SAXS Study of the Nucleation Process of Glycine Crystals from Supersaturated Solutions

S. Chattopadhyay,[1,2] J.M.B. Evans,[2,3] J. Ilavsky,[4] H. Amenitsch,[5]
D. Erdemir,[2] C.U. Segre,[1,6] A.S. Myerson[2]
[1]Advanced Photon Source (APS), Argonne National Laboratory (ANL), Argonne, IL, U.S.A.
[2]Department of Chemical Engineering, Illinois Institute of Technology, Chicago, IL, U.S.A.
[3]New Products and Innovations, Diosynth BV, Montrose, Angus, Scotland
[4]Department of Chemical Engineering, Purdue University, West Lafayette, IN, U.S.A.
[5]Institute of Biophysics and X-ray Structure Research, Austrian Academy of Sciences, Graz, Austria
[6]Physics Division, Illinois Institute of Technology, Chicago, IL, U.S.A.

Introduction

Crystallization from solution has been the subject of intense research in recent years. The nucleation of crystals from solution is of great importance in industries, since it is the primary method for the preparation and purification of industrially important chemicals, such as pharmaceuticals, explosives, dyes, and photographic materials. Crystallization is also used for the synthesis of single crystals of proteins, amino acids, etc., as well as very small crystals whose large surface area make them important for gas sensing and catalysis, among other purposes. The early stages of the formation of the particles from solution can play a decisive role in determining the properties of the solid in its final state. Our group previously reported that supersaturated solutions of glycine crystallized into the polar ?-polymorph when subjected to plane polarized laser light [1]. Recent studies suggest that classical nucleation theory is not even qualitatively correct and that crystallization from solution is, in fact, a two-step process. The first step involves the formation of a liquidlike cluster of solute molecules. The second step involves the organization of the cluster into an ordered crystalline structure [2-4]. However, not much is known about the processes of nucleation and crystallization. In recent years, significant progress has been made in experimental techniques, and there has been full utilization of the entire spectrum of x-ray scattering capabilities (ultrasmall-angle small-angle, and wide-angle x-ray scattering [USAXS, SAXS, and WAXS]) to characterize the nucleation and crystal growth of a broad range of compounds, including zeolites, polymers, and colloids [5-7]. There have been reports of small-angle neutron scattering (SANS) and SAXS studies of supersaturated solutions of lysozyme (8-10), but there has been little work on crystallization studies of other proteins, peptides, or amino acids. In fact, the vast majority of work has been done on dilute systems; supersaturated solutions have been ignored, for the most part. We report here on the preliminary results obtained by our group in studying the nucleation and crystallization of the amino acid glycine from its supersaturated aqueous solution by using SAXS.

Methods and Materials

Glycine (CH_2NH_2COOH) has three polymorphs (α-, β-, and γ-glycine) that can be formed under different solution concentrations. β-glycine and γ-glycine crystals spontaneously convert to α-glycine in the presence of α-crystals in a saturated solution. α-glycine forms spontaneously from aqueous solution. γ-glycine is the most stable phase at room temperature.

Solution Preparation

The supersaturated solution of glycine at a concentration of 3.6 M was prepared by combining solid glycine (99.8%, NH_2CH_2COOH, purchased from Sigma) and deionized water (resistivity = 18 MΩ-cm) in a Pyrex® test tube with a screw-on cap. The glycine was completely dissolved by sonicating and heating the test tube in an ultrasonic water bath at 60°C for several days. A micro syringe was used to transfer 200 μL of solution into a 3.5-mm special glass capillary tube. The syringe and the syringe needle had been placed in an oven beforehand to bring them above the solution temperature in order to prevent the solution from crystallizing during this process. The capillary was then immediately sealed and placed into the sample holder stage in the SAXS beamline. The latter was preheated to 60°C before the sample was transferred. The temperature controller was used to bring the temperature down to 10°C at a rate of 2°C per minute, and the data collection process was started immediately. The supersaturated solution was cooled to enhance the process of crystallization and to achieve it within a few hours.

SAXS Measurements

SAXS measurements were performed at the Bio-CAT undulator beamline (ID-18) at the APS by using a camera length of 1.8 m. We developed a sample stage whose temperature could be controlled within ±2°C by

a water bath. The sample holder has a hole for inserting the thermocouple close to the capillary. The capillaries (3.5 mm in diameter) were placed in a vertical position in the path of the beam. X-rays at 12 keV were used in conjunction with the charge-coupled device (CCD) detector, and scattering data were collected as a function of q for about 6 hours. Exposure time was 20 seconds with a delay of 60 seconds between exposures to prevent heating and radiation damage of the glycine solution. Measurements were done until crystallization was achieved. The supersaturated solution of glycine is a weak scatterer; hence, the first file of the series was used for background subtraction. The SAXS patterns were also corrected for the intensity of the x-ray source.

