Towards an Understanding, Control and Possible Use of X-Ray Radiation Damage in Macromolecular Crystals

Long-term project (LTP) ls-2047, awarded 2001/II to 2003/I (to Ravelli, McSweeney, Garman and Caffrey)

Since the LTP started, there has been a sharp increase in the profile of research into radiation damage processes in protein crystals. Many protein crystallographers are realising that their experiments are being limited by radiation damage, and there is a keen interest in the topic. Several factors have contributed to this; not least the burden of experience of failed MAD structure determinations attributable to radiation damage. The very fact that an LTP into this area is ongoing at the ESRF has also given an impetus to the research, which would have been difficult to sustain without this long term commitment to dedicated beamtime.

Research efforts to understand damage were fully discussed at the Second International Workshop on Radiation Damage to Crystalline Biological Samples held on 1st and 2nd December 2002 at the APS in Chicago. This was organised jointly by Elspeth Garman of this LTP, Colin Nave (SRS, Daresbury) and Gerd Rosenbaum (Uni of Georgia). Results of experiments performed under the LTP at the ESRF were reported in four talks given by James Murray (Uni of Oxford), Martin Weik (IBS, Grenoble), Raimond Ravelli (EMBL, Grenoble) and Sean McSweeney (ESRF) on radical scavengers, the temperature dependence of specific structural damage, Radiation Damage Induced Phasing, and dose/dose rate effects respectively. The availability of carefully collected data on statistically significant numbers of samples was only possible due to the LTP, which has given us the beamtime and long term planning potential to carry out such experiments.

7 days were allocated in the first year, 4 days on ID14-4 (14-11-01, 16-11-01, 18-4-02, 2-10-02/2 shifts), 2 days on ID14-2 (06/07-02-02) and 1 day on ID29 (21-04-02). In retrospect, some experiments were initially setup somewhat naively, and as was anticipated in the original LTP-proposal, and had to be redesigned. Other experiments, however, worked out better than had been hoped, and have resulted in a number of publications covering all of our 3 major aims:

Towards an Understanding of X-ray Radiation Damage

Upon exposure to X-rays, radicals are formed within the cryo-cooled crystal [1,2]. Whereas primary absorption will occur linearly with dose and independent of temperature, the formation and possible recombination of radicals will be temperature and time-dependent.

In order to assess the temperature-dependence of specific radiation damage we collected a series of data sets on a single trigonal crystal of the enzyme *Torpedo californica* acetylcholinesterase (*Tc*AChE) at two temperatures, one below and one above the glass transition of the crystal solvent, at 100 and at 155 K, respectively [3]. A buried disulfide bond, a buried cysteine and solvent exposed methionine residues show drastically increased radiation damage at 155 K in comparison to 100 K. More importantly, we observe irradiation-induced conformational changes in the catalytic triad at the active site at 155 K but not at 100 K. These changes lead to an inactive catalytic triad conformation and thus represent the observation of radiation-inactivation of an enzyme at the atomic level. Our results show that at 155 K the protein has acquired, at least locally, 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.

We have examined disulfide bond lengths in irradiated crystals of *Tc*AChE based on quantum simulations and on a series of data sets collected at 100 K [4]. Our experimental data suggest that one disulfide bond elongates by ~ 0.7 Å upon X-ray irradiation. Simulation of the same

bond suggests elongation by a similar value if a disulfide radical anion is formed by the trapping of an electron. The absorption spectrum of a crystal irradiated under similar conditions shows a peak at ~ 400 nm, which in aqueous solution has been attributed to disulfide radicals. From the spectra, it can be clearly seen that the formed radicals have a finite lifetime. Our results suggest that the formation of disulfide radicals in protein crystals owing to X-ray irradiation can be observed experimentally, both by structural means and by absorption spectroscopy.

[1] Garman, E. and Nave, C. (2002). Radiation damage to crystalline biological molecules: current view. *J. Synchrotron Radiat.* In press.

[2] O'Neill, P., Stevens, D.L., Garman, E.F. (2002). Physical and chemical considerations of damage induced in protein crystals by synchrotron radiation: a radiation chemical perspective. *J. Synchrotron Radiat.* In press.

[3] Weik, M., Ravelli, R.B.G., Silman, I., Sussman, J.L., Gros, P. & Kroon, J. (2001). Specific protein dynamics near the solvent glass transition assayed by radiation-induced structural changes. *Protein Sci* 10, 1953-1961.

[4] Weik, M., Bergès, J., Raves, M., Gros, P., McSweeney, S., Silman, I., Sussman, J.L., Houée-Levin, C. and Ravelli, R.B.G. (2002). Evidence for the formation of disulfide radicals in protein crystals upon X-ray irradiation. *J. Synchrotron Radiat.* In press.

Towards Controlling X-ray Radiation Damage

While screening for putative scavengers, one would like to have a fast way of identifying the effect of each scavenger on the lifetime on the crystal in the X-ray beam. Several metrics for monitoring radiation damage have been considered and unit cell volume expansion was systematically investigated [5] using crystals of three different types. Unfortunately, it was found to be too variable to be a useful metric.

More promising results were obtained using an off-line microspectrophotometer. Different radical scavengers have been tested, all on hen egg white lysozyme. Styrene was found to be ineffective. However, in conjunction with systematic crystallographic studies of specific structural damage, tentative but suggestive evidence was observed that ascorbate could extend the lifetime of the crystal in the X-ray beam [6].

[5] Ravelli, R.B.G., Theveneau, P., McSweeney, S., and Caffrey, M. (2002). Unit-cell volume change as a metric of radiation damage in crystals of macromolecules. *J. Synchrotron Radiat.* In press.

[6] Murray, J. and Garman, E. (2002). Investigation of possible free-radical scavengers and metrics for radiation damage in protein crystallography. *J. Synchrotron Radiat.* In press.

Towards Making Use of X-ray Radiation Damage

Radiation damage is in general seen as a problem that should be avoided. However, we have shown that far from being a hindrance to successful structure determination, radiation damage provides an opportunity for phasing macromolecular structures. This has been successfully demonstrated for both a protein and an oligonucleotide, by way of which complete models were built automatically. We have named the method Radiation damage-Induced Phasing (RIP) [7].

[7] Ravelli, R.B.G., Schrøder Leiros, H.K., Pan, B., Caffrey, M., and McSweeney, S. (2002). Specific Radiation-Damage Can Be Used To Solve Macromolecular Crystal Structures. *Structure*. accepted.