Investigation of Possible Free Radical Scavengers

James Murray and Elspeth Garman, Laboratory of Molecular Biophysics, Department of Biochemistry, Rex Richards Building, University of Oxford, South Parks Road, OXFORD, OX1 3QU, U.K.

Although little can be done to reduce the primary damage to protein crystals caused by X-rays, the effects of the secondary free radicals can be mitigated. The now-standard practice of cooling crystals to 100K reduces the mobility of these radicals and leads to vastly increased crystal lifetime. However even at 100K, specific damage to protein crystals occurs, e.g. to disulfide bonds, indicating that secondary damage processes are occurring. Free radical scavengers in the crystal could compete with the protein for the secondary radicals. Documented scavengers include styrene, ascorbic acid, cysteine, ethanol and glucose. It may also be possible to mitigate damage by de-oxygenating the crystal before it is cooled.

We have performed preliminary experiments with hen egg-white lysozyme crystals soaked with styrene at ID14-EH4 at the ESRF. We have observed specific disulfide bond cleavage and unit cell expansion, both classic symptoms of radiation damage. Although the diffraction pattern showed no visible degradation, structural damage was clearly visible in the elctron density maps. Unfortunately it has not yet been possible to determine whether styrene has had a reproducible effect on these variables, due to the large inter-crystal variation and lack of statistically significant quantities of data. We are currently in the process of collecting more data to remedy this.

We are also investigating unit cell expansion as a possible on-line metric of radiation damage. The effects observed are marginal, and care must be taken over the data processing parameters. These issues, and in particular the Mosflm and Denzo parameter refinement and post-refinement, will be discussed.