Ligand-mediated and tertiary interactions cooperatively stabilize the P1 region in the guanine-sensing riboswitch

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Riboswitches, short genetic regulatory elements, are commonly found in the 5' untranslated region of bacterial mRNA. These regulatory mRNA segments usually consist of two domains: the aptamer domain, which binds small ligand molecules with high specificity, and the expression platform, which, upon binding of the ligand to the aptamer domain, undergoes a conformational change and subsequently alters the expression of the downstream genes.

Transcriptionally acting riboswitches, like the guanine-sensing riboswitch from the *xpt-pbuX* operon of *Bacillus subtilis*, typically only have a short time window for folding, binding a ligand molecule, and transferring the information about the bound ligand to the expression platform and the subsequent conformational change. If this process is not fast enough, the conformational change will happen too late to influence the transcription by the RNA polymerase. In order to understand the decision for one of the two mutually exclusive folding pathways, detailed knowledge about the unbound state of the riboswitch is required.

While crystal structures of guanine-sensing and related riboswitches are available in their ligand-bound state, atomic level information about the unbound state and its dynamics is still scarce. Furthermore, knowledge about the interplay of tertiary interactions in the loop region and ligand binding site on the stabilization of the terminal P1 region would be beneficial towards the understanding of the regulatory decision.

In order to shed light on the complex network of long-range interactions in the unbound state of the guanine-sensing riboswitch aptamer domain (Gsw), we performed molecular dynamics (MD) simulations in explicit solvent of the wildtype Gsw and a mutant, which exhibit different stability of the tertiary interactions in the loop region. We simulated six variants of the system with three replications each, yielding a total simulation time of more than 10 µs. Using the wildtype and the mutant of Gsw, we are able to observe a dynamic coupling between the tertiary interactions in the loop region and the ligand binding region, which is located ~25 Å away. Furthermore, we found this coupling to be dependent on the presence of Mg^{2+} ions. We performed rigidity analyses with a modeled ligand in the binding site in order to investigate the influence of the presence of a ligand on the rigidity of the aptamer domain. Results from these rigidity analyses indicate a cooperative effect between the tertiary interactions in the loop region and the distant ligand binding site on the terminal P1 region of the aptamer domain.

Our results indicate that information on ligand binding to the binding site, in connection with that on the stability of the tertiary interaction, is transferred across the aptamer domain via changes in the domain's dynamics, rather than by conformational changes. This would allow for a quick response of the riboswitch upon ligand binding, in accordance with the kinetic control mechanism found for this riboswitch.