Physical and Chemical Considerations of Damage Induced in Protein Crystals by Synchrotron Radiation: A Radiation Chemical Perspective

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Radiation-induced degradation of protein or DNA samples by synchrotron radiation is an inherent problem in X-ray crystallography especially as the 'brighter light' sources become available. The aim of the presentation is to give a radiation chemist's perspective on some of the physical and chemical processes which need to be considered in understanding potential pathways leading to the gradual degradation of the samples. The talk will focus on the primary radiation chemical events and how damage may localise at specific sites.

Absorption of synchrotron photons by the sample induce ionisation and excitation processes. In the ionisation process and electron loss centre is formed and a secondary electron is produced with several keV energy, the amount of energy depending on the energy of the incoming photon. This secondary electron will induce further excitation and ionisation events in the crystal. Initially, electron loss centres and electron gain centres are formed. These events may occur directly in the protein together with its primary water of hydration (direct events) or in the support material. If the radicals induced within the support material diffuse and interact with the protein, damage may also occur (indirect effects). These latter effects, mainly at the surface of the protein, are minimised by irradiating at low temperature, to minimise diffusion of the radicals, by having support material, which intercepts any radicals prior to their interaction with the protein and by ensuring the appropriate electrostatics around the protein molecules. Under conditions of X-ray crystallography, the indirect effects are generally minimised.

The direct effects produce electron gain and electron loss centres. These centres may recombine to lead to an excited state, which may or may not cause damage. These recombination processes are in competition with charge separation through migration. More importantly these centres may migrate by tunnelling (essentially non-temperature dependent) or hopping (temperature dependent) to given sites in the protein. Carbonyl groups and disulphide bridges are two major sites for localisation (trapping) of the electron. Other sites of charge trapping are aromatic residues (e.g. tryptophan, tyrosine) and metal redox centres. Deprotonation of electron loss centres may lead to persistent damage. Ionisation of water molecules in the first hydration shell of the protein may need to be considered as precursors to oxidation of specific amino-acid residues in competition with reaction of $H_2O^{+\cdot}$ in an ion molecule reaction with a neighbouring water molecule.

Some of the chemical processes, which may occur at these protein centres, such as bond scission, will be discussed. These latter processes may lead to degradation of specific amino-acid residues. Degradation of the samples may lead to conformation changes, the timescale of which with respect to the photon fluence, i.e. time of irradiation may need to be considered.

The potential use of agents known to minimise radical induced degradation in radiation chemistry will be discussed. Through developing the themes above, it is hoped that through discussion ideas may evolve on ways to interfere with these damaging pathways reduce radiation degradation.