FINAL TECHNICAL REPORT

NASA Grant No. NAG8-1408 "Ground based program for the Physical Analysis of Macromolecular Crystal Growth" (Dr. Alexander J. Malkin, PI)

In a reported period in situ atomic force microscopy was utilized in our laboratory to study mechanisms of growth and kinetics of crystallization of ten protein and virus crystals. These included canavalin, thaumatin, apoferritin, lipase, catalase, t-RNA, lysozyme, xylanase, turnip yellow mosaic virus (TYMV) and satellite tobacco mosaic virus (STMV). We have also designed and constructed in our laboratory both *in situ* conventional two-beam Michelson and phase shift Mach-Zenhder interferometers. Computer software for the processing of the interferometric images was developed as well. Interferometric techniques were applied for studies of growth kinetics and transport phenomena in crystallization of several macromolecular crystals. As a result of this work we have published 21 papers and have given many presentations at international and national meetings. A list of these publications and conference presentations is attached. A brief summary of the main results is presented below.

Mechanisms of Growth

For most of the protein and virus crystals investigated the dominant source of the growth layers was exclusively two-dimensional (2D) nuclei. This was true for thaumatin, STMV, TYMV, lipase and catalase over a broad supersaturation range. For catalase it was demonstrated that because of the weak association of successive layers of tetramers in the unit cell, 2D nuclei with step heights one half of the unit cell forms exclusively. For lysozyme, t-RNA and xylanase crystallization 2D nucleation dominated as well at relatively high supersaturation. Remarkably, no dislocations were found on literally dozens of catalase, STMV and thaumatin crystals. Dislocation-free crystals always grow at relatively high supersaturation. It is possible that the well-known phenomena of cessation in growth of macromolecular crystals occur as a consequence of a steep supersaturation dependence of the normal growth rate, whereby a relatively small decrease in supersaturation would result in termination.

The sources of growth steps on the surfaces of canavalin crystals were single and double screw dislocations Growth proceeded in a manner entirely consistent with the classical model of conventional crystal growth on dislocations. Unlike that model, however, multiple dislocation sources generated steps without any one dislocation becoming dominant. Activities of dislocation sources changed during the crystallization process, resulting in fluctuations of the normal growth rate. Similar phenomena were previously observed in Michelson interferometric studies of canavalin crystallization. Screw dislocations were also present on the surfaces of lysozyme, thaumatin and xylanase crystals. In these cases, screw dislocations acted as a source of growth steps to relatively high supersaturation, at which time 2D nucleation began to dominate growth.

Adsorption of 3D clusters was observed on to the surfaces of all macromolecular crystals investigated so far. Upon interaction with the underlying lattice the 3D clusters

restructured themselves into multilayer stacks, which then advanced tangentially and in the normal direction due to 2D nucleation. In all of those cases, multilayer stacks (typical height 2-15 layers) assumed characteristic morphologies and orientations consistent with the lattice of the underlying crystal. Based on a number of experimental observations we proposed that multilayer stacks were formed upon adsorption of 3D molecular clusters with short-range order. These clusters appear to form in bulk solution due to fluctuations in density. Upon adsorption, 3D clusters transform into crystallites, the process being guided by the underlying lattice. As a result, the lattices of 3D crystalline nuclei are consistently aligned with the lattices of the underlying crystals, and stack merge flawlessly both with other steps on the crystal surface and with each other. When a 3D cluster becomes crystalline, its lattice occasionally misaligns with respect to the lattice of the underlying crystal. In those cases misaligned 3D nuclei develop into discreet microcrystals.

In the case of apoferritin crystallization, surfaces of the growing crystals were extremely rough and grew by intensive random nucleation. This process, known as normal growth, is practically unheard of for growth of conventional crystals from solution but has been described for a number of systems growing from a melt or from the vapor phase.

Growth parameters.
From the supersaturation dependencies of tangential step rates, the kinetic coefficient of the steps for thaumatin and catalase crystallization were determined to be in range of 10^{-4} cm/sec. The supersaturation dependence of the rate of two-dimensional nucleation was measured. growth rate. From these data the surface free energy of the step edge were calculated to be in range of 0.4 – 0.8 erg/cm^2 These parameters are two-three orders of magnitude lower than for inorganic crystals grown from solution.

Influence of Impurities
Cessation of step advancement at low supersaturations, kinetic anisotropy and non-linear step kinetics were observed for thaumatin and catalase crystallization. We were also able to directly observe filaments formed by impurity molecules on the surfaces of macromolecular crystals. These filaments adsorb intact from the bulk solution and orient themselves on the crystalline surface rather than grow on it. We demonstrated the cessation of growth of macromolecular crystals as a consequence of the formation of an impurity adsorption layer. This suggests that macromolecular crystals do not stop growing upon reaching a certain "terminal" size because of accumulation of defects, but because of adsorption of impurities.

Molecular Resolution
The molecular resolution images of the surface layer for several macromolecular crystals were observed. Cell dimensions and the number of molecules per asymmetric unit can be deduced from AFM images. Additional information useful for X-ray crystallographers was also revealed.

Classes of Defects.

In the course of AFM studies of macromolecular crystal growth different kinds of defects were observed. Among these are point defects, or vacancies, in the surface layer of the crystalline lattices. The size of these point defects varied from only single molecule absences to the entire volume of several unit cells. Point defects represent about 10^{-4} of the entire volume of a surface layer on crystalline surface. Another type of defect, one of great importance to crystal growth since it serves, as a source of growth a step is the screw dislocation. Planar defects or stacking faults were also observed. These derive from a fractional unit cell shift of the lattice on one side of the fault with respect to that on the other. Stacking faults are severe in terms of long-range disorder because they propagate through the entire volume of the crystal, and they occur with relatively high frequency. Stacking faults generate significant lattice distortion and strain in their immediate neighborhood, and the misorientation of portions of the lattice by distances of tens to hundreds of angstroms may have serious implications for diffraction quality. Microcrystals, foreign particles such as dust and amorphous debris are also incorporated into growing macromolecular crystals. In those cases one or more defects are always produced, along with severe lattice strain in the immediate proximity of the included particle. The defect density for different macromolecular crystals is consistently in the range of 10^4 to 10^6 defects/cm^2. There are preliminary data that inherent diffraction limit of macromolecular crystals may be correlated to this property of the crystals, and the variation in resolution limit among macromolecular crystals may be as well.

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