IECBM 2022

Abstract

Space and Terrestrial Temperature-Induced Nucleation and Growth of Protein Single Crystals†

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† Presented at the 2nd International Electronic Conference on Biomolecules: Biomacromolecules and the Modern World Challenges, 1–15 November 2022; Available online: https://iecbm2022.sciforum.net/.

Abstract: A method is described for high-quality protein crystal solution growth with the help of localized action of a thermal control field. Two techniques for the nucleation and growth of single crystals of biological macromolecules have been proposed. The first one utilizes a very slow temperature shift at a capillary point where the crystal is to be grown. This allows to suppress an undesirable multiple nucleation. The second technique includes several local rapid temperature changes forcing the nucleation at the given point. These techniques were successfully tested while growing single crystals of lysozyme, xylanase and human serum albumin (HSA) respectively.

Keywords: protein; crystal; growth; control; temperature; mathematical modeling

Citation: Bezbakh, Z.I.; Safronov, V.; Zakharov, B.G. Space and Terrestrial Temperature-Induced Nucleation and Growth of Protein Single Crystals. *Biol. Life Sci. Forum* **2022**, *2*, x. https://doi.org/10.3390/xxxxx

Academic Editor:

Published: date

1. Introduction

Single crystals of biological macromolecules, primarily proteins, are obtained mostly for determining their spatial structure by X-ray diffraction methods. Due to a broad diversity of such macromolecules there is no standard growth recipe for obtaining well-ordered crystals of all the range of these complicated substances. Most widely used protein crystallization methods, such as the vapour diffusion and the bulk crystallization [1], rely on the dependence of protein solubility on concentration either of the protein itself, or of a precipitating agent such as a salt or polyethylene glycol. Unfortunately, none of such methods can guarantee a single-crystal nucleation because the required supersaturation is formed throughout the entire volume of a crystallization solution. In most cases this induces a multiple nucleation that results in the growth of numerous tiny or twinned crystals, which often appear to be unsuitable for X-ray diffraction studies. Although with modern synchrotron sources one can study quite small crystals or even focus on parts of polycrystals, larger single crystals may be measured using more available laboratory X-ray sources and are especially necessary for neutron diffraction studies.

To overcome the multiple nucleation problem, many techniques have been developed, such as microseeding [2], counter-diffusion [3], laser ablation with spatially precise ultrashort pulses [4] and a method based on a protein solubility dependence on temperature [5,6] rather than on concentration. We consider the last one especially perspective because it provides good means to control the nucleation and growth processes and try to elaborate it further in this paper. Unlike the concentration, the temperature can be easily changed locally providing the possibility to spatially limit the nucleation zone. A desired temperature gradient is achieved by putting a thermostated conical or cuneiform metal object into thermal contact with a thin-walled capillary holding the crystallization

solution. However, conducting the entire crystallization experiment at a constant temperature of the nucleation zone leads to a multiple nucleation as well, although in a smaller volume. The reason is that the nucleation requires a substantial (several-fold) protein supersaturation [5] while crystallization solutions always contain multiple potential nucleation centers. It is practically possible neither to detect the moment of a protein crystal nucleus formation, nor to change the temperature promptly after that. In this work we propose a technique that allows to get rid of the multiple nucleation using a gradual and slow temperature shift.

Another problem, that makes the temperature-driven crystallization method less attractive for experimentalists, is that it is applicable only to the proteins showing a substantial temperature dependence of solubility. In this work we make an attempt to overcome this difficulty by combining the temperature-driven and the bulk crystallization methods to a technique that forces the nucleation by local and rapid temperature changes. This technique can be useful to crystallize proteins with an unknown temperature dependence of solubility or even proteins not exhibiting such dependence at all. Therefore, one can skip preliminary protein-consuming studies of solubility against temperature. We, however, should note that a single crystal nucleation in supersaturated protein solutions can be induced by other means as well. The temperature was shown to be a factor, capable to initiate and drive the crystallization, and also to influence the nucleation stage and, hence, the protein crystal morphology. This may be useful for obtaining good quality single crystals of biological macromolecules. One or the other technique may be chosen. The stronger the dependence of protein solubility against the temperature, the better chances one has to succeed with any of the approaches.

A mathematical model has been developed and computational investigation has been performed of the processes of protein crystallization from a homogeneous aqueous solution in the crystallization volume. It describes crystal nucleation and growth depending on the local supersaturation and temperature as well as heat-and-mass exchange within the entire volume of the solution including the protein crystals. This mathematical model developed describes the process of nucleation and growth of protein crystals from solution under the control action of a thermal field based on an intermediate phase concept, the intermediate phase consisting of a mixture of solid and liquid phase fractions. This model was used to calculate an experiment on growing a protein crystal from a homogeneous aqueous solution, with the process of crystal nucleation and growth being acted upon by the precipitant and the thermal field. The calculations showed that this model is adequate to the processes being modeled and can be used for parametric investigations and predictive calculations of protein crystallization processes under thermal control field conditions both under terrestrial and space conditions.

These techniques were successfully tested while growing single crystals of lysozyme, xylanase and human serum albumin (HSA) respectively.

Author Contributions:

Funding:

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Acknowledgments: This work was supported by the Ministry of Science and Higher Education within the State assignment FSRC "Crystallography and Photonics" RAS.

Conflicts of Interest: The authors declare no conflict of interest.

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