SAXS Data Analysis

SAXS data provide information about particles of different sizes and shapes that are present in a system. They also give information about the presence of different particle populations and types of interactions. Data reduction was done by using the Igor Pro, Wavemetrics, Inc.-based programs developed by the Intense Pulsed Neutron Source (IPNS) Group at ANL. The unified exponential/power law approach to small-angle scattering (SAS) was used for fitting the scattering data [11]. Data evaluation was done by using the Igor Pro package of SAS data modelling and evaluation routines called Irena [12], which contains an implementation of the unified model.

Results

We observe from the normalized and background-subtracted scattering pattern that initially there is an increase in the intensity at low q values with a decrease in temperature and an increase in time, as expected with a van der Waals potential. However, after about 3 hours, the intensity decreases in both the low and high q regions, and a decrease in the glycine content of the solution and consequently a decrease of the actual supersaturation is seen. Unified fit theory [11] was used to fit all the curves. The power law exponent value (P) and radius of gyration (R_g) were calculated for each of the curves. They are plotted in Fig. 1 at an interval of 15 minutes. R_g could not be determined for the first 20 minutes. R_g was found to increase from a value of 3.7 Å at 22 minutes to 8.25 Å at ≈4 hours.

The dimer size for glycine is 3.72 Å. Hence, we observe from our SAXS studies that glycine shows a tendency to exist as dimers in supersaturated solutions. This is in accordance with the prediction of Myerson and coworkers on the basis of diffusion coefficient measurements of supersaturated solutions [13, 14]. After reaching a maximum, R_g decreases steadily to a value of 4.78 Å. The decrease of R_g correlates with the decrease in supersaturation caused by crystal growth. The maximum in R_g could correspond to the onset of

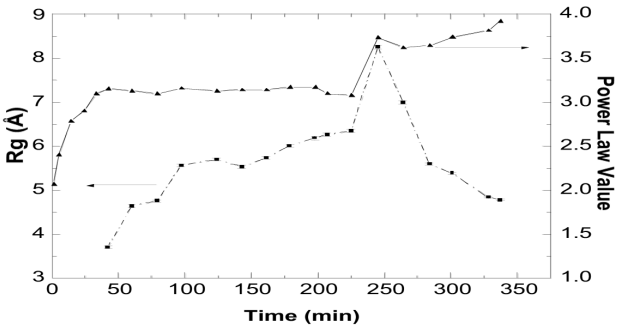

FIG. 1. Variation of the radius of gyration (Rg) and the power law exponent (P) with time. Both the parameters were calculated from the slope of the log(I) versus log(q) plots.

nucleation. Glycine crystals tend to move to the surface of the solution or sink to the bottom of the capillary tube when they form. On the basis of this information, we propose that beyond the point of the maximum R_g, some particles grow rapidly and subsequently leave the portion of the solution illuminated by the x-ray beam. The solution then contains particles that have a smaller R_g. Hence, the beam sees only the smaller particles.

To interpret the change in the power law behavior of our glycine solution, we employed the fractal approach to SAS. An aggregation is a typical example of a complicated random process that may display typical fractal characteristics, such as self-similarity, scaling, and universatility [15]. Figure 1 shows the variation of the scattering exponent with a change in time. The power law exponent value is initially –2.068 at ≈2 minutes, and it increases gradually to a value of ≈–3.1 at 22 minutes. It remains here until ≈3 hours and 40 minutes. It then makes an abrupt jump to a value of –3.7. This corresponds to the decrease in the intensity in the low and high q regions. The power law value then gradually increases to a value of –3.93 and tries to approach the value of –4 that corresponds to the Porod law. Since a power law value between –2 and –3 corresponds to the formation of mass fractals in the solution, this might indicate that the first step of crystallization is the formation of liquidlike clusters. An increase of the power law exponent value to –3 would indicate the formation of surface fractals, which could imply that the clusters are forming aggregates. The increase of the power law exponent value from –3 to –3.93 would indicate a transformation of these aggregates from rough fractal structures to smooth nonfractal structures. The latter is indicative of the fact that the liquidlike clusters are organizing themselves and forming the glycine crystals. The proposed separation of size would agree with the observed change in the slope of the power law, which occurs at

exactly the same time as when the R_g values start decreasing.

Discussion

With the help of SAXS, we have begun to study the nucleation and crystallization of the amino acid glycine from its aqueous supersaturated solution. Our preliminary results indicate that glycine molecules exist as dimers in the supersaturated solution. The structure factor and the form factor follow a power law behavior that is a signature of fractal structures. The increase of the power law exponent value indicates a transformation of the system from mass fractals to surface fractals. Further studies on supersaturated solutions of glycine and urea are in progress to investigate if the cluster size depends on the concentration of the supersaturated solution and if the size is altered by the presence of additives.

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