Radiation Damage in Protein Crystals at Low Temperature

By A. Gonzalez and C. Nave

DRAL Daresbury Laboratory, Warrington WA4 4AD, England

Abstract

This paper describes the study of the effects of radiation damage on the quality of data collected from a protein crystal at 100 K. It is shown that radiation damage causes measurable effects in the diffraction pattern. This implies that, even at liquid nitrogen temperatures, there is a limit to the size of a crystal from which a complete data set can be collected.

1. Introduction

One of the major problems of X-ray diffraction from biological materials in general and protein crystals in particular is that the samples are susceptible to radiation damage. Protein crystals are particularly sensitive because the lattice contacts are relatively weak and easily disrupted by radiolytic products resulting from absorbed photons. Nave (1994) has reviewed the different processes taking place in the crystal in the wavelength range commonly used in protein crystallography and how they are likely to induce direct or indirect radiation damage in the sample.

There are at least two components to the radiation damage. Primary damage is caused by energetic electrons resulting from the photoelectric, Auger or Compton effect. Secondary damage is caused by the reactions of the resulting radiolytic products. These propagate through the crystal and cause further reactions and damage to the lattice contacts. These secondary processes vary greatly between different proteins and even different crystal forms of the same protein. At cryo-temperatures the secondary effects are much reduced and a large improvement in the crystal lifetime is obtained (see, for instance, Hope *et al.*, 1989; Young, Dewan, Thompson & Nave, 1990; Young, Dewan, Tilton & Nave, 1993).

Nevertheless primary radiation damage keeps on accumulating in the crystal during exposure to X-rays. Using experience from electron microscopy, Henderson (1990) has predicted a limit of around 1.3 $\times 10^{17}$ keV mm^{-3} absorbed energy in the specimen before significant radiation damage occurs at cryo-temperatures. This prediction is essentially independent of the sample considered, as only primary radiation damage is significant. It would

take several days on a monochromatic source to obtain this dose and much longer for a systematic study to be carried out. Therefore, Gonzalez, Thompson & Nave (1992) used the white beam to test this prediction. Using visual estimation of the Laue patterns they observed radiation damage after an absorbed dose of approximately 4 $\times 10^{17}$ keV mm^{-3}. However, no attempt was made to quantify the radiation damage or to investigate how data are affected at different resolutions.

The present paper is an extension of this work and has three aims.

(1) To obtain a more quantitative estimate of the radiation damage in terms of the effect on the diffraction pattern as a function of resolution and absorbed dose.

(2) To perform experiments at different incident intensities, to verify that the radiation damage was not due to heating of the crystal in the intense X-ray beam.

(3) To estimate the maximum size of crystal from which it will be possible to collect a data set at cryo-temperatures.

The experiments, therefore, are intended to give practical information which is relevant to data collection at cryo-temperatures. The X-ray intensities in the white beam are similar to those which could be obtained on future undulator sources of X-rays in a monochromatic beam. The results are, therefore, relevant to the utilization of these sources for X-ray protein crystallography.

2. Experimental

2.1. *Methods*

Tetragonal crystals of lysozyme were prepared by the following method. 400 mg of hen egg-white lysozyme (Sigma Chemical Company) were dissolved in 5 ml of 0.04 M acetate buffer, pH 4.7 and stirred gently for 5 min. 5 ml of 10%(w/v) NaCl was then added dropwise over 5 min and continuously stirred for another 5 min. The solution was filtered into a plastic container and left at room temperature for 2 d until suitably sized crystals appeared. Crystals of lysozyme prepared in this manner can be successfully

frozen with only a small increase in mosaic spread (Young, Dewan, Thompson & Nave, 1990).

A crystal was suspended in a thin film of mother liquor supported by a loop made of human hair and frozen to a temperature of 100 K in a cold nitrogen stream (Oxford Cryosystems). This technique was first described by Teng (1990). The crystal was irradiated with the focused white beam on the SRS beamline station 9.5 (Thompson *et al.*, 1992).

The radiation damage was monitored by comparing Laue diffraction patterns recorded after each exposure with a reference pattern collected at the beginning of the experiment. The radiation dose received by the crystal after the exposures was estimated by calculating the number of photons absorbed by a thin slice of the crystal multiplied by the energy deposited by each photon at regular wavelength intervals in a 0.1% bandwidth. The resultant energy was then integrated over the wavelength spectrum. In order to compensate for the decay of the current in the storage ring during the experiment the estimates for the dose were adjusted using the readings from an ion chamber.

