Microcalorimetric and Small-Angle Light Scattering Studies on Nucleating Lysozyme Solutions

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Abstract: We have investigated the properties of nucleating lysozyme solutions, at various lysozyme and NaCl concentrations at pH 4.2, by isothermal conduction microcalorimetry and small-angle static light scattering. Both types of experiments were performed under stirring and differ drastically from their counterparts where stirring was not applied. Pronounced heat−power peaks, that can be attributed to nucleation and growth, appear at finite times which in turn depend on the supersaturation level. The calorimetric results are in qualitative accordance with the nucleation behavior deduced from small-angle scattering experiments. The implications of these experiments in the nucleation process of lysozyme are (i) without stirring, large fractal clusters with dimensionalities lower than ∼2.00 form and, after 1−2 days, large crystals appear and (ii) with stirring, nuclei of several micrometers in size appear within 2−13 h depending on conditions. Their dimensionalities vary between 2.5 and 3.0, indicating dense morphology; their appearance is accompanied by showers of microcrystals.

Introduction

Predicting the solution conditions where proteins aggregate and successfully crystallize remains a significant obstacle in the advancement of structural molecular biology. Most proteins have been crystallized under virtually random conditions, a practice that often fails to yield crystals suitable for X-ray diffraction. Therefore, understanding the crystallization process *per se* and the development of methods for, even approximately, predicting adequate conditions remains a worthwhile task. Most of the problems arising in protein crystallization are due to our incomplete understanding of protein−protein interactions and lack of kinetic data. It is gratifying that in recent years, there is an ongoing interest in gaining more insight in the issue of nucleation and growth of colloidal crystals using light scattering techniques.[1−3] Recently, the van Megen group[4] has examined the growth properties of colloidal crystals and aspects of the classical nucleation theory[5,6] have been reexamined by Ackerson and Schätzel.[7] Colloidal crystals can be obtained either charge or steric stabilization and crystallize at high volume fractions, these properties have been reviewed in ref 8. Proteins crystallize by screening with suitable electrolytes, or in the presence of low molecular weight inert polymers (dextrans, polyethylene glycols), but at much lower volume fractions. At present there are no easy ways to correlate the properties of proteins and colloidal crystals.

Static light scattering (SLS) may yield valuable information on protein aggregation and nucleation. The range of conventional equipment allows the determination of the scattering function for ensembles of particles comparable in size with that of the wavelength of the employed radiation.[9] For large nonequilibrium clusters, time-resolved measurements at small scattering vectors are mandatory. From the time evolution of the scattered intensities one can then deduce (i) the mean size and weight-average mass of the evolving clusters and (ii) the exponents that typify cluster morphology.

We have focused on the study of lysozyme for obtaining a picture of the events involved in nucleation and growth and on the morphology of the resulting microstructures. Molecular interactions and dynamics of fractal structures evolving in nucleating lysozyme solutions have been explored by us[10−15] and others[16,17] using light and neutron scattering techniques. We have pursued in our previous works the study of fractal structures, which appear at intermediate supersaturation levels. Fractal clusters[18] are formed far from equilibrium, and they can be conveniently studied by scattering techniques.[19] A major

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drawback in light scattering work with crystallizing proteins is the inability to work with isorefractive conditions, since most proteins denature and precipitate in organic solvents. Therefore, studies are limited to those volume fractions that ensure absence of significant multiple light scattering contributions.

Lysozyme is a suitable model system for investigating crystallization of biomolecules since it fulfils some important requirements: (i) the protein is stable and its monomeric state well defined and (ii) aggregation and concomitant crystallization can be easily induced by simple electrolytes, *i.e.*, NaCl upon screening the net positive surface charges. The role of the fractal clusters (amorphous precipitate) that appear in nucleating lysozyme solutions is not yet understood in quantitative terms. We assume that a catastrophic nucleation burst occurs within very short times (probably within milliseconds) when protein and NaCl are coming into contact. The majority of the nuclei formed during the burst are consumed in the formation of fractal clusters and only a minority forms crystals.

A reasonable, albeit indirect, way to test this hypothesis is to subject the fractal clusters to a controlled degree of shear with the hope that by continuously disrupting them, the solutions will exhibit characteristics expected for classically nucleating systems. Another question concerns the degree of order of the evolving clusters. Since this question cannot be unambiguously answered by scattering techniques, due to the low number density and small size of the nuclei, alternative methods should be sought.

