

**MS01.01.06 THE COOLING RATE DURING SHOCK FREEZING IN MACROMOLECULAR CRYSTALLOGRAPHY** T.-Y. Teng and K. Moffat, Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL 60637.

Cryocrystallographic study of macromolecules has grown rapidly in recent years<sup>1</sup>. Data collection of single protein crystal x-ray diffraction at liquid nitrogen temperatures is now routinely conducted at x-ray labs and synchrotron beamlines. Cryocrystallography greatly reduces radiation damage on the crystals, increases diffraction resolution, simplifies sample handling in the experiment and saves valuable protein crystals when compared with conventional data collection around room temperature, although slightly increased mosaicity is observed in frozen crystals. But, there always remains doubt about the identity between the structure revealed at cryo-temperatures and the structure at physiological temperatures. Further, the average structure observed at low temperature is an ensemble of series structures frozen at different stages in the shock freeze process<sup>2</sup>. This is especially important when time-resolved x-ray diffraction studies are interpreted.

To answer the question of how shock freezing proceeds, the cooling rates of different cryoprotectant solutions (sucrose, PEG) with different volumes (0.2 - 0.8 mm<sup>3</sup>) by different cooling agents (cold nitrogen or helium gas, liquid nitrogen and liquid propane) were measured. The result shows, indeed, macromolecular crystals are not instantaneously frozen to their final stage. Freezing is usually complete within the 1 - 2 second and the cooling speed varies from ~50 to 700 °/s depending on the volume of the sample, the cooling agent used and the temperature at the time of measurement.

Detailed data will be presented along with some guidelines for shock freezing of protein crystals.

<sup>1</sup> Rogers, D. W. (1994). *Structure*, 2, 1135-1140.

<sup>2</sup> Moffat, K. and Henderson, R. (1995) *Current Opin. in Structural Biology*, 5, 656-663.

**MS01.01.07 A CRYO-CRYSTALLOGRAPHIC REDOX STUDY OF THE STRUCTURAL CHANGES CAUSED BY MUTATION OF KEY AMINO ACIDS AT THE FMN BINDING SITE OF A FLAVODOXIN** M.A. Walsh,<sup>1,2</sup> A. McCarthy,<sup>2</sup> T. Higgins,<sup>3</sup> P. O'Farrell<sup>3</sup> and S.G. Mayhew.<sup>3</sup> <sup>1</sup>European Molecular Biology Laboratory, Hamburg, Germany. <sup>2</sup>Department of Chemistry, University College, Galway, Ireland <sup>3</sup>Department of Biochemistry, University College, Dublin, Ireland

The flavodoxins are a group of small flavoproteins which function as electron carriers in low potential redox reactions. They contain a single molecule of non-covalently bound flavin mononucleotide, (FMN) as their sole prosthetic group. The FMN cofactor can exist in three oxidation states: oxidized, semiquinone and hydroquinone. When bound to protein, the redox potentials of FMN for both the oxidized/semiquinone, (E2) and semiquinone/hydroquinone, (E1) are significantly shifted. These shifts in redox potential, are essential for flavodoxin to carry out its physiological role. The interactions between the protein and FMN which cause the large shifts in redox potential are not yet fully understood. A number of site directed mutagenesis studies have therefore been carried out to assess the role of specific amino acids at the FMN binding site of flavodoxin.<sup>1-3</sup> We have carried out an extensive crystallographic study of a number of the site mutations in their differing oxidation states at the FMN binding site. The results show the FMN binding site to be more flexible than previously envisioned and further structural studies should allow a more clearer understanding of the protein/FMN functioning.

