Molecular dynamics simulations of the Trp repressor–DNA complex and the AV77 mutant

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Abstract

Molecular dynamics simulations were performed on E. coli wild type (WT) Trp repressor and the AV77 mutant in interaction with DNA. Alanine to valine mutation at position 77 results in a repressor with enhanced activity at low concentrations of the L-Trp co-repressor. However, WT and AV77 mutant show an identical crystallographic structure, preventing to devise a clear structure-function correlation and suggesting that the different activity could be ascribed to a varied dynamical behavior. Root mean square deviations, dynamical cross correlation and hydrogen bond analysis have been performed to detect specific differences between the two repressor forms, showing that both ligand and DNA binding is different in the two complexes.

Keywords: Trp repressor; Protein–DNA interaction; AV77 mutant; Molecular dynamics simulation; Hydrogen bond

1. Introduction

Trp repressor of E. coli is a paradigmatic example to bring to light the effects of protein–ligand (co-repressor binding), protein–protein (dimerization), protein–DNA (operator binding) interactions and the effects of single point mutations on protein structure and dynamics. The primary function of this protein is to control the intracellular concentration of L-tryptophan (L-Trp), through a feed-back mechanism, acting as repressor inhibiting the transcription of several enzymes involved in L-Trp anabolism [1,2]. The protein is a 25 kDa symmetric homodimer [3], each monomer containing six \( \alpha \)-helices labeled A to F. Helices A–C and F of both monomers constitute the interlaced protein core domain and helices D–E the DNA binding regions. Binding of L-tryptophan (co-repressor) induces a conformational change that switches the protein from the apo form, characterized by a weak DNA binding affinity, to the holo form that is able to bind the target DNA sequence with high affinity [4–6]. Mutation of an alanine at position 77 (in the middle of the DNA binding motif) with a valine (AV77) makes the repressor a super-repressor [7], due to its ability to repress the TrpEDCBA operon at limiting L-Trp concentrations, conditions under which the
 WT repressor does not work. The aim of this work is to characterize, by means of molecular dynamics simulations, possible structural and related functional differences between the two repressor–DNA complexes, one involving the WT protein and the other the AV77 mutated one.

2. Materials and methods

The starting coordinates of the repressor–DNA complex were taken from NMR structures (PDB ID: 1CO0) in the Protein Data Bank [8]. The AV77 mutated form was generated starting from the native one by fitting the backbone of a valine onto the backbone of the substituted alanine at position 77. The DNA was simulated by a symmetrical 20-base-pair duplex. Each system was immersed in a water filled parallelepiped box with edges 59.14, 78.85 and 78.85 Å. TIP3P water molecules [9] were introduced starting from a FCC crystal with a lattice parameter equal to 4.96 Å, to reproduce a density of 1 gr/cm^3. Water molecules overlapping with the solute were discarded. As a result, each protein was solvated by about 12.000 water molecules. The systems were simulated under periodic boundary conditions using the force field of MacKerell et al. [10] (CHARMM), one of the most used force fields for the simulation of biological systems, by means of the molecular dynamics program ORAC [11]. A multiple time step scheme was adopted to integrate the equations of motion, according to the r-RESPA integration algorithm [12]. Long range electrostatic interactions were computed using the Smooth Particle Mesh Ewald (SPME) procedure [13] to calculate the reciprocal part of the Ewald sum [14]. We assigned all bending, stretching and improper torsion terms to a fast reference system, with an associated time step of 0.5 fs, the proper torsions and the V_{1-4} term to a slower reference system, with a time step of 1.0 fs. The non-bonded interactions were shared among three different shells, corresponding to the “short”-, the “medium”- and the “long”-distance ranges in the direct space. The “short” shell covers the range between 0 to 4.7 Å, with an associated time step of 2 fs, the “medium” shell the one between 4.7 to 7.3 Å, with a time step of 4 fs while the “long” shell extends up to 9.7 Å, with a time step of 12 fs. Both the Van der Waals and direct-space Coulomb interactions give a contribution in each of these shells. The reciprocal-space coulomb term is assigned to the medium-range shell. Each protein monomer carries a net charge of $-3e$, being $e$ the proton charge, while each DNA strand contributes with $-19e$ to the system total charge, in correspondence of the phosphate groups. The DNA charge was neutralized placing a diffusible Na^+ counterion on the bisector of the OPO angle at a 5.25 Å distance from each phosphorus atom. This method is satisfactory as long as only double-stranded structures are studied. The protein charge was instead neutralized replacing 6 randomly chosen water molecules with as many diffusible Na^+ counterions. Molecular dynamics simulations of each system were carried out for 6 ns, after the equilibration performed initially by means of the conjugated gradient method [15]. Equilibrium properties were computed averaging configurations sampled in the canonical ensemble (NVT), by coupling the system to a Nosé–Hoover heat bath [16] at 300 K. Coordinates of all atoms were stored every 0.12 ps and used for the analysis.