We have combined isothermal microcalorimetric experiments and small-angle SLS. The experiments described below differ strongly from those of our previous works since they are conducted under continuous stirring. In terms of aggregation theory, the two categories of experiments can be distinguished as perikinetic (due to Brownian motion) and orthokinetic (involving stirring and concomitant shearing) aggregation kinetics.[20,21] In the latter case, the collision frequency between clusters is a sensitive function of the applied shear. Stirring breaks and redisperses the fragile fractal clusters. The formation of the new microstructure depends on (i) the Brownian motion of the clusters, (ii) on sedimentation and mass transport induced by thermal convection currents, and (iii) on dragging of smaller by larger clusters during sedimentation. Pronounced differences are found in both the calorimetric and the light scattering experiments, depending on whether stirring was applied or not.

Materials and Methods

Three times crystallized lysozyme was purchased from Sigma Chemical Co. (Deisenhofen, Germany), dialyzed against water, lyophilized and stored at 4 °C. All experiments were performed in a buffer containing 0.1M sodium acetate, pH 4.25. Monodispersity of the preparations was controlled by dynamic light scattering (DLS) without added electrolyte before each series of experiments, as previously described.[10−12] Protein and salt concentrations were determined for the calorimetric experiments with a precision balance (Mettler 261).

Calorimetric experiments were performed with a conduction differential microcalorimeter (Zielenkiewicz and Utzig, in preparation) with a nominal sensitivity of 0.103 mV/mW using 3 mL vessels equipped with a mechanical stirrer rotating with a constant speed of 60 rpm. Lysozyme was dissolved into the buffer−NaCl system, and signal registration started about 40 min after mixing. Typical duration of each measurement was 24 h, and all measurements were performed at 20 °C. Temperature differences between the vessel and the isothermal shield were determined by a semiconductor thermopile. The stability of baseline in a 24 h measurement corresponds to ±0.3 mV (or 2.9 mW). This noise level determined the lower limit of the measurable heat−power changes. Heat effects were determined from the integral $-\int_{t_2}^{t_1} \mathrm{d}H/\mathrm{d}t = Q$ where t_1 and t_2 denote suitably selected time limits. Two series of calorimetric experiments were performed: In the first, the concentration of lysozyme was varied from 2.60 to 3.16 mM and the concentration of NaCl was kept constant at 0.640 M (Table 1A). In the second, the concentration of NaCl was varied between 0.199 to 0.802 M and the concentration of lysozyme was kept constant at 2.90 mM (Table 1B).

Table 1. Summary of Calorimetric Results[a]

C_{NaCl} (M)	C_L (mM)	t_{max} (h)	Q (kJ mol^{-1})	after 24 h
		A. Figure 1A		
0	3.16		nhpp	baseline
0.639	2.60	13.17	16.3	mc
0.639	2.78	6.86	23.6	mc
0.639	2.95	4.15	29.1	mc
0.640	3.16	2.1	30.9	mc
0.642	3.14		nhpp	mc−ns
		B. Figure 1B		
0.199	2.91		nhpp	
0.394	2.91		nhpp	
0.563	2.90		nhpp	mc
0.642	2.91	3.0	21.79	mc
0.722	2.90	2.6	34.50	mc
0.802	2.89	1.9	41.77	mc

[a] Key: t_{max}, denotes the elapsed time between mixing of the components and the appearance of the heat−power peak maximum in the thermograms; nhpp, indicates that the heat−power peak does not appear in the microcalorimetric experiment; mc, denotes the appearance of the microcrystalline phase; ns, denotes no stirring.

Small-angle SLS experiments were conducted with a homemade CCD-based device. The apparatus and the data acquisition procedures were identical to those previously described.[22] In these experiments, lysozyme and NaCl were rapidly mixed in the appropriate ratio and filtered through Minisart sterile filters, 0.2 μm pore size, into standard 1cm × 0.5 cm black glass cells. Measurements were initiated within 30 s after mixing. The content of the light scattering cells was continuously stirred with a commercially available miniature magnetic stirrer.

Results and Discussion

To our knowledge microcalorimetric results on crystallizing lysozyme solutions were first reported by Takizawa and Hayashi.[23] Sibille and Pusey[24] have used calorimetry to determine ion binding enthalpies on lysozyme. During the course of their measurements, they observed a slowly evolving exothermic reaction taking place along the initial ion-binding signal. The authors postulated that the peak was due to lysozyme aggregation. However, they did not mention whether stirring was applied in these experiments or whether the experiments were extended to the times required for the observation of the nucleation heat−power peaks. It is therefore not unexpected that the appearance of the exothermic effect reported below may have escaped their attention.

