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Studying disulfide damage at low X-ray doses with an engineered protein approach

A significant problem in biological X-ray crystallography is the radiation chemistry caused by the incident X-ray beam. This causes both global and site specific damage. Global damage manifests itself in the decay of the diffraction pattern and data processing parameters. Site specific damage targets glutamates, aspartates, methionines, and disulfide bonds. This can misdirect the biological interpretation of the structural models produced. Cryo-cooling crystals has been successful in mitigating but not eliminating damage; however, cooling has also been shown to limit functionally relevant protein conformations. Due to this, and the difficulty in cryoprotecting some systems, there has been interest in the return to near-physiological temperature crystallography. Radiation chemistry at these conditions is less well studied. The doses used for X-ray crystallography under both cryocooled and near physiological temperature are in the kGy to MGy range. X-rays are used therapeutically at much lower doses. Disulfide bonds are among the most significantly affected species in a protein in the crystalline state but limited information is known about their response to damage in vivo. In this work we engineered a protein that dimerizes through a vulnerable disulfide bond to understand if radiation damage processes seen in structural studies translate to conditions closer to physiology, specifically in solution. We monitored monomerization with small angle solution X-ray scattering (SAXS), simultaneously pumping the sample with X-rays and while probing for structural impact using doses that are therapeutically relevant and a fraction of that required for crystallographic studies. Our results show that X-ray radiation drives a dose dependent fragmentation of the engineered protein that can be explained by a dimer to monomer transition, indicating disulfide bond cleavage. This supports the crystalline mechanism and suggests that crystallographic damage results can be extrapolated to physiologic conditions. Fragmentation was observed to be pH dependent, suggesting radiation damage processes and future routes for investigation and mitigation. The engineered protein approach represents a promising tool for advancing radiation damage studies and studying physiologically relevant radiation-protein interactions.

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