3. Results

In a previous work [17], we studied the apo and holo repressors without DNA, both in the WT and AV77 forms. Ligand binding in the WT protein results in a great stabilization of the DNA binding motif, while the mutation stabilizes this region even in absence of the co-repressor, making the mobility of the apo and holo structures very similar. Here, we focus our attention on the DNA-bound repressors. Analysis of the secondary structure elements shows that there are no significant differences between the two systems. Fig. 1 shows the protein and DNA root mean square deviations (rmsd) obtained from the simulation. Even if the all-chain values for the WT and AV77 proteins (see Fig. 1 caption) are very similar, helices B and E are markedly more flexible in the mutated protein, while helix D is slightly more mobile in the WT form. Differences are more striking for DNA, being the one in the AV77 system clearly more fluctuating than the WT counterpart. In both cases the major groove (the effective protein binding region) is more rigid than the minor groove. Informations about the dynamical correlation between different protein regions are provided by the dynamical cross correlation factors (dccf), eval-
Fig. 1. rmsd calculated for the protein residues, averaging on the backbone atoms (N, Cα, C and O) and on the two monomers, and for the DNA nucleotides, averaging on the heavy atoms (N, C, O and P) and on the two strands, excluding the highly mobile N-terminal protein residues and the terminal DNA nucleotides. Here and afterwards, wild-type results are shown in blue and AV77 ones in red. Horizontal bold lines mark the position of the α helices along the protein sequence and the nucleotides forming the DNA major (C7-A12) and minor (C13-T17) grooves. WT average values: (2.0 ± 1.2) Å, (2.6 ± 1.2) Å, AV77 average values: (2.2 ± 1.2) Å, (3.4 ± 0.9) Å (for the protein and DNA, respectively).

Fig. 2. Ligand–protein dccf. The dotted vertical line separates the two protein subunits. Evaluated for the α carbons of each pair of residues i and j and defined as

\[ c_{ij} = \frac{\langle (r_i - \langle r_i \rangle) \cdot (r_j - \langle r_j \rangle) \rangle}{\sqrt{\langle (r_i - \langle r_i \rangle)^2 \rangle} \sqrt{\langle (r_j - \langle r_j \rangle)^2 \rangle}}. \] (1)

In Fig. 2 the dccf between the co-repressor and the two protein subunits are shown. The WT ligand shows relevant positive correlations (i.e. \( \geq 0.5 \)) with helices A–C and E in one subunit and helices A and E of the other one. The situation is quite different in the mutated structure where, on average, the co-repressor is less correlated with the protein and relevant correlations comprise helix B of one monomer and helices C and E of the other one. High negative anticorrelations (i.e. \( \leq -0.5 \)) involve helix D of one subunit in both WT and AV77 structures and, only for WT, the terminal regions of the other one.

In Fig. 3 we show the ligand–protein hydrogen bonds (HBs). In both structures the ligand is hydrogen bonded with residues in the core (mainly helix C) and DNA binding region (helix E) of both chains. The binding with core residues is on average stronger in AV77, while the interaction with residues in the DNA binding domain is stronger in the WT structure. In Fig. 4 we show the DNA-protein HBs. The AV77 repressor makes only two direct HBs with two DNA nucleotides. One of these involves helix E and the DNA major groove, the other helix C and a terminal nucleotide. Many more HBs are present between the WT protein and DNA, namely, the same observed in AV77 plus others with terminal nucleotides, but all of them are less persistent. Between the two complexes are detected in the water mediated HB network between the repressor and DNA. In the WT complex we observe several strong water mediated interactions mainly between the DNA minor and major grooves and protein helices D and E, forming the DNA binding motif, and the core helix C. In particular, residues Thr53 (helix C) and Arg84' (he-
lix E) engaged in these contacts are also directly hydrogen bonded to the co-repressor, that makes a medium-strength water mediated HB with Ade12' of the DNA minor groove, also interacting with Arg84'. These results point out the relevant role played by the L-Trp ligand in the WT protein–DNA interaction. The number and the persistence time of the water mediated HBs between the AV77 repressor and DNA are much lower and none of them involves the co-repressor.
4. Conclusions

Although the two Trp repressors show very similar structures, their interaction with the ligand and DNA is different. The co-repressor motion is more correlated to the WT protein dynamics, but its HB network with the protein core is stronger in the AV77 complex, probably allowing an anchorage of the ligand to the protein structure even when the mutation occurs. The WT repressor forms many direct HBs with the DNA nucleotides, but their permanence along the trajectory is very poor, while those in the AV77 complex are fewer but much stronger (i.e. persisting). On the contrary, strong water mediated HBs between the repressor and DNA are only formed in the WT complex. The ability of the WT protein in repressing the transcription of several operons can therefore be ascribed to the rapid formation and breakage of many direct HBs with DNA, while the strength of the interaction is gained through highly persistent water mediated HBs, also involving the ligand. The AV77 protein interacts with DNA by means of two direct HBs, which may account for repression of just one Trp operon, while the lack of persistent water mediated interactions may explain the enhanced flexibility of the complex. Finally, the ligand appears to be much less involved in the AV77 protein–DNA interaction, in accord with the experimental finding that, upon this mutation, the regulation mechanism become possible even at limiting L-Trp concentrations.

References


