Structure and Dynamics of a Ribosome Regulatory Peptide

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Introduction

Proteins are biomolecules which participate in virtually all processes in cells ranging from signaling, regulation, catalysis, to structure support, or storage. In all living organisms, the proteins are synthesized by large biomolecular complexes called ribosomes (Fig 1). Ribosomes read the genetic information temporarily stored in a strand of ribonucleic acid (RNA), and translate it into a sequence of amino acids. The amino acids are connected by so called peptide bonds. The ribosomes catalyze the formation of peptide bonds making it possible under conditions common in living matter. Because the catalytic center is buried deep in the ribosome, all nascent proteins leave the ribosome through a tunnel.

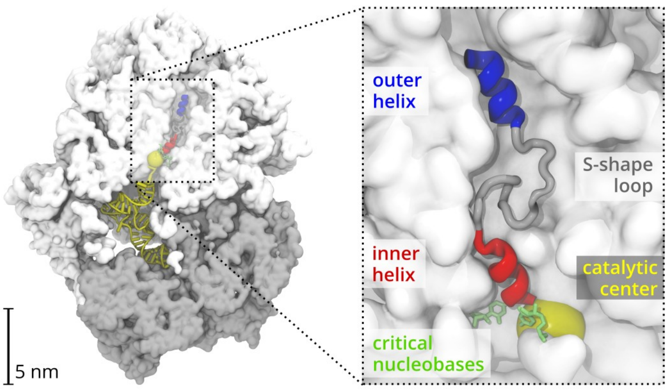

Figure 1: On the left, a cross-section of a bacterial ribosome is shown with the large ribosomal subunit in white, small ribosomal subunit in gray and transfer RNA in yellow. On the right, the VemP in the ribosome tunnel features two helical segments.

The catalytic center with the two critical nucleobases are highlighted in yellow and green, respectively. Based on PDB 5NWY [4].

The enormous importance of ribosomes requires a precise control of their action. Some regulation mechanisms involve ribosome stalling [1], where the affected ribosome pauses translation, but no protein is released. The stalled ribosomes remain assembled and cannot be recycled. There are many physiologic consequences of the stalling: for instance, a large class of antibiotics acts through ribosome stalling [2].

In a genus of marine bacteria *Vibrio*, stalling events regulate how proteins are exported out of the cell. The regulation involves a short peptide called VemP [3]. Recently, the structure of the peptide-ribosome complex has been determined experimentally [4]. VemP is uniquely compact and its structure is well classified. It folds into an α-helix near the catalytic center which is connected, through an S-shape loop, to another α-helix further in the ribosome tunnel (Fig 1). The inner helix inactivates two nucleobases at the catalytic site, which prevents peptide bond formation.

Using all-atom molecular dynamics (MD) simulations, we studied VemP structure and dynamics on a microsecond time scale. We have attempted to answer what determines the VemP structure. In a broader context, we want to understand, how the tunnel content modulates ribosome function.

Results and Methods

We have performed MD simulations of several VemP constructs in aqueous environment. Namely, we simulated entire VemP, and the inner and outer helices. The Newton's equations of motion were numerically propagated using GROMACS software package [5], a well established C++ code which uses mixed MPI/OpenMP parallelization and scales up to a few thousands of cores [6].

The peptides were dissolved in a rhombic dodecahedron box filled with explicit water and ions, and simulated at 310 K and 1 bar using periodic boundary conditions. In order to assess, how our conclusions depend on the choice of the empirical potential energy function, two sets of parameters were used to describe the smaller of the systems (inner or outer helix): either AMBER peptide model with SPC/E water model, or CHARMM peptide with TIP3P water model. For the entire VemP simulations, the AMBER parameters were used. The ensembles of peptide conformations were analyzed and compared with those from the in-ribosome simulations done previously.

Despite the simulations are shorter than the anticipated folding and unfolding times, they suggest that VemP is less stable in water than inside the ribosome tunnel. Starting from the in-ribosome conformation, the entire VemP collapsed down into a more compact conformation within less than 25 ns. The helical character was partially preserved for the outer VemP part, whereas the inner part lost its helicity more notably (red and orange bars in Fig 2). The inner and outer helices, when simulated alone and initiated from the helical structure, lost their helical character and most of the simulation time appeared unstructured. The data for AMBER and CHARMM sets are qualitatively comparable. Insufficient conformational sampling (i.e. simulation time) prevented us from assessing the quantitative correspondence.

