Structural Changes and Binding Characteristics of the Tetracycline-Repressor Binding Site on Induction

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Abstract: The binding motif (pharmacophore) for induction and the changes in the structure of the binding site that accompany induction have been determined from molecular-dynamics simulations on the tetracycline-repressor signal-transduction protein. The changes and the induction mechanism are discussed and compared with conclusions drawn from earlier X-ray structures. The differences in inducer strength of tetracycline and 5a,6-anhydrotetracycline are discussed with respect to their interaction in the MD simulations.

One of the best characterized and important signal-transduction proteins is the tetracycline repressor; TetR.[1] Not only is its role in the regulated resistance of Gram-negative bacteria to tetracycline antibiotics of therapeutic importance,[2] but also its use as a "gene switch"[3] that allows genes to be regulated by administering tetracycline have made TetR and its operons *tetO1* and *tetO2* key regulatory systems in current research. TetR regulates both its own expression and that of the tetracycline antiporter TetA, an intrinsic membrane protein that pumps tetracyclines out of the bacterial cell and therefore renders the bacterium tetracycline resistant.[1] The experimental situation with respect to the structure of TetR is excellent. X-ray structures of several induced forms[4−6] and one noninduced form complexed to DNA[7] are available. The allosteric change that accompanies induction involves the two DNA-binding heads moving apart so that the distance between them is no longer ideal for binding in consecutive turns of the major groove. The TetR/DNA binding energy therefore decreases and the protein dissociates from the complex, freeing access to the two operons and allowing expression. However, what turn out to be key regions of the sequence are not resolved because of their flexibility. Thus, although a mechanism for the allosteric change that occurs on induction was suggested on the basis of the X-ray structures,[7] this did not include some key contributions arising in the unresolved parts of the sequence. The question as to the microscopic interactions that cause the allosteric change remained largely open, and a clear picture of the cause of induction on the molecular level did not emerge. Our recent long time-scale molecular-dynamics (MD) simulations, however, revealed the probable mechanism of induction, which involves the inducer (usually a tetracycline) displacing an aspartate (Asp156′, Asp156 of the second monomer) from the magnesium ion in the receptor to initiate a chain of salt-bridge rearrangements. As an end result of these changes, salt bridges between the two DNA-binding heads are broken, freeing up a scissors-like very-low-frequency vibration that results in the previously fixed distance between the two DNA-binding heads increasing and becoming very variable.[8]

This mechanism represents what is becoming an important class of protein structural rearrangements; those induced by the addition or removal of a metal ion. Such processes are thought to be important in the development of diseases caused by protein misfolding, which are clearly affected strongly by divalent metal ions.

As a second part of our analysis of TetR-induction, we have now used the results of the MD-simulations to define and interpret the changes in the binding-site in order to determine the requirements necessary for a small molecule to be able to induce TetR.

This information results in an "induction pharmacophore", which can either be used to try to avoid inducing resistance to tetracycline-antibiotics or to design nonantibiotic inducers that can be used to regulate the TetR/*tetO* system.

The aim of this work is to define as closely as possible the prerequisites for an inducer of TetR and to provide some rationalization for differences in inducer strength observed for the tetracyclines. We also discuss changes in the protein structure in the immediate vicinity of the binding site.

Time-averaged structures were calculated for the last 30 ns (20−50 ns) of the simulations reported previously.[8] These were superimposed[9] using the α-carbons of all residues for which any atom was within 6 Å of the magnesium ion in either structure in order to detect changes in the binding sites themselves, rather than global changes in the protein structure. The details of the simulations are given in the Supporting Information.

Figure 1 shows the overlay of the time-averaged structures from MD-simulations.[8] The largest movement was found for a single residue, Asp156′, which initiates the allosteric change on induction by being displaced from the magnesium ion by the inducer.[8] The cascade of salt bridges caused by this change has been discussed in detail in ref 8 and will not be repeated here.

The consequence of the Asp156′ movement in the binding site is that the magnesium ion, its ligand His99, and the sequence Thr102-Arg103-Pro104 move 3−3.5 Å toward the center of the binding site on induction, although Glu146′ moves little and is therefore displaced from the magnesium ion. On the opposite side of the binding site, the largest movement is that of His63, which leaves its solvent-exposed position in the noninduced form and swings into the binding site on induction to hydrogen-bond with the amide group of the inducer. Figure 2 shows that this movement is simply a reorientation of the side chain and that it does not affect the conformation of the backbone significantly.

Thus, His63 serves as a recognition residue that enters a very specific hydrogen bond with the enol/amide substituents of the tetracycline but does not cause any backbone rearrangement between the noninduced and the induced forms of the receptor. Note that only one of the two monomers adopted the "non-induced" conformation in the simulation,[8] so that the reorientation of the His63 side chain, which is observed in both monomers, does not have any causal relationship with the induction rearrangement.

