THIRD SEMI-ANNUAL PROGRESS REPORT

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CONVECTIVE FLOW EFFECTS ON PROTEIN CRYSTAL GROWTH

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Summary of Work Performed and Results Obtained under this Grant

A high-resolution microscopic interferometric setup for the monitoring of protein morphologies has been developed. Growth or dissolution of a crystal can be resolved with a long-term depth resolution of 200 Å and a lateral resolution of 2 microns. This capability of simultaneously monitoring the interfacial displacement with high local depth resolution has yielded several novel results. We found with lysozyme that (a) the normal growth rate is oscillatory, and (b) depending on the impurity content of the solution, the growth step density is either greater or lower at the periphery of a facet than in its center.

The repartitioning of Na\(^+\) and Cl\(^-\) ions between lysozyme solutions and crystals was studied for a wide range of crystallization conditions. A nucleation-growth-repartitioning model was developed, to interpret the large body of data in a unified way. The results strongly suggest that (a) the ion to lysozyme ratio in the crystal depends mostly on kinetic rather than crystallographic parameters, and (b) lysozyme crystals possess a salt-rich core with a diameter on the order of 10 \(\mu\)m. Preliminary synchrotron white beam x-ray and scanning electron microscopy results appear to confirm this finding, which could have far-reaching consequences for x-ray diffraction studies.

A computational model for diffusive-convective transport in protein crystallization has been applied to a realistic growth cell geometry, taking into account the findings of the above repartitioning studies and our kinetics data for the growth of lysozyme. The results show that even in the small cell employed, protein concentration nonuniformities and gravity-driven solutal convection can be significant. The calculated convection velocities are of the same order of magnitude as those found in earlier experiments. As expected, convective transport enhances the growth rates. However, even when diffusion dominates mass transport, i.e. at 0g, lysozyme crystal growth remains kinetically limited. The salt distribution in the crystal is predicted to be non-uniform at both 1g and 0g, as a consequence of protein depletion in the solution.

Static and dynamic light scattering studies in undersaturated and supersaturated solutions have been performed. Diffusivities in undersaturated solutions were found to vary with lysozyme concentrations. Depending on the salt concentration, the diffusivities either increase or decrease. Interestingly, the corresponding static scattering intensities behave oppositely. Our current analysis indicates that these changes are inconsistent with aggregation in undersaturated solutions. However, the data are compatible with concentration-dependent changes of the interactions between protein and salt.
Since our crystal growth kinetics studies strongly suggest the presence of contaminants in the investigated lysozyme solutions, we have begun to characterize their composition by gel electrophoresis. We found, depending on the protein source, various high molecular weight impurities. Removal of these impurities by Fast Protein Liquid Chromatography is being pursued.

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1. Introduction
Our main research objectives under this grant are:

1. To investigate the effect(s) of defined convective transport and supersaturation conditions on the growth kinetics and morphology of selected proteins.
2. To establish a correlation between the growth conditions, crystallographic quality and x-ray diffraction resolution for some of these proteins.

As outlined in the original proposal, these objectives are to be pursued through work on the following tasks:

1. Refinement of our earlier morphology and kinetics studies without forced flow. In particular, this will be a continuation of our investigations of lysozyme crystal growth.
2. Morphology and kinetics studies under forced flow.
3. Determination of the repartitioning of the precipitant between solution and crystal.
5. Preparation, purification and compositional analysis of proteins.
7. Lights scattering studies of aggregation and nucleation in protein solutions.
9. X-ray diffraction studies of crystallographic quality.
2. Work Performed

During the report period, work was performed on tasks 1 - 7. Details are given in the following sections. Work on task 8 has been concluded; see the First Semi-Annual Progress Report.

2.1. Refinement of Morphology and Kinetics Studies Without Forced Flow

The main objectives of the work in this area for the report period were investigations of:

- the influence of impurities, shown to be present by gel electrophoresis (Sect. 2.5), on the growth processes of tetragonal lysozyme crystals;
- the growth kinetics oscillations, reported in the previous progress report.

