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A new approach to finding the protein crystal growth conditions.

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Nowadays protein crystallization is an extensive area of the scientific research. Currently the crystallization conditions are usually obtained by trial-and-error methods and sometimes takes months and even years. So the possibility to obtain protein crystals with high X-ray diffraction (XRD) quality is not so predictable. Recently we proposed new approach for predicting crystallization conditions and controlling crystallization process, based on finding conditions the creation of special protein oligomers at solutions [1].

The objective of present reports to describe our results to protein crystallization mechanisms, identify the structures of possible crystal growth units, study a correlation between crystal formation in the solution containing certain oligomer types and the optimal crystallization conditions.

We hypothesize that at the initial step of the crystallization process there had to be an intermediate phase between disordered solution of protein molecules and ordered crystalline phase in solutions. According to our assumption, the formation of ordered oligomers from monomers is preceded the nucleation process. The mutual arrangement of molecules in the crystal structure due to oligomers formation and oligomers themselves are the building blocks of the future crystal [2].

We studied the various states of protein molecules in the crystallization solution by SAXS (Small-Angle X-ray Scattering) and SANS (Small-Angle Neutron Scattering) techniques. SAXS experiments were performed at DICSY beamline (NRC "Kurchatov Institute", Moscow) and BM-29 beamline (ESRF) [3], SANS experiments were performed at YuMO beamline (IBR-2, Dubna). We observed the structural changes of protein solution due to the presence of various types of the precipitant added. We also studied temperature, protein and precipitant concentrations, the ionic composition of the precipitant influence on the protein solution structure at early stages of crystallization. The study of protein conformation was performed under various temperatures. And also the effect of substitution in the crystallization solution of ordinary water (H2O) to heavy water (D2O) was investigated.

The oligomers models obtained from the crystalline molecular packing analysis were used in the experimental SAXS/SANS data processing. We extracted some elements (parts or blocks) in a protein crystal structure, which are ordered oligomers, and assumed that these oligomers could be 'building blocks' in the crystal growth process (fig.1).

The most detailed our research was the study of tetragonal HEWL (Hen-Egg White Lysozyme) crystal growth. The results showed a noticeable presence of lysozyme dimers and octamers under the crystallization conditions, and the total absence of oligomers under the conditions when crystal growth was impossible. We identified the structures of possible crystal growth units of tetragonal HEWL and revealed the correlation between the specific types of oligomers formation in solution and the optimal crystallization conditions.

Experimental evidence of our assumption was obtained in our studies of the molecular complexes presence in the solution, which had provided the growth of a few protein crystals (thermolysin, proteinase K, various forms of lysozyme).

Efficiency of proposed approach helped us to performing a modification of the Langmuir-Schaeffer technique for the high quality protein films making.

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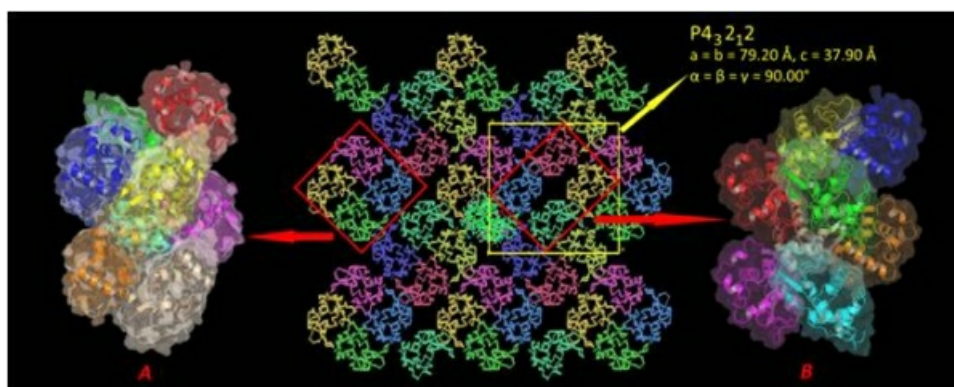
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