MS16.01.05 GROWING PROTEIN CRYSTALS BY HOOK OR BY CROOK: FROM MOLECULAR BIOLOGY TO LIPIDS. Aled M. Edwards, Cancer Research Group, Institute for Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario, Canada.

We have used two general approaches to facilitate the growth of protein crystals for X-ray diffraction experiments. The first approach is called SAF, for smallest, active fragment. SAF is simply a logical extension of the old observation that proteolytic fragments of proteins often both harbour complete biological activity and form useful crystals more readily than the full length protein. However, rather than depend on the fortuitous position of protease recognition sites, SAF couples partial proteolysis with deletion analysis in order to refine the boundaries of the domain of interest. In practice, an iterative cycle of partial proteolysis, deletion analysis, biochemical assays, protein over-expression and crystallization is used to identify a domain that forms diffraction-quality crystals. SAF capitalizes on the ease and rapidity of subcloning, over-expressing and purifying proteins as well as the technical ease and availability of N-terminal sequencing and mass spectrometry. We have used the SAF approach for three proteins, and we have generated fragments of each that were suitable for structure determination.

The second approach. lipid-layer seeding, uses lipid monolayers to seed crystal growth. We observed several years ago that two-dimensional protein crystals grown on lipid layers effectively nucleate the epitaxial growth of three dimensional protein crystals. In this way, crystals suitable for X-ray crystallography can be grown more rapidly, and at substantially lower protein concentrations and precipitants than for conventional crystal trials. The methodology has now been developed such that the procedure takes only a few seconds per crystal trial. We are now applying the method to an assortment of proteins in an effort to demonstrate the generality of the approach.

PS16.01.06 THE RELATION BETWEEN DIFFERENT POLYMORPHIC MODIFICATIONS OF RHAMNO-GALACTURONAN ACETYLESTERASE. Anne B. Gjerlov and Sine Larsen, Centre for Crystallographic Studies, University of Copenhagen, Denmark

Rhamnogalacturonan acetylesterase (RGAE) is a novel enzyme involved in the the enzymatic degradation of the polysaccharide rhamnogalacturonan, one of the major components of pectin. RGAE has been isolated from *Aspergillus aculeatus* and overexpressed in *Aspergillus oryzue*[1]. The structure determination of the enzyme showed that it has an open twisted α/β fold.

The protein crystallizes readily, despite substantial glycosylation, and has been crystallized under several different conditions, yielding three different crystal forms. Using the hanging drop method and the conditions 19% PEG 4000, 18% isopropanol, 40 mg/ml RGAE and 0.1M citrate buffer, a tetragonal form is obtained around pH 4.1, an orthorhombic form around pH 4.7 and a trigonal form at pH 5.1. The orthorhombic crystals can also be obtained with the conditions 1.4M $(NH_4)_2SO_4$ or 1.4M Li_2SO_4, 0.1M Na acetate, 40 mg/ml RGAE and pH 5.0.

The structure of the orthorhombic form was solved by Multiple Isomorphous Replacement, using two heavy atom derivatives. The spacegroup is $P2_12_12_1$ with cell parameters a=52.14Å, b=56.87Å, c=71.89Å. There is one molecule in the asymmetric unit and the water content is 35%. Data collection was performed with a rotating anode as X-ray source operating at room temperature. The structure was refined using data to a resolution of 1.55Å. The results from investigations of the two other modifications by the molecular replacement method will also be presented and related to the difference in crystallization conditions.

[1]Kauppinen, S., Christgau, S., Kofod, L.V., Halkier, T., Dörreich, K. and DalbØge, H. (1995) J.Bio.Chem., **270**, 27172-27178.

PS16.01.07 THE POLYETHYLENE GLYCOL (PEG) POSITIVE EFFECT IN PROTEIN CRYSTALLIZATION. Qing Han and Sheng-Xiang Lin, MRC Group in Molecular Endocrinology, CHUL Research Center and Laval University, Québec, G1V 4G2, Canada

The polyethylene glycols (PEG) are one of most frequently utilized group of precitating agents for macromolecular crystallization. The structure-stabilizing effects of PEG for macromolecule have been described in many biochemical literatures. However, the mechanism of PEG in macromolecular crystallization is relatively ignored. The PEG action in crystallization was studied using PEG 4K, hen egg lysozyme and human 17ß-hydroxysteroid dehydrogenase (17ß-HSD) here. The lysozyme crystallization was established at 14 different pH values from 4.0 to 10.5 with 0.5 interval. The protein solution was prepared in four different compositions: lysozyme 10 mg/ml, lysozyme 10 mg/ml with PEG 4K 15%, lysozyme 10 mg/ml with 1.5 % NaCl, lysozyme 10 mg/ml with 1.5% NaCl and PEG 4K 15 %. The quality of crystallization was evaluated with score from 0 to 5. When the protein solution contained 1.5% NaCl, lysozyme crystals could be found with pH values from 4.0 through 10.5. If PEG was added to the protein solution, the quality of almost all the crystals was greatly improved. If the protein solution with PEG did not contained NaCl, crystallization occurred only when the pH is from 4.0 to 7.5; at pH from 8.0 to 10.5, which is around the pI of lysozyme, no crystals of lysozyme were found. These results were compared with each other. The electrical conductivity under different crystallization solutions was determined and analyzed too. Besides, the effects of PEG for stabilizing protein structure were studied by 17ß-HSD in different concentrations and temperatures. The result showed that the PEG can keep 17ß-HSD activity very well. This is necessary for crystal growth of 17ß-HSD.

PS16.01.08 PROTEIN CRYSTAL SEEDING STUDIED BY PHASE DIAGRAMS. Qing Han and Sheng-Xiang Lin, MRC Group in Molecular Endocrinology, CHUL Research Center and Laval University, Québec, G1V 4G2, Canada

Though different seeding techniques of macromolecular crystallization were explored and successful examples were reported, the seeding technique is not yet a standard method in macromolecule crystallization. The main reason is that we lack basic knowledge about macromolecule seeding process. In this report the seeding phase diagrams were determined by the batch experiments using hen egg lysozyme and human 17ß-hydroxysteroid dehydrogenase. On the basis of the growth of microcrystals, the phase diagram could be divided into five areas: damage region, equilibration region, unobvious growth region, obvious growth region, nucleation region. The ideal seeding, in which the microcrystal can grow evidently without dissolution and new nucleation, was only achieved in a narrow domain of phase diagram. The solution of high protein concentration with low salt concentration is shown to be beneficial for seeding. The broken mincrocrystal and aggregation that were introduced to new drops could cause nucleation at low level of supersaturation. The techniques of microcrystal wash, crystal transfer and crystal soaking are also described. These results will be useful for rational seeding in macromolecular crystallization.