The solution used in the growth experiments and the stock solution were characterized by gel electrophoresis (see Fig. 1). We found that both solutions contain comparable amounts of protein impurities with molecular weight of ~20, 67 and 94 kD.

For small crystal size and low supersaturation, $\sigma = \ln(C/C_{\text{sat}})$, we found that growing (101) faces remained flat within ~ 1,100 Å. This corresponds to one interference fringe, that is, as the crystal grew, the interference contrast changed across most of the face from bright to dark and so on, as Figs. 2a and 2b show. At higher $\sigma$, several fringes appeared on the interface, indicating morphological height differences of several thousand Å. The fringes originated at the edges of the crystal and moved inwards. Figures 3a and 3b were captured 20 minutes apart, at $\sigma = 2.42$. They show that the fringe pattern constantly changed, indicating the absence of a fixed step source on this face. These morphological observations indicate the dominance of step generation by 2 dimensional (2D) nucleation. One may be tempted to interpret the appearance of fringes on a previously flat face as evidence of an emerging dislocation. This conclusion, however, might only be justified if a large number of consecutive fringes originate at the same location.

At still higher supersaturations and larger crystal size, fringes always appeared on the facet edges, Fig. 4a. During inward motion, the fringes slowed down (see below) thus forming a depression with lower slope in the facet center, Fig. 4b. This depression is similar to the one we observed before on a (110) face (see Second Semi-Annual Progress Report).

The $\sigma$-dependence of the average normal growth rate $\mathbf{V}$, measured in this run is shown in Fig. 5a. It is vanishingly low for supersaturations less than 1.5. The corresponding average local slope $\mathbf{p}$ and average tangential velocity $\mathbf{v}$, determined as described in the Second Semi-Annual
Progress Report, are presented in Figs. 5b and 5c. The high value of the lowest \( v \) likely indicates that the vanishing \( V \) at lower \( \sigma \) is not due to impurity poisoning, but rather a lack of step sources. This interpretation is further supported by the sharply rising \( p(\sigma) \). This sharp rise is expected for steps originating from 2D nucleation events outside the pixel pair used for the slope determination (see earlier report). This further supports the interpretation of the above morphological observations. Figures 5b and 5c also show that \( p \) is smaller and \( v \) is larger in the facet center, although the differences are much smaller than those observed earlier for the (110) face. The decrease in \( v \) at high supersaturations, leading to the local minimum in \( V(\sigma) \) of Fig. 5a, is not understood at this point.

We expanded our study of the novel interface topography (higher slope/step density at the edges than at the face center) to (101) faces. Fig. 6a (taken from the earlier report, and augmented with a height profile along the diagonal of the face, Fig. 6b) shows this topography for a (110) face. The concavity of the shape increases with crystal size, see Fig. 4 for (101) and Fig. 7 for (110). This is the result of an increase of the difference in tangential velocity at the edge and the center of the facet with crystal size, see Figs. 5c and 8. Furthermore, note from Figs. 4 and 7 that the (101) face is considerably less concave than the (110) face. However, in a run in which we had indication of higher impurity concentration, we observed a strongly concave (101) face. Thus, the greater depression depth of the (110) face discussed here, is likely due to higher impurity contents in this run.

The dependence of depression depth on the crystal size suggests a bulk transport effect. As we have speculated earlier, the retardation of the growth steps, i.e., the lower tangential velocity near the face edges is probably due to the transport-conditioned higher availability of impurities in these regions. We have now further evidence for this supposition. As depicted in Fig. 9, growth from a higher purity solution lead to a quite different topography: higher slope in the center and lower slope at the edges. This topography has been extensively discussed in the past, and is due to the more ready supply of nutrient at the face edges.

