Small Protein Crystals

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Introduction

The field of structural biology is vitally dependent on people's ability to grow crystals of the desired biomolecules. Most structures of biological macro-molecules are solved by using x-ray diffraction from high-quality crystals [1]. The preparation of these crystals can often be tedious at best. There is no definite way to predict under which conditions a crystal will grow or even whether one can grow at all. There is a clear need for a better understanding of the processes involved in the preparation of high-quality protein crystals. We are interested in the very early stages of crystallization — the moment when a supersaturated solution of protein nucleates into stable aggregates that, in time, grow into macroscopic-size crystals [2]. In addition to the technological importance of understanding protein crystal nucleation and growth, the problem is also interesting in itself. The nucleation process is generally not well understood, and proteins, because of their large size and slow growth kinetics, are an excellent system for such a study.

Methods and Materials

The protein chosen for study was horse spleen ferritin, which resembles a spherical shell with an outer diameter of 130 Å. The function of the protein is to store iron inside its shell. Horse spleen ferritin usually crystallizes in a sodium acetate 0.2 M buffer to which $CdSO_4$ is added as a precipitant. The crystals produced are faced-centered cubic (FCC) with a lattice parameter a = 184 Å. Our goal here was to produce a large amount of small crystals instead of one or a few large ones, as is usually desired for protein crystallography. This was achieved by adding a quantity of precipitant much larger than usual, increasing the supersaturation of the system. This system being prepared very far out of equilibrium is quickly driven toward equilibrium, and numerous crystals are rapidly formed.

The reason why it was desirable to proceed this way is that small crystals of the order of 1 μm would be almost impossible to orient accurately. So by creating a lot of them, we can increase the probability that a least one of them will have the proper orientation to meet the Bragg condition.

The experimental geometry is shown in Fig. 1. A drop of roughly 4 μL of ferritin and the precipitant is placed into the beam on a glass slide. The beam used was

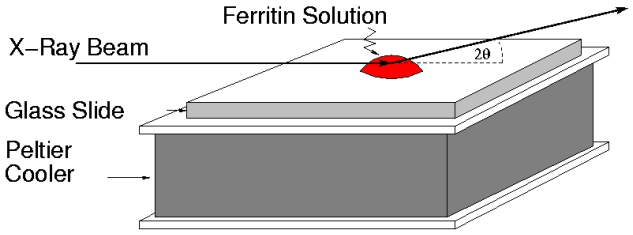

FIG. 1. Diagram of the experimental geometry.

produced by an undulator, and a diamond double crystal monochromator was used to select an energy of 9.5 keV. The temperature was controlled with a Peltier cooler. The drop was found to contain ~10^5 crystals almost immediately after it was prepared. The diffraction was measured with a charged-coupled device (CCD) camera placed 2.2 m away at an angle 2θ corresponding to the (111) Bragg peak. The sample was placed in a closed cell to avoid evaporation or condensation.

With a large number of crystals present, we expect to see something resembling a powder diffraction ring. If that number isn't too large, we should be able to resolve individual Bragg peaks coming from distinct small crystals.

Results

Diffraction patterns (an example is shown in Fig. 2) were collected for a few different crystallization conditions. The first ring observed corresponds to the (111) ring of FCC ferritin, while the second is the (200) ring. Individual peaks can be easily resolved. The time series shown indicates there is a evolution of the peaks in time. One of the Bragg peaks is seen to get "blown away" in time.

The first thing to notice is that the peaks do not lie perfectly on rings. There are variations in the radial position. This suggests there may be variations in the crystal structure from one crystal to the next.

The evolution in time of the peaks suggests a few things. First of all, some of the peaks are seen to appear, stay a while, and then disappear, or are even seen to rotate around the ring. This suggests the crystals are rotating. Also, the entire pattern gets weaker with time, indicating overall radiation damage.

But the most striking feature is the Bragg peak getting blown away in time. It would seem to be attributable to

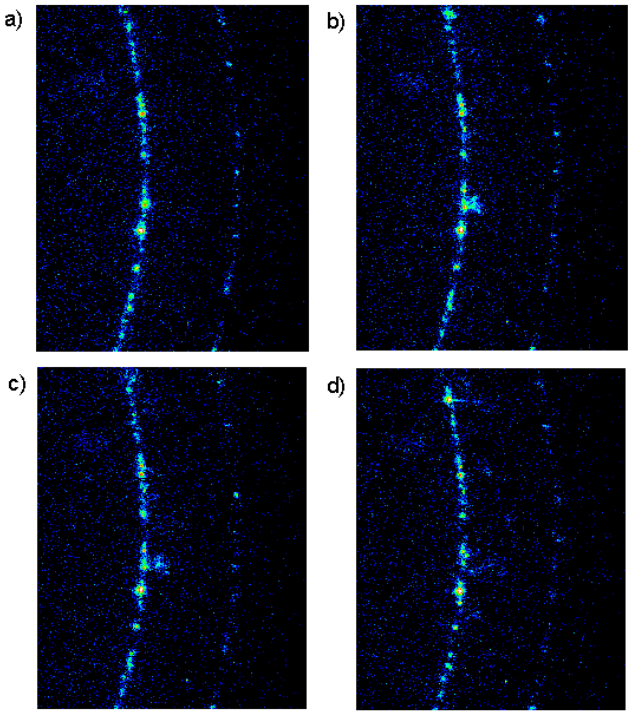

FIG. 2. Time series of diffraction patterns from solution containing many ferritin small crystals: a) t = 0, b) t = 5 min, c) t =7 min, and d) t = 10 min.

radiation damage. However, the peak just doesn't get weaker in time. It is very surprising that the intensity just moves toward larger angles, which would mean a smaller lattice spacing. It seems the crystal structure is shrinking as it disintegrates. It could be that the radiation causes the solvent inside the crystal to evaporate, thus collapsing the crystal.

Another interesting feature is that the widths of most of the peaks are very similar, i.e. ~30-40 μrad. There would seem to be a fairly uniform distribution of sizes of the crystals in the drop. We estimate this size to be ~5 μm.

Discussion

The size of the crystals formed is ultimately just a little too large for using coherent diffraction methods to study the small crystals. If the small crystals can be made smaller than the lateral coherence length of the beam (~5 μm), it would be possible to observe fringes from the coherent interference of the edges of the crystals [3]. The measurement of such coherent diffraction patterns could provide information about the shape of small crystals closer to the critical nuclei size and how these evolve in time.

Acknowledgments

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