Study of Static Magnetic Field on Crystallization of L-alanine

（高强磁场对丙氨酸结晶成长的影响研究）

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PREFACE

Today, in life science and bioengineering the most important problem is probably to understand the relationship between the primary sequence, the higher order structure and the function of proteins. One of the most critical aspects of the problems, is understanding the structure of different protein itself which constitutes different lives. Two methods are now commonly used to determine the atomic structural models for proteins: nuclear magnetic resonance and X-ray crystallography. Although nuclear magnetic resonance allows the direct, noninvasive determination of protein structure, it is hampered by low sensitivity and is limited to protein less than 30,000 molecular weights. Until April 1999, about ten thousands of kinds of protein structures have been registered by the Protein Data Bank. Among them, about eight thousands of kinds were analyzed and obtained by X-ray crystallographic method which has become more and more important for protein structure analysis. However, X-ray crystallographic method is not only limited in some respects requiring a single, high order, three-dimensional crystal, but also is the availability of high-quality crystals in order to perform structural characterization-besides sufficient size (typically 0.1mm in all dimensions), the quality of protein crystals determines the accuracy and reliability of the three-

dimensional structure analysis.

On the other hand, actual requirement of new functional crystal materials in various areas such as pharmaceutics, foods and optics also make the same request to crystallization. For industrial crystallization, these requirements mean high quality, ideal habit, size and structure etc.

There are many advances in the methods for crystallizing, growing and determining all kinds of biophysical or biochemical parameters that have been taken into account in order to obtain high quality crystals. Until now, several methods considered to control the crystal habit, size, structure and quality have been attempted to solve these "bottleneck" problems in crystallization. These conditions include microgravity, high pressure (or hydrostatic pressure) and external electric field, additives and the magnetic field.

This work was set about with the most basic problem of crystallization-crystal growth rate. With a number of numerical data of growth rate, it was headed for investigating crystal growth rate change within different magnetic field intensity and different magnitude of magnetic force. We also investigated additives effect within the magnetic field, as far as we know, until now there is no research about additives within the magnetic field. All these research about crystal growth rate was respected to be useful for clarify the whole growth progress of L-alanine grown from its pure aqueous and solutions with additives used within the magnetic field and its mechanism.

Previous studies on crystallization and existing problems were stated in **Chapter 1**. And in this book, we mainly pick up of the influence of magnetic field on crystal growth rate.

Chapter 2 deals with the magnetic field intensity effect on growth

rate of L-alanine crystal from its pure aqueous solution. The growth rate of the $(\bar{1}20)$ and (011) faces of an L-alanine crystal from aqueous solution was investigated in situ using an optical microscope system within a static and homogenous magnetic field from 0T to 5T. A clear inhibition in the growth rate of the two faces was observed within the magnetic field. Crystal growth rate decreased with increased magnetic field intensity from 0T to 1T, while it had no clear change when magnetic field intensity changed from 1T to 5T. The growth inhibition induced by the magnetic field was attributed to a lower mass transfer, which was assumed be caused by a decreased flow velocity of buoyancy convection and crystal orientation in suspending state in solution.

Chapter 3 deals with magnetic gradient effect on the growth rate of L-alanine crystal. Crystal growth rate of the $(\bar{1}20)$ and (011) faces of an L-alanine from aqueous solution was investigated in situ using an optical microscope system. The measurement were carried out at different positions of 0, 60, 125 and 160mm upper or lower from the center of the magnet where magnetic field intensity was 5T and the solution will experiences upward or downward magnetic force equal to 2.4%~5.7% of its gravity. Crystal growth rate was found decreased with experiencing upward magnetic force and increased when the force is downward. Convection rate change by magnetic force was considered as one reason: upward magnetic force decrease solution convection rate thus decrease crystal growth rate, and vice versa.