We have further investigated the growth rate oscillations under various conditions of supersaturation and crystal size. We found that the relative amplitude of the oscillations \( \Delta V/\bar{V} \) increases with supersaturation and growth rate, Fig. 10, and with crystal size, Fig. 11. Furthermore, it appears that \( \Delta V/\bar{V} \) is somewhat greater at the face center than at the edges, Fig 11. These observations indicate that the oscillation magnitude depends on bulk transport. Allégret et al. (Nature 294 (1981) 223) deduced from a general growth rate model that growth rate oscillations will be strongest under mixed kinetics and diffusion control. Growth of lysozyme is mostly kinetics controlled. Hence, even without understanding of the underlying
mechanism, it is not surprising to find that a shift towards transport control, through increased growth rate and crystal size, leads to an increase in $\Delta V/V$.

To characterize the step patterns associated with growth rate oscillations, we monitored the local slope and the tangential velocity at several supersaturations. Figure 12 shows that the relative amplitude of the oscillations of $p$ and $v$ are several times greater than the $\Delta V/V$ of about 0.4. The tangential velocity tends to decrease with rising slope. A lower tangential velocity at higher slope likely indicates overlapping of the bulk or surface diffusion fields feeding the growth steps. Variations in $v$ should reflect the variations of either interfacial supersaturation or adsorbed impurity concentration. Since the surface supersaturation differs only slightly from the bulk $\sigma$ (see Sect. 2.4), it is unlikely that it could vary to such a large extent. Therefore, we assume that the growth rate oscillations are related to impurity effects.

Based on the above observations, we propose the following working model for the growth rate oscillations. We assume partially transport controlled, time-dependent impurity adsorption on the interface. A fluctuation in the concentration of adsorbed impurities leads to a change in $v$ and thus, in $V$. For instance, a reduction in $V$, increases the exposure time of the terraces between steps and, thus, results in still higher impurity adsorption. This provides the *inertia* needed in an oscillator model. Since $\partial p/\partial t \approx -\partial v/\partial x$, the locally reduced $v$ will lead to a gradual increase in $p$. Eventually, since

$$ V = p \cdot v , $$

$V$ begins to increase again. This, in turn, leads to a reduction in adsorbed impurity concentration and, hence, higher $v$. This provides the *restoring force* which depends on “displacement”. Because of the overlapping step diffusion fields, $p$ and $v$ are not in exact counterphase (see Fig. 12). That is why their oscillations do not result in a steady $V$, see Eq. (1). The resulting step pattern on the crystal surface will consist of waves of higher step density (step bunches) with steps dashing forward from the bottom of a bunch and new steps joining a bunch at the top.

The proposed growth kinetics oscillations mechanism will be extensively tested with growth experiments in (a) highly purified solutions, (b) on crystals with dislocation-generated growth layers and (c) under transport conditions modified by forced flow.

### 2.2. Morphology and Kinetics Studies With Forced Flow

Parallel to the above efforts, we began to design the flow-through crystallization cell needed for the studies with forced flow. A peristaltic pump, that can deliver flow rates between 0.003 and 6.7 ml/min, has been received and tested. Assuming a $2 \times 2$ mm\(^2\) cross-section of the
crystallization cell, this range corresponds to average flow velocities between 0.012 and 28 mm/sec.

2.3. Repartitioning of Precipitant

As reported in the Second Semi-Annual Progress Report, our segregation results support the concept of coring in lysozyme crystal growth. We hypothesized that small crystals $O(20 \times 20 \mu m^2)$ incorporate much higher amounts of Na and Cl ions per lysozyme molecule (40-100) than during their ensuing growth. Salt-rich cores in protein crystals have not been observed previously and could introduce lattice strain, possibly reducing x-ray diffraction resolution.

In order to either prove or disprove the "coring hypothesis" we sought a technique which directly yields compositional information for a selected region of a lysozyme crystal. It was suggested by M. Pusey (MSFC) that a core could be resolved by microtoming of a crystal and application of a Cl- sensitive fluorescence dye. This idea and available resources, lead us to perform energy dispersive spectroscopy (EDS) using a Jeol JSM-820 scanning electron microscope with a KEVEX attachment. This technique provides compositional information with high spatial resolution.