In a previous experiment Gonzalez, Nave & Thompson (1992) estimated that the dose absorbed by the front face of the crystal was 1.2×10^{16} keV mm^{-3} s^{-1} for the wavelength range 0.45–4 Å. Because absorption by the sample is quite strong for the longest wavelengths other parts of the crystal will receive a lower dose. Thus, the total dose received by the crystal could be overestimated. Also, the high dose per second in this experiment might have caused the crystal to overheat. In order to solve both of these potential problems, the present experiment was performed for weaker incident intensities and shorter wavelength distributions. These conditions were achieved by inserting from one to three 0.2 mm-thick aluminium foils between the source and the crystal.

The data collection details are given in Table 1.

2.2. Data analysis

The intensities of the singlets in the Laue patterns were calculated using the programs *LAUEGEN* and *INTLAUE* (Helliwell *et al.*, 1989). Laue patterns are more susceptible to crystal mosaicity than monochromatic patterns. The spots in the patterns were a little elongated due to the small increase in the mosaic spread of the crystal during freezing and the 'streak' option had to be used to obtain good profiles. Normalization curves were calculated for the first pattern with the program *LAUENORM* (Helliwell *et al.*, 1989).

We used the correlation coefficient to assess the changes which occurred in the diffraction patterns at different resolutions due to radiation damage. This is

Table 1. *Total exposure time and absorbed dose for each recorded pattern using one and three aluminium attenuators*

The dose received after each exposure was calculated as explained in §2.

One attenuator

	Reference	1	2	3	4	5	6	7
Exposure time (s)	0.3	30.6	50.9	71.2	91.5	111.8	132.1	162.4
Dose absorbed ($\times 10^{17}$ keV mm^{-1})	0	0.7	1.1	1.5	1.9	2.3	2.6	3.1

Three attenuators

	Reference	1	2	3	4	5
Exposure time (s)	1.6	92.4	183.2	274	364.8	485.6
Dose absorbed ($\times 10^{17}$ keV mm^{-3})	0	0.8	1.6	2.4	2.9	3.6

given by $[\Sigma(F_o - \langle F_o \rangle)(F_n - \langle F_n \rangle)]/[\Sigma(F_o - \langle F_o \rangle)^2 \Sigma(F_n - \langle F_n \rangle)^2]^{1/2}$. F_o refers to the amplitudes obtained from the first Laue pattern, F_n to the amplitudes from the nth pattern, $\langle F_o \rangle$ and $\langle F_n \rangle$ to the means over the data points and the sum is over the reflections in a resolution bin common to both data sets. This criterion is independent of the scale factor between different images and is therefore insensitive to any overall decrease of intensity in the crystal due to radiation damage. As the analysis is carried out independently at different resolutions, it is also insensitive to any decrease in average intensity at high resolution, characteristic of an increase in temperature factors. A large decrease in the average intensity at high resolution will, however, lead to greater statistical errors and a consequent reduction in the correlation coefficient. The correlation coefficient will also be sensitive to changes in the relative intensity between reflections in any resolution bin as a result of radiation damage. The correlation coefficient as applied here, therefore, assesses how well subsequent data sets can be scaled with the original data set. It, therefore, gives a good indication of the effects of radiation damage in a practical situation.

2.3. Results

Fig. 1 shows the correlation coefficient as a function of the absorbed dose for data at different resolutions and with different foils. The calculated absorbed dose for the different foils is shown in Fig. 2. Radiation damage occurs at a dose in good agreement with the prediction of Henderson (1990). There is no evidence of increased radiation damage (due to heating effects) for the higher intensity experiment with one foil. As expected, the high-resolution data are affected more than the lower resolution data. The correlation coefficient changes gradually with the absorbed dose. There is no evi-

dence of a sudden loss in resolution which can occur with secondary radiation damage.

With three foils in the beam, the wavelength distribution is shifted to shorter wavelengths (Fig. 2). From Fig. 1, the radiation damage does not appear to decrease under these conditions.

3. Concluding remarks

The results can be summarized as follows.

(1) Radiation damage occurs at 100 K after a dose in agreement with the prediction of Henderson (1990).

(2) The cooling procedures (using a flow of cold nitrogen gas) are adequate with incident X-ray intensities present in the white beam on station 9.5. These intensities are comparable with those obtainable on the most powerful monochromatic beamlines on synchrotron radiation sources in operation or under construction. Comparison of the results with different numbers of attenuators gave no indication that crystal heating occurred.

(3) There will be a limit to the size of crystal which can be used to collect a complete data set.

