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Studying Aggregate in Lysozyme Solution by Atomic Force Microscope

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Abstract: The aggregates in lysozyme solution with different NaCl concentration were investigated by Atomic Force Microscope (AFM). The AFM images show that there exist lysozyme monomers, n-mers and clusters in lysozyme solution when the conditions are not suitable for crystal growth. In favorable conditions for crystal growth, the lysozyme clusters disappear and almost only monomers exist in solution.

Keywords: Lysozyme, aggregate, atomic force microscope.

Protein crystal growth has been studied for several decades. The result of investigation showed that it depends on many parameters, such as pH, precipitant agent, supersaturation, *etc*. However, for lacking of the criteria of diagnosing crystal growth, many efforts have been failed.

The aggregates presented in protein solution affect the protein crystal growth[1]. To our knowledge, few of researchers have investigated this aspect. Martin Zulauf *et al*. had studied several proteins (excluding lysozyme) dilute solution (all samples were at concentrations between 0.1 and 0.5 $mg \cdot mL^{-1}$) and concluded that the crystals may be obtained if there are only monomers in protein solution[1]. Yannis Georgalis *et al.* had studied the aggregate behavior in lysozyme (15 $mg \cdot mL^{-1}$) solution with NaCl concentration higher than 0.5 $mol \cdot L^{-1}$ [2]. However, neither of the above researches had referred to the high protein concentration (such as higher than 20 $mg \cdot mL^{-1}$) and low NaCl concentration (such as lower than 0.5 $mol \cdot L^{-1}$). The high protein concentration and low precipitant concentration are often used in crystal growth when using vapor diffusion method (one of the most popular methods in protein crystal growth). Atomic force microscope (AFM) is a powerful tool in studying surface topography of protein aggregates with nanometer lateral resolution[3]. For the above reasons, aggregates in 25 and 50 $mg \cdot mL^{-1}$ lysozyme solution with 0-0.5 $mol \cdot L^{-1}$ NaCl were investigated by AFM to find the change of morphology of aggregates, and the effect of aggregates on crystal growth under the above conditions.

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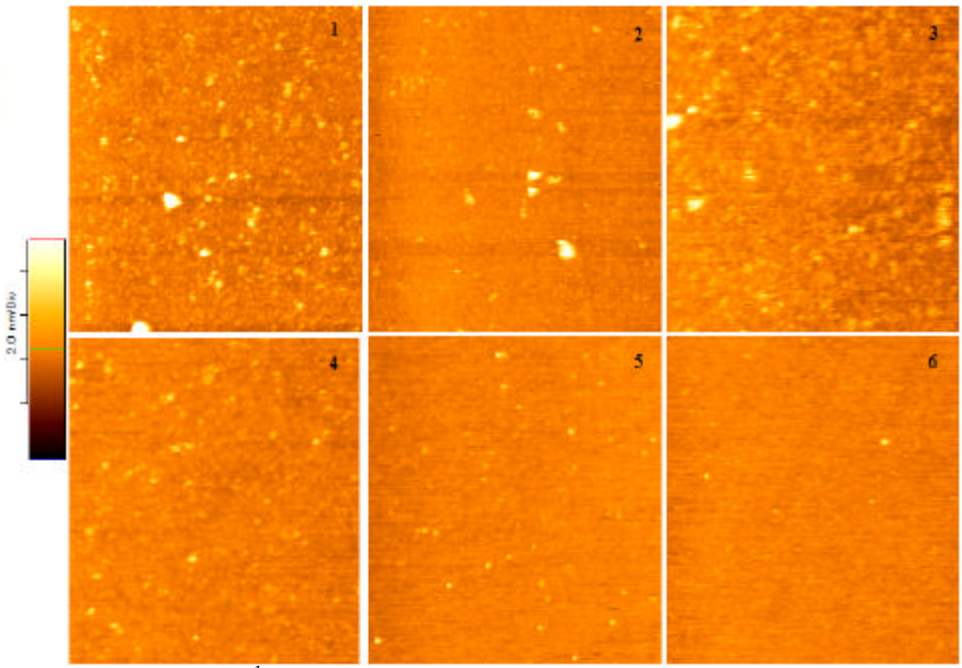
Experimental

