The Binding Site Interactions between 7-Chlortetracycline and the Tet Repressor

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ABSTRACT

Tetracyclines (Tc) are a family of broad-spectrum antibiotics. The most abundant resistance mechanism for Tc in gram-negative bacteria is efflux-based resistance, which is regulated by the TetR protein. In this work we are concerned with investigating the electronic structure related to the interaction between 7-Chlortetracycline and the Tet Repressor. For this purpose, we use density functional theory (DFT) calculations to obtain the interaction energies for each of the residues in the $TetR^D$ binding site with $[Mg\text{-}7ClTc]^+$. Atomic positions were obtained from available crystallographic data. Results give a general view of the interactions in the TetR pocket and indicate an area with potential for bypassing TetR-mediated resistance.

Keywords: tetracycline, antibiotic, tet repressor, density functional theory, drug design

1 Introduction

Tetracyclines (Tc) are a family of broad-spectrum antibiotics that exhibit activity against a wide range of gram-positive and gram-negative bacteria and atypical organisms. The absence of major side-effects and favorable characteristics of its action has led to their extensive use in the therapy of both human and animal infections [1]. The most common resistance mechanism for Tc in gram-negative bacteria is efflux-based resistance, which consists of the active export of the drug out of the bacterial cell. The mechanism is regulated at the level of transcription by the Tet repressor protein (TetR). Upon binding $[Mg\text{-}Tc]^+$ (complex of Tc and a cationic magnesium that occurs under physiological conditions), the TetR protein undergoes a conformational change, and as a consequence the gene encoding for the efflux protein is expressed. In theory, Tc analogs that do not bind effectively to TetR and retain antibiotic activity, could be used against all bacteria with solely this mechanism of resistance. The sensitive regulation provided by TetR and high affinity for Tc revealed the potential of the Tet repressor as a specific and efficient gene regulator [2]. For all these reasons, a deeper understanding of the Tc-TetR interaction would be invaluable.

In this work we are concerned with investigating the electronic structure related to the interaction between tetracycline and its receptor. For this purpose, we use density functional theory (DFT) calculations to obtain the interaction energies for each of those residues in the $TetR^D$ binding site with $[Mg\text{-}7ClTc]^+$. Also, the mapped electron density and electrostatic potential of the binding site was obtained. Therefore, we have obtained a means to compare quantitatively the interaction of the tetracycline with the amino acid residues of TetR.

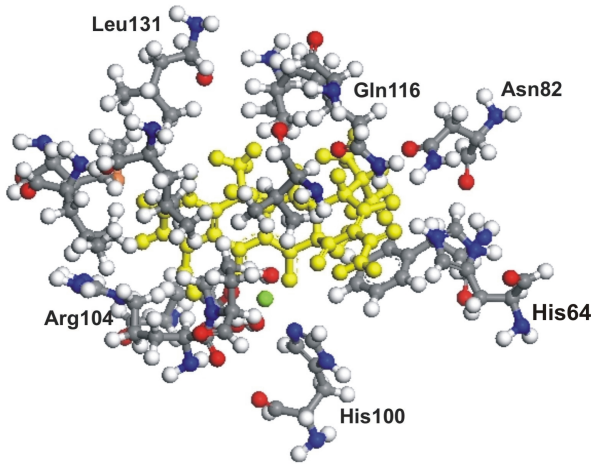

Figure 1: View of the Tc binding site on TetR. The antibiotic is displayed in yellow.

2 Methods

Our calculations were performed with the 7ClTc-TetR complex (PDB ID 2TCT) protein structure. We expect the results to be fairly general since the selected residues are mostly conserved or type-conserved throughout the TetR classes [3]. Due to computational limitations relative to density functional approach, we were constrained to use only the residues thought to have relevant interactions. All hydrogen addition to 7ClTc,

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water molecules and residues was made using standard bonds lengths and angles.

To study the 7ClTc-TetR, we have used the DMol3 code to perform DFT energy calculations within the generalized gradient approximation (GGA) adopting the Becke - Lee-Yang-Parr (BLYP) hybrid exchange and correlation functional. To save numerical processing time due to our limited computational resources, all calculations were carried out without inclusion of solvent effects, although water molecules pertinent to the binding site were included. To determine the interaction between tetracycline and each amino acid we first obtained the interaction of each residue with the rest of the binding site by calculating the energy change upon removal of one amino acid at a time and then we determined the interaction of that residue with the other residues by determining the energy change upon removal of one amino acid at a time in the binding site without 7ClTc. The difference between these two energy changes results in the interaction between the residue and 7ClTc. This way we account for the vicinity's influence on the binding energy of each residue. As a rule, we have used the following scheme to obtain the interaction energy (ΔE_{Tc-R}):

$$\Delta E_{Tc-R} = (E_T - E_{T-R} - E_R) - (E_T^* - E_{T-R}^* - E_R) = (E_T - E_{T-R}) - (E_T^* - E_{T-R}^*)$$

where E_T, E_{T-R}, and E_R stands for the complete binding site energy (all residues plus 7ClTc), the complete binding site minus selected residue energy, and the isolated residue energy, respectively. The asterisk denotes the aforementioned energies after 7ClTc removal.

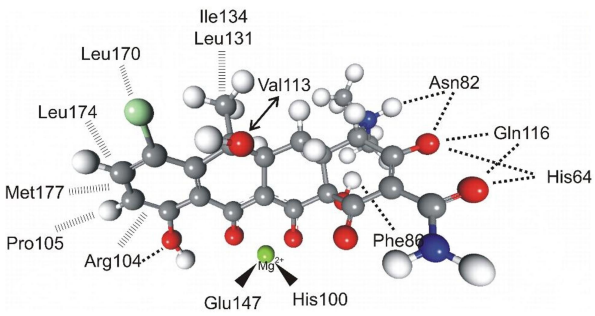

Figure 2: Schematic representation of 7ClTc-TetR binding site interactions.

3 Results

According with the current biochemistry knowledge of the 7ClTc-TetR complex, the key interactions are expected to be the chelation sites of Tc, namely the hydrogen bonds of the ring A and bonds involved in the magnesium coordination. Alteration of groups in these points of Tc are detrimental to its antibiotic activity [4]. Figure 2 gives a schematic view of the binding site. Residues near ring A are involved in hydrogen bonding and are expected to give the strongest bonds. On the other hand, residues interacting with 7ClTc aromatic ring D and other hydrophobic groups should give less intense bonds. Val113 was to give a somewhat significative repulsion, since removal of the hydroxyl group on C6 gives a 500-fold increase in TetR affinity [5].

In general, our results indicate that the binding site interaction was highly favorable. The interaction energy results from our calculations correlate well with the picture described above. Gln116, His64 and Asn82 indeed revealed strong bonds, being responsible for just over 50% of the overall binding energy. Magnesium coordination also revealed strong bonds with Glu147 and His100, the first one being water-mediated. As expected ring D revealed weak interactions and even a few repulsions. The only residue with clearly unexpected nature of interaction was Met177, which revealed a strong bond energy. This could be an overestimation due to the density functional method, but one should not entirely dismiss specific interactions such as this except after further evaluation by different methods. In conclusion, our results describe the polar interactions of residues with ring A as the most important for binding and TetR affinity, particularly the hydrogen bonding network involving residues His64, Asn82 and Gln116.

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