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Study of Growth Mechanism of Lysozyme Crystal by Batch Crystallization Method

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Abstract: The lysozyme crystals were made by batch crystallization method and the distribution of aggregate in solution were measured by dynamic light scattering. The results showed that the dimension of aggregate increased with the increase of the concentration of lysozyme and NaCl, lysozyme molecules aggregated gradually in solution and finally arrived at balance each other. The higher the concentrations of lysozyme and NaCl were, the faster the growth rate of (110) face was. The growth rates of lysozyme crystal were obtained by a Zeiss microscope, and the effective surface energy (α) of growing steps were calculated about 4.01×10^{-8} J·cm^{-2} according to the model of multiple two-dimensional nucleation mechanism.

Keyword: Protein crystal, growth rate, dynamic light scattering, lysozyme.

Space environment is regarded as the perfect environment for the production of higher quality protein crystals since the sedimentation movement and convective flow due to the gravity is negligible under the microgravity condition of space environment. A number of experiments involving protein crystal growth have been carried out in space under microgravity condition to obtain larger and better ordered crystals than those grown on earth. However, there have also been a lot of crystals produced in space experiments that have no noticeable improvement in the quality and internal structure as compared to those grown under the normal gravity on earth[1]. The reason is that the process of protein crystallization is not clear, it is a complicated process controlled by multi-parameter. The influence actors include temperature, pH values, the protein concentration and flow field. The studies of growth mechanism of protein crystal were carried out by using optical microscopy, atomic force microscopy. Though the understanding about the whole process of protein crystallization become more deep, the protein crystallization is still not clear completely. In this paper, the growth dynamics of lysozyme crystal produced by batch crystallization method was studied.

Experimental

An amount of lysozyme powder and NaCl were dissolved in 50 mmol/L HAC-NaAC

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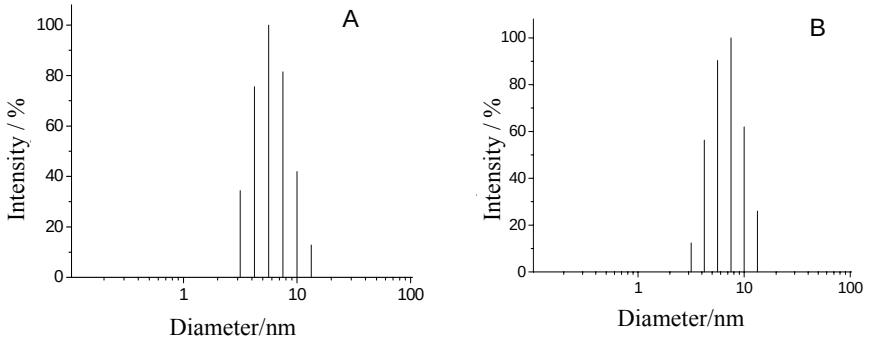
buffer solution (pH 4.5), respectively. Then these two kinds of solution were mixed in the growth cell. The lysozyme was purchased from Sigma Company (U.S.A.). Other reagents are in analytical grade. The growth rates of lysozyme crystal were observed by Zeiss microscope (Zeiss Company, Germany) and the distributions of aggregate in the solution were determined by BI-200SM dynamic light scattering DLS Instrument (Brookhaven Instruments Company, U.S.A.).

Results and Discussion

Figure 1 showed that the dimension of aggregate which was the biggest distribution in solution were 5.62 nm and 7.50 nm at $\sigma = 2.58$ and $\sigma = 4.68$, respectively. It was thought that the dimension of aggregate increased with the increasing of the concentration of protein.

Figure 1 Distribution of aggregates in solution of different lysozyme concentration

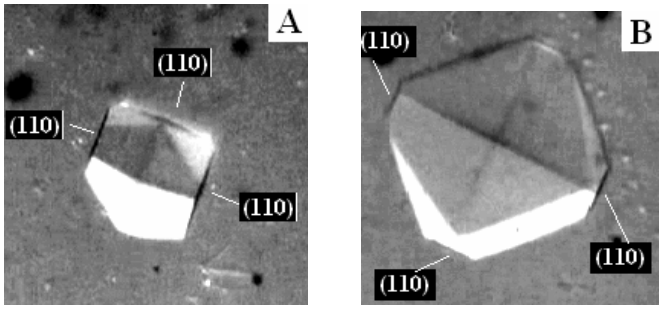


50 mmol·L^{-1}HAC-NaAC, 7% NaCl，26.5℃, the distribution of aggregates in solution were measured by DLS after 40 minutes. (A) $\sigma = 2.58$；（B）$\sigma = 4.68$

Figure 2 showed that, the (110) face of crystal at $\sigma = 4.68$ was smaller then that at $\sigma = 2.58$. But with the growth of crystal, the (110) face of the crystal at $\sigma = 4.68$ became larger gradually. It will look like the morphology in **Figure 1** (A) finally.