Chapter 4 deals with the magnetic field effect on growth rate of L-alanine crystal from aqueous solution with L-glutamine and L-aspartic acid as additives which concentration was 2%. The crystal growth rate was measured at different supercooling of 2℃, 5℃, 7℃ and 10℃ within the

magnetic field intensity of 5T and without the magnetic field. Crystal habit modification under different supercoolings within and without magnetic field was also investigated. It was found that without magnetic field, crystal growth rate of L-alanine was decreased with L-glutamine or L-aspartic acid was added. While within magnetic field, this inhibit effect become weaker. On the other hand, when L-glutamine or L-aspartic acid was added, crystal growth rate within magnetic field was faster than that without magnetic field.

Finally the general conclusions were summarized in **Chapter 5**. Further studies of this subject were also stated.

This book is edited by Zhao Bin, an associate professor of Industrial and Commercial University of Chongqing. Thanks to the Prof. K. Shimizu's guidance and valuable advice in the process of writing and other people including E. Suzuki and L. A. Guzmann, K. Ogawa and Y. Mitobe. This book is full of content and full citation, and it is a rare reference book for the research field of crystal growth and seed cultivation.

Because of the hasty time, the book is unavoidable or could not meet the needs of the reader, please peer criticism.

CONTENTS

CHAPTER 1 INTRODUCTION

1.1 Previous review

Today, in life science and bioengineering the most important problem is probably understanding the relationship between the primary sequence, the higher order structure and the function of proteins. One of the most critical aspects of the problem, is understanding the structure of different protein itself which constitutes different lives. Two methods are now commonly used to determine the atomic structural models for proteins: nuclear magnetic resonance and X-ray crystallography. Although nuclear magnetic resonance allows the direct, noninvasive determination of protein structure, it is hampered by low sensitivity and is limited to protein less than 30,000 molecular weight. Until April 1999, about ten thousands of kinds of protein structures have been registered by the Protein Data Bank. Among them, about eight thousands of kinds were analyzed and obtained by X-ray crystallographic method which has become more and more important for protein structure analysis. However, X-ray crystallographic method is not only limited in some respects requiring a single, high order, three-dimensional crystal, but also is the availability of high-quality crystals in order to perform structural characterization-

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There are many advances in the methods for crystallizing, growing and determining all kinds of biophysical or biochemical parameters that have been taken into account in order to obtain high quality crystals. Until now, several methods considered to control the crystal habit, size, structure and quality have been attempted to solve these "bottleneck" problems in crystallization.

1.1.1 Crystallization under several kinds of conditions

Recently crystallization especially protein crystallization has been investigated under several kinds of conditions by many researchers. These conditions include microgravity, high pressure (or hydrostatic pressure) and external electric field, additives and the magnetic field. Studies under these conditions are reviewed briefly as following:

Crystallization under microgravity

Gravity is generally considered unfavorable for crystallization since it gives rise to solution convection and in many cases, to sedimentation of small crystal. In order to counterbalance gravity to eliminate crystal flaws caused by convection and sedimentation also keep the growing crystals from crashing to the bottom of the vessel, microgravity environment was attemped to apply in crystallization. Since the 1980's many protein

crystallization studies have been carried out under microgravity. However, until now different groups have reported different even conflicting results. Nowak (1995) reported that there's a common perception that crystal growing in space has failed to live up to its promise of producing protein crystals that are bigger, better and more suitable for gleaning a protein's 3D structure than those grown on Earth. Erdmann (1989) reported that a number of space-grown crystals were larger in size and of a better quality to diffract X-rays using a Chinese re-entry system than the corresponding ground control crystals grown at the Chinese launch site. But the space-grown crystals have not reached the X-ray diffraction quality of the crystals obtained under condition in the home laboratories. Helliwell (1993), reported that the mosaicity of the microgravity crystals, minimum 0.0010(1) degrees, where three times smaller than the corresponding earth controls, minimum 0.0032(1) degrees. Also Helliwell (1995) reported that the monochromatic data collection on beamline gave microgravity rocking widths of 0.0017 at minimum compared with 0.0067 for earth grown controls. It was noted that the decrease in rocking width is proportional to the increase in peak height of reflections with, after corrections for volume in the beam, the microgravity crystals displaying peak intensity levels three to four times that of the earth grown counterparts. These two studies suggested that the resolution of X-ray diffraction for microgravity-grown crystals is much better than Earth-grown crystals. The latter are actually composites of not so well aligned blocks of crystal that result in a broadening, or even a splitting, of the x-ray reflections. In space-grown crystals, these cells tend to be better aligned throughout the crystal, a feature known as low mosaicity which can improve the signal-to-noise ratio and should result in improved precision in determining crystal