Both calorimetric and small-angle SLS experiments deliver usable observables within reasonable times, say 1−2 days of experimentation, only within a narrow protein and electrolyte region. At high protein−electrolyte levels the exothermic peaks

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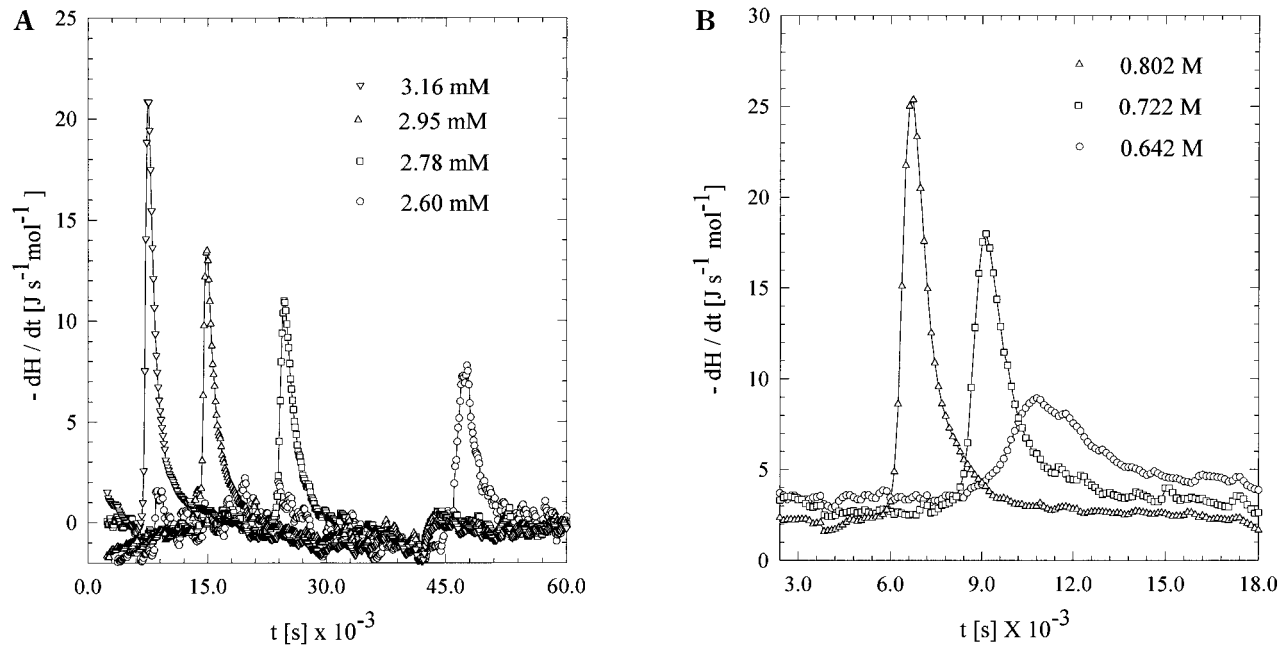

Figure 1. Microcalorimetric signals of isothermal nucleation and growth, after baseline subtraction. In A experiments, the NaCl concentration was kept constant at around 0.64 M (Table 1A) and lysozyme concentration was varied as indicated, whereas in B the lysozyme concentration was kept constant at 2.9 mM (Table 1B) and the NaCl concentration was varied as indicated; 0.1 M sodium acetate buffer, pH 4.25. Note the appearance of the exothermic heat−power peaks at finite times modulated by varying the supersaturation conditions.

appear within the times required for sample thermostation. At low levels the peak may be observed after several days but with an amplitude that hardly differs from the background. We faced more drastic problems during the course of the small-angle SLS experiments under stirring. The concentrations of lysozyme and NaCl employed in the calorimetric experiment cannot be studied by small-angle SLS due to formation of microcrystals at the walls of the scattering cells and extreme transmittance losses. Therefore, the protocol used in the small-angle SLS experiments unavoidably differs from that of the microcalorimetry in the sense that the lysozyme concentrations had to be reduced by ∼1.5 times. Except for differences in the employed concentrations, the (i) the volumes of the reaction vessels were 3 times smaller in the small-angle SLS experiment and (ii) the stirring speed required for measurable effects in the SLS experiments was some 5 times faster than that applied in the calorimetric experiment. These changes influence aggregation so that the complete coincidence of the events observed by each technique is not warranted. For these reasons the comparisons made below should be considered qualitatively.

The isothermal calorimetric signals are displayed in Figure 1 after baseline subtraction. At sufficiently high lysozyme and NaCl concentrations, a characteristic exothermic heat−power peak appears in the thermograms. The time of appearance of the peak depends strongly on the supersaturation. In the first series of experiments (constant NaCl concentration, 0.640 M) t_{max} varied between 13.2 and 2.1 h while the concentration of lysozyme was varied between 2.60 and 3.16 mM. The heat effect, Q, associated with the crystallization, varied between 16.3 and 30.9 kJ mol^{-1} depending on lysozyme concentration (Table 1A). Microcrystals were visually observed in all samples upon completion of the experiments. The heat−power peak in a control experiment involving a high lysozyme concentration 3.14 mM and 0.642 M NaCl was *not observed* under nonstirring conditions, indicating that the undisturbed evolution of fractal clusters obscures the direct manifestation of nucleation. This is an important aspect which corroborates our postulate[15,25,26] namely, that the seed particles involved in the formation of the fractals are not lysozyme monomers but nuclei.