Crystals about 100 \mu m in size were grown from 50 mg/ml lysozyme, 2.5 % (w/v) NaCl solutions with pH = 4.5. To render the crystals suitable for electron microscopy, they were cross-linked by addition of 6 % gluteraldehyde solution. The crystals became yellow-tinged and were readily dislodged from the walls of the growth tubes. For first attempts with EDS the crystals were not microsectioned since we expect that the historical core (as opposed to geometrical core) would be close to the crystal face that was in contact with the glass since nucleation.

Preliminary qualitative EDS measurements, using beam energies of up to 10 keV, gave adequate signals from Na, Cl and S, while not damaging the crystals. Based on the assumption that the area of the salt-rich core is approximately 20 x 20 \mu m, x-ray dot mapping was chosen as the most advantageous manner in which to analyze the crystals. In this mode, the electron beam that scans the specimen is used for both the CRT image and the x-ray elemental analysis. Thus one can clearly identify the analysis location on the CRT image. Signal intensity derived from beam specimen interactions is used to adjust the brightness of the spot appearing on the CRT.

Thus far, we have obtained two composition maps. While we are still in the process of optimizing the signal collection procedure, our first results indicate that over most of the specimen surface, Na, Cl, and S are present in comparable amounts. In non-core areas, for which our segregation results indicate between 2-4 Na and Cl atoms per lysozyme molecule, these
results are expected since there are 4 sulphur atoms for each lysozyme molecule. However, in two small areas (10 X 10 μm) we found increased concentrations of all three elements. Further analysis of these areas is underway. In case the historical core is somewhat recessed in the crystal, we will slice the crystal and systematically map sections.

2.4. Numerical Modelling of Diffusive-Convective Transport

In the previous Semi-Annual Progress Reports, we have given a detailed account of our lysozyme crystal growth model and numerical approach, together with some preliminary results. In order to estimate the error caused by the assumption of fixed crystal size, we have obtained solutions for pure diffusion (g = 0) for both moving and stationary boundary cases. The resulting lysozyme concentration in the solution after 5 hours of crystal growth is shown in Fig. 13, (a) for moving crystal surface and (b) fixed crystal size. While the crystal size changes from 400 μm x 600 μm to 466 μm x 738 μm after 5 hrs, thus increasing 43.3%, the solution “volume” decreases from 5.76 mm^2 to 5.656 mm^2, i.e. by only 1.8%. Hence, as illustrated by Fig. 14, the difference in local solute mass concentration resulting from the two approaches is negligible. As a consequence, our previous assumption of a fixed crystal size is reasonable for the description of concentration distributions and their time dependence for crystals of the order of 100 μm.

In order to describe the precipitant (salt) distribution in the solution, we have incorporated another species equation in the model. For the segregation coefficient k we used the value 0.01, which is a representative value obtained in our lab for the NaCl:lysozyme system. Fig. 14 shows the isoconcentration lines in the solution after 1 hour of crystal growth for (a) g = 0 and (b) g = 1. Both cases gave a rather uniform salt distribution. The slight increase of salt concentration is mainly due to the loss of solvent from the solution due to incorporation in the crystal. (Note that one-third of the volume of a lysozyme crystal consists of water.) In view of the uniform salt concentration, even in the interfacial solution region, one can not expect any significant effect on the crystal growth rate due to precipitant segregation.

Our numerical simulation work on transport in protein crystal growth is summarized in an extensive publication that has been submitted to the Journal of Crystal Growth; See Sect. 3.