structures. Besides, Normil (1995), McPherson (1999), Otálora (1999), Carter (1999), Eschenberg (2000) and Kitano (2000) also reported somewhat different findings. These findings have made research under microgravity one of the hottest challenges in crystallization. So far as these reports, most investigation reported results similar to Erdmann (1989): better resolution of X-ray diffraction of crystal grown under microgravity was got but it is not good enough for structure analysis with X-rays yet.

Crystallization under high pressure

This condition was carried out with exerting pressure directly to solution to produce hydrostatic pressure. Until now different conclusions about pressure effect on crystallization were found, also different machenism was explained as the reason respectively. The pressure effect on solid-liquid equilibrium has been known as early as 1914, but its application was begun in the 1960's (K. Visuri, 1990). It has been observed that high pressure sometimes denature and inactivate proteins (Morild, 1981). Suzuki (1994) measured the change of the solubility, the nucleation rates and the normal growth rates of lysozyme crystal with applying hydrostatic pressure to solution and made the following conclusions: ① Solubility under high pressure was larger than low pressure; ② Nucleation rates decreased when pressure was increased; ③ Crystal growth rate was also decreased when pressure was increased; ④ Ratio of crystal growth rate of two faces was also changed when pressure was increased which led to crystal habit modification. Here increase of solubility was explained on the basis of law of mobile equilibrium (Le Chatelier's law):

$$\frac{\partial \ln K}{\partial p} = -\frac{\Delta V}{RT}\left(\Delta V = V_{solute} - V_{crystal}\right) \tag{1}$$

K, p, R, T, and $V_{crystal}$ represent the equilibrium constant for the crystal-solution equilibrium, the pressure, the gas constant, the absolute temperature, the partial molar volume of the solute in the solution and the molar volume of the crystal, respectively,. According to eq. (1), if V_{solute} is smaller than $V_{crystal}$, the crystal dissolves into solvent when pressure is increased that is coincide with experimental data of this study. The nucleation rate, J, can be expressed by

$$J = vaq_1 \exp\left(-\frac{\Delta G}{kB}\right) \tag{2}$$

where v, a, q_1, ΔG, k and B represent the collision rate of monomers with critical nuclei, the sticking probability, the concentration of monomers of lysozyme in solution, the activation energy for a lysozyme molecule to form a critical nucleus, the Boltzmann constant and the absolute temperature, respectively. In the case of a spherical nucleus, ΔG can be expressed as:

$$\Delta G = \frac{16\pi \Upsilon^3 \Omega}{3\left[kT\left(\ln C - \ln C_e\right)\right]^2} \tag{3}$$

Where Υ, Ω, C and C_e represent the surface free energy of the lysozyme crystal, the average volume occupied by a lysozyme molecule in the crystal, the concentration of the lysozyme solution and the equilibrium concentration, respectively. Since Ω changes little with pressure and Υ is constant with respect to pressure, it is strongly suggested that pressure increased the solubility; hence the nucleation rate was decreased by applying pressure.