In the second series of experiments, the heat−power peaks were not observed at all at and below 0.563 M NaCl (Table 1B). This concentration is in agreement with the previously determined[14] NaCl concentration (>0.50 M) required for triggering significant aggregation of lysozyme. Increment of NaCl concentration led to pronounced heat effects. For NaCl concentrations between 0.642 and 0.802 M, heat−power peaks appeared at times between 3.02 and 1.92 h. The heat effect, Q, which is associated with the crystallization varied in this series of experiments between 21.8 and 41.8 kJ mol^{-1}, depending on NaCl concentration. Above 0.60 M NaCl, microcrystals were again visually observed in all samples upon completion of the experiments.

We have plotted both t_{max} and Q as a function of lysozyme and NaCl concentration in Figure 2. The range of observations is limited, but it still delivers useful insights to the times required for the onset of nucleation. We find a linear dependence for both t_{max} and Q on lysozyme and NaCl concentration. From the regressions it can be predicted that a 0.1 mM (1.43 mg/mL) reduction of lysozyme concentration will cause 114 min delay (!!) in the appearance of the peak. Somehow less drastic is the influence of NaCl concentration where 0.1 M reduction will cause 45 min delay. Similar considerations apply for the trends observed in Q if the latter is understood as a measure of nucleation success. We expect that in typical crystallization attempts without stirring, these estimates will be even more pronounced and longer delays should accompany small changes in supersaturation. It is, therefore, understandable why small variations of the supersaturation often lead to arbitrarily long expectation times before protein crystals appear.

Examination of the exothermic peaks indicates that the underlying transformation may not be a simple process. Both

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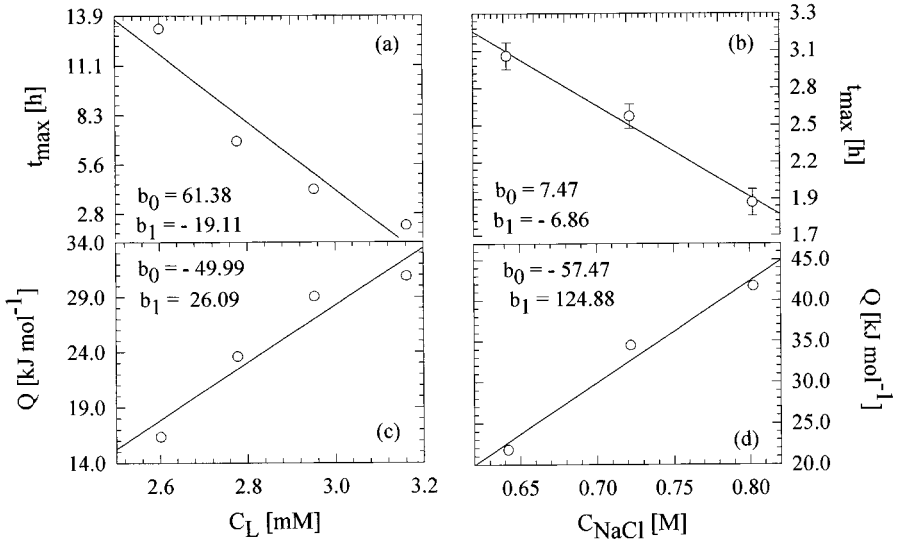

Figure 2. Variations of time of appearance of peak maximum, t_{max} and heat effect, Q, as a function of lysozyme concentration in a and c and NaCl concentration in b and d, according to Figures 1A and 1B. Note the differences in the slopes indicated in a and b. Except for the data in b, the error bars in the other plots do not exceed the symbol size.