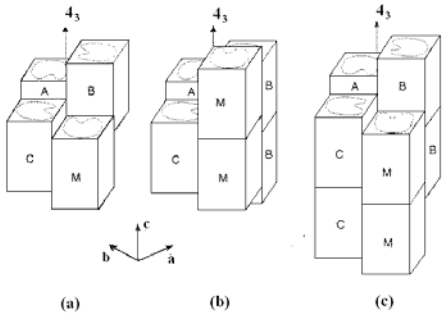
Nadarjah *et al.*[2,3] consider that lysozyme molecules formed higher ordered aggregates in solution before they come into crystals. Those aggregation reactions were at equilibrium in solution. The reversible doubling reactions are: monomer⇌dimmer ⇌tetramer⇌hexamer⇌octamer⇌higher order n-mers. The aggregates corresponding 4_3 helix are the growth unit of lysozyme crystal. **Figure 3** showed the aggregate growth units corresponding to the 4_3 helix. The octamer was thought to be the growth unit of (110) face and tetramer was thought to be the growth unit of (101) face [2]. The dimensions of monomer is 3.79 nm × 2.8 nm × 2.8 nm. So the aggregate, whose dimension was about 5.62 nm, should be accordant with tetramer. The aggregate, whose dimension was about 7.50 nm, should be accordant with octamer. It was proposed that the number of tetramer is largest at $\sigma = 2.58$, which led to the (101) face of the crystal growing faster, and the number of octamer was small that the (110) face of the crystal grew slowly. The result of the initial supersaturation $\sigma = 4.68$ is opposite to that of the initial supersaturation $\sigma = 2.58$.

Figure 2 The shape of lysozyme crystal (after 45 mins)



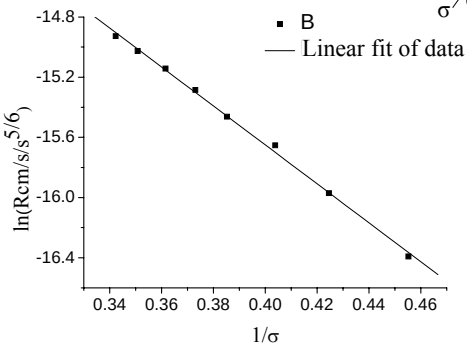
50 mmol/L HAC-NaAC, 7 %NaCl, 26.5 ℃, (A) σ = 2.58 (B) σ = 4.68

Figure 3 The aggregate growth units corresponding to the 4_3 helix [2]



(a) tetramer, (b) hexamer and (c) octamer

Figure 4 The linear relationship between $\ln(\frac{R}{\sigma^{5/6}})$ and $\frac{1}{\sigma}$



50 mmol/L HAC-NaAC, 7 %NaCl, 28.8 ℃

When σ was higher than 1.22, the growth of lysozyme crystal proceeded by multiple two-dimensional nucleus formation[4]. The formula between the growth rate R of (110) face and supersaturation σ was as follows [5].

$$\ln(\frac{R}{\sigma^{5/6}}) = \ln\{hc_e\beta_l[\omega^2\pi h a n_s]^{1/3}\} - \frac{\pi\omega\alpha^2 h}{3(kT)^2}\frac{1}{\sigma} \quad (1)$$

The volume of growth unit[2] ω is 2.38×10^{-19} cm^3 and the step height[4] h is 5.6×10^{-7} cm. T is temperature. C_e is the equilibrium concentration of the dissolved lysozyme. n_s is the surface density of lysozyme. a is the distance between the neighbouring molecules in the <110> direction and β_1 the kinetic coefficient for the (110) face [5]. So the effective surface energy α is calculated about 4.01×10^{-8} J/cm^2 by the slope of the fit line in **Figure 4**.

Acknowledgments

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