TheTemperature-Dependence of Specific Radiation Damage to Proteins

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Intense synchrotron radiation produces specific structural and chemical damage to proteins [1-3]. This happens even at 100 K, at which data are routinely collected in cryo-crystallography. Disulfide bonds break, acidic residues are decarboxylated and the active site of enzymes appear particularly radiation-sensitive. Chemically identical groups in a given protein are not equally radiation-sensitive. Differences in solvent accessibility and in the chemical and dynamical nature of the close environment are most likely among the crucial factors that determine radiation-sensitivity of a specific residue. Another factor that plays an important role in radiation damage is the physical state of the crystal solvent. Amorphous at 100 K, the solvent crystallizes at a higher temperature, which depends on its composition and confinement within the crystal. Solvent in trigonal crystals of the enzyme *Torpedo californica* acetylcholinesterase (*Tc*AChE) crystallizes upon warming at 155 K [4], thus providing circumstantial evidence for the occurence of a glass transition at or below 155 K. That water in the close vicinity of a protein surface indeed melts into a highly viscous liquid prior to crystallisation was shown be neutron diffraction (Weik, Lehnert, Zaccai, unpublished results). To assess the temperature-dependence of specific radiation damage we collected a series of data sets on a single crystal of *Tc*AChE at two temperatures, one below and one above the glass transition of the crystal solvent, viz. at 100 and at 155 K, respectively [5]. A buried disulfide bond, a buried cysteine and solvent exposed methionine residues show drastically increased radiation damage at 155 K, in comparison to 100 K. Moreover, the irradiation-induced unit cell volume increase is linear at 100 K, but not at 155 K, which can be attributed to the increased solvent mobility at 155 K. Most importantly, we observe conformational changes in the catalytic triad at the active site at 155 K but not at 100 K. These changes lead to an inactive catalytic triad conformation and thus represent the observation of radiation-inactivation of an enzyme at the atomic level. Our results show that at 155 K the protein has acquired, at least locally, sufficient conformational flexibility to adapt to irradiation-induced alterations in the conformational energy landscape. They reveal the influence of both protein and solvent dynamics on specific radiation damage to proteins.

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