein RQ, Sigler PB. 1989. Stereochemical effects of L-tryptophan and its analogues on *trp* repressor's affinity for operator-DNA. *J Biol Chem* 264:9149-9154.

- Marmorstein RQ, Sprinzl M, Sigler PB. 1991. An alkaline phosphatase protection assay to investigate *trp* repressor/operator interactions. *Biochemistry* 30:1141-1148.
- Morse DE, Yanofsky C. 1969. Amber mutants of the *trpR* regulatory gene. *J Mol Biol* 44:185-193.
- Otwinoski Z, Schevitz RW, Zhang RG, Lawson CL, Joachimiak A, Marmorstein RQ, Luisi BF, Sigler PB. 1988. Crystal structure of *trp* repressor/operator complex at atomic resolution. *Nature* 335:321-329.
- Pearlman DA, Case DA, Caldwell JC, Seibel GS, Singh UC, Weiner P, Kollman PA. 1991. AMBER 4.0. San Francisco, California: University of California.
- Pittard J, Camakaris J, Wallace BJ. 1969. Inhibition of 3-deoxy-D-arabinoheptulosonic acid-7-phosphate synthetase (*trp*) in *E. coli*. *J Bacteriol* 97:1242-1247.
- Rose JK, Squires CL, Yanofsky C, Yang HL, Zubay G. 1973. Regulation of in vitro transcription of the tryptophan operon by purified RNA polymerase in the presence of partially purified repressor tryptophan. *Nature New Biol* 245:133-137.
- Rose JK, Yanofsky C. 1974. Interaction of the operator of the tryptophan operon with repressor. *Proc Natl Acad Sci USA* 71:3134-3138.
- Schevitz RW, Otwinoski Z, Joachimiak A, Lawson CL, Sigler PB. 1985. The three-dimensional structure of *trp* repressor. *Nature* 317:782-786.
- Sharp KA, Honig B. 1990. Electrostatic interactions in macromolecules: Theory and applications. *Annu Rev Biophys Biophys Chem* 19:301-332.
- Shimizu Y, Shimizu N, Hayashi M. 1973. In vitro repression of transcription of the tryptophan operon by *trp* repressor. *Proc Natl Acad Sci* 70:1990-1994.
- Singh UC, Kollman PA. 1984. An approach to calculating electrostatic charges for molecules. *J Comput Chem* 5:129-145.
- Somers WS, Phillips SEV. 1992. Crystal structure of the *met* repressor-operator complex at 2.8 Å resolution reveals DNA recognition by β -strands. *Nature* 359:387-393.
- Squires CL, Lee FD, Yanofsky C. 1975. Interaction of the *trp* repressor and RNA polymerase with the *trp* operon. *J Mol Biol* 92:93-111.
- Staacke D, Walter B, Kisters-Woike B, von Wilcken-Bergmann B, Muller-Hill B. 1990. How *trp* repressor binds to its operator. *EMBO J* 9:1963-1967.
- Warwicker J, Watson HC. 1982. Calculation of the electric potential in the active site cleft due to α -helix dipoles. *J Mol Biol* 157:671-679.
- Yanofsky C. 1971. Tryptophan biosynthesis in *Escherichia coli*: Genetic determination of the proteins involved. *JAMA* 218:1026-1035.
- Zhang RG, Joachimiak A, Lawson CL, Schevitz RW, Otwinoski Z, Sigler PB. 1987. The crystal structure of *trp* aporepressor at 1.8 Å shows how binding tryptophan enhances DNA affinity. *Nature* 327:591-597.