Free energy profile of tRNA dissociation from ribosome studied by coarse-grained molecular dynamics simulations

Yoshiharu Mori
ymori@landscape.kobe-u.ac.jp

Shigenori Tanaka
tanaka2@kobe-u.ac.jp

Graduate School of System Informatics, Kobe University, 1-1, Rokkodai, Nada, Kobe, Hyogo 657-8501, Japan

Keywords: Protein synthesis, Molecular simulation, Free energy calculation

Ribosomes are large protein-RNA complexes that are responsible for protein synthesis in cells. The process of protein synthesis in ribosomes mainly consists of three steps: initiation, elongation, and termination. The elongation process is essential for protein synthesis, while the initiation and termination processes are also essential for maintaining the high quality of protein synthesis. In this study, we focused on the initiation process of translation in ribosomes. During the translation initiation phase, a pre-translational complex consisting of ribosomes and initiation-related proteins is formed to ensure the initiation of protein synthesis. These initiation-specific proteins are called initiation factors (IFs), and in the case of bacteria, there are three initiation factor proteins; IF1, IF2, and IF3. In bacteria, protein synthesis is initiated by methionine bound to an initiator tRNA. One of the roles of initiation factors is to enable the selective binding of such tRNAs. This study aims to elucidate the roles of these factors and ribosomal proteins using computational methods.

Because the pre-translation complex consists of more than 20 protein and RNA molecules, it is difficult to perform an all-atom molecular dynamics simulation. In this study, we performed molecular dynamics simulations of the protein-RNA complex using a coarse-grained model to elucidate the roles of initiation factors and ribosomal proteins in the complex. The complex consists of a small ribosomal subunit, a messenger RNA (mRNA), an initiator transfer RNA (tRNA), and initiation factors IF1, IF2, and IF3. The used structures of the complexes were obtained by cryo-electron microscopy, and these structures were treated with the Martini coarse-grained model. The pre-initiation complex is further classified into various states, and in this study, we focused on the incomplete (state *a*) and nearly complete (state *b*) states of the complex. The coarse-grained pre-translational complex using the Martini model consisted of about 17,000 particles, and the total number of particles, including solvent, was about 173,000. Molecular dynamics calculations were performed by GROMACS.

In order to understand how the presence of the initiation factors and the ribosomal proteins contribute to the stability of the initiator tRNA, coarse-grained molecular dynamics simulations using umbrella sampling were performed for each of the treated states *a* and *b*. The free energy profile of tRNA dissociation from the complex was calculated. Furthermore, by analyzing the structure of the ribosome-RNA complex, we revealed how the IFs and ribosomal proteins are related to the accurate recognition of the initiator tRNA by the ribosome. Comparing the free energy profile of the complex in the incomplete state with that in the nearly complete state (state *b*), the tRNA-bound state is stable and the activation barrier for dissociation is high in the nearly complete state. One of the reasons for these different free energy profiles is that the interactions between ribosomal proteins and tRNAs are different. The interactions between the C-terminal region of the ribosomal proteins and tRNA may stabilize the ribosome and tRNA. This interaction could be necessary for the accurate recognition of tRNA by the ribosome. The structure of ribosomal proteins and their interactions with tRNA will be discussed in the presentation.