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Protein crystallization in a magnetic field

Da-Chuan Yin*

Key Laboratory for Space Bioscience & Biotechnology, Institute of Special Environmental Biophysics, School of Life Sciences, Northwestern Polytechnical University, Xi'an 710072, China

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Abstract

The rapid advance in superconducting magnet technology enables more and more applications for the use of high magnetic fields in scientific researches and industrial manufacturing. These applications include material processing, separation, chemical reaction, nuclear fusion, high energy physics, and many more. Generally, a superconducting magnet provides both homogeneous and inhomogeneous magnetic fields simultaneously, and both can affect the samples in the field so that the magnetic field can be utilized for various purposes. A homogeneous or inhomogeneous magnetic field will exert a torque on suspending particles in a solution if the particles have anisotropic magnetic susceptibility, which will further influence the properties of the solution; in an inhomogeneous magnetic field, a repulsive force will act on a diamagnetic solution so that the levels of apparent or effective gravity of the solution can be tuned in a vertical magnetic field. These effects can be utilized to govern the physical and chemical processes in solution like crystallization. In recent years, high magnetic fields have been applied in protein crystallization. It was found that a magnetic field can align the crystals along the field direction, decrease the diffusivity of macromolecules in the solution, and increase the viscosity of the solution; a suitable inhomogeneous magnetic field can damp the natural convection substantially, which resembles the case in a space environment. Both homogeneous and inhomogeneous magnetic fields have been found to improve the quality of some protein crystals. These discoveries showed that the researches on protein crystallization in high magnetic field is potentially valuable, because obtaining high quality protein crystals is important for 3-dimensional structure determination of proteins using X ray crystallography. This paper will review the background and more recent progress and discuss the future perspectives in this research field.

Keywords: Protein; Nucleation; Crystal growth; Crystallization; Microgravity; Magnetic field; Levitation; Biomacromolecules

1. Introduction

1.1. Why study protein crystallization?

Studies on the structure and function of biological macromolecules (e.g., proteins, nucleic acids) and their complexes are of essential importance for understanding the nature of life. It is well known that the biological macromolecules (especially proteins) play important roles in all biological processes. Typical examples of these processes are generation of energy, synthesis of complex compounds, transformation of complex compounds to simple ones, transmission of neural signals, and growth of bones, etc. Diseases are also closely related with the functions of the proteins.

* Tel./fax: +86 29 88460254.
E-mail address: yindc@nwpu.edu.cn.

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Rational drug design is to find suitable molecules to bind to and block an active site of a disease-related protein so as to cure the disease. Since the function is determined by the structure, to deeply understand their functions and further succeed in rational structure-based drug design, it is very important for researchers to obtain the structural information of the biological macromolecules.

There are several methods to obtain the 3-D structural information of proteins. Nuclear magnetic resonance (NMR) has been used as one promising technique to study the structure of biological macromolecules (including membrane proteins [1,2]) in solution [3,4], and it is also possible now to study relatively large biological macromolecules; molecular modelling (computer-aided model building) has been used to predict the 3-D structure of proteins [5–7]; neutron diffraction has been used to resolve the structure (with emphasis on identifying hydrogen atoms) of biological macromolecules in single crystals [8]; cryo-electron microscopy (Cryo-EM) has been applied to determine the structure of macromolecules [9,10] with emphasis on membrane proteins and large protein complexes which are hard to crystallize. More recently, Atomic Force Microscopy (AFM) has been also explored to probe protein conformations [11]. Apart from the above methods, the major technique to determine structure of biological macromolecules is X-ray diffraction (XRD) [12–15], which requires the samples in a crystalline state. Among the 105,499 entries deposited in Protein Data Bank (Data on Jan 11, 2015, Protein Data Bank, www.rcsb.org), approximately 88.85% of the solved structures were obtained by the XRD crystallography, while approximately 10.23% were determined by NMR, and only less than 1% were resolved by all the other methods (Cryo-EM and others). For protein structures, the percentage of the structures determined by XRD is even larger (89.59%). Thus, utilization of XRD to determine the structure of biological macromolecules is unquestionably important.

The diffraction techniques (X-ray, neutron and electron diffraction) require single crystals of high structural perfection as diffraction samples. For many years, however, the success rate in obtaining high quality protein crystals, which is critically important to obtain reliable and high resolution structure information of macromolecules, has been low. An estimated overall success rate is approximately 30–40% [16] from purified soluble proteins to diffracting crystals when concentrated efforts are made on each individual crystallization target, while in structural genomics projects the success rate tends to be even lower (from purified soluble proteins to diffracting crystals, the success rate is about 15%; from selected target to structure, the success rate is about 2.5%). Thus, though the total number of released entries in PDB database has exceeded 100,000, and is still rapidly growing, the trend should be attributed to the increasing total number of studied targets and the widely applied automated systems that enable efficient high-throughput structure determination. The crystallization of biological macromolecules is still a "bottleneck" for structural biology.

Normally the difficulties in crystallization are due to the following three problems: (1) how to prepare highly purified and mono-dispersed solution of macromolecules, (2) how to screen the crystallization conditions to obtain the first crystals; and (3) how to improve the crystal quality so as to guarantee the successful collection of X-ray diffraction data. In only a few cases the above mentioned problems can be solved without much trouble. While in most cases, it is difficult to find ideal solutions to these problems owing to many different parameters (pH, type of crystallization agent and concentration, protein concentration, temperature, etc.) are to be determined for a specific protein. Protein type itself is also one important factor. A number of proteins, like lysozyme and proteinase K, are easy to be crystallized, while many others are difficult. To increase the success rate, the mechanism and methodology studies on protein crystallization are necessary.

1.2. Why use magnetic field?

Among the problems mentioned in Section 1.1, preparing high quality protein crystals is of great importance for collecting high resolution X-ray diffraction data, because high quality protein crystals yield more detailed structural information. Then how to improve the quality of protein crystals?

There are many parameters which may affect the crystal quality of proteins. The quality of the protein sample (purity, homogeneity, mono-dispersity), level of supersaturation (concentration, temperature, temperature stability), mass transport (convection, diffusion), physical and chemical environment (magnetic field, electric field, gravitational field, pressure, pH level, types of crystallization agents), etc. are possible reasons affecting the crystal quality of proteins. To obtain high quality protein crystals, one shall take good control of these parameters.

Mass transport is one of the most frequently addressed issues when studying protein