The normal growth rate, G, is proportional to the velocity of step movement and is expressed as

$$G = s\beta_{st}\Omega\left(C_{surf} - C_e\right) \tag{4}$$

Where s, β_{st} and C_{surf} represent the slope of a crystal surface to a selected singular face, the kinectic coefficient for a step and the local concentration at the crystal interface. According to eq. (4), the normal growth rate, G, decreases when C_e is increased. In one word, the increase of solubility was assumed to be responsible to the reduction of the nucleation rates and crystal growth rate. Besides, Schall (1994), Saikumar (1995), Gross (1996), Lorber (1996), Takano (1997), Webb (1999), Suzuki (2000), Suzuki (2000), Waghmare (2000), Waghmare (2000) and Nagatoshi (2003) measured the solubilities of lysozyme and subtilisin crystal and found the same results that with increasing pressure, their solubilities were increased that led to the decrease of crystal growth rate under the same supercooling.

However, there are also some researchers such as Miller (1988) who found that both growth and activities are accelerated when pressures increases. K. Visuri (1990) reported that high pressure affected the crystallization of glucose isomerase dramatically: ① With increasing pressure a rapid onset of nucleation of glucose crystal occurred in such a short time as 2 minutes; ② The rate of crystallization increased with increasing pressure; ③ The certainty of the onset of the crystallization was also increased: at high pressure crystals were formed in every experiment while at low pressure often no crystallization was observable within several hours despite highly supercooled conditions. No special understandable reason for these changes was stated, but the researcher

assumed that the controlling mechanism for the crystal growth rate might be the surface reaction when the molecules fit into a grid. This assumption is based on the fact that the ordinary mass transfer rate from the bulk liquor to the surface of the crystal is not greatly dependent on pressure.

Sazaki (1999) stated that pressure act differently on the same crystal with different crystal habit: the solubility of tetragonal crystals increased with pressure while that of orthorhombic crystals decreased. This difference was explained that different crystal habits have different volume change of water-hydrated molecules that led to different solubility change.

Crystallization under external electric field

Proteins are polymers of amino acids containing dipoles and ionic groups suscesptible to be influenced by an external electric field. In order to obtain crystals, protein molecules are dissolved in a "mother liquor" containing some salts and precipitant agents at define pH values. The net charge of the biological molecule depends on the pH value of the solution and it is possible to obtain migration of these molecules in the solvent when an electric field is applied. In this way, it could be possible to create some supersaturated areas leading to crystals. Based on this consideration, research about protein crystallization under an external electric field were first set up by Taleb (1999). Taleb (1999) showed that the electric field suppressed lysozyme crystal nucleation and simultaneously improved the diffraction quality of lysozyme crystals. Nanev (2001) showed that preferred orientation along c-axis of lysozyme crystals has been found in an external electrical field. Besides, the crystals grew predominantly on the cathode side of the growth cell. A plausible explanation was given on the basis reported by Darby (1993) that the lysozyme molecules are

positively charged in the solution which makes the special distribution.

Crystallization within magnetic field

So far as recognitions of microgravity effect on crystallization, application and research of microgravity effect become hotter in recent years. People hope to carry out more and more experiments under microgravity to develop their recognitions also make better protein crystals. It is commonly considered that microgravity can suppress convection and sedimentation of solution to grow high quality crystals. However many problems such as its dramatically high cost, usage of cosmos, limitation of experiment time etc. make it impossible to research in many kinds of conditions that retard the recognition of microgravity effect on crystallization inevitably.

In recent years, with rapid development of superconducting magnetic field, application of the magnetic field become very simple and easy. There are two kinds of the magnetic field effects on the behavior and properties of materials. One is the microscopic effect on the dynamics of electron and nuclei through their magnetic moments, which gives the foundation to magnetic resonance, magnetic properties of solids, and the magnetic field effects on chemical reactions. These are subjects for basic studies in solid state physics and physical chemistry. The other kind of effects arise from the influence of the magnetic force acting on the materials due to their macroscopic magnetic properties.

The magnetic field can be classified into two kinds according to its homogeneity: uniform field (magnetic field intensity was the same in different positions) and gradient field (magnetic field intensity was different in different positions). All proteins are diamagnetic substances whose atoms have no permanent magnetic dipole moment. Within uniform