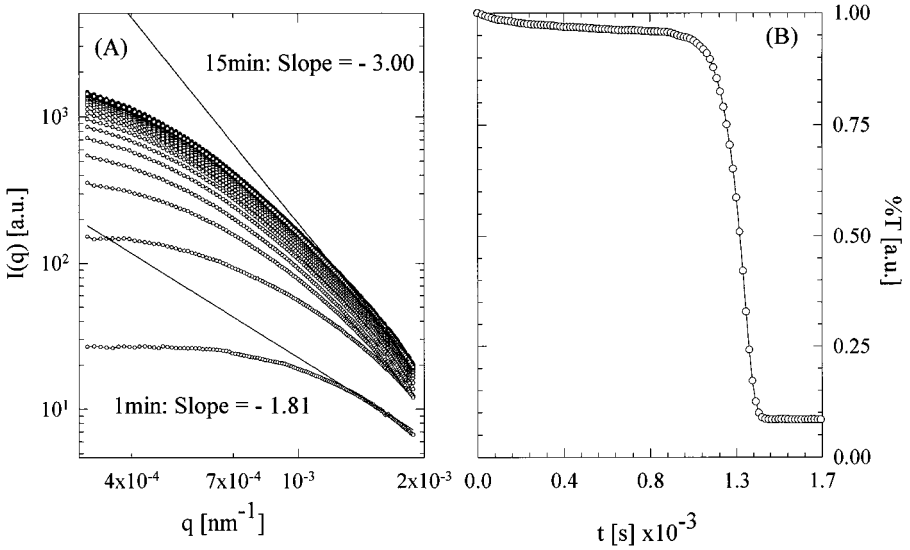

Figure 3. (A) Time-resolved intensity distributions $I(q)$ plotted as a function of scattering vector q. Lysozyme, 2.84 mM, was incubated with 0.640 M NaCl under the conditions described in Materials and Methods. For clarity, only every fourth distribution is plotted. In the time interval between 1 and 15 min aggregation typical for mass fractal clusters proceeds normally. Note the progressive decrement of the slope typifying the power-law region indicative of restructuring (fractal clusters with a dimensionality $d_f = 1.81$ undergo restructuring and cross rapidly to compact structures with a dimensionality $d_f = 3.00$). At later stages a drastic loss of the transmittance accompanied by cessation of growth and differential cluster sedimentation is observed (B). Transmittance loss (% T), recorded simultaneously with the scattering experiment plotted as a function of time. The 50% loss around 75 min is accompanied by a rapid sedimentation of microcrystals.

DLS and small angle-SLS indicate the appearance of fractals at the initial stages of the reaction under nonstirring conditions.[13,16] Small-angle SLS, under stirring (see discussion below), indicates also that the evolving structures assume gradually a more compact morphology. The overall picture is quite complex and may involve a combination of processes.

If the cluster sizes are small, the process may not be captured by microcalorimetry. After the nucleation burst, the number of small clusters that adhere on larger clusters is relatively small and the heat effect is below the sensitivity limits of the calorimeter. After some time, their number increases and the effect becomes measurable, showing an increasing heat-power amplitude. We expect that the nuclei attain their maximum size around the times where the peak maximum appears. Above this size, further growth ceases and the peak amplitude becomes again vanishingly small.

Growth Rates and Cluster Morphology Deduced from Small-Angle SLS Experiments

Small-angle SLS experiments indicated the growth of clusters under the chosen conditions. The scattered intensity distributions have been fitted with a Fisher−Burford[27] function of the form

$$I(q) = I(0)\left[1 + 2\frac{(qR_g)^2}{3d_f}\right]^{\left(-\frac{d_f}{2}\right)} \quad (1)$$

which yields good results for fractal clusters.[15,28,29] $I(0)$ denotes the zero-angle extrapolated scattered intensity which is propor-

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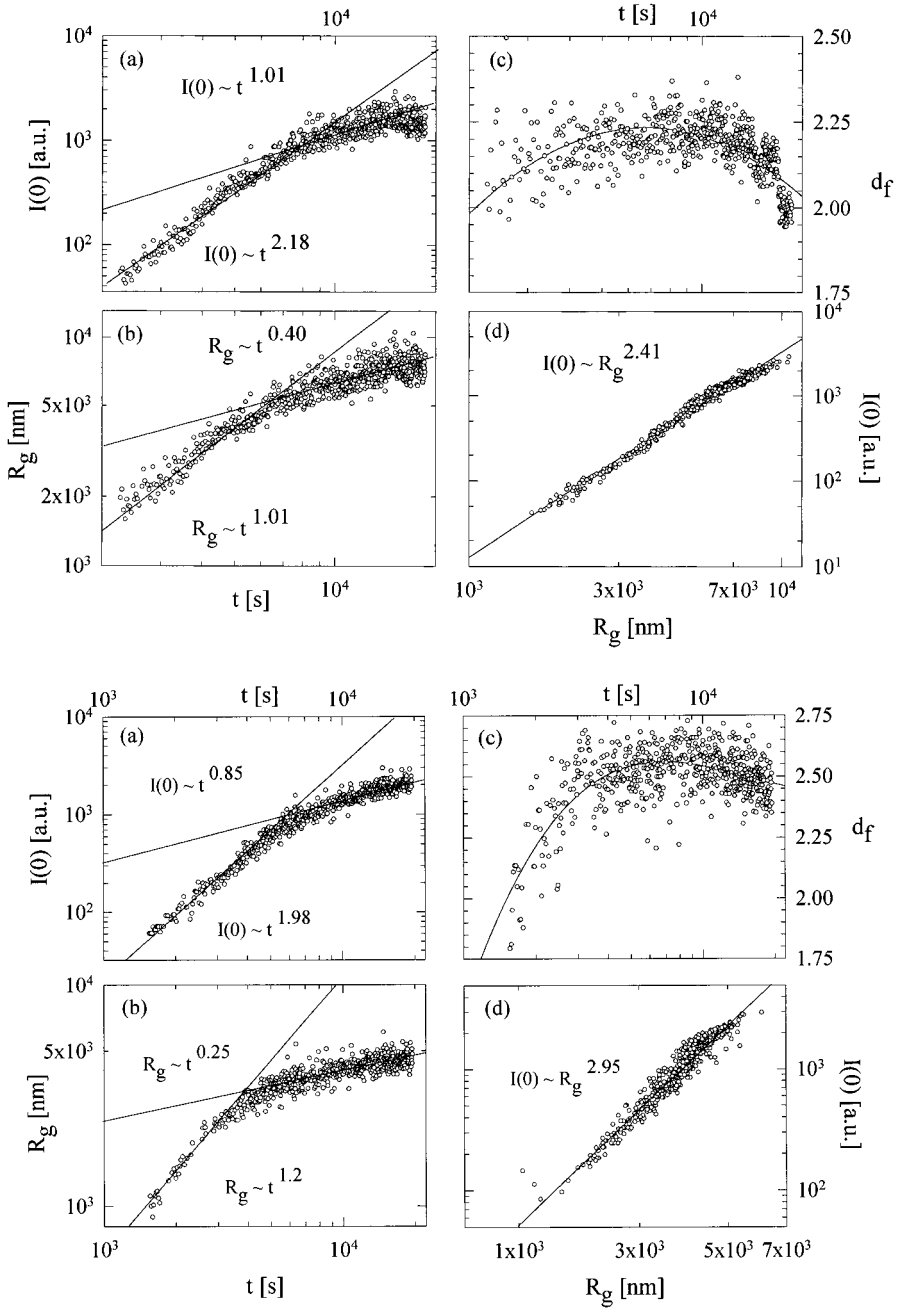