Generally, the effect of induction is to cause the binding site to shrink inward. His99 and the segments Thr102-Pro104 and Ala172′-Leu173′ each move several angstroms toward the center

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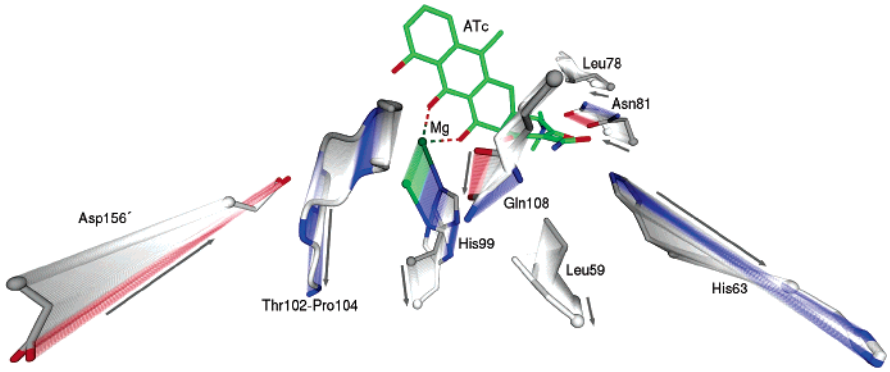


Figure 1. Overlay of the induced and noninduced time-averaged structures of the binding site of one monomer of the dimeric tetracycline-receptor. The arrows indicate the direction of the movement of the residue on removal of the inducer. Only the backbone atoms of the Thr102-Pro104 segment are shown.

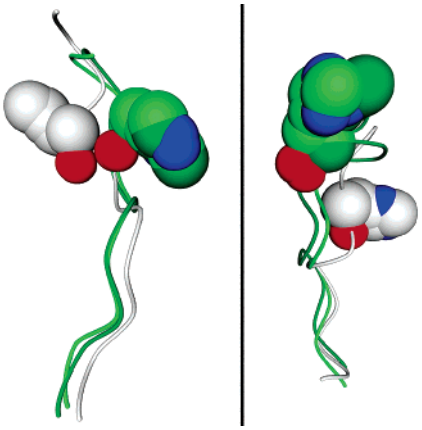


Figure 2. Overlay of the time-averaged structures of the Ala60-Ala70 segments of both monomers of induced (with 5,6a-anhydrotetracycline, dark green; with tetracycline, light green) and noninduced (gray) TetR. His63 and His63′ of the noninduced structure and that with 5,6a-anhydrotetracycline are shown as space-filling models with carbons shown in gray and green, respectively.

Table 1. Calculated Cavity Volumes (Å^3) and Surface Areas (Å^2) for the Binding Sites of the Two TetR Monomers in the Three Simulations

	no inducer		tetracycline		5a,6-anhydrotetracycline	
monomer	**1**	**2**	**1**	**2**	**1**	**2**
volume	1691	1310	1453	1378	990	986
total	1208	989	988	1049	729	776
hydrophilic	831	661	597	698	364	383
lipophilic	376	327	391	350	365	393

of the cavity, but leucines 59, 78, and 130 all move slightly outward. Thus, this shrinking of the binding site serves to surround the inducer more closely. Exceptions to this general trend are Val112 and Leu168′, which move away from the cavity center on induction. The volume of the cavity[10] and the hydrophilic and hydrophobic components of the cavity-surface area are shown in Table 1.

As observed previously,[8] the differences between the induced and noninduced forms of TetR are clearest if the simulation without inducer is compared with that including 5a,6-anhydrotetracycline, which is known[11] to be a stronger inducer than tetracycline. Table 1 shows quite clearly that the shrinking of the cavity on binding the inducer occurs almost exclusively at the cost of the hydrophilic surface and that the lipophilic surface area remains essentially constant in all three simulations. These observations are consistent with a water-filled cavity becoming occupied by a relatively lipophilic ligand. However, the degree of cavity shrinking is probably larger than for most receptors because the geometry change is part of the allosteric rearrangement on induction. Figure 3 shows a comparison of the calculated cavities for the three simulations.

