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Estimated effects of silicone glue on protein crystal growth

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ABSTRACT

Silicone glue (modified silicone polymer) is widely used for both experiments involving inorganic crystal growth and those involving organic materials like proteins. This material is very useful for building a hand-made experiment setup or for fixing protein crystals to specific locations. Though silicone glue is regarded as harmful to proteins, no systematic verification was performed to investigate its impurity effects on protein crystal growth. We focused on and estimated the impurity effects of silicone glue on protein crystal growth.

Hen egg white lysozyme (HEWL) was used as a model protein. Surface morphology and step velocity of tetragonal lysozyme crystals in the presence and absence of silicone glue were investigated by laser confocal interference contrast microscopy (LCM-DIM). The surface morphology of a tetragonal lysozyme crystal in the presence of silicone glue corresponded to that grown in a lysozyme solution without silicone glue. The dependency of step velocities on supersaturation in the presence of silicone glue also exhibited the same tendency as that of a glue-free system. These two phenomena indicate that the silicone glue did not act as an impurity on lysozyme crystals. Therefore, we conclude that silicone glue is an effective material for various unique experiments involving protein crystals or for applying new methods to create large, high-quality protein crystals.

1. Introduction

Protein molecules have various functions related to their three-dimensional (3D) structure. Understanding 3D structures and the specific functions of proteins gives us wonderful benefits, such as new drug discoveries and improved medical care. Diffraction methods like X-ray and neutron crystallography are powerful tools for determining the 3D structures of proteins. Though large crystals (e.g. >1–$10\,mm^3$) with high quality are, required especially for neutron crystallography [1], to grow such crystals is still a significant impediment.

Several protein crystallization techniques, including sitting-drop vapor diffusion, hanging-drop vapor diffusion, and batch methods, are well known techniques for producing protein crystals for diffraction methods, though these are not able to produce large crystals of sufficiently high quality [2,3]. Our group developed novel methods, such as a laser-irradiation technique [4,5], a two-liquid system [6,7], and a solution-stirring technique [8–11], and succeeded in improving the quality and size of the resulting protein crystals. The latest and most effective method for making large, high-quality protein crystals is the top-seeded solution growth method (TSSG) [11]. To use this method, we need to fix protein crystals to a special holder using silicone glue (modified silicone polymer) [11]. This type of silicone glue has been used in many research studies related to protein crystal growth because the researchers needed to construct a specially designed experimental system or in-situ observation cells [12–15]. This type of silicone glue was regarded as a non-polluting material for inorganic materials like anorthite and calcite because it is very stable in solution [16–18]. Van Driessche et al. [19] compared surface morphology of Hen egg white lysozyme (HEWL) crystals grown in their observation cells with or without the silicone glue, and concluded that the material is also non-polluting for proteins. However, systematic evaluation of the material has not been performed. The growth of protein crystals can be affected by slight environmental change or by impurities contained in the growth solutions. The impurity effects of silicone

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glue must be estimated before utilizing a novel method like TSSG to obtain large, high-quality protein crystals or to construct specially designed experiment systems of protein crystal growth.

In this study, we focused on and estimated the impurity effects of silicone glue on protein crystal growth. HEWL was used as a model protein. We monitored the surface morphology and step propagation of the HEWL crystals to judge the effects of silicone glue on them.

2. Experimental procedures

Commercial grade lysozyme (98.5% purity, recrystallized six times, Seikagaku Co.), sodium chloride (99.5% purity, Wako Pure Chemical Industries, Ltd.), and sodium acetate (98.5% purity, Wako Pure Chemical Industries, Ltd.) were used to prepare lysozyme solutions. Impurities contained in the commercial grade lysozyme of Seikagaku Co. were reported as 0.5% of lysozyme dimer, 1.0% of an 18 kDa polypeptide, and less than 0.1% of a 39 kDa polypeptide [20]. Tetragonal seed crystals of lysozyme were grown from a solution containing 60 mg/ml lysozyme, 4% NaCl, and 0.1 M sodium acetate buffer (pH 4.5) at 20 °C (± 0.1 °C).

The seed crystals were transferred to two kinds of observation cells (Fig. 1). Fig. 1(a) is a schematic image of a normal observation cell made with micro-cover glasses (Matsunami Co., Ltd.) and plastic bars (evergreen scale models, Kyosyo Co.). The plastic bars were fixed on a cover glass using silicone glue (modified silicone polymer 100%, Konishi Co., Ltd.). We paid extreme attention not to allow the silicone glue to enter the observation cell. This normal observation cell was named Cell 1. Fig. 1(b) is a schematic image of a special observation cell. For this, we put silicone glue into a normal observation cell by design to test the effects of silicone glue on protein crystal growth. There was sufficient silicone glue inside the observation cell compared to the lysozyme crystal volume. This special cell was named Cell 2. The two observation cells were filled with a lysozyme solution containing 20 mg/ml lysozyme, 3% NaCl, and 0.1 M sodium acetate buffer. Fig. 2(a) and (d) shows images of the lysozyme crystals after enclosure into each observation cell, taken by a polarizing microscope. Both of the crystals were tetragonal single crystals. One lysozyme crystal in Cell 2 was located near the silicone glue (Fig. 2(d)).

