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RADIATION DAMAGE TO PROTEINS STUDIED BY TEMPERATURE-CONTROLLED CRYO-CRYSTALLOGRAPHY

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Intense synchrotron radiation produces specific structural and chemical damage to proteins even at 100 K. Disulfide bonds break or elongate, acidic residues are decarboxylated and the active site of enzymes appear particularly radiation-sensitive. Chemically identical groups in a given protein are not equally radiation-sensitive. Differences in solvent accessibility and in the chemical and dynamical nature of the close environment are most likely among the crucial factors that determine radiation-sensitivity of a specific residue. Another factor that plays an important role in radiation damage is the physical state of the crystal solvent. Solvent in trigonal crystals of the enzyme Torpedo californica acetylcholinesterase (TcAChE) crystallizes upon warming at 155 K, thus providing circumstantial evidence for the occurrence of a glass transition at or below 155 K. The temperature-dependence of specific radiation damage was assessed by collecting a series of data sets on a single crystal of TcAChE at two temperatures, one below and one above the glass transition of the crystal solvent, viz. at 100 and at 155 K, respectively [1]. Besides increased damage to sulfur-containing groups, conformational changes in the catalytic triad at the active site were observed above the solvent glass transition. These results show that at 155 K the protein has acquired sufficient conformational flexibility to adapt to irradiation-induced alterations in the conformational energy landscape. They reveal the influence of both protein and solvent dynamics on specific radiation damage to proteins.

References

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Keywords: RADIATION DAMAGE, CRYO-CRYSTALLOGRAPHY, CRYSTAL SOLVENT

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PROTEIN-CRYSTAL WATER RECONSTRUCTION IN A LOW-TEMPERATURE PHASE TRANSITION

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Flash-cooled crystals of the orthorhombic I222 form of crystalline concanavalin A undergo a sharp, non-destructive, non-reversible phase transition upon warming to around 160 K. The phase transition is characterized by an anomalous increase in cell volume. This expansion is anisotropic, being primarily associated with the b and c axes. X-ray diffraction data extending to 1.7 Å were collected at 130 K on a flash-cooled crystal of concanavalin A. The crystal was then warmed slowly to 170 K, where a second data collection was performed. After this, the crystal was slowly cooled back to 130 K for a third data collection. To investigate the structural origin of the phase transition, each dataset was collected under identical (aside from temperature) conditions. The structure (starting coordinates from PDB file 1jbc), was refined against each of the three datasets using modern methods for reducing phase bias.

Structural rearrangements in the protein molecule itself were negligible. Changes in the water structure as a result of the phase transition will be described. Details of the cooling experiments and the importance of maintaining control over experimental parameters during crystal manipulation and data collection will be stressed. In addition, the small (but clear) differences in data quality statistics for each dataset will be described.

Keywords: CRYOCRYSTALLOGRAPHY, PHASE TRANSITION, WATER STRUCTURE

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AUTOMATED MOUNTING OF CRYO-COOLED CRYSTALS ON STANFORD SYNCHROTRON RADIATION LABORATORY BEAMLINE 11-1

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A robotic system for auto-mounting crystals is now operational on SSRL BL11-1. Its compact sample cassettes can each accommodate up to 96 samples mounted on Hampton Research magnetic cryo-pins and are easily transported inside a standard shipping Dewar. Up to three cassettes (288 crystals) at a time are placed within a liquid nitrogen-filled Dewar at the beam line. Samples selected for screening or data collection are transferred to the goniometer using a small industrial 4-axis robot with a custom built actuator. The system uses permanent magnets to extract samples from and return samples to the cassettes. The robot mounts and dismounts samples using a cryo-tong tool. Sample visualization hardware enables automated alignment of the cryo-loop to the x-ray beam. Control of the robot and other beamline hardware is integrated within the distributed control system architecture developed at SSRL.

The system was developed to increase the throughput of the SSRL macromolecular crystallography beamlines, particularly with respect to the demands of the Joint Center for Structural Genomics (JCSG) program and the scheduled commissioning of SPEAR 3 in Winter 2003. Plans are underway to replicate the production system on the remaining beamlines.

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Keywords: CRYOCRYSTALLOGRAPHY, HIGH-THROUGHPUT, AUTOMATION

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PHASING IN THE PRESENCE OF RADIATION DAMAGE

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The brightest SR sources create havoc with macromolecular crystals when used at design specifications. The problem of radiation damage is not only severe in studies involving kinetics and mechanism where cryotechniques are not always viable, but is also significant for cryo-cooled (100 K) crystals. In the course of the data collection, the diffraction power of the crystal is reduced, the mosaicity and overall B-factor go up, and eventually one will loose all higher order reflections. In addition to these general effects, some highly specific changes might occur, such as breakage of disulphide bonds and loss of definition of carboxyl groups. Both the non-specific and the specific effects result in loss of perfect isomorphism throughout the data collection.

The multiple-wavelength anomalous dispersion (MAD) method has become the standard technique for structure determination. However, its main advantage, perfect isomorphism, can easily be swamped by radiation damage. In practice at the brightest SR sources, the problem of radiation damage is often overcome by attenuating the beam or by the exclusive use of single-wavelength anomalous dispersion (SAD) experiments.

We will report on systematic experiments aim at understanding the consequences of radiation damage for MAD and SAD data collections. We will report on the consequences of specific and non-specific changes for the structure determination process. It will be shown that at current date, not only radiation damage itself is detorial for the phasing process, but more importantly our handling of its consequences. Examples will be shown on how to improve dramatically on this last issue.

Keywords: RADIATION DAMAGE,PHASING,SYNCHROTRON RADIATION