Radiation damage to crystalline biological molecules: current view†

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Radiation damage to crystalline biological molecules under investigation using X-rays is a problem which limits the structural information that can be extracted from the samples. In macromolecular crystallography, the widespread introduction of cryogenic cooling (to around 100 K) of crystals in the 1990s appeared to have largely alleviated the problem of radiation damage for data collection at synchrotrons. However, by the mid-1990s there were already indications that most crystals still had a finite lifetime, even at 100 K, particularly in the more intense beams which became available from the large multi-GeV third-generation synchrotrons, *e.g.* ESRF, APS and SPring-8. Thus, it became clear that during the time necessary for completion of the diffraction experiment, damage to some cryocooled samples (by nitrogen at around 100 K) was significant. Not only was sample damage compromising the full utilization of the intense flux beams available from undulators, but it was also limiting the quality of the data that could be collected.

In the damage process, the high-resolution data disappear first; these are the very data which provide the fine details of the macromolecular conformation. The damage also causes problems on several other fronts: it prevents a complete data set from being obtained from a single crystal while the protein is in the same 'functional state', multi-wavelength anomolous dispersion (MAD) phasing methods fail, it causes specific structural alterations resulting from radiation damage (Weik *et al.*, 2000; Burmeister, 2000; Ravelli & McSweeney, 2000), and may result in incorrect conclusions being drawn for the structure-based mechanism.

A consensus thus emerged that it was imperative for the research community to gain a better understanding of the radiation damage process, and to investigate possible methods of mitigating, or at least minimizing, the effects.

As a result, several studies of the problem have been carried out at various synchrotron radiation facilities over the period from 1992 to date. These studies require significant amounts of beam time and much scientific staff effort. To increase our general understanding of the problem, the studies must be very carefully designed and control-experiments performed. The results are often hard to interpret. It was clear that disparate efforts round the world could be more productive if the available expertise and experience was brought together to discuss the problems and define the useful experiments more precisely. Thus the First International Workshop on X-ray Damage to Crystalline Biological Samples, funded by the ESRF and attended by about 30 interested researchers, was held in June 1999 at the ESRF in Grenoble. The Second Workshop, funded by DoE-OBER and NIH-NCRR and attended by around 50 people, was held in December 2001 at the APS in Chicago. In this issue of *Journal of Synchrotron Radiation* we have assembled eight papers written following the presentations made at the second workshop. We believe that these papers provide the current status of the research activity in this highly topical area of radiation damage to crystalline macromolecules and will constitute a valuable resource to the structural biology community.

The specific topics discussed at the second workshop include:

(*a*) Experience of data collection at very high intensities.

(*b*) Radiation damage at specific sites in a protein.

(*c*) Effect of radiation damage on structure determination by MAD and SAD.

(*d*) Modelling studies of putative heating effects at high intensities.

(*e*) Experience of the use of helium for cryocooling.

(*f*) Assessment of the onset of increasing degrees of radiation damage as a function of dose and dose rate.

The following conclusions are drawn by us from the various presentations given at the meeting and the papers presented in this issue. These conclusions are necessarily ours but are based on collective experience.

(i) A large number of damage sites per protein molecule are created during typical X-ray experiments (O'Neill *et al.*, 2002). These can cause effects at specific sites, observed by analysis of protein crystals. The identification of 'sensitive' sites is important as it means that caution should be used in interpreting structural features in these regions if the specimen has been subject to a high dose. This is especially true of active sites of enzymes which by their nature are usually solvent accessible and therefore more susceptible to damage. However, ionization and free-radical formation is likely to occur elsewhere without producing identifiable changes in the positions of the atoms.

(ii) Radiation damage can cause reduction at metal sites. This is an important consideration during X-ray spectroscopy (and diffraction) investigations of metalloproteins.

(iii) The radiation damage can alter phase transitions (in lipid systems), even though it is not always possible to identify the onset of radiation damage from individual X-ray diffraction patterns (Cherezov *et al.*, 2002).

(iv) It is possible to model heating effects in protein crystals subject to intense X-ray beams (Kuzay *et al.*, 2001; Nicholson *et al.*, 2001). Measurements of actual heating can be made by observing small changes in d spacing (Müller *et al.*, 2002) or by thermal imaging (Snell *et al.*, 2002). It would be useful to combine all these methods.

(v) No clear evidence for a dose-rate effect at flux densities below 10^{15} photons s^{-1} mm^{-2} was presented.

(vi) The effect of ascorbate as a scavenger to reduce radiation damage at cryotemperatures (Murray & Garman, 2002) appears promising and merits further investigation.

(vii) Cooling to 16 K helium temperature appears to have some benefits in terms of reducing the increase in atomic displacement factors (B values) during X-ray exposure (Hanson *et al.*, 2002). However, it is not clear whether the potential gains merit adoption of helium cooling as a routine technique. A recent paper by Teng & Moffat (2002) concluded that the benefit of cooling to 40 K was only significant at doses greater than 1×10^7 Gy.

(viii) Radiation damage can have a serious effect on MAD experiments and this has led to the suggestion that collecting complete high-quality data at the 'peak' wavelength should be a priority (Rice *et al.*, 2000). Other wavelengths could then be used as an 'insurance' if SAD methods fail. This advice will depend on the details (*e.g.* is there a strong 'white line'). Some work on using the changes produced by radiation damage at specific sites as a phasing method was presented.

(ix) There is still no clear evidence that collecting data at very short (or very long) wavelengths will reduce radiation damage. There is

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some indication in the work of Müller *et al.* (2002) that radiation damage could increase for wavelengths above 1.3 Å. It has been pointed out by R. Henderson (personal communication) that, for very small crystals, the photoelectron could escape before it has deposited all its energy in the crystal. This effect would be more pronounced for higher energy photoelectrons (and therefore higher energy photons) as the path length would be longer.

(x) The expansion of the unit cell with dose can be used to monitor the progress of radiation damage in a particular crystal (Ravelli *et al.*, 2002). However, different crystals of the same protein can show different behaviour (Murray & Garman, 2002) so this indicator should be used with caution.

(xi) Spectroscopic measurements can provide useful information about the extent of radiation damage (Weik *et al.*, 2002). In principle, there is no reason why such measurements should not be carried out routinely on each crystal after X-ray data collection.

Clearly the issue of radiation damage to biological specimens still merits further investigation. It is possible in the future, as for electron microscopy, that low-dose techniques with multiple specimens will become more popular for examining weakly diffracting crystals. However, the X-ray structural biology community are aware of the issue and, like the electron microscopists, are designing their experiments to take account of the effect of intense doses on the specimens.

The question of experimental practice for radiation damage investigations was also addressed. Necessary information for systematic studies to be fruitful is: the careful estimation of incident and absorbed radiation dose for all measurements, all parameters of experiment (flow rate of cryogen, exact cryo-protocol of crystal treatment, crystal size) to be recorded to allow isolation of the variable in question, and comparison experiments where possible to be performed under identical physical conditions (beamline, slits, wavelength, attenuation).

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