15-Crystal Growth

MS-15.01.05 CRYSTALLIZATION OF PROTEINS BY TEMPERATURE CONTROL.

By G. I. Birnbaum*[1], W. Kung[2], D. R. Uffen[2] and H. Scholaert[2]. [1]National Research Council of Canada, Ottawa, Ontario, Canada; [2]Aastra Aerospace Inc., Downsview, Ontario, Canada.

Protein crystallization can sometimes be accomplished by a change of temperature. This method has the advantage of being non-invasive, reversible, easily reproducible and requiring lower protein and salt concentrations. To take advantage of this method, it is necessary to determine three-dimensional phase diagrams, varying temperatures as well as protein and salt concentrations. On the basis of such diagrams it will be possible to determine conditions that are optimal for nucleation and crystal growth. This method has not been used extensively in the past owing to the absence of a commercially available apparatus suitable for such experiments.

We have developed an instrument which is capable of controlling the temperature of a protein solution, in a volume as small as 30 μL, to within 0.1°C in the range 1-60°C. The solubility of a protein at a given concentration and temperature can be deter-mined by detecting laser light scintillation caused by protein crystallites in the stirred solution. The experiment can be controlled either manually or by a personal computer.

We have tested our instrumentation by determining solubility dia-grams for lysozyme (whose solubility in the presence of NaCl decreases with decreasing temperature) and horse serum albumin (whose solubility in the presence of $AmSO_4$ increases with decreasing temperature) and by comparing them with published values. Future experiments will be aimed at determining the nucleation and metastable zones of the supersaturated protein solutions in order to establish conditions for growing high-quality crystals.

MS-15.01.06 THE ORSAY CRYSTALLISATION AUTOMAT

N. Sadaoui, J. Janin*§ and A. Lewit-Bentley#
ABCx, Bât. 403, Université Paris-Sud, 91405 Orsay, France
§L.B.P.C., Bât. 433, Université Paris-Sud, 91405 Orsay, France
#LURE, Bât. 209D, Université Paris-Sud, 91405 Orsay, France

We have designed, built and tested an automat for protein crystallisation. Our design is based on a classical vapour diffusion experiment with hanging, sitting or "sandwich" drops. We are more concerned with the accuracy and reproducibility of the crystallisations than the speed of the automat, which can prepare approximately 10 crystal plates a day. It can use either the ACA Crystal plate or the LINBRO 24 well tissue culture plate. Up to 12 different solutions can be pipetted to form the reservoir solutions and up to 3 solutions can be used to form the drop. The protein is maintained at a constant temperature selected by the user in a thermostated stand.

At the centre of the automat is a 5-axis prototype robot which was fitted with a pipetting tool, a suction pad for handling coverslips and a tool for manipulating the crystal plates. There is a separate post on which the coverslips are turned over and another one on which the silicone seal is prepared.

The PC controlling the automat has been programmed extensively to help the user to design and follow a crystallisation experiment. Any number of protocols can be designed with the programme optimising the volumes and concentrations of all the solutions, while simple designs are equally possible. All protocols used are archived, including all the details concerning the solution preparations, the execution of the protocol and all subsequent results. This should ultimately help to devise strategies for the optimisation of the crystal quality.

The first tests of the automat, using model proteins, are very satisfactory, showing its reliability and good reproducibility.

PS-15.01.07 SPHERULITIC GROWTH OF CHOLESTEROL CRYSTALS IN SILICA GEL.

By Thomas Abraham and M A Ittyachen*, School of Pure and Applied Physics, Mahatma Gandhi University Kottayam, Kerala ,India.

