[MS12–03] X-ray induced disulfide bond cleavage studied with EPR, optical absorption, and X-ray diffraction.

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Electron Paramagnetic Resonance (EPR) and online UV/visible absorption microspectrophotometry with X-ray crystallography have been used in a complementary manner to follow X-ray induced disulfide bond cleavage. On-line UV/Visible spectroscopy showed that upon X-irradiation, disulfide radicalization appeared to saturate at an absorbed dose of approximately 0.5~0.8 MGy in contrast to a saturating dose of ~0.2 MGy observed using EPR at much lower dose rates. The observations suggest that a multi track model involving product formation due to the interaction of two separate tracks is a valid model for radiation damage in protein crystals. The saturation levels are remarkably consistent given the widely different experimental parameters, and the range of total absorbed doses studied. Our results indicate that even at the lowest doses used for structural investigations, disulfide bonds are already radicalized. Multi-track considerations offer the first step in a comprehensive model of radiation damage that could potentially lead to a combined computational and experimental approach to identifying when damage is likely to be present, to quantitate it, and provide the ability to recover the native unperturbed structure.

Keywords: radiation damage; disulphide bond; multi-track model