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Review

Photochemically-induced crystallization of protein

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Abstract

I demonstrate photochemically induced crystallization of metastable hen egg-white lysozyme solution by weak UV irradiation for several tens seconds. The most effective irradiation time range is 10–60 s, and in this range the enzyme activity is maintained. Intermediates, neutral radicals at tryptophan residual produced by one-photon absorption, enhance nucleation. When the intermediate is selectively excited by visible light, the intermediate is denatured. At that time the light-induced nucleation is inhibited. This result indicates the intermediate induces nucleation. The radical forms lysozyme dimer that is detected by an SDS-PAGE electrophoresis experiment. An addition of polyethylene glycol (PEG) greatly enhances light-induced nucleation. PEG affects to shorten the intermediate radical lifetime, which suggests that PEG assists to form dimer. We consider that the photochemical dimer behaves as smallest cluster to grow critical nucleus. The smallest cluster formation is the rate determining step in classical nucleation theory due to surface energy disadvantage. The photochemical dimer is formed by a covalent bond, and the nucleation is initiated from stable dimer. The nucleation enhancement is reasonably explained. The present researches results point out the development of a new method for controlling nucleation and growth that could be applied for structural genomics and pharmaceutical industry for instance.

Keywords: Protein crystallization; Lysozyme; Nucleation

Contents

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Tetsuo Okutsu was born in 1961 in Kanagawa prefecture. He graduated from Tokyo Institute of Technology (BSc, 1985), and his DS degree (1991) was awarded by Tokyo Institute of Technology under the direction of Professor Kinichi Obi. He had worked at Fuji Photo Film for three years as research and studied preparation of high-sensitive silver halide AgBr grain for color photo film. He moved to the laboratory of Professor Hiroshi Hiratsuka at Gunma University as a research associate on 1994. Then he was promoted lecture (2000) and associate professor (2004). From 2003 to 2004, he studied protein crystal growth at CRMCN-CNRS at France as postdoctoral fellow. His scientific work has focused on the photochemically induced crystallization.

1. Introduction

An important area of post-genomic research is the determination of 3-D protein structures. The main technique used for this purpose is X-ray crystallography, which has become a valuable tool for the analysis of crystals with the use of synchrotron radiation. Techniques for the preparation of high-quality single crystals are being developed continuously. Protein crystallization experiments are carried out in the presence of crystallization agents, e.g., inorganic salts, non-adsorbing polymers, and alcohols, which reduce protein solubility and increase intermolecular interactions to form cluster, nucleus and crystal. In these experiments, account needs to be taken of the protein concentration, nature and concentration of the crystallization agent, pH, buffer constitution, and temperature. Screening, i.e., the incremental variation of these parameters in order to optimize the crystallization conditions, is time-consuming.

Since protein molecules have highly anisotropic features, nucleation does not take place spontaneously, even in supersaturated solutions. Fig. 1 shows the solubility curve for lysozyme in the presence of NaCl as the crystallization agent at 20 °C [1]. The supersaturation values, calculated according to the formula:

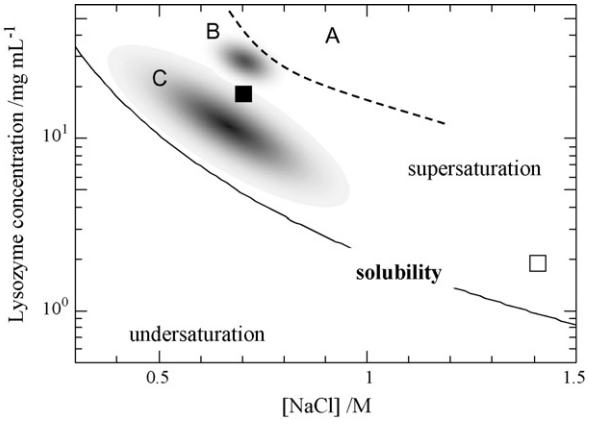

Fig. 1. Solubility curve of lysozyme against NaCl concentration after Ref. [1]. At the highest supersaturation (region A), lysozyme aggregation and/or liquid–liquid phase separation occurs. At intermediate supersaturation (region B), nucleation takes place spontaneously. At lowest supersaturation (region C), the so-called metastable zone, nucleation does not takes place but growth by seeding take place. Open square: supersaturation of the photo-irradiated solution in a liquid-seeding experiment. Closed square: supersaturation of the solution for growth process after mixing solutions.

$\beta = C/C_e$, where C and C_e are the concentration and solubility, respectively, of lysozyme, can be classified into three regions. At the highest supersaturation, i.e., $\beta > 12$ (region A, Fig. 1), the lysozyme molecules aggregate immediately; single crystals cannot be obtained from this region. At the supersaturation level of $9 < \beta < 11$ (region B, Fig. 1), moderate nucleation takes place. Although this level of supersaturation is sufficient for nucleation in region B, coagulation of the nucleus, invagination of the crystal or polycrystallization frequently occurs during the growth process due to the high-level supersaturation of the solution. In region C (Fig. 1), in which supersaturation is too low for protein crystallization, i.e., $\beta < 9$, the solution is metastable and nucleation does not occur, although the nucleus can grow slowly if it is seeded. The growth of crystals in this region is, to the best of our knowledge, the optimal method for obtaining high-quality single crystals without lattice defects.

Thus, one strategy to obtain a high-quality single crystal is to form the nucleus at a higher level of supersaturation (region B) and to grow the nucleus at a lower level of supersaturation (region C). Controlling supersaturation to establish conditions for nucleation and growth is a challenging task; temperature control is sometimes used to control the nucleation and growth [2–5]. Seeding techniques are often effective in metastable solutions [2]. An alternative strategy is to induce crystallization from metastable solutions using an external field, such as a magnetic or electrical field [6,7].

Light-induced nucleation has also been reported. Two types of light-induced nucleation, photochemical and photophysical, have been described. Photochemically induced nucleation in solution was first reported by Tyndall [8] and this type of nucleation in the vapor phase has also been reported [9–17]. Recently, I have demonstrated laser-induced control of organic crystal morphology using a photochemical method [18,19].

Photophysical light-induced nucleation and control of polymorphism of supersaturated aqueous glycine solutions has been reported [20–24]. Recently, laser-induced growth of protein crystal with exposure to a very high-intensity femtosecond laser (Gigawatt power) has been reported [25–27]. The mechanism behind this type of nucleation is thought to be photophysical.

I have reported light-induced nucleation of hen egg-white lysozyme under light produced by a 300 W continuous Xe lamp [28]. In this instance, the mechanism of light-induced nucleation is photochemical. The irradiation of supersaturated protein solutions in NaCl at pH 4.5, $\beta = 7$–10, from 10 to 60 s increased the number of lysozyme crystals in the droplet. The most effective irradiation time range was 10–30 s, and in this range enzyme activity was maintained. The enhancement of nucleation by light depends on the irradiation wavelength associated with the electronic transition of lysozyme. Furthermore, the resulting crystal lattice parameters are identical to those normally obtained. I propose a mechanism whereby intermediates produced by one-photon absorption produce lysozyme radicals that enhance nucleation [29,30].

In this review article, I describe photochemically induced crystallization of lysozyme and the mechanism underlying this process.