One shortcoming of our current model is that it does not possess sufficient spatial resolution to resolve the concentration distributions associated with very small crystals. Hence, in order to illuminate, in particular, the evolution of “concentration boundary layers” that are expected by many workers during the initial crystallization process at g = 0, we will recast our numerical approach correspondingly.
2.5. Preparation, Purification and Compositional Analysis of Proteins

In the past, we had numerous indications in our growth kinetics studies, that the protein solutions used contained significant amounts of unidentified impurities and/or protein heterogeneities. In order to put our studies on a firmer footing, we have expanded our preparatory and analytical capabilities. An experienced biochemist was hired. In addition, we have acquired state-of-the-art instrumentation for Fast Protein Liquid Chromatography (FPLC) and Gel Electrophoresis (Phastgel system). These instruments will allow us to highly purify and thoroughly characterize the protein solutions to be used in the crystal growth and nucleation studies.

Electrophoresis data have revealed that high molecular weight (HMW) impurities exist in the stock solutions purchased from both Sigma and Seikagaku companies. The samples were electrophoresed on a 12.5% homogenous polyacrylamide gel under reducing conditions to obtain separation according to molecular weight. Approximately 0.5 μg of lysozyme sample was loaded in each lane of the gel. The Sigma lysozyme sample appears to contain four bands: 14.4 kD (lysozyme), 20 kD, 67 kD and 94 kD. The Seikagaku lysozyme sample appears to contain three bands: 14.4 kD (lysozyme), 20 kD and 94 kD. These bands are shown in Fig. 15.

The removal of these HMW proteins by FPLC is now being pursued. Preliminary results have been obtained for Sigma lysozyme fractions from a cation exchange column, which separates proteins according to charge. As illustrated by the chromatogram of Fig. 16, the profiles show a dominant peak which is preceded by two shouldering peaks. The fractions across the peak(s) were analyzed electrophoretically under reducing conditions. It appears that at least one of the HMW proteins coelutes with lysozyme, see Fig. 17. Hence, as a next step in the purification procedure, we will attempt to remove the HMW components by sieve chromatography, which separates according to molecular weight. Probably, the optimal procedure will then consist of a combination of the above techniques.

The preparation of highly homogeneous lysozyme samples will be crucial for our growth kinetics studies (Sects. 2.1, 2.2) and investigations of nucleation and aggregation (Sect. 2.7).

2.6. Measurements of Protein/Precipitant Diffusivities

During this report period we have completed the construction of a interferometric setup for diffusivity measurements in liquids. A high-resolution Mach-Zender interferometer, acquired under an earlier NASA grant, was combined with a novel, readily dismountable diffusion cell and a PC for data acquisition. The filling of the cell with two solutions of slightly different diffusant concentration was automated with motor-driven syringes. A novel integral algorithm
for accurate fringe analysis has been developed. Extensive tests of this setup have produced
diffusivity data for undersaturated aqueous NaCl solutions, that agree with published values to
within 0.5%. Extension of the measurements to saturated solutions, yielded diffusivity data
which are considerably lower than the few results obtained earlier. This is likely due to the
smaller concentration difference used in our sensitive setup.

A publication on this work is in preparation; see Section 3. Measurements of diffusivities
of the lysozyme:NaCl:H2O system will begin shortly.

2.7 Aggregation and Nucleation Studies

We use light scattering as a tool to investigate aggregation and nucleation in protein
solutions. In a series of recent experiments we explored scattering from undersaturated
solutions. These experiments have produced some unexpected and exciting results with
important implications for the future direction of our work.

The investigated solutions contained 50mM sodium acetate buffer (pH=4.7) and
lysozyme concentrations between 2 and 50 mg/ml, with either no or 1% (w/v) NaCl added. The
lysozyme used was 6-times recrystallized, lyophilized stock from Seikagaku Chemicals and, for
this preliminary round of experiments, was dissolved in our buffer without prior dialysis or
sample purification.

We performed simultaneous static and dynamic light scattering measurements at multiple
angles ranging between 23° - 120°. This allowed us to measure the molecular weight, the second
virial coefficient and the diffusion coefficient in one measurement for each sample. Between
protein samples we measured the intensity of a toluene sample as reference standard for our
static measurements and to correct for slow drifts of the laser intensity. All measurements were
made in VV-polarization with a Glan Thompson prism as polarizer of the incoming beam and as
analyzer in front of the PMT-tube optics.