Figure 4. Typical time-resolved small-angle SLS experiments: (A, top panels) 1.75 mM and (B, lower panels) 1.98 mM lysozyme were incubated with 0.640 M NaCl under the conditions described in Materials and Methods. Note the biphasic character of the plots: (a) scaling of the zero-angle extrapolated scattered intensity, *I(0)*; (b) of the cluster radius of gyration, R_g; and (c) of the fractal dimension d_f of the evolving clusters on the elapsed reaction time, *t*. In c, the estimates of d_f range between 2.25 and 2.60. Close to the cross-over times, where both *I(0)* and R_g change slope, d_f attains its maximum value. In d, the scaling of *I(0)* on R_g provides an independent determination of the cluster dimensionality.

tional to the weight-average molecular weight of the clusters, R_g the radius of gyration and d_f the dimensionality of the clusters. The scattering vector, *q*, is a function of the wavelength λ of the employed radiation, of the refractive index of the medium, *n*, and of the scattering angle θ:

$$q = \frac{4\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right) \qquad (2)$$

and defines the extent of the spatial resolution. The parameters of interest, $I(0)$, R_g and d_f can be determined from eq 1, using a three parameter nonlinear least-squares fits to the individual distributions.

In Figure 3 we display an extreme example of small-angle SLS intensity distributions collected with 2.84 mM lysozyme in 0.640 M NaCl at pH 4.25 under stirring. This protein concentration resembles a case intermediate to that examined by microcalorimetry. At the beginning of the experiment, the scattered intensities increase as expected for typical fractal clusters, Figure 3A. After some 20 min however, they level-off, indicating the onset of transmittance losses and differential settling. Around 75 min the beam transmittance has reached one-half of its initial value. Above this time the transmittance decays very rapidly and beam attenuation is complete within the next few minutes (Figure 3B). A shower of microcrystals is observed under a microscope. This may be associated with the critical behavior of lysozyme[30,31] examined at lower temperatures and 5-fold higher concentration.

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Table 2. Summary of the Exponents Derived from Small-Angle SLS Experiment[a]

C_L (mM)	*I(0) vs. t*		R_g *vs t*		d_f		t_c (h)	R_g (μm)
1.47	1.70	1.00		0.53	~2.10	3.00		
1.75	2.18	1.01	1.01	0.40	~2.25	2.41	~2.10	~3.8
1.98	1.98	0.85	1.20	0.25	>2.50	2.95	~1.67	~2.3
2.12	2.05	0.96	0.78	0.31	>2.55	3.01	~1.53	~4.0
2.32	2.12	>0.00	1.10	>0.00	>2.60	2.95	~1.25	~5.5
2.39	2.81	>0.00	0.80	>0.00	>3.00	3.14	~0.45	~6.0

[a] Parameters typifying the growth of lysozyme nuclei deduced from small-angle SLS experiments. All plots are biphasic and scaling exponents were derived with a precision better than 15% from linear regression fits in the data segments corresponding to the fast (second and fourth column) and slow (third and fifth column) processes. The dimensionality of the clusters was determined either from the Fisher-Burford fits (cf., eq 5, sixth column) or from the scaling relation between *I(0)* and R_g (seventh column). t_c denotes the time at the crossover (completion of linear growth, eighth column). Estimates for the corresponding radii of gyration are given in the ninth column. They were obtained with a precision of better than 20% by averaging 20 points around the crossover. They exhibit a nearly linear dependence when plotted as a function of lysozyme concentration.

