Computational Study of Ligand-dependent Oligomerization of Ribonucleotide Reductase

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Abstract
Certain protein oligomerization can be strongly influenced by its ligand-binding status. We conducted a computational method to investigate how ligand-binding and oligomerization can be coupled. We tackle this issue using an approximate approach of studying the properties of individual monomers and how they associate. By connecting the dynamics at monomeric level and the information of oligomer interface, we quantify the synchronization of two types of contact dynamics: (1) between the ligand and its binding pocket, and (2) the contact dynamics at interface. In this work, we applied our methodology on protein ribonucleotide reductase (RNR), which is an essential enzyme for DNA de novo synthesis. The study of RNR’s regulatory mechanism could lead to new designs of antimicrobial drugs targeting allosteric control of RNR function. We first performed atomistic simulation of RNR with different ligand binding status, and then used statistical analysis to gather the contact dynamics. We observed and quantified the level of resonance between S-site (Specificity allosteric site) ligand binding and the dimer interface formation, where we also revealed insights on RNR dimerization mechanism and potential druggable site. We also studied the (de)activation mechanism via ligand-induced hexamerization at the A-site (Activity allosteric site). There is a drastic change in dynamics of protein when ATP vs. dATP is bound at A-site. ATP-bound protein has a complex and delocalized dynamics, whereas dATP-bound protein has a relatively simple and localized motion around S-site.

RNR Structure and System Setup

The coarse-grained segment-segment contact analysis [1] was utilized to avoid running a computationally expensive algorithm on large protein systems such as RNR. Four monomeric systems were created (000, 010, A10, DAT0) depending on ligand occupancy at either A-site or S-site. The segments are defined based on their secondary structures, consisting of 38 a helices, 21 b strands, and 30 other structural regions. The size of each segment corresponds to the sphere volume displayed on the figure; red spheres represent the starting segments while blue spheres show the ending segments.

Activation and Deactivation processes correspond to different oligomeric states

(a) A simplified model of RNR hexamer. (b) A contact map scheme is displayed for inter-contact (labeled A), for dimer (B) or hexamer (C) interface interaction. (c) An intra-chain contact matrix. (d. e) Interprotein contact matrices at dimer and hexamer interface, respectively.

S-site Ligand Binding and Dimerization: Correlated motion in dTTP-bound state promotes interface formation

A relatively simple motion localized at the S-site is revealed in the dominant eigenvector (PC1) of 000 and 010. This motion involves a prominent correlated binding dynamics, anti-sync for 000 and sync for 010, between the two regions B8-G9-B9 and G10 with respect to their strong self-interaction. In 010, the contact formation between B8-G9-B9 (red sphere) and the ligand dTTP makes G10 (red sphere) become more ordered and more likely to contact with another dTTP, thus facilitating RNR dimerization. The stability and conformation differences of these regions are supported by B factor simulation data and the distance differences between 000 and 010’s mean structures.

A-site Ligand Binding and Hexamerization: Drastic dynamics changes between ATP-bound and dATP-bound states

The dominating mode (PC1) of A0 and dAT0 displays a significant difference in their dynamic motion. A0 correlated motion is dispersed throughout the structure, indicating a strong dynamic communication between many different segments which is achieved by ATP binding at the A-site. However, with bound dATP, dAT0 motion becomes much simpler and more localized, specifically at the two regions B8-G9-B9 and G12. The region G12 is speculated to be an ideal drug target binding site for allosteric regulation; specific binding to G12 can promote RNR dimerization by enhancing G10 stability in the S-site at A0, or disrupt the ligand binding capability at dimer interface due to B8-G9-B9 instability in dAT0. This monomeric study, however, lacks the dimer simulation data so it limits our ability to make assertive conclusions on RNR hexameric dynamics.

Concluding Remarks
RNR contains multiple nucleotide sensors (nucleotide binding sites) to regulate its function. In response, the structure changes even at the quaternary level. Our simulation and contact analysis provide detailed mechanisms of how dTTP-bound S-site promotes the dimer interface formation and vice versa. How the two types of contacts (ligand-binding contacts and interprotein contacts) correlate can also be influenced by the dynamics of the other parts of the protein, which potentially leads to the identification of new allosteric sites. Our results also revealed drastic dynamics changes of the whole protein between ATP-bound vs. dATP-bound states, which, however, offers some clues to the elusive hexamerization question and the sensitive ATP vs. dATP control at the A-site.

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References