Atomistic simulations of GabR transcription factor at microsecond scale

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The *Bacillus subtilis* transcriptional regulator GabR is a chimeric protein possessing a N-terminal DNA binding domain (containing a Helix-Turn-Helix motif) and a C-terminal aspartate amino transferase-like domain (AAT) [1]. In the presence of γ-aminobutyric acid (GABA), GabR activates transcription of the gabTD operon coding for enzymes of the GABA catabolism. The enzymes reduce GABA to succinic semialdehyde and then to succinate. Amino transferases are widespread enzymes that catalyze the transfer of an amino group from a donor (usually an amino acid) to an acceptor (typically a keto acid or an aldehyde). All amino transferases contain pyridoxal 5'-phosphate (PLP) as a cofactor essential for catalysis [2]. The cofactor is generally bound to the ε-amino group of an active site lysine forming an internal aldimine. Likewise, GabR can form an internal aldimine with PLP. Spectroscopic studies [3] have shown that GABA binds as an external aldimine to the PLP in the GabR amino transferase domain. Although GabR can interact with DNA in apo (without PLP) and holo (PLP internal aldimine) forms, it must bind PLP and GABA to activate gabTD transcription [2]. Presence of GABA at GabR AAT active site likely causes a conformational change that alters the mode of binding to DNA and switches on gabTD transcription [4]. The crystallographic structures of the entire dimeric apo GabR (GabR with no PLP at the active site) and holo GabR (PLP internal aldimine) have been recently solved [1]. Later on, the structure of the GabR AAT-like domain in complex with GABA external aldimine has been made available by two independent groups[4-5]. Although structural and functional characterization of GabR is proceeding at a fast pace, virtually nothing is know about the molecular details of the changes induced by the effector binding and on how these modifications can influence the functional properties of the regulator.

To understand the underlying molecular mechanism of action of GabR, an extensive molecular dynamics (MD) study on the apo (without PLP), holo (with PLP), and holo-GABA (complex with the GABA external aldimine) forms of the GabR from *Bacillus subtilis* was undertaken. The missing structure of the entire GabR dimer in its GABA external aldimine form has been reconstructed by merging the Protein Data Bank (PDB) structure code 5t4j (reporting the AAT-like GabR domains complexed with GABA external aldimine) with the HTH and linker domains extracted from the structure 4n0b (GabR internal aldimine). Domain merging has been carried out using homology modeling techniques as implemented in the program Modeller v9.17.

A molecular dynamics study on the apo- and holo-GabR has been already reported [6]. The simulations covered a 350 ns time span. The presence of PLP in the binding pocket of GabR apparently destabilizes the interaction between the HTH domain of one subunit and the AAT domain of the other. The linker appears to play a significant role in the HTH conformational change in the holo-GabR form.

In the present study, the conformational changes of the three GabR forms (apo-, holo- and holo-GABA) are analyzed by explicit solvent 1μs molecular dynamics simulations carried out at T=298K and P=1atm. Preliminary results suggest that the presence of the ligand (PLP or PLP/GABA) induces some structural rearrangements in the GabR structure. In particular, the root mean square and the radius of gyration calculations show larger movements in the holo-protein at an earlier timescale with respect to the apo-form. Further investigation carried out within the protein structure network analysis framework also shows that in the absence of the ligand the correlation patterns are different and the protein domains have an in-phase interaction (being it positive or negative) at a later time. Detailed analysis of the differences at the active site of the three GabR forms will add further data.

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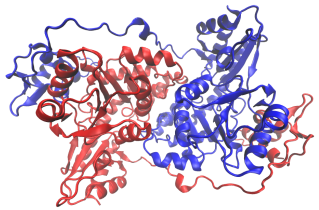

Figure 1. GabR apo structure

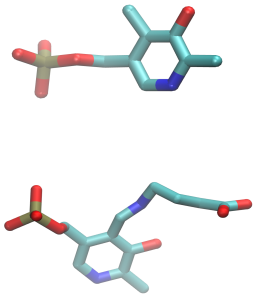

Figure 2. GabR ligands: PLP (top) and PLP/GABA complex (bottom).

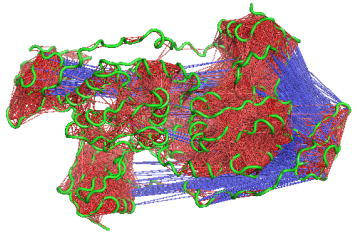

Figure 3. GabR apo structure: correlation (red) and anti-correlation (blue) analysis.

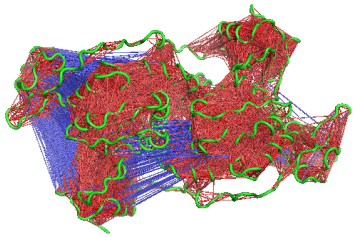

Figure 4. GabR holo structure: correlation (red) and anti-correlation (blue) analysis.