We employed laser confocal interference contrast microscopy (LCM-DIM) to observe the surfaces of the lysozyme crystals. LCM-DIM has high vertical resolution, sufficient to observe elementary steps of nanometer height. Details of the LCM-DIM setup are

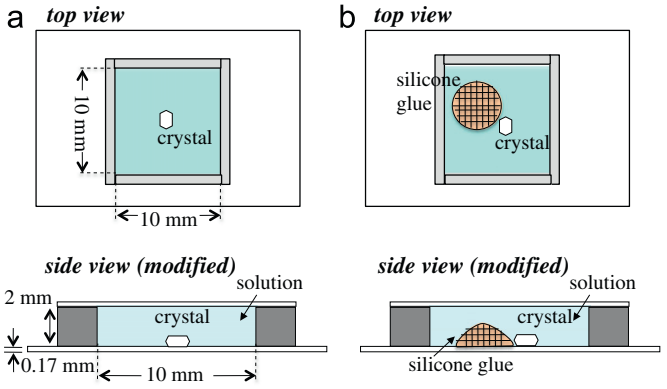

Fig. 1. Schematic images of observation cells. (a) Normal observation cell designed for lysozyme crystal growth in glue-free lysozyme solution. (b) Special observation cell designed for testing silicone glue effects on lysozyme crystal growth. Sufficient silicone glue was added to the observation cell as compared to the lysozyme crystal volume.

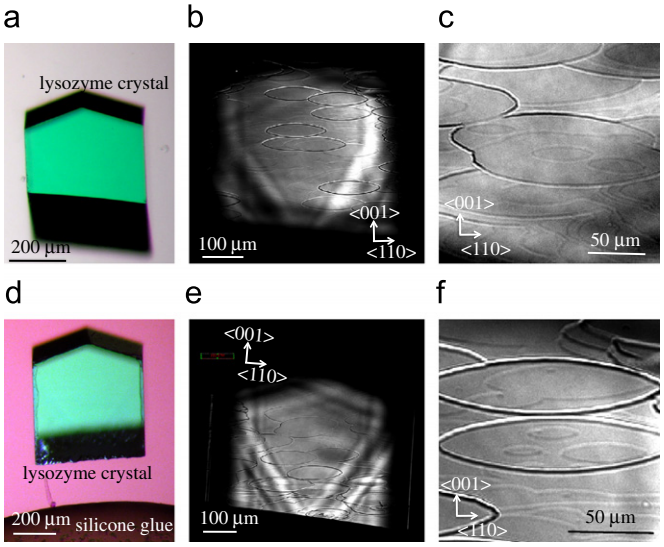

Fig. 2. Pictures of {1 1 0} faces of tetragonal lysozyme crystals grown in Cell 1 ((a)–(c)) and Cell 2 ((d)–(f)). The {1 1 0} surface images of tetragonal lysozyme crystals were taken by a polarizing microscope ((a) and (d)). Details of the crystals grown in Cell 1 and Cell 2, taken by LCM-DIM ((b) and (e)). Morphologies of the 2D islands as a function of silicone glue ((c) and (f)).

presented in Refs. [15,19,21,22]. LCM-DIM images, such as the pictures in Fig. 2(b) and (e), were acquired automatically every minute, for a total duration of 60 min under each condition. The observation cells were set on a temperature-controlled stage. We introduced a temperature controller (MT702-10P12, NetsuDenshi Co.), which automatically switches from heating to cooling mode to keep temperatures at a set point within an error of ± 0.1 °C. Two sets of peltier elements were used to heat or cool the observation cell.

Absolute Supersaturation (σ) of the lysozyme crystal is given in the form

$$\sigma = C - C_e \quad (1)$$

Here C is the bulk concentration of lysozyme and C_e is the solubility. Solubilities are from Refs. [23,24]. We changed the supersaturation by changing the experimental temperature from 20 to 30 °C.

3. Results and discussion

Fig. 2(b) and (c) shows LCM-DIM images of a {1 1 0} face of a tetragonal lysozyme crystal grown in Cell 1 (without silicone glue, glue-free system). Many 2D islands were observed on the surfaces of the crystal (Fig. 2(b)). These 2D islands have an elementary step height of 5.6 nm, which is the characteristic height of a {1 1 0} face of tetragonal lysozyme crystals [25–28]. The aspect ratio (long axis to short axis) of the 2D islands is used as an index for evaluating impurity effects because the aspect ratio corresponds to the ratio of step velocity between the ⟨1 1 0⟩ and the ⟨0 0 1⟩ directions [12,29]. Step velocity in the ⟨1 1 0⟩ direction is more sensitive to impurities than in the ⟨0 0 1⟩ direction. Therefore, impurities contained in the solution reduce the aspect ratio of the 2D hillocks [12]. When purified lysozyme (99.99% purity) is used, the shape of the 2D islands becomes very sharp and exhibits a high aspect ratio (≈ 5–6). When commercial lysozyme (98.5% purity) is used, the aspect ratio reduced to ≈ 2 – 4, and it strongly depends on impurity concentrations and effects [12,15,19,28,30]. We measured the aspect ratio of the 2D islands on a lysozyme crystal grown in Cell 1 at 20 °C, estimating it to be 4.12 ± 0.06. Fig. 2(c) illustrates the typical shape of the 2D islands when