

Dose Rate vs. Dose Dependency of Radiation Damage to Protein Crystals

Piotr Sliz*, Stephen C. Harrison*, Gerd Rosenbaum**

* Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, Harvard University, Cambridge MA 02138, USA

** Dept. of Biochemistry, University of Georgia, SER-CAT at the APS, Argonne, IL 60439, USA

Anecdotal observations of crystal damage non-linear with dose have raised concerns about the usefulness of full intensity at 3rd-generation synchrotrons. The possibility of increased radiation damage due to sample overheating was considered during the previous workshop on radiation damage. Temperature increase of only a few °C, even with fluxes of the order of 10^{13} ph/sec, were shown by James Nicholson's finite element thermal analysis which included heat removal by the cold stream, and by Gerd Rosenbaum's calculation of the adiabatic temperature increase per exposure. Nevertheless, some stations, including SBC 19-ID routinely attenuate radiation. In response, a few crystallographers have questioned the need to operate high intensity beamlines and plans to improve them.

To clarify the situation, Gerd Rosenbaum, among others, was charged with the design of experiment to assess the dose rate effect.

We present here the results of radiation damage experiments using crystals of five quite different proteins: HLA-A2 Class I MHC, BTB domain, US2/HLA-A2 complex, reverse transcriptase and the reovirus RNA polymerase. To determine whether radiation damage is a function of dose rate or just of total dose, we exposed each of the five sample protein crystals to alternating regimes of high and low flux density, while keeping the dose per exposure constant. The study was carried out at the SBC beamline 19-ID. The high flux density was $1.8 - 2.5 \cdot 10^{15}$ photons/s/mm² (flux: $2.2 - 3.2 \cdot 10^{12}$ photons/s; beam size: 0.065 mm FWHM horizontal, 0.020 mm FWHM vertical; slit size: 0.10 mm horizontal, 0.05 mm vertical). The low flux density was 1/10th of the high flux density (by insertion of a 0.5 mm thick Al-filter). The exposure time per frame was 0.5 s for the high flux density and 5.0 s for the low flux density for all specimen except the BTB domain where the exposure times were 0.05 s and 0.5 s, respectively. At the end of the alternating exposures, a set of frames with 10x dose/frame (high flux density, 5.0 s exposure, (0.5 s for BTB)) was collected.

There was significant radiation damage observed in frozen samples exposed to the intense X-ray beams. The rate of damage observed as a function of accumulated dose did not depend on the flux density nor on the dose per frame. Damage was solely proportional to the cumulative dose absorbed by each sample. We conclude that the full strength of undulator sources at 3rd-generation synchrotrons can be used to advantage by protein crystallographers.