FERRITIN-BASED FUSION PROTEIN SHOWS OCTAMERIC DEADLOCK STATE OF SELF-ASSEMBLY

***V. V. Sudarev*[1], *M. S. Gette*[1], *S. V. Bazhenov*[1], *O. M. Tilinova*[1], *E. V. Zinovev*[1], *I. V. Manukhov*[1], *A. I. Kuklin*[1,2], *Yu. L. Ryzhykau*[1, 2], *A. V. Vlasov*[1, 2]**

[1]Research center for mechanisms of aging and age-related diseases, Moscow institute of physics and technology, 141701, Dolgoprudny, Institutskiy per., 9;
Sudarev.vv@phystech.edu

[2]Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, 141980, Dubna, Joliot-Curie, 6

Ferritin is a unique self-assembling globular protein, which is present in the majority of living organisms. One ferritin globule consists of 24 identical subunits, which rapidly and strongly interact with each other in natural living systems. Due to its unique structural and functional features ferritin is widely used in nanotechnology as a drug carrier. In particular, the drug can be loaded directly inside the globule, or the globule could be used as a carrier for a drug or vaccine itself. Nevertheless, both applications face with ferritin self-assembly – the process of globule formation from monomers, which molecular mechanisms still remain enigmatic.

The process of ferritin self-assembly is not completely clear nowadays. Only a few works speculate on the kinetics and intermediate oligomers of the process. Authors of different works claim contradictory statements. Thus, Kim et al. shows 10-meric and 20-meric oligomers, while Sato et al. suggests 6- and 12-meric oligomers. Such oligomers are mentioned only as possible intermediates in the pathway of globule formation, but not as a separate fraction in protein solution. There is only one type of stable intermediates except the globule itself, the dimer. The existence of so-called «deadlock» intermediate states is discussed in a number of works; however, they were not observed directly in experiments. One of such commonly described «deadlock» states is an octamer. Despite the mentions in the starting works on ferritin self-assembly, an octamer wasn't observed in literature before.

In this study we reveal missing octameric state of ferritin self-assembly. We fused a ferritin subunit with a SMT3 protein tag, a homolog of human Small Ubiquitin-like Modifier (SUMO-tag), which was taken to destabilize ferritin 3-fold channel contacts and increase ferritin-SUMO subunits solubility. We first obtained the octameric protein complex of ferritin-SUMO (8xFer-SUMO) and studied its structural organization by small-angle X-ray scattering (SAXS). Obtained SAXS data correspond well with the high-resolution models predicted by AlphaFold and CORAL software showing an octameric assembly around the 4-fold channel of ferritin without formation of 3-fold channels. Interestingly, three copies of 8xFer-SUMO do not assemble into 24-meric globules. Thus, we first obtained and structurally characterized ferritin-based self-assembling oligomers in a deadlock state. Deadlock oligomeric states of ferritin extend the known scheme of its self-assembly process, being new potential tools for a number of applications. Finally, our results might open new directions for various biotechnological platforms utilizing ferritin-based tools.

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