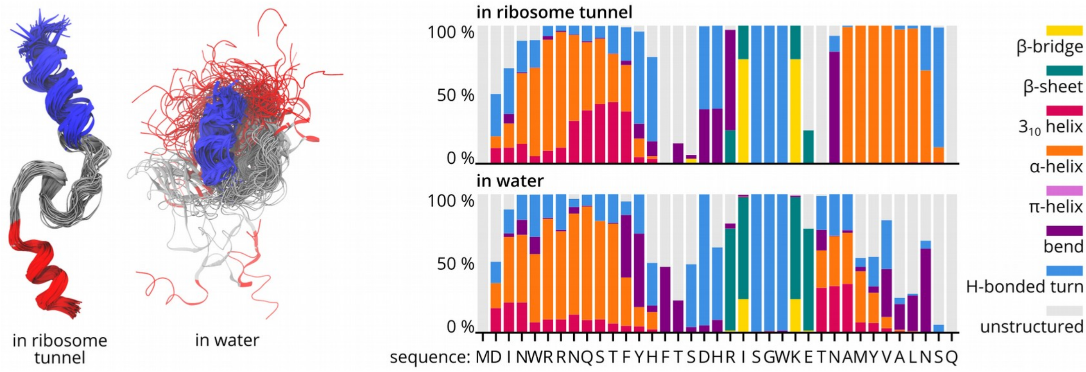

Figure 2: Left: multi-microsecond structural ensembles of the VemP inside and outside the ribosome tunnel. Right: fractions of various secondary structure motives, as captured by multi-microsecond MD simulations with AMBER parameters.

Conclusions

On a microsecond time scales, VemP proved stable inside the ribosome tunnel. On the other hand, VemP secondary structure elements are less stable in water, which renders the tunnel walls being the structure determining factor. For the inner helix, the tunnel plays a critical role. For the outer helix, the interactions with the tunnel are less important, because the outer helix is somewhat stable even in the water. It agrees with the observation that the tunnel is narrow around the inner helix near the catalytic center, but wider closer to the exit, where the outer helix ix located

Outlook

Structural information about what happens inside the ribosome tunnel is rather sparse. Traditional biophysical techniques such as X-ray crystallography or cryo-electron microscopy suffer from the inherent nascent peptide flexibility. Consequently, the electron density of the tunnel content is often blurred and difficult to interpret. MD simulations may provide a valuable insight into functional motions of the ribosome [7] possibly triggered by the tunnel content.

VemP is able to sense an external mechanical force. The MD simulations (together with the VemP experimental structure) offer a good opportunity to study how VemP responses to such a force, and, most importantly, how the nascent peptides pass through the tunnel. These topics are natural extensions of our current efforts and will be addressed in some of our future simulation projects.

References

1. D. N. Wilson, R. Beckmann, Curr. Opin. Struc. Biol. 21, 274 (2011) DOI: 10.1016/j.sbi.2011.01.007

2. J. Poehlsgaard, S. Douthwaite, Nat. Rev. Microbiol. 3, 870 (2005) DOI: 10.1038/nrmicro1265

3. E. Ishii, et al., Proc. Natl. Acad. Sci. U. S. A. 40, E5513 (2015) DOI: 10.1073/pnas.1513001112

4. T. Su, et al., eLife 6, e25642 (2017) DOI: 10.7554/eLife.25642

5. M. J. Abraham, et al., SoftwareX 1-2, 19 (2015) DOI: 10.1016/j.softx.2015.06.001

6. C. Kutzner, et al., J. Comput. Chem. 36, 1990 (2015) DOI: 10.1002/jcc.24030

7. L. V. Bock, M. H. Kolář, H. Grubmüller, Curr. Opin. Struc. Biol. 49, 27 (2018) DOI: 10.1016/j.sbi.2017.11.003