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The insight into structural properties of unique tardigrade radioprotective damage suppressor protein (Dsup)

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Motivation and Aim: Extraordinary stress-tolerance of the most radioresistant animal *Ramazzottius varieornatus* being promoted by the spread of specific families of tardigrade intrinsically disordered proteins (IDPs) as CAHS, MAHS, SAHS and Dsup. Active investigation of Dsup protein (UniProt P0DOW4) had already revealed it's direct DNA protecting role, moreover this effect was transferred to transgenic model human cells HEK293 [1, 2]. At this point, the mechanism of stress tolerance remains uncertain, but currently is interpreted through formation of macromolecular aggregate of DNA with highly charged and putatively intrinsically disordered Dsup protein (445-residues, 42.8 kDa), which leads to the promotion of defense from reactive oxygen spices (ROS). The goal of our work is an *in vitro* investigation of Dsup protein structure, that improves both an understanding of Dsup-involving mechanism of radioprotection and it's applications.

Methods: Dsup protein was expressed in *E. coli* and purified by the size exclusion chromatography (SEC). Structural features and stability of Dsup, it's self-assembling and complex formation were studied under native, chemically denatured and elevated temperature conditions by small angle X-ray (SAXS) and neutron (SANS) scattering techniques at FLNP JINR, MIPT and ESRF Grenoble. Secondary structure of Dsup protein and it's dynamical properties were determined by combination of circular dichroism (CD) spectroscopy, SAXS and computational approaches.

Results: In PBS solutions Dsup protein in 1.4–2.5 mg ml^{-1} concentrations is a flexible rod-like monomer (R_{flex}~0.85) of ~10 nm with presence of secondary structure in the middle region. Notably, the formation of globular and rod-like fiber oligomers of 4–6 molecules under native conditions was observed and we outline the structurization of oligomers is specific for Dsup protein. Additionally, chemical denaturation (6 M urea) reduced this process and the form of native oligomers differed from temperature-induced aggregates of Dsup protein (20–60 ºC).

Conclusion: Obtained data evidences intrinsically disordered properties of Dsup protein and approves the presence of secondary structure, also we report the formation of higher-order globular and rod-like oligomers through self-assembling. We expect, this topic is of special interest for neurodegeneration studies [3] as gives a special outlook into aggregation and fibrillization processes of DNA-binding IDPs.

References

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