15-Crystal Growth

15.01 - Crystallization of Biological Macromolecules

MS-15.01.01 Rational Crystallization of Biological Macromolecules
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Fifth ICCMB* held in San Diego, August 8-13, was focused on the integration of molecular biology, biochemistry and biophysics in crystallization of biological macromolecules. In addition, a satellite meeting-membrane crystallization workshop has been organized in order to integrate membrane protein crystallization in one unifying concept of modern technology for crystallization of macromolecules.

Four major developments will be reviewed:

a) expression vectors and design of proteins for crystallization. This is the most rapidly developing field and the review will be supplemented by examples of single step purification of proteins.

b) development of detergents for membrane protein crystallizations.

c) rapidly developing applications of SAXS and dynamic light scattering for monitoring the intermediates present in early stages of crystallization.

d) new fast screening methodologies for searching for crystallization conditions.

Each of these topics will be illustrated with examples of crystallization of protein kinase (catalytic subunit of protein kinase A) as reported from author’s laboratory and p21ras as reported in Poger Goody’s (MPI Heidelberg) and Fred Wittinghofer’s (MPI Heidelberg) laboratories.

*ICMB-International Conference on Crystallization of Biological Macromolecules

MS-15.01.02 SMALL ANGLE NEUTRON SCATTERING STUDY OF THE INITIAL STAGE OF THE SYNTHESIS OF THE MICROCRYSTALLIZATION PROCESS. By N. Nimura*, Y. Minezaki, M. Ataka, and T. Katsura, Advanced Basic Research Center, Japan Atomic Energy Research Institute, Tokai, Ibaraki-ken, Japan, and Laboratory of Nuclear Science, Tohoku University, Sendai, Japan, Research Institute For Polymers and Textiles, Tsukuba, Japan

Despite the enormous amount of information obtained from atomic resolution crystal data, the difficulties encountered in growing crystals preclude structural X-ray studies for the majority of known isolated proteins.

We have conducted small angle neutron scattering (SANS) experiments on the time evolution from the initial stage to the visible size of crystallization of hen egg-white lysozyme. SANS from several kinds of solutions has been carried out. One example of solutions, where single crystals of lysozyme grow for a day, is that the concentration of lysozyme and NaCl is 20 mg/ml and 0.1 M, respectively, pH 4.6 and the temperature is 18°C, and the SANS results showed the distinctive change of time evolution. On the other hand, another example of solutions, where single crystals never grow, did not show any change of SANS time evolution at all. We have also conducted the experiments under various unsaturated conditions. From these experiments, we could observe how the aggregation of lysozyme molecules transferred into the crystallization.

MS-15.01.03 INTERACTION OF CRYSTALLIZING AGENTS WITH PROTEINS IN THE CRYSTAL
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Among the many parameters which affect protein crystallization, the nature and concentration of the salt used as a precipitating agent is known to influence strongly the solubility and polymorphism of the proteins. In the case of lysozyme, we have shown (Ries-Kautz, M. & Ducruix, A. (1989). J. Biol. Chem., 264, 742-746) that the main effect is due to anions following reverse Hofmeister series. Thiocyanate and para-toluene sulfonate anions are the most efficient to crystallize lysozyme at low concentration at 18°C and pH 4.5. These results were extended to other basic proteins and specific interactions between anions and proteins were postulated (Ries-Kautz, M. & Ducruix, A. (1991). J. of Crystal Growth, 101, 20-25).

In order to localize potential targets at the surface of the proteins, we crystallized lysozyme in the presence of SCN-, para-toluene sulfonate, N3-, F- salts of sodium after removing all counter-ions from the protein. Crystal data were recorded at high resolution. Presence of salt molecules interacting with the protein and their influence on packing will be discussed.

MS-15.01.04 PROTEIN CRYSTALLIZATION IN MICROGRAVITY. By R.-C. Br, L.-L. Guo, K. Shi, Y.-F. Wang, S.-Z. Chen, G. Pan, Y.-L. Hu, F.-L. Shen, X.-Z. Niu, Z.-Q. Huo, G.-Y. Li, J. Zhang, S.-L. Li, W.-M. Gong, L.-W. Niu and C.-Q. Huang, Institute of Biomedical Engineering, Academia Sinica, Beijing 100080, Department of Biology, University of Science and Technology of China, Hefei 230026, Department of Chemistry, Peking University, Beijing 10087, China

Microgravity environment of space is an ideal place to study the complicated protein crystallization and to grow good-quality protein crystals. We have carried out a series of crystal growth experiments of different proteins on Chinese re-entry satellite FSW-II in space. This mission lasted for about two weeks in 1992 and the experiments were performed at 65±5°C using a tube crystallization equipment made in Shanghai Institute of Technical Physics, Academia Sinica. More than half of the samples provided crystals, and among the 9 proteins crystallized in space, hen-egg white lysozyme and an acidic phosphatase type A2 from venom of Agkistrodon halys Dallos yielded larger, more uniform and less crystals in space than in ground controls. X-ray analysis of these-dimensional diffraction data for tetragonal lysozyme crystals showed that the space-grown crystals possessed better internal order than that of the crystals grown on earth.