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

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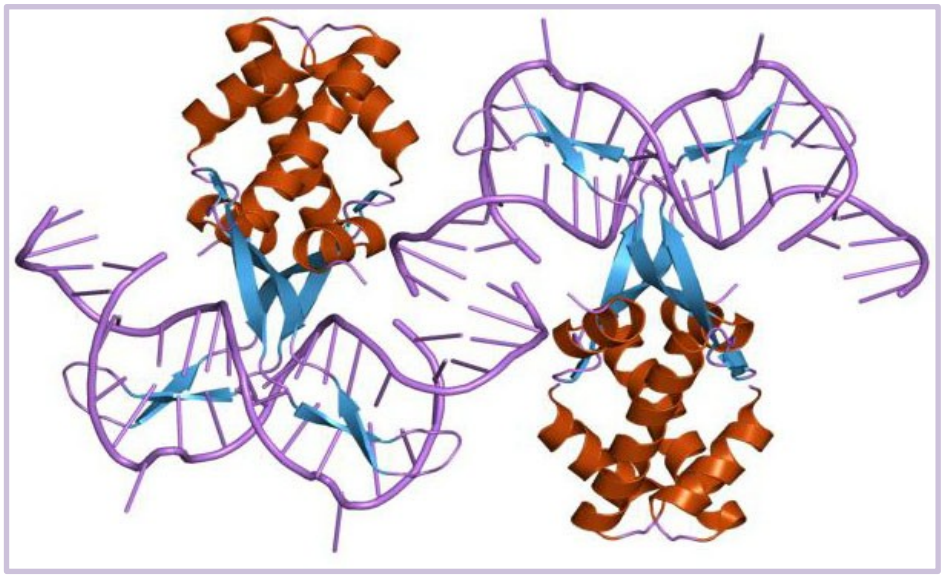
Motivation and Aim:

HU proteins are DNA-binding structural proteins of prokaryotes - analogues of histones in eukaryotic organisms.



Main functions:

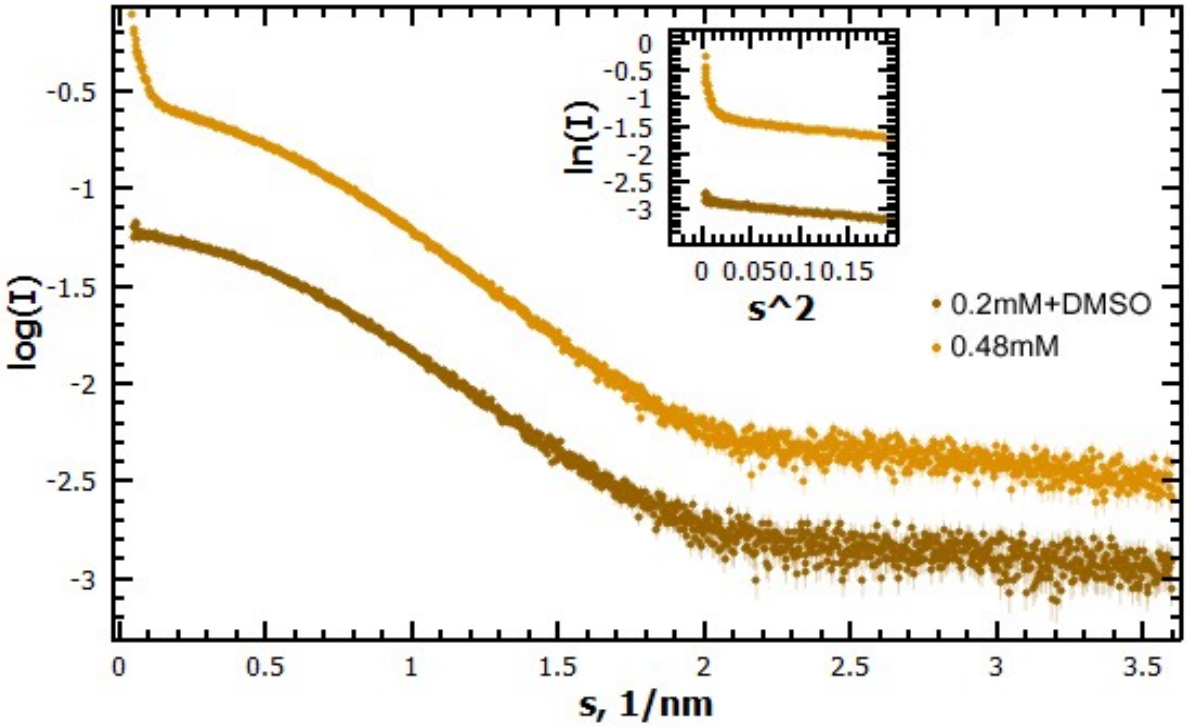
- Support for DNA supercoiling;
- Condensation of nucleoid;
- Regulation of transcription and replication processes;
- Participation in the processes of genomic DNA repair;
- Adaptation to stress conditions.

