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Small-angle X-ray scattering study of histone-like protein from *Spiroplasma melliferum* in solution

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Motivation and Aim: Histone-like proteins (HU) are the most abundant and ubiquitous nucleoid associated proteins in bacteria. These small dimeric proteins bind, bent, and wrap genomic DNA playing important roles in regulation of the major DNA-dependent transactions. Despite the well-known involvement of HU in the regulation of viability and virulence of pathogenic bacteria, only few chemical inhibitors of HU with antibacterial effects were reported until now. One of the numerous putative reasons preventing the successful development of such inhibitors is the inconsistency between the crystal structures of HU used for structure-based drug design and the shape, size and folding of the protein and in a solution.

Methods and Algorithms: In this study, a solution of HU from *Spiroplasma melliferum* (HUSpm) was studied by small-angle X-ray scattering (SAXS). The experimental SAXS curves were compared with theoretical curves calculated for both the HUSpm crystal structure (PDB ID 5L8Z) and dynamic models of solution structure obtained by the combination of the NMR spectroscopy and molecular dynamics (PDB ID 5OGU). The values of following parameters were analyzed: *hi2* -reduced chi-squared, R_g – Guinier radii, D_{max} – maximum distances in the molecule calculated using coordinates of the atoms, D_{mon} – distance between mass centers of the monomers in the dimer, *MW* – molecule weight.

Results: In all cases, both *Rg* and *MW* confirmed dimeric conformation of HUSpm. The 15 models from the NMR structure 5OGU were grouped into 2 clusters. All structures from the cluster-1 have smaller *hi2* (better agreement with experimental SAXS curve) and D_{mon} than those from cluster-2. R_g, and D_{max} of the cluster-1 structures were also smaller. One can conclude that shorter D_{mon} corresponds smaller values of R_g, and D_{max}. The best fitting to the experimental SAXS curve was obtained for structure 2 from cluster-1 with *hi2* = 1.28, while the crystalline structure fitted to the experimental curve with *hi2* = 1.36 (Fig. 1A). Values of *Rg*, *Dmax*, *MW* of the best theoretical curve corresponds to the parameters of the experimental SAXS curve within the experimental errors. Since, the best structure from cluster-1 has smaller D_{mon} than that of the crystal structure, one can conclude that in solution monomers of the HUSpm dimer probably are closer to each other compare to the crystalline state. Analysis of the interface between the monomers in the HUSpm dimer revealed two aromatic clusters of stacking interactions, which, as NMR-data analysis can suggest, are responsible for structural

plasticity of the molecule and can provide a possibility of mutual sliding of the monomers toward each other (Fig. 1, B and C) [1].

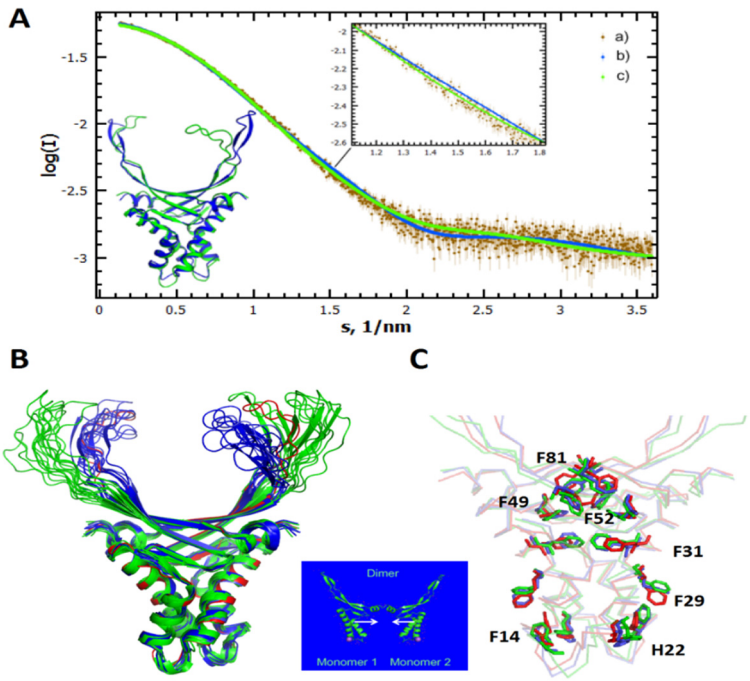

Fig. 1. Variations in the HUspm folding according to X-Ray, NMR and SAXS studies. A – An experimental SAXS curve (a) and its fitting with calculated SAXS curves for the 5L8Z crystal structure (b) and the best model from the 5OGU NMR structure (c). Superposition of the corresponding structures is shown at the bottom. B – Superposition of two clusters of the 5OGU structure (green and blue) and the 5L8Z crystal structure (red). C – Two aromatic centers of staking interactions in the interface between monomers of HUSpm. The color-coding is similar to B

Conclusion: Upon comparative structure analysis of the HUSpm dimer in solution and crystalline state and the analysis of calculated and experimental SAXS curves, we conclude that the distance between mass centers of the HUSpm monomers in solution is shorter compare to the distance in crystalline state; the difference of distances is about 0.1 nm.

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References

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