Radiation Damage in Macromolecular Cryocrystallography.

E. F. Garman

Department of Biochemistry, University of Oxford, Rex Richards Building, South Parks Road, Oxford OX1 3QU, UK.

E-mail: Elspeth@biop.ox.ac.uk

Structural biologists utilise crystallography to determine the three-dimensional structures of large biologically interesting macromolecules. Advances in dealing with radiation damage in this field are intimately intertwined with the development of cryotechniques in the 1990s in which data collection is performed with the crystal in an open flow 100 K nitrogen stream which significantly reduces secondary damage allowing much more data to be obtained from each crystal. The loop mounting innovation of Teng (1) made the cryo-method much more straightforward and gave impetus to many laboratories to experiment with it.

However, it was not long before high flux density synchrotron beams were observed to cause radiation damage even for cryocooled crystals. Researchers started to try to understand the physical and chemical processes involved in this damage (reviewed in 2), which manifests itself in a number of different ways, including: changes in crystal colour, decreasing diffraction power with dose, noticeable first in decreasing values of $I/\sigma(I)$ for the highest resolution reflections, a small linear increase in unit cell volume, and specific structural damage to covalent bonds in the protein in a reproducible order (3-5). This specific structural damage can lead to incorrect conclusions on biological mechanisms being drawn from structures, especially as enzyme active sites and metal binding sites seem particularly sensitive to change by X-ray irradiation. Thus the issue of radiation damage has recently come to the fore as a concern for all structural biologists.

This contribution will summarise the current state of our understanding of radiation damage in macromolecular cryocrystallography, including putative mitigation strategies and the experimental determination of an upper dose limit of 3×10^7Gy for the biological veracity of the information obtained from 3-D structures to be maintained (6).

References

(1) Teng T-Y (1990) *J Appl Cryst* **23**, 387-391.
(2) Ravelli RGB and Garman EF (2006) *Curr Opin Struct Bio*, **16**, 624-629.
(3) Weik M, Ravelli RBG, Kryger G, McSweeney S, Raves ML, Harel M, Gros P, Silman I, Kroon J, and Sussman JL (2000) *PNAS* **97**, 623-628.
(4) Burmeister WP (2000) *Acta Cryst.* **D56**, 328-341.
(5) Ravelli RGB and McSweeney S (2000) *Structure* **8**, 315-328.
(6) Owen RL Rudino-Pinera E and Garman EF (2006) *PNAS* **103**, 4912-4917.