In Figure 4 we display two selected experiments conducted with (A) 1.75 and (B) 1.98 mM lysozyme in 0.640 M NaCl, pH 4.25. The time evolution of $I(0)$, R_g, and d_f is given in panels a−c. The plots are biphasic, indicating a fast and a slow growth process (or complete cessation of growth). We have determined the slope of each segment by linear regression; these results are summarized in Table 2. The cluster radius of gyration at the crossover ranges between 2.3 and 6.0 μm. An independent measure of the apparent cluster dimensionality can be derived from the scaling of $I(0)$ over R_g as shown in panels d. The dimensionality of the clusters varies, for the majority of experiments, between 2.50 and 3.00, indicating the evolution of structures with a compact morphology.

At early times the nuclei grow linearly. The data reproducibly indicate an $R_g \sim t$ scaling as expected from classical nucleation theory.[6,32] The slow growth at later times depends more strongly on the lysozyme concentration. There, growth rates vary as $R_g \sim t^{1/4}$ or $R_g \sim t^{1/3}$ before cessation of growth occurs. These exponents could be attributed to a Lifschitz-Slyojov[33] type of growth whereas scaling with larger exponents, *e.g.*, $R_g \sim t^{1/2}$ could reflect a Lifschitz−Allen−Cahn type of growth.[34] The growth at long times could be compatible with ripening where further cluster growth occurs at expenses of smaller clusters. Further, $I(0)$ exhibits for the fast regime quadratic scaling on time, $I(0) \sim t^2$ and only for the 2.39 mM lysozyme sample, scaling proceeds nearly as $I(0) \sim t^3$. For the slow regime, linear scaling, $I(0) \sim t$, is exhibited for all cases before the cessation of growth.

As shown in Table 2, the interplay between the growth exponents characterizing the temporal evolution of both cluster molecular weight and radius of gyration, indicates that clusters exhibit a quasicompact morphology with values of d_f close to 3.00. This is in clear distinction to cluster morphologies determined without stirring where d_f values clearly below 2.00, typically between 1.75 and 1.85, are found at comparable NaCl and protein concentrations[10−12] at the initial stages of the reaction. Such estimates are indicative of diffusion limited cluster−cluster aggregation[35] (DLCA). However, the homogeneity exponents[36,37] typifying the reactivity between clusters deviate[15] from those expected for pure DLCA, and we have termed this type of aggregation DLCA like. These deviations have been attributed to solvent-mediated interactions, and the attempts to understand them are part of wider theoretical research.[38]