Cholesterol, which is universally distributed in animal tissues, is both a vital and a lethal sterol. Cholesterol monohydrate constitutes the major component of most gallstones and cholesterol monohydrate together with cholesterol esters constitute the lipid lesion of atherosclerosis. Less commonly cholesterol esters may form liquid crystalline accumulations in liver, gall bladder wall and reticulo-endothelial systems (Small, D.M., J. colloid Interface Sci, 1977, 58:581m Garris, J.R., Micron. Microsc. Acta, 1988, 19:19). The cholesterol monohydrate crystals deposited from biological membranes exhibited plate-like morphology. On the other hand needle shaped crystals of cholesterol grown from organic solvents were anhydrous in nature. Crystals of cholesterol monohydrate was first grown in silica gel by Narayana Kalkura (Narayana Kalkura, J. Mat. Sci. Lett. 1986, 5:741 ,J. Cryst. Growth, 1991, 110, 265-269). In this paper we present an improved growth procedure. Cholesterol can be easily dissolved in water soluble organic solvents like acetone, ethanol and methanol. Cholesterol is practically insoluble in water. Water was used to reduce the solubility of cholesterol which was initially dissolved in a gel solution alongwith any one of the above mentioned solvent. Calcium and oxalate ions are used as additives to change the morphology of the crystals. Incorporation of these ions resulted in the growth of a spherulitic type of cholesterol of size 1-2 mm. The crystals were characterised using the usual investigating probes. It became clear that the spherulite crystals are composed of multiple plate single crystals of cholesterol monohydrate with well defined c-planes and are arranged radially.

PS-15.01.08 TWINNING CRYSTAL IN THE GROWTH OF PROTEIN CRYSTAL.

By Lin Yujuan. Fujian Institute of Research on the structure of Matter, Academy Sciences, Sinica, Fuzhou, Fujian, 350002, P.R.China.

In a system of biological macromolecule such as protein, enzyme, and nucleic acid, the interaction are very complex and poorly understood, thus it is not always easy for a protein crystallographer to obtain a perfect crystal. A. Mcpherson has in detail described many methods and steps for crystallization attempt[1] (Alexander Mcpherson "preparation and analysis of protein crystals" (1982) pp.82 New york). Unfortunately many protein crystals are often twinned or disordered. Here some ways to overcome the crystal twinning are described.

Earlier, it has been reported that NH_4NO_3 can improve crystal twinning[2](Osserman, E.F., code, S.J., Swain, I.D.A. & Blare, C.C.F.(1969), J.Mol.Biol. 46, 211-212). In the research on Trichosanthin $NaNO_3$ has been used to overcome crystal twinning[3](Pan, K.Z. et al., (1978), Academia Sinica(china) 23, 176-178.) Recently, twinning crystals of neuraminidase type B Human influenza virus have been also overcome by the addition of $NaNO_3$, the crystal belongs to space group $P2_1$, with unit cell dimension a=72.94Å, b=124.64Å, c=124.40Å, β=98.75°, diffracted X-ray at 3.0Å resolution.

Dioxane has been helpful to avoid crystal twinning in chymotrypsin[4](Sigler, P.B., B.W. Mathews, and D.M. Blow (1969), J. Mol.Biol.15, 175-192.) and phosphoglycerate[5] (Watson, H.C., P.L. Wandell, and R.K. Scopes (1971), J. Mol.

 Copyright © 1993 International Union of Crystallography

15-Crystal Growth

Biol. 57, 623-625.) before. Adding dioxane to the previous buffer system, the author has also obtained perfect crystals of neuraminidase, with space group $P42_12$.
Some divalent metal ions, particularly metal ions of the transition series have been found that stimulate crystal growth. In order to be free of serious twinning problems in crystal growth of neuraminidase type B, we added $CaCl_2$ to the previous buffer system, obtained very ideal crystals and collected a dataset at 2.3Å resolution [6](Lin, YuJuan, et al., (1990), J. Mol. Biol. 214, 639-640.).
Decreasing the protein concentration is of great advantage to eliminate crystal twinning. For example, single crystals of Trichosanthin with C2 space group are often found on the edge of the drop or on end with low protein concentration.
As before, we might draw the conclusion that it is unnecessary to give up the previous buffer system to overcome the crystal twinning if only trying the ways described above.

PS-15.01.09 **IMPROVEMENT OF EGF RECEPTOR CRYSTALLIZATION BY EXPOSURE TO MICROGRAVITY AND BY ISOFORM SEPARATION**

E. Wenisch[2], N. Günther[1], L.J. DeLucas[3], C.E. Bugg[3], P.G. Righetti[2], Ch. Betzel[4] & W. Weber[1]*

[1] Universitätskrankenhaus Eppendorf, Hamburg, Germany • [2] University of Milano, Italy • [3] Center for Macromolecular Crystallography, UAB, Birmingham, Alabama, USA • [4] EMBL-Outstation, DESY, Hamburg, Germany

The EGF receptor plays an important role in normal and pathological growth control. A secreted form of the receptor (sEGFR) representing its 100 kd external domain (Weber, Gill, Spieß, Science, 1984, 224, 294-7) had been crystallized in the presence of the ligand, and a 1:1 molar complex of receptor and ligand could be recovered in the crystals. Diffraction, however, had been only about 10 Å, probably due to the high extent (30%) of complex receptor glycosylation and to the limited volume of the crystals obtained so far (Günther, Betzel, Weber, J. Biol. Chem., 1990, 265, 22082-5).

