

Influence of high temperature and high pressure on the Nip7 proteins from the hyperthermophilic archaea *P. abyssi* and *P. furiosus*: molecular dynamics simulation analysis

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High temperature and hydrostatic pressure are severe for living cells. However some organisms can survive and proliferate at deep-sea hydrothermal vents with high temperatures (above 100°C) and pressures (hundreds times greater than atmospheric). The molecular mechanisms of the adaptation of these organisms to extreme environments remain unclear.

In this work we apply comparative analysis of the molecular dynamics simulation of Nip7 proteins from the hyperthermophilic archaea *P. abyssi* and *P. furiosus* species. *P. furiosus* inhabits shallow hydrothermal vents, it can exist under pressures not above 20 MPa (~200 atm; 1 atm≈0.1MPa). *P. abyssi* exists at the depth of about 2200 m (~22 MPa) but it can tolerate pressures up to 50 MPa.

Nip7 proteins are involved in ribosomal biogenesis, participate in 27S pre-rRNA processing and exosome function [1, 2]. It contains two domains, C-terminal PUA domain, involved in non-specific RNA-binding, and N-terminal supposed to be involved in protein-protein interaction with exosome. Recently, we observed that Nip7 proteins accumulates radical amino acid substitutions at high rates during *P. furiosus* species divergence from deep-sea common ancestor with *P. abyssi* [3].

Here, we investigated changes of the Nip7 polypeptide chain conformation and solvent accessibility at different pressures (0.1 - 300 MPa) and temperatures (300 and 373 K).

Both proteins do not unfold under all evaluated temperature/pressure conditions. We found that high temperature increase fluctuations of the polypeptide chain of both proteins. High pressure does not affect structure fluctuation but result in conformational changes of the proteins. Interestingly, the RMSD of the *P. abyssi* Nip7 model from the X-ray structure decrease with increasing pressure and temperatures.

Obtained data demonstrated that the RNA-binding domain of the *P. abyssi*/*P. furiosus* Nip7 protein is more flexible than N-terminal one. This flexibility may provide C-terminal domain functionality: non-specific binding of the poly-U/poly-AU RNA sequences. N-terminal domain demonstrates stable hydrophobic core and flexible loop regions. Additionally we found that segment of the Nip7 sequence 45-55 (helices $\alpha 2$ - $\alpha 3$) demonstrate high structure fluctuation with increasing temperature in proteins from both species. Interestingly, the sequence of this segment is highly conserved in archaeal Nip7 proteins. We suggested that hydrophobic patch formed by this segment may be involved in protein-protein interactions.

Our data also suggests that the interactions of these proteins with solvent are different and could be important for adaptation to high-pressure conditions at the protein structure level.

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