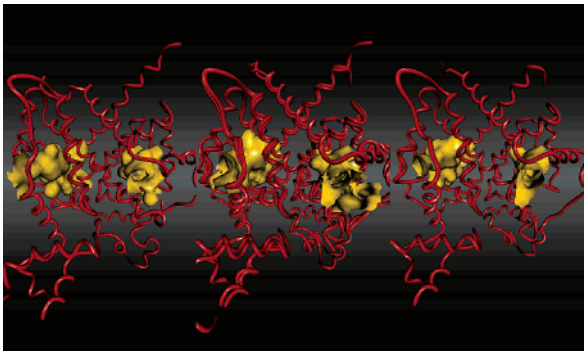


Figure 3. The calculated binding pockets (yellow) for the time-averaged structures obtained from the simulations without inducer (left) and with tetracycline (center) and 5a,6-anhydrotetracycline (right). The volume change is most obvious if the inducer-free and 5a,6-anhydrotetracycline structures are compared. The definition of the cavity is to some extent arbitrary as it depends heavily on where the border is chosen in the canal leading to the exterior of the protein.

The movements indicated in Figure 1 correspond fairly closely to those described by Hinrichs and Fenske[12] on the basis of the X-ray structures. In particular, the shift of the Thr102-Pro104 segment and of helix α4 is found in the MD simulations. However, this change, in a region that is well resolved in the X-ray structures is suggested by the MD simulations to be a consequence, rather than the cause, of the allosteric rearrangement on induction.

Table 2 shows an analysis of the occupation of the hydrogen bonds to and from the inducers tetracycline (Tc) and 5a,6-anhydrotetracycline (ATc) during the last 30 ns of the 50 ns simulations.[8] This analysis differs somewhat from the conclusions derived from the X-ray structures of induced TetR[4−6,12] and will be discussed in detail here as it provides the basis for understanding tetracycline binding to TetR.

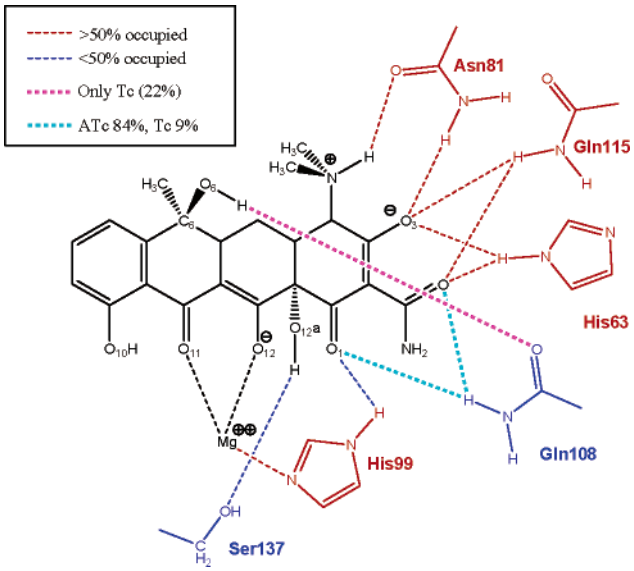
Chart 1 shows a summary of the information contained in Table 2 (using average values for the two monomers in each case). The strongest interactions are those belonging to the pharmacophore outlined previously.[12] Thus, Asn81 acts as an oxygen-centered H-bond acceptor for 100% of the simulation and as an NH-donor for a mean of 77.2% of the TetR-Tc simulation and 88.9% for the stronger inducer[11] ATc. His63 forms a hydrogen bond to the inducer 96.8% (average of the two monomers) of the time for Tc and 99.9% for ATc. The hydrogen bond between the inducer and Gln115 is not as strongly conserved, but is still occupied as donor 63% and 72.1% of the time and 15.5% and 18.7% as acceptor for Tc

Table 2. Hydrogen-Bonding Analysis for All Interactions in Which Tetracycline or 5a,6-anhydrotetracycline Act as the Donor or Acceptor. The Percentage Occupancies Are Calculated from the Last 30 ns of the 50 ns Simulations Described in Ref 8

		% occupancy[a]			
donor	acceptor	tetracycline		5a,6-anhydro-tetracycline	
inducer	Asn81	100.0	100.0	100.0	100.0
inducer	His99	24.3	7.7	25.1	29.9
inducer	Gln108	5.9	38.9	0.0	0.0
inducer	Gln115	5.0	26.0	16.0	21.4
inducer	Ser137	2.3	5.7	10.2	26.4
His63	inducer	100.0	93.6	100.0	99.7
Ser66	inducer	1.6	12.3	5.7	3.0
Asn81	inducer	56.0	98.3	100.0	77.7
Gln108	inducer	11.9	5.3	97.5	71.1
Arg103	inducer	5.9	0.0	0.0	0.0
Gln115	inducer	32.6	93.4	73.1	71.1
Ser137	inducer	3.2	6.3	0.4	5.5

[a] D−H···A hydrogen bonds were indicated if the D−A distance was less than 3.5 Å, irrespective of the D−H−A angle. The % occupancy is defined as the percentage of the snapshots in which these criteria were satisfied for the hydrogen bond in question. The two columns for each inducer refer to the two separate TetR monomers in each case.