A significant improvement of crystal quality was achieved in a crystallization experiment under microgravity conditions on US space shuttle mission STS47. In 8 out of 9 set-ups prepared under different conditions crystal growth was observed after 8 days in space. One of the space-grown crystals (0.6 x 0.43 x 0.2 mm^3) showed higher diffraction quality than all earth-grown crystals before, and it allowed for the first time partial data collection: diffraction extended for the first 5 exposures to 6 Å resolution, followed by a rapid decrease of diffraction patterns occurring during the following exposures. 2585 reflections were merged to R.symm = 9.3%.

A further improvement of crystal quality was realized in the laboratory by a novel technique included as an additional purification step of the protein: Whereas receptor preparations used for crystallization so far, consisted of more than 10 isoforms resolved by isoelectric focusing, selected single pI-species were prepared by exploiting the unique resolving power of a multicompartment electrolyzer with isoelectric membranes (Wenisch, Righetti, Weber, Electrophoresis,1992, 13, 668-73). From one of these isoforms a crystal could be grown to a size of 1.3 x 0.5 x 0.3 mm^3. It allowed data collection up to 6 Å for the first 10 images; then diffraction patterns decreased to 10 Å during the following 10 exposures because of increasing radiation damage. 2942 reflections were merged to R.symm = 5.4%. The reduced data set showed a completeness of 26%.

The space group of both, the space-grown 'wild-type'-receptor as well as the earth-grown single-pI isoform, was assigned to be orthorhombic $P2_122$ or $P2_12_12$ with pseudo-tetragonal unit cell parameters of a =116.3 Å, b = 119.5 Å, and c = 204.5 Å. These values yield a unit cell volume of 2.8 x 106 $Å^3$ and a packing parameter V_M of 3.58 $Å^3$/Dalton assuming 2 molecules each of receptor and ligand in the asymmetric unit. The fractional volume occupied by solvent was calculated to be 65%.

Since either approach, microgravity conditions and isoprotein selection, has improved crystal growth, further benefit may be expected from a combination of both: crystallization of selected EGF receptor isoforms in space.

15.02 – Crytal Growth of Inorganic Compounds

PS-15.02.01 **RAPID GROWTH OF TGS AND ATGSP CRYSTALS** By Wang Qingwu*, Fang Changshui, Zhuo Hongsheng and Wang Min, Institute of Crystal Materials, Shandong University, Jinan 250100, P.R.C.

At present,TGS (triglycine sulphate) series crystals are excellent pyroelectric materials being widely used to make infrared detectors and vidicons. We have engaged in the study of the rapid growth of these crystals.
First, we studied the solution status of TGS and ATGSP (L-alanine and phosphoric doped TGS) crystals and selected the optimum solution conditions for rapid growth. By adding a rapid rotating impeller to enhance the convection of solution in the crystal growth process, we have realized the rapid growth of these crystals . The growth rate of the (010) face,whose cross section is 40mmx40mm, reached 5mm/day by using single face growth technique. The growth rate has been raised 3-5 times of the normal rate.
Second, we studied the perfection of the as-grown crystals by x-ray topography, and found that there are more defects in the rapid-growth crystals than those grown by normal speed.
Finally, the properties of TGS and ATGSP crystals grown by using high speed have been measured. The results show that pyroelectic coefficient and dielectric constant of the rapid-growth crystals are icreased slightly, but their figures of merit are almost the same as those of the crystals grown by normal speed. Especially in the case of the ATGSP crystal, when its growth rate is 5mm/day, its bias field reached 10 Kv/cm, and its dielectric loss at room temperature is 2-3 times smaller than that of the crystals grown by normal speed. The peak of dielectric loss near the Curie point disappears. These changes are due to the high bias field in crystals. The high bias field and low dielectric loss are very important for making infrared detectors and vidicons.