Despite the well-known involvement of HU in the regulation of viability and virulence of the pathogenic bacteria, only a 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 proteins in solution.

The aim of the study was comparative analysis of the HUSpm dimer structure in the crystalline state and solution.

Methods

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).

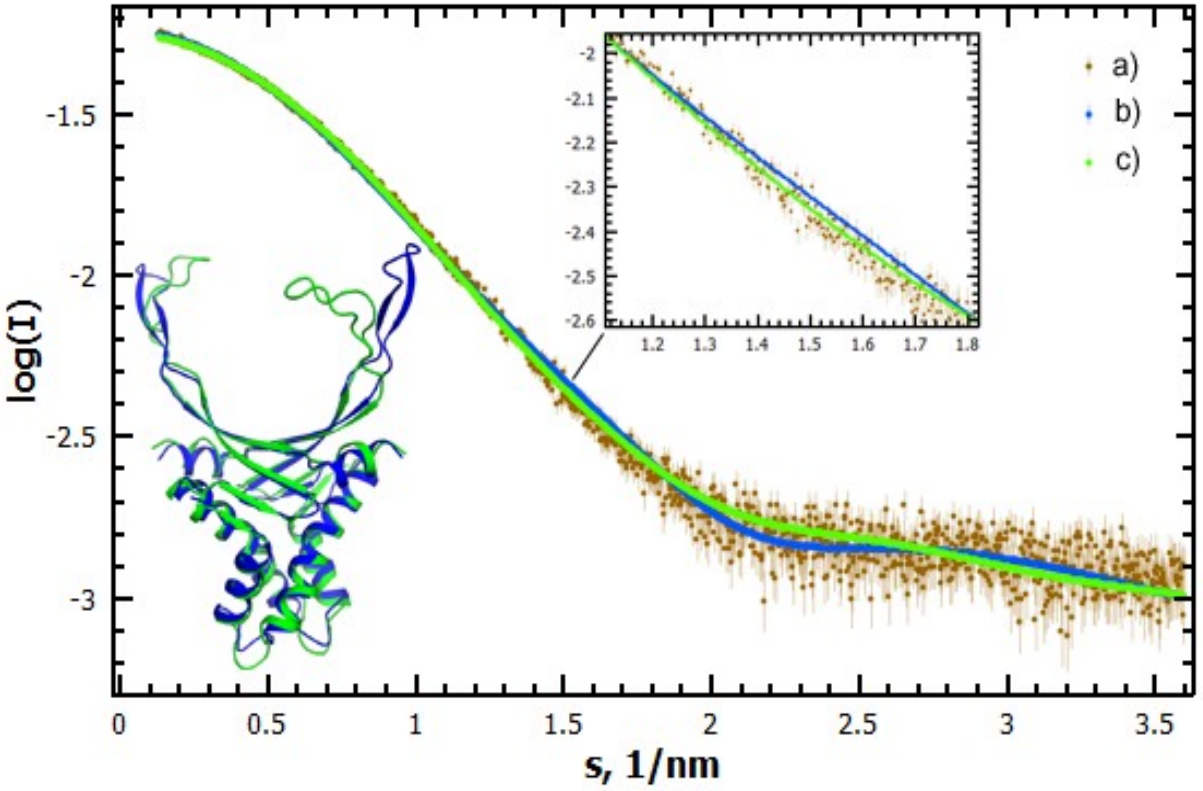


Comparison of SAXS curves obtained for different HUSpm samples. At the top of the main plot is a Guinier plot. All plots were created by PRIMUS software

Results

The comparative analysis of the following parameter values: *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, were performed.

It revealed two cluster, containing different type of structures among the HUSpm NMR structures. The structure with the best *hi2* value was structure 2 from cluster 1

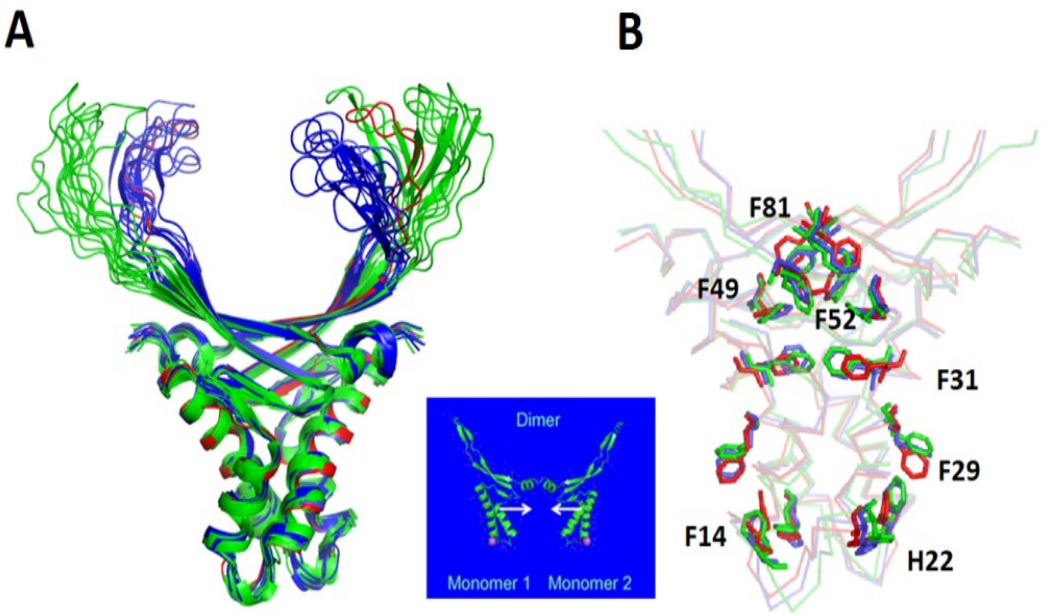


Experimental SAXS curve (a) and its fitting with calculated SAXS curves for 5L8Z X-Ray structure (b) and 5OGU NMR structure 2 from cluster 1 (c).

The inserts demonstrate the curves in more details and a superposition of the crystal structure 5L8Z (blue) and structure 2 from cluster 1 of 5OGU (green).

Conclusion

Upon comparative analysis of calculated and experimental SAXS curves complemented with structural analysis of the HUSpm dimer in solution and crystalline state, we conclude that the distance between mass centers of the HUSpm monomers in solution is shorter compared to the distance in the crystalline state.



HUspm folding according to the X-Ray and NMR structures.

A. Superposition of two clusters of the 5OGU structure (green and blue) and the 5L8Z crystal structure (red).
B. Two aromatic centers of staking interactions in the interface between monomers of HUSpm, which, according to the NMR-data, are responsible for structural plasticity of the molecule and can provide a possibility of mutual sliding of the monomers toward each other.

Acknowledgment

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