学 位 論 文 審 査 の 要 旨
Doctoral Dissertation Evaluation Review

博士の専攻分野の名称	博士（生命科学）	氏 名	李 龍
Degree requested	Doctor of Life Science	Applicant name	LI Long

審査担当者			
Examiners	主査 / Chief examiner	教 授	姚 閔
	副査 / Associate examiner	教 授	玉置 信之
	副査 / Associate examiner	准教授	尾瀬 農之
	副査 / Associate examiner	助教	中島 祐

学 位 論 文 題 名
Title of Doctoral Dissertation

Developing novel methods for protein crystallization
（新規タンパク質結晶化法の開発)

博士学位論文審査等の結果について（報告）

The structure of proteins is essential for understanding the structure-function relationship of proteins which play an important role in biological and medical science. To determine the 3-D atomic structure of proteins, X-ray diffraction or neutron diffraction of crystals is a powerful method. However, the preparation of protein crystals good enough for diffraction experiments is still the main problem because the theory of crystallization is still unclear, resulting in no efficient and practical method for protein crystallization. Generally, the process of crystallization can be divided into three steps: (1) the nucleation, (2) crystal growth, and (3) termination. In this study, LiLong focus on the first two steps.

I. Enhancement of protein crystallization through the heterogeneous nucleation

Nucleation is classified into homogeneous and heterogeneous cases. Homogeneous nucleation occurs in the solution without preferential nucleation sites. Many approaches of homogeneous nucleation have been developed, however, those methods still largely depend on trial and error, and none of them has been proven as an efficient method in X-ray/neutron crystallography. Unlike homogeneous nucleation, heterogeneous nucleation happened at the surface of nucleants. A variety of nucleants has been reported in the crystallization of some typical proteins. So far, the development of efficient and practical nucleants is still underway.

Considering the advantages of homogeneous and heterogeneous nucleation, LiLong planned to develop nucleants for initializing protein molecular packing. For this purpose, LiLong chose the

lattice-surface of nucleants for inducing the nucleation of protein crystals. Furthermore, according to experimental results, LiLong found that nucleants with short lattice distance (< ~10 Å) does not promote crystallization of proteins because of large volume size (~30 Å to 75 Å~) of proteins. Subsequently, LiLong selected and designed novel nucleants with large lattice distance as possible as he can synthesize. As a result, he found that a metal coordination framework, Co-cage-1 with 37.5 Å lattice distance, facilitates the nucleation of the protein crystal. Among 13 tested proteins (nine of them are very difficult to crystallize and have been tried for long time), successful crystallization of 11 proteins (11kDa~110kDa, six crystal structure were determined) proved the capability of Co-cage-1. In addition, he found that the surface topography also plays a key role in nucleation besides the lattice. Furthermore, in situ the observations of nucleation on Co-cage-1 surface were performed using Cryo-TEM and HS-AFM. Based on the results, a heterogeneous nucleation mechanism was proposed. The findings of this study lead to design rationally the efficient and practical nucleants for crystallizing proteins.

II. Growth of huge protein crystals for neutron protein crystallography

Neutron macromolecular crystallography (NMC) is a powerful method to obtain the accurate position of key hydrogen atoms, which are difficult to visualize by X-ray crystallography lonely. However, among over 13 x 10^4 crystal structures deposited in PDB, only about 120 structures (MW < 24 kDa) are determined by neutron diffraction. One of the challenges in NMC is to grow crystal of large volume (> 1 mm^3), specifically for large protein (MW > 60 kDa) with large unit cell (> 150 Å).

Our target protein is glutamine amidotransferase CAB (GatCAB), in which the ammonia is proposed to transfer through the inner channel of 30 Å in length. To visualize the ammonia in the channel, neutron diffraction studies are indispensable. However, the following three reasons make GatCAB becomes to a difficult sample for neutron diffraction: (1) the largest parameter of unit cell c = 180 Å; (2) MW is 110 kDa; (3) needle sharp of GatCAB crystal. LiLong adopted a temperature-responsive copolymer (TRCP) to adjust the crystal growth. As a result, he successfully obtained the large GatCAB crystals up to a volume of ~1.8 mm^3, collected neutron diffraction data and solved the neutron structure. These results represent a challenge in current neutron diffraction technology.

In conclusion, in this study, the applicant did not only develop a method for growing large protein crystals for NMC and developed also efficient nucleants for crystallizing proteins and proposed a heterogeneous nucleation mechanism. These developments will provide important contribution to the field of structural biology. In addition, through this study, the applicant has acquired a scientific intellectual power and acting power.

Therefore, we acknowledge that the applicant is qualified to be granted the Doctorate of Life Science from Hokkaido University.