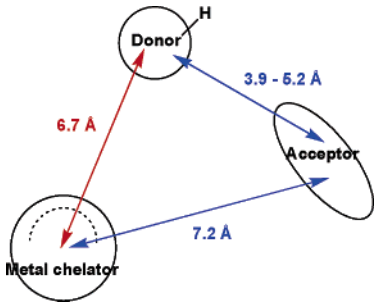
Chart 1. Analysis of the Hydrogen Bonding Interactions between Tc, ATc, and the Tetracycline-Repressor



and ATc, respectively. An interesting difference between the two inducers is that O_1 in Tc is mostly (48.8%) occupied by internal hydrogen bonding (to O_{12a} and amide NH_2) and only sporadically (16%) with His99, which is also coordinated to the magnesium ion, whereas the same oxygen in ATc interacts with His99 27.5% of the time and 18.8% with Ser137. Gln108 coordinates weakly for Tc but not for ATc and Ser137 weakly in both cases. Gln108 changes its preferred interaction mode between Tc and ATc. It interacts with the O_6H group of the parent tetracycline moderately (22%), but also to a small extent with O_1 and the oxygen atom of the amide group. This second interaction becomes much more prevalent (84%) for ATc, which lacks the O_6H group. The most obvious discrepancy between the binding picture derived from the X-ray structures and that given by the MD simulations is that Arg103 is suggested to H-bond to O_{10} by the static structures but that this interaction is only found about 3% of the time for Tc and not at all for ATc.

Table 2 and Chart 1 suggest some reasons for the fact that ATc is a stronger inducer than Tc. The differences occur in the region O_{12}−O_1-amide and are a consequence of a slight

Chart 2. Schematic View of the "Induction Pharmacophore" for TetR. The Diagram Shows the Essential Features of the Inducer Derived from the Bonding Interactions between the Inducer and TetR Taken from the MD Simulations



realignment of the inducer relative to tetracycline so that interactions in this region can become more favorable. This realignment is possible because the O_6H group is missing in ATc. As might be expected from the very different occupancies for interactions with His99 and Gln108 given by the two monomers in the ATc simulation, the positions of ATc in the binding pocket differ significantly between the two monomers. In monomer 1, the ATc molecule is tipped toward Gln108 in order to optimize interactions with the side-chain amide group. In monomer 2, however, a more drastic shift of the inducer molecule with its magnesium ion and His99 is found, leading to increased hydrogen bonding between His99, Gln115 and Ser137, and ATc. No X-ray structure of TetR:ATc is yet available, so that the exact binding mode of ATc in TetR is not known experimentally. In either case, however, the lack of a hydroxyl group at C_6 leads to an optimization of the hydrogen bonding in the $O_{12}H$−O_1-amide region, either with Gln108 or His99 as partner.

The above results allow us to define the binding features (the pharmacophore) needed to induce TetR. Strictly speaking, it is only necessary to pull the magnesium ion away from Asp156′ in order to break the complexation and free the acidic residue to induce the rearrangement cascade. The large change in the conformation of His63 is not necessary for induction, so that the minimal pharmacophore is given by a hydrogen-bond donor and a metal chelator 6.7 Å apart, as shown by the red double arrow in Chart 2.

However, this interaction would need to be extremely strong to be able to displace the aspartate from the magnesium ion alone. In reality, the H-bond donor must probably be a positively charged residue in order to be able to exert enough strain on the CO_2^-···Mg^{2+} linkage to break the coordination. Therefore, to strengthen the strain on the magnesium coordination, a second interaction that pulls in the same general direction is probably necessary. Thus, the extended pharmacophore also contains a hydrogen-bond acceptor, also roughly 7 Å from the center of the metal chelator and only 4−5 Å from the H-bond donor. This acceptor can interact with His63 to increase the strain on the magnesium coordination and eventually to dissociate the CO_2^-···Mg^{2+} bond and initiate the allosteric change. Hinrichs and Fenske[12] have called the region occupied by the H-bond donor and acceptor in our pharmacophore the "anchor region". This description is particularly fitting because specific bonding interactions in this region are the prerequisite for initiating the allosteric change, even though the residues involved are not directly part of the structural rearrangement. Binding to TetR is strengthened by the interaction of the hydrophobic D-ring with Pro104, Leu130′, Ile134′, Leu169′, Leu173′, and Met176′, as outlined by Hinrichs and Fenske.[12]

Thus, the TetR receptor combines the classical elements of ligand-binding. The anchor region provides specificity and the necessary strain to cause induction, whereas the hydrophobic region helps to provide the strong binding needed for the induction mechanism to protect the cell against tetracyclines.

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Supporting Information Available: Details of the calculation of the cavity volume and surface areas and the assignment of hydrophilic and lipophilic surface areas. Full page versions of the figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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