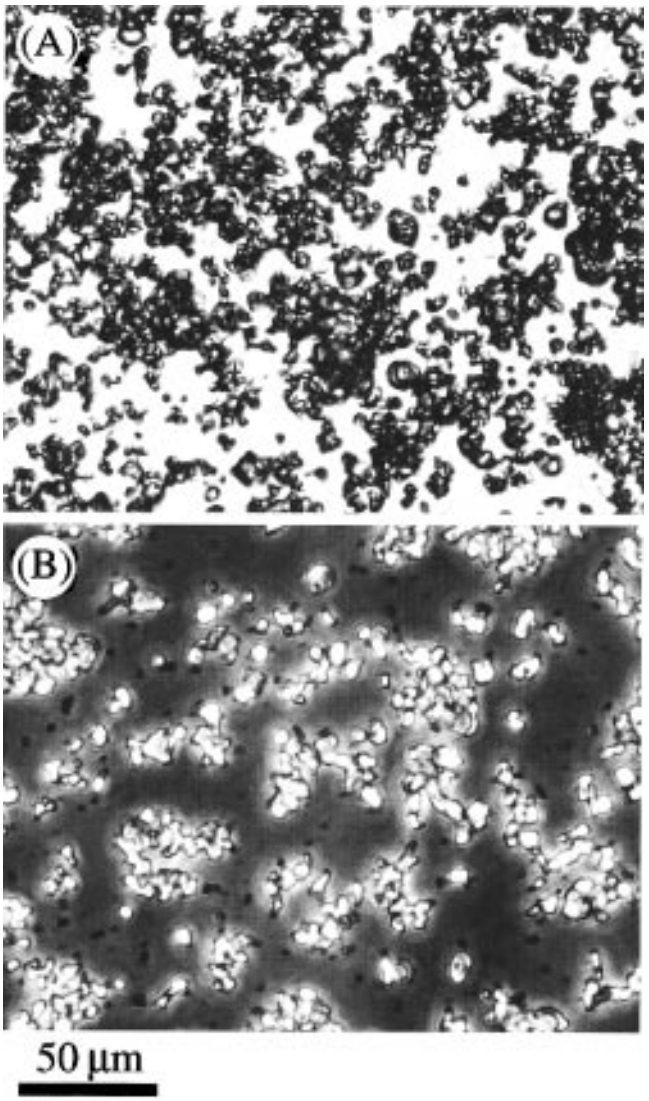

Figure 5. Light microscopy image of (A) 1.98 mM lysozyme incubated with 0.64 M NaCl. The solution was subject to continuous stirring during the light scattering experiment (Figure 4B) for 5 h before the image was recorded. Large clusters exhibiting strong birefringence under crossed polarizers sediment within a few minutes to the bottom of the light scattering cell. Images were recorded with the use of using phase contrast optics. Part B, 2.10 mM lysozyme with 0.64 M NaCl, was recorded with differential interference contrast optics.

The times typifying the approach to the crossover (Table 2) drop with increasing protein concentration in a manner similar to t_{max} determined by calorimetry. We believe that small-angle SLS captures events similar to those of microcalorimetry, although the time scales of their appearance may not necessarily be identical. Below the crossover region, the clusters radii of gyration grow linearly and attain sizes of several micrometers, From classical nucleation theory we expect the precritical nuclei

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to grow up to a critical size above which growth proceeds spontaneously via coalescence.[39] We find that the radii which could be associated with a nucleus size that triggers crystallization may vary between 2.0 and 6.0 μm at the cross-over. Up to this size the clusters grow linearly, and coalescence with smaller exponents is observed at the later stages of the reaction. This behavior correlates qualitatively with the onset of the heat−power peaks in the microcalorimetry experiments.

The evolving clusters were further examined by light microscopy under an inverted microscope (Zeiss Axiovert-100, Germany) upon completion of the light scattering experiments. A typical ensemble of microcrystalline clusters, corresponding to the experimental conditions of Figure 4B is displayed in Figure 5. The clusters resemble micrometer-sized crystals and exhibit strong birefringence. Prolonged microscopic observations showed that they do not grow further. Fractal clusters, as observed in our previous works,[25,26] were not visualized in any of the examined solutions. Larger, high quality crystals are obtained at these NaCl and protein concentrations after a few days without stirring. It is also worth mentioning that the drastic changes in nucleating solutions, observed by both calorimetry and light scattering, occur only in a very narrow "window" of protein and NaCl concentrations.

Concluding Remarks

The combination of microcalorimetric and small-angle SLS experiments can provide useful insights in the sequence of events governing the crystallization of lysozyme. The appearance of the heat−power peak signifies the onset of nucleation and growth. At comparable time scales, for both techniques the parameters typifying cluster growth undergo drastic changes. The heat effect observed in the microcalorimetric measurement is associated with the initial stages of growth of globular and ordered clusters. The growth of these clusters depends strongly both on protein and electrolyte concentration. Growth ceases after attainment of a terminal cluster size within finite times (that compare well for both techniques).

Microcalorimetric and light scattering experiments under stirring conditions exhibit completely different behaviour than without stirring. With stirring, lysozyme clusters are compact and globular, but if growth is allowed to take place without stirring, fractal clusters are formed and persist for several days in the solutions. The differences observed in the present work are attributed to disruption and redispersion of the fragile fractals caused by stirring. The fact that shear is necessary for observing, in both experiments, the characteristic nucleation behavior clarifies to some extent the role of fractals in nucleating solutions under nonstirring conditions (the usual case encountered in protein crystal growth attempts). Under stirring, nucleation proceeds with faster rates since oligomeric seed nuclei either do not bind onto or they are steadily removed from the fractals. This procedure leads to rapid microcrystal formation as judged by optical microscopy.

Shear has been shown to destroy order in colloids,[40,41] whereas our findings indicate that structure formation may be favored by shear. This behavior is not easy to explain. It could be attributed to different modulation of the interactions when stirring is applied. The issue requires further experimentation, combining scattering and rheological methods. Mandatory, however, is a better understanding of the set of interactions typifying protein crystallization. The variety of species present, under the conditions examined so far, may prohibit meaningful design of experiments and comparisons.

This research is currently directed toward a better understanding of nucleation phenomena in proteins as such. The long term task is, however, the development of high-capacity screening techniques for the identification of optimal crystallization conditions. The combination of small-angle SLS and microcalorimetry provides essential information on the parameters governing nucleation and crystallization which can be utilized as a first platform for further investigations.

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