PRACTICAL, RAPID SCREENING OF PROTEIN CRYSTALLIZATION CONDITIONS BY DYNAMIC LIGHT SCATTERING

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As crystallization is now the primary barrier to three-dimensional structure determination of new proteins, there is a need to replace empirical recipes with a logical framework within which standard methods can be routinely applied. Two cornerstones of such a framework are the systematic survey of crystallization conditions and quantitation of how these conditions affect crystallization. Incomplete factorial designs can efficiently screen many different conditions (1). Specific conditions can be evaluated by measuring the protein concentration dependence of aggregate size distributions in solution (2). Using dynamic light scattering, size distributions can be measured from 30 μl samples within minutes of sample preparation. Here we present our initial findings using this procedure.

MATERIALS AND METHODS

Egg white lysozyme was from Sigma Chemical Co., St. Louis, MO. Bovine serum albumin (BSA) was from Boehringer Mannheim, Indianapolis, IN. Salts and buffers were of highest commercial grade. All buffers and protein solutions were filtered through a Millex-GV 0.22 μm filter unit (Millipore Corp., Bedford, MA). Doubly distilled, deionized water was used in all experiments.

Airfuge

Dust was removed from small (30 μl) protein solutions by centrifugation 100,000 × g for 20 min in the Airfuge (Beckman Instruments, Palo Alto, CA). Cellulose propionate centrifuge tubes (Beckman) were preferred over Ultra-Clear centrifuge tubes (Beckman). After the sample was clarified, the airfuge tube was mounted in a square black Teflon mask designed to fit within a 1-cm^2 glass cuvette. On each face the mask had 3-mm wide slots exposing the airfuge tube. The glass cuvette was filled with filtered water to a level above the protein solution meniscus. The cuvette and airfuge tube assembly was placed in a temperature controlled spectrophotometer block cooled by a Haake 516/ED circulator and water bath (Brinkmann Instruments, Westbury, NY). The block height was adjusted so that the laser beam passed through the sample just below the meniscus.

Dynamic Light Scattering Instrumentation

Light-scattering data were collected at 90°C in the photon-counting mode (3). A Spectra-Physics model 120 S He-Ne (633 nm; 5mW) laser (Spectra-Physics, Inc., Mountain View, CA) served as the light source. A Pacific Photometrics model 124 digital photometer was used (Pacific Photometric Instruments, Emeryville, CA). The autocorrelation function was constructed by a Langley-Ford autocorrelator. The autocorrelator is interfaced to a Heath microcomputer (Heath/Schlumberger Instruments Div., Heath Co., Benton Harbor, MI). The nonlinear autocorrelation function G, is fit by a quadratic least-squares routine and the z-averaged diffusion coefficient, D_z, calculated. The solvent-airfuge tube scatter is on the order of 5 mg/ml lysozyme. Data collected near this limit tend to be noisy.

RESULTS

Our model studies of lysozyme suggest that when attempting to crystallize a protein for the first time it is helpful to characterize the protein by a standard curve of the monomer diffusion coefficient, D_1 vs. temperature. Because the viscosity of solutions is very temperature sensitive (4), the monomeric diffusion coefficient, D_1, measured at 23°C cannot be used to calculate the ratio D_z/D_1 for a dilution curve at 14°C (Fig. 1 *A*). Factors that affect this standard curve have not been systematically characterized. An experiment with 3% lysozyme at 14°C suggests that changes in protein concentration do not affect the diffusion coefficient in 0.1 M Tris, pH 7.5 (data not shown). It is probable that changes in buffer composition, for example, the addition of glycerol, will significantly alter the standard curve. BSA, when cooled, exhibits similar behavior to that of lysozyme (Fig. 1 *C*). As far as we know, no systematic study of the effect of temperature on the diffusion coefficient of concentrated protein solutions has been recently undertaken. We are attempting to apply previous theoretical analysis to this data (5).