For a given concentration, the intensity correlation functions from different angles are fit
to a single exponential plus background. The corresponding diffusion constant is extracted by
fitting the decay rates \( \Gamma \) obtained at different angles to the expected \( q \)-dependence for diffusing
particles

\[
\Gamma = 2D q^2 ,
\]

where \( q = 4\pi n / \lambda \sin(\theta/2) \) is the magnitude of the scattering vector and \( \theta \) is the scattering angle. There were no detectable deviations from the \( q^2 \)-dependence over the whole range of
measurement angles. This is not surprising given that the radius of the protein is approximately
2 nm and therefore much smaller than $1/\theta_{\text{max}} \sim 30.1$ nm - the upper size limit for Rayleigh scattering.

The results of the diffusion constant measurements are summarized in Fig. 18. Note that for the solution without added NaCl, the apparent diffusion constant $D_{\text{app}}$ sharply increases from its zero concentration value $D_0 = 11.1 \times 10^{-7}$ cm$^2$/sec to a value of $18.9 \times 10^{-7}$ cm$^2$/sec at 50 mg/ml. This result is in stark contrast to the most commonly used reference for lysozyme: Dubin, Clark and Benedek [1] measured the diffusion constant in a 0.1M sodium acetate buffer solution at pH 4.2. For protein concentrations ranging between 10-100 mg/ml at $T=20^\circ$C they report a concentration-independent value of $D = 10.6 \times 10^{-7}$ cm$^2$/sec.

The observed concentration dependence in our measurements is reproducible and compares favorable with unpublished data obtained in low-concentration solutions by Prof. W. Wilson from Mississippi State University, who kindly made his results available to us. Furthermore, similar observations have been made by several authors in bovine serum albumin (BSA) [2,3]. Hence, we are confident that our data are reliable and represent a real effect. Salt-protein interaction models used to interpret such changes in diffusivity [3] also predict the sublinear behavior at low salt levels clearly visible in Fig. 18 for no NaCl added.

However, at 1% NaCl concentration the diffusion constant slightly decreases with increasing protein concentration. The zero-concentration diffusivity value extrapolated from both zero and 1% added NaCl curves is, within our experimental accuracy, the same value $D_0 = 11.1 \times 10^{-7}$ cm$^2$/sec. Again, both the downward slope at increasing salt concentration and the value of $D_0$ compare well with observations by Wilson.

The static intensity data were processed as follows. The count rate for any given protein concentration were compared to the count rate in toluene and the sample is assigned a Rayleigh ratio

$$R_{\text{sample}} = \frac{I_{\text{sample}}}{I_{\text{toluene}}} R_{\text{toluene}}$$

where $I$ stands for the total number of PMT-counts for a fixed measurement duration. The Rayleigh ratio of toluene for VV-detection was taken to be $R_{\text{VV}} = 24.4 \times 10^{-5}$ cm$^{-1}$. The resulting Rayleigh ratios for the protein solutions are displayed in two different ways, depending on whether interactions between the scatterers is taken into account.

In the absence of interactions in the sample the scattered intensity for a collection of Rayleigh scatterers is
\[ R = K \langle M \rangle_w c. \]  

Here \( K \) is a constant equal to \( K = \frac{2\pi n_w dn}{dC} \) \( N_A \lambda^4 \)  

which, with \( n_w = 1.33 \) the refractive index of the solvent (buffer), \( dn/dc = 0.2 \) ml/g the refractive index increment for lysozyme, Avogadro’s number \( N_A \), and \( \lambda = 514.5 \times 10^{-7} \) cm the wavelength of the incident laser beam, takes on the value \( 6.62 \times 10^{-7} \) cm\(^2\) mol\(^{-2}\). The \( \langle M \rangle_w \) in Eqn. (3) is the weight-average molecular weight of the scatterers \[ \langle M \rangle_w = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i} , \]  

where \( N_i \) is the particle concentration of \( i \)-mers.