1. Curley GP, Carr MC, O'Farrell PA, Mayhew, SG Voordouw, G (1991). *Flavins and Flavoproteins*, 1990, Berlin: Walter de Gruyter, 429

2. Swenson, RP. & Krey, GD. (1994) *Biochemistry* 33, 8805

3. Swenson, RP & Zhou, Z. (1995) *Biochemistry* 34, 3183

**PS01.01.08 LOW TEMPERATURE STRUCTURE OF THE THROMBIN-HIRUGEN COMPLEX AT 1.7Å RESOLUTION.** S. P. Bajaj\*#, I. I. Mathews\*, J. H. Matthews\*, A. Tulinsky\*, \*Department of Chemistry, Michigan State University, East Lansing, MI-48824; and #Departments of Medicine and Biochemistry, St. Louis University Medical School, St. Louis, MO - 63110

Thrombin is a serine proteinase that converts fibrinogen to fibrin. Hirugen is a 12-residue hirudin-like peptide that binds to thrombin in the fibrinogen exosite. The room temperature structure of active site D-PhePro Arg chloromethyl ketone inhibited thrombin at 1.9Å resolution and the thrombin-hirugen complex at 2.2Å resolution have been reported. Since thrombin is an important target of anticoagulation therapy, we determined its structure at 150K to further refine localization of the protein and solvent atoms. Such information could be useful in improving the design of potent thrombin inhibitors.

The thrombin-hirugen complex at 150K has been refined to a current R-value of 0.176 at 1.7Å resolution. Although the folding is strikingly the same as that determined at room temperature (RMS Δ for Cα atoms ~0.28Å), the average B-factor of the protein and solvent atoms is about 8Å<sup>2</sup> lower and some residues show alternate conformations. Both the active site serine and the S1 aspartate are held in optimal configurations poised for catalysis by a chain of highly ordered water molecules that link up to the protein. A water molecule is hydrogen bonded to the OG of Ser195 and Gly193N in the oxyanion hole that must be displaced on substrate binding. Glu192 is pointing away from the active site and at low temperature is held in position by a hydrogen bonding network of water molecules which extends to the exosite. Additional water molecules stabilize several β-strands near turns or in regions apparently missing hydrogen bonds. The octahedrally coordinated Na<sup>+</sup> -binding site of the fast form of thrombin has four well-defined water molecules, while an octahedrally coordinated intermolecular Na<sup>+</sup> -site has three. A tetrahedral cluster of five peaks around crystallographic 2-fold rotation axis could be a phosphate ion. In addition, many other solvent molecules have been located that were not noted in the room temperature structure. Thus, the low temperature structure reveals biologically important features that were not apparent in the room temperature structure.

**PS01.01.09 COMPOSITION OF CRYSTALLITES RESULTED FROM FREEZING OF WATER SOLUTIONS OF BIOMOLECULES AT CRYOGENIC TEMPERATURES.** Kosevich M.V., Shelkovsky V.S., Boryak O.A., Stepanov I.O.; Institute for Low Temperature and Engineering NAS Ukraine, Kharkov, Ukraine.

Chemical composition of the micro crystallites formed due to phase separation of the multi-component water solutions of biomolecules and inorganic compounds during freezing can be directly determined by the recently proposed method of the low temperature fast atom bombardment (FAB) mass spectrometry. It was observed, that in addition to powerful analytical potential, FAB mass spectrometry appeared to be extremely sensitive to the energetic and structural parameters of the frozen solutions at the cryogenic temperatures. The patterns of the clusters sputtered from the frozen solid, reflects the nano-scale arrangement of molecules and ions in crystal and glassy phases. The hydrate clusters (H<sub>2</sub>O)<sub>n</sub>\*M are recorded only for crystalline hydrates and compounds with strongly bound water. Phase transitions in the samples with temperature change (dehydration of crystalline hydrates, sublimation, glass-crystal transitions) are clearly reflected in the changes in mass spectral pattern. The possibility of the study of phase composition and transitions in the frozen solutions, which contain cryoprotectors and biomolecules, are of exceptional value for cryobiology. The formation of complexes of biomolecules in the frozen solid, prohibited in solutions, is also elucidated from the corresponding clusters. The design of the dedicated cryogenic block for the secondary emission FAB ion source of a magnetic mass spectrometer, which allows to control the sample temperature in the 77-300K range, is described.