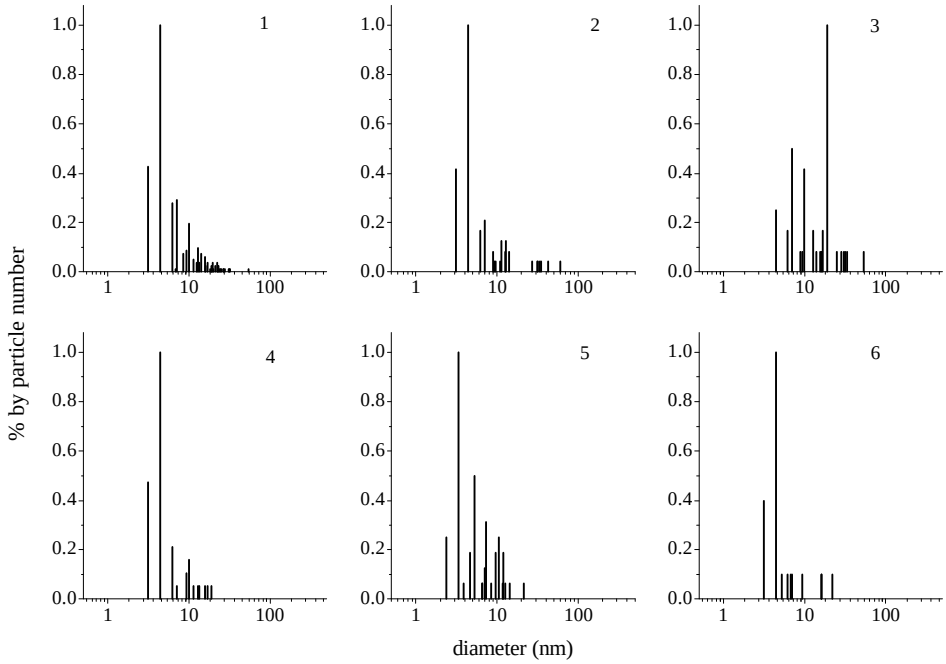
Lysozyme: purchased from Sigma Corporation. (product No.: L6876, three times crystallized without further treatment). The concentrations of lysozyme in our experiments were 25 and 50 mg·mL^{-1}. Buffer was acetate buffer solution, 0.1 mol·L^{-1}, pH 4.5, with different NaCl concentrations. The concentrations of NaCl used in this experiment were 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mol·L^{-1}, respectively. The lysozyme was dissolved in buffer solution and preserved in 4°C for 24 h and then a drop of lysozyme solution was added onto the surface of silicon wafer. After 5 minutes, the lysozyme solution on silicon wafer was washed away by deoined water and then dried by nitrogen. The method of silicon wafer treatment can be found in reference 4. AFM: Park 1000, Park Scientific Instrument Corporation. The AFM images were gotten from AFM AutoProbe CP Research Scanning Probe Microscope. IC-AFM mode and Ultralevers 20 microcantalever were used. All AFM experiments were under the conditions: 20°C and humidity 50-60%. The aggregate size was represented by the length of major axis of aggregate, which was measured using the software named "Image Processing and Data Analysis" from ThermoMicroscope company accompanied by AFM.

Figure 1 AFM images of aggregates absorbed on the surface of silicon wafer.



The 25 mg·mol·L^{-1} lysozyme solution (with different NaCl concentrations in it) had been absorbed for 5 minutes. Size: 1×1 μm. 1, 2, 3, 4, 5 and 6 are AFM with 0, 0.1, 0.2, 0.3, 0.4 and 0.5 mol·L^{-1} of NaCl solution, respectively.

Figure 2 The size distribution of the aggregates presented in lysozyme solution with different NaCl concentration in it.



All diameters are measured by software "Image Processing and Data Analysis" from the AFM images. 1, 2, 3, 4, 5 and 6 are the size distribution with 0, 0.1, 0.2, 0.3, 0.4 and 0.5 mol· L^{-1} NaCl solution, respectively.

Results and Discussion

Although the aggregates in silicon wafer surface are different to some extent with that in lysozyme solution, the AFM investigation is still helpful for the information of aggregates because it can give us the morphology of the aggregates which may help us to know the formation of the aggregates easier.

In our experiments, the results of 50 mg· mL^{-1} lysozyme solution are almost the same as 25 mg· mL^{-1}. So, only the results of 25 mg· mL^{-1} are showed here. The concentration of NaCl was varied between 0 and 0.5 mol· L^{-1} in our experiment. According to our previous works, crystals were not observed at all when the concentration of NaCl was varied between 0 and 0.3 mol· L^{-1}. For batch method in lysozyme crystal growth, the optimum concentration of NaCl for crystallization of lysozyme was found between 0.50 and 0.70 mol· L^{-1} [5]. **Figure 1** is the AFM results and **Figure 2** shows the distribution of aggregates, it is derived from **Figure 1.** From **Figure 1** and **Figure 2**, we find that there always exist 2-3 nm and about 10 nm aggregates in solution under the experimental conditions. These aggregates correspond to lysozyme monomer and n-mers (n is about 2, 3, or 4). Otherwise, the aggregates with diameter between 50 and 70 nm existed in the solution when NaCl concentration was less than 0.4 mol· L^{-1}. We named these aggregates as clusters. These clusters correspond to the oligomeric lysozyme.

The results obtained from **Figure 1** showed that the clusters are formed by small aggregates with diameter varying between 5 and 10 nm. Under the unfavorable conditions, such as when the concentration of NaCl was varied between 0 and 0.3 mol·L^{-1}, the monomers, n-mers and clusters all existed in the lysozyme solution and the ratio of each component is almost relatively constant. However, when the condition is suitable for crystal growth, such as the concentration of NaCl was varied between 0.4 and 0.5 mol·L^{-1}, the clusters disappeared and only monomers and n-mers existed in solution, and the content of monomers was higher in 0.4 mol·L^{-1} NaCl solution than in 0.5 mol·L^{-1}. That is to say, for crystallization of lysozyme with NaCl, more and more clusters are disassociated when the condition changes towards the favorable condition. Once the condition is suitable for crystal growth, the clusters disappear and most of the n-mers are disassociated. This result provides us a proper criterion for lysozyme crystal growth when using batch method, *i.e.*, lysozyme crystals can not grow in the solution existing clusters. There are almost only monomers in solution under suitable lysozyme crystal growth conditions.

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