Figure 19 displays the measured Rayleigh ratios for the two salt concentrations. First we notice the strong increase in scattering intensity with increasing salt concentrations. In addition, we see that based on Eqn. (5) one deduces an average molecular weight for the zero salt concentration about half the value for lysozyme of \( M = 14\,700! \) When applying the Einstein-Stokes relation for free Brownian motion to extract a radius from the corresponding diffusion constant \( D \) \( r = \frac{k_B T}{6\pi \eta D} , \)  

the particle radius \( r \), as well, falls well below the size of 2nm for monomeric lysozyme.

This type of analysis might lead one to the erroneous conclusion that the protein is “decomposing” into fragments well below its monomeric size! There is, however, no indication from gel electrophoresis to support such a claim (see Section 2.5). Furthermore, the variation in molecular weight and diffusion constant are both reversible and concentration dependent.

However, there is another way to analyze our results, which takes into account the salt-concentration dependent interactions to be expected for the highly charged proteins. Under these circumstances the intensity data should be plotted in the form of a virial expansion \[ \frac{K_c}{R} = \frac{1}{M} + 2 B c \]
where B is the second virial coefficient (note that we recover Eqn. (3) for $B = 0$). The corresponding plots of the data are presented in Fig. 20. We can see that this presentation resolves the discrepancies of the previous analysis: The average molecular weight $M = 18\,000$ is within the experimental accuracy identical for both salt concentrations and about 20% higher than the monomeric weight for lysozyme $M_{\text{mono}} = 14\,700$. This difference could be due to the presence of a fixed fraction of higher molecular weight impurities detected in our gel electrophoresis measurement (see Sect. 2.5) and/or a salt-independent fraction of higher n-mers of lysozyme itself. Further sample purification and characterization, which we are currently performing, will help to answer this question.

The magnitude of the virial coefficient B for different amounts of added salt is the criterion proposed by W. Wilson and coworkers [4] in their work on “optimal crystallization conditions”. To gain more insight into the origins and relevance of different interactions to the protein crystallization process, we plan to take a slightly different approach. We will attempt to connect the current light scattering measurements in undersaturated solutions with extensive theoretical and experimental results obtained from colloidal aggregation studies over the last decade.

We have only started to explore these connections and it is too early to draw any definite conclusions on the general applicability of these colloidal models. Their relevance to protein crystallization cannot, however, be overstated. Already our measurements, as well as W. Wilson's results, indicate that the scattering in undersaturated protein solutions is dominated by interaction effects and not aggregation. One will have to account for the presence of these interactions for work in supersaturated solutions: Thus, changes in the diffusion constant and scattering intensity cannot be readily related to particle sizes. Most importantly, however, these measurements are of great value when trying to elucidate the role of different interactions in the nucleation and crystallization process. We will therefore focus our future efforts on this crucial question.

References:
3. Presentations, Publications and Other Professional Activities

Presentations


L.A. Monaco, F. Rosenberger, *Growth kinetics and morphology of tetragonal lysozyme*; invited talk at the Protein Crystal Growth in Microgravity Conference, Panama City Beach, Florida, April 23-26, 1993.

L.A. Monaco, Hong Lin, A. Nadarajah, F. Rosenberger, *Convective flow effects on protein crystal growth diffraction resolution - A research plan*; The Protein Crystal Growth in Microgravity Conference, Panama City Beach, Florida, April 23-26, 1993.


L. Monaco, P. Vekilov, F. Rosenberger, *Kinetics, morphology and segregation in lysozyme crystallization*; invited talk at Fifth International Conference on Crystallization of Biological Macromolecules, San Diego, California, August 8-13, 1993.