Each crystallizing condition is characterized by a dilution curve showing the concentration dependence of D_z. Dilution curves are fitted to the thermodynamic model of Kam et al. to refine parameters K_∞ and K_1. K_∞ is the association constant for adding a monomer to a crystal,

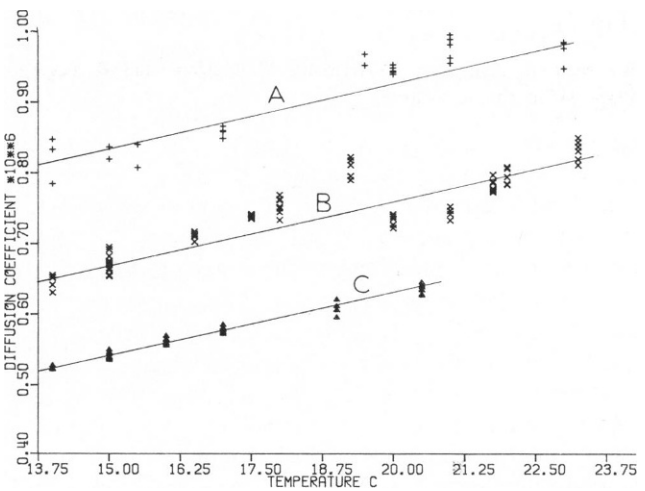

FIGURE 1 Temperature dependence of the diffusion coefficients of protein solutions. (*A*) 0.5% lysozyme, 0.1 M Tris, pH 7.5. (*B*) 3% lysozyme, 3% NaCl, pH 4.6; (*C*) 3% BSA in 0.1 M Tris, pH 7.5.

and K_1 is that for monomer association. Their ratio reflects the tendency of a solution to crystallize. Theoretical curves for selected ratios are shown in Fig. 2. Ratios near one are predicted to correlate with precipitation and ratios tending to larger values correlate with increasing tendency to crystallize. We repeated the experiments of Kam et al. and confirmed that the dilution curves for crystal forming and precipitate forming conditions have different shapes (Fig. 2).

In our hands the Kam et al. experimental conditions yielded macroscopic results within 15 h. Because this time is very short compared with the time most proteins take to crystallize, we analyzed dilution curves of lysozyme solutions, which crystallize over a much longer time frame. To this end a 3% lysozyme, 3% NaCl solution autobuffered at pH 4.6 was prepared. At all temperatures tested, this solution diffuses more slowly than monomeric lysozyme, as shown by the standard curves in Fig. 1 *A* and *B*. When the 3% lysozyme solution is diluted, the diffusion coefficient increases to a value consistent with the pattern of curve *A*. The dilution curve for this solution at 14°C is shown in Fig. 2. The dilution curve at 23°C is superimposable with a horizontal translation (data not shown). However, the time of appearance of macroscopic crystals differed. At 14°C crystals were observed after 24–48 h. At 23°C crystals were observed only after 10–14 d.

DISCUSSION

The utility of incomplete factorial experiments for crystallizing new proteins has been previously shown (1). Our results indicate that measurement of the concentration dependence of D_z can accurately evaluate crystallization experiments well in advance of actual macroscopic crystal formation and somewhat independently of pH or precipitant concentration. We have discussed the use of a standard curve specific for the protein to be crystallized (Fig. 1). This standard aids in the construction of dilution curves (Fig. 2).

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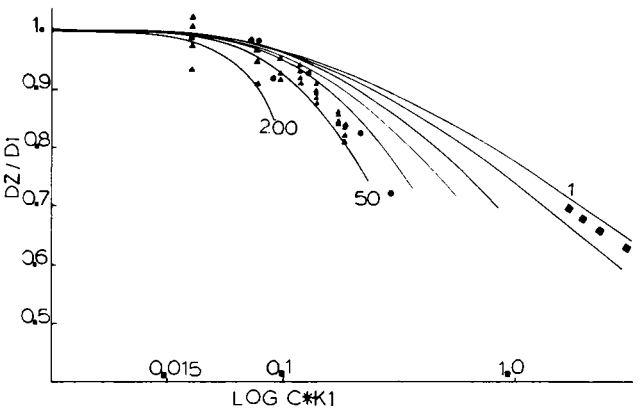

FIGURE 2 Dilution curves: D_z/D_1 vs. lysozyme concentration times K_1. Solid lines represent theoretical fits for different values of K_∞/K_1. Theoretical dilution curves to the right predict precipitation and curves to the left predict crystallization. ♦ represent the dilution curve data for 2% lysozyme, 30% saturated ammonium sulfate in 0.1 M sodium acetate buffer, pH 4.2, 20°C; $K_\infty/K_1 = 1.5$. ● represent the data for 5% lysozyme, 5% NaCl in 0.1 M sodium acetate buffer, pH 4.2, 20°C; $K_\infty/K_1 = 28$. ▲ indicate the dilution curve for 3% lysozyme, 3% NaCl, pH 4.6, 14°C; $K_\infty/K_1 = 27$.

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