The final point can be illustrated by assuming a dose of 8×10^{17} keV mm^{-3} as the maximum the sample can absorb while still giving processable and useful data. On an insertion device beamline on a third generation synchrotron source about 10^{14} photons mm^{-2} would be available in a monochromatic beam. It would take approximately 2 min exposure time at 0.9 Å to collect a good quality data set from a crystal with a 100 Å unit cell and 0.3 mm in each dimension. Approximately 10^{16} keV mm^{-3} will be absorbed during this time allowing 80 data sets to be collected before reaching the limit given above. For a crystal of 0.03 mm in each dimension the time required would be 1000 times greater and one would need about 13 crystals to collect a whole data set. These estimates are, of course, dependent on the required accuracy and resolution of the data set.

Our results seem to indicate that a change in the wavelength distribution to shorter wavelengths does not lead to a large improvement in the lifetime of the crystal at cryo-temperatures. This is consistent with the radiation damage being proportional to the energy deposited in the crystal rather than the number of photons absorbed (see, for example, Fig. 2 of Arndt, 1984). More studies, using the most intense monochromatic beams are necessary to confirm this result.
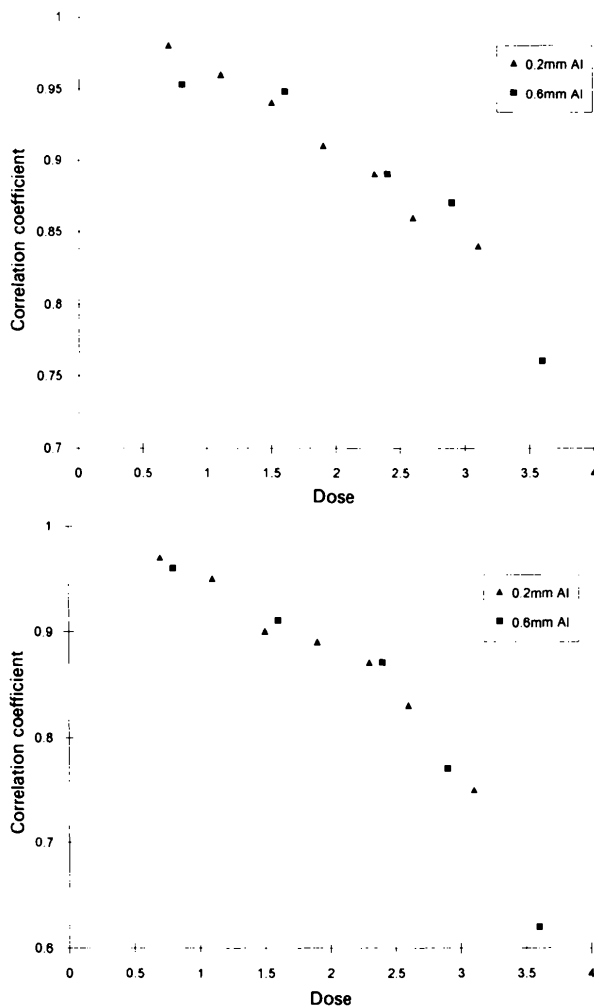
Fig. 1. Correlation coefficient as a function of dose absorbed for reflections in two resolution bins collected with different aluminium attenuators in the beam. Horizontal scale in units of 10^{17} keV mm^{-3}. (a) 3.6–2.8 Å resolution, (b) 2.1–1.9 Å resolution.
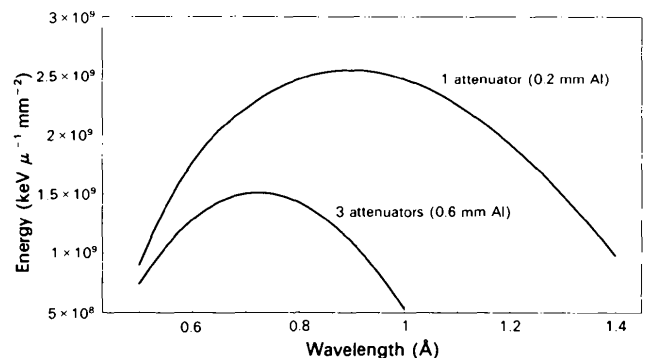

Fig. 2. Dose absorbed by the front face of the crystal in a 0.1% bandwidth. Integration under these curves gives an absorbed dose per second of 2.2×10^{12} keV μ^{-1} mm^{-2} (one attenuator) and 0.9×10^{12} keV μ^{-1} mm^{-2} (three attenuators).

University) for their help with Laue data processing.

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