Publications


Other Related Professional Activities

Franz Rosenberger has organized and chaired a session on Physics of Crystallization at the Fifth International Conference on Crystallization of Biological Macromolecules, August 8-13, 1993 in San Diego, CA. He has also been elected president of the Advisory Board for the Sixth International Conference on Crystallization of Biological Macromolecules, to be held in October 1995 in Kyoto, Japan. Furthermore, Rosenberger has been invited to present a plenary lecture on "Protein Crystallization - What We Should Know, and What We Do Know" at the Eleventh International Conference on Crystal Growth in Arnhem, Holland, in June 1995.
**FIG. 1**

SDS-PAGE (12.5%)

Markers

Lysozyme growth cell soln with increasing concentration

Lysozyme stock soln

Markers

kD

94

67

30

20

14.4
(101) Face

$\sigma = 1.59$  \hspace{1cm} $\sigma = 1.65$

(a) \hspace{2cm} (b)

100 $\mu$m

FIG. 2

(101) Face, $\sigma = 2.42$

(a) \hspace{2cm} (b)

100 $\mu$m

FIG. 3
(101) Face, $\sigma = 3.28$

FIG. 4
(101) Face

- Center

- Periphery

\[ \text{SUPERSATURATION } \sigma = \ln \left( \frac{C}{C_{\text{sat}}} \right) \]
Average Tangential Velocity [μm/s] vs. Supersaturation $\sigma = \ln \left( \frac{C}{C_{\text{sat}}} \right)$

- (101) Face
- Open Circles: Center
- Black Circles: Periphery

Increasing Crystal Size

FIG. 5c
(110) Face, $\sigma = 2.35$
(110) Face, $\sigma = 2.8$

**FIG. 7**

![Images showing crystal growth on (110) face](image)

**FIG. 8**

Graph showing tangential velocity vs. supersaturation.

- **Tangential Velocity [\(\mu m/sec]\)**
- **Supersaturation \(\sigma = \ln(C/C_{sat})\)**

Legend:
- ○ Center
- ● Periphery

Increasing crystal size with supersaturation.
(110) Face, $\sigma = 1.7$

Layer Generation

**FIG. 9**
(110) Face

\[
\begin{align*}
\sigma &= 1.7 & \Delta V/V &= 0.12 \\
\sigma &= 2.0 & \Delta V/V &= 0.21 \\
\sigma &= 2.8 & \Delta V/V &= 0.31 \\
\sigma &= 3.2 & \Delta V/V &= 0.36
\end{align*}
\]
(101) Face, $\sigma = 3.28$
(101) Face, $\sigma = 3.28$
FIG. 13
FIG. 15

SDS-PAGE (12.5%)

Markers | Sigma | Seikagaku | Blank | Markers

kD
94
67
30
20
14.4
Buffer: 40 mM Na-acet., pH 4.5
Eluent: buffer + 1 M NaCl

FIG. 16
SDS-PAGE (12.5%)
Lysozyme, $T = 20 \, ^{\circ}C$
50 mM NaAc, pH = 4.7

- $\triangle$ No NaCl added
- $\bigcirc$ 1% NaCl (w/v)
- $D_{\text{app}} = D_0 + K_D \cdot c$

$D_0 = 11.08 \times 10^{-7} \, \text{cm}^2/\text{sec}$
$K_D = -30.9 \, \text{ml/g}$
(first 6 points fitted)

$D_0 = 11.15 \times 10^{-7} \, \text{cm}^2/\text{sec}$
$K_D = +261.9 \, \text{ml/g}$

FIG. 18
Lysozyme, $T = 20 \, ^\circ C$
50 mM NaAc, pH = 4.7
- No NaCl added
- 1% NaCl (w/v)

$R = KMC$

$M = 7,360$

$M = 15,700$

FIG. 19
Lysozyme, T = 20 °C
50 mM NaAc, pH = 4.7

- No NaCl added
- 1% NaCl (w/v)

\[ \frac{Kc}{R} = \frac{1}{M} + 2Bc \]

M = 18,200
B = 13.5 \times 10^{-4}

M = 18,500
B = 0.89 \times 10^{-4}

FIG. 20