UV related cataract formation: insights from serial synchrotron crystallography

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Cataracts are a leading cause of blindness, characterised by opacification of the eye lens, due to the aggregation of crystallin proteins. Human γ-D-Crystallin (HGD) is the most abundant monomeric crystallin, a long-lived, small compactly folded protein of 173 amino acids. Localised to the lens fibre cells, which lack the cellular machinery for translation, HGD must therefore remain correctly folded and soluble for the entire human lifespan (Slingsby et al. 20). Although highly stable repeated exposure to UV-radiation has been implicated in the aggregation of HGD and the subsequent formation of cataracts. UV radiation has been reported to destabilise HGD via two mechanisms; primary photodamage caused by cleavage of the indole ring leading to the conversion of tryptophan to kynurenine, partial unfolding and inevitably aggregation and secondary photodamage caused by the formation of reactive oxygen species (ROS) is thought to accelerate cataract formation through oxidation of surface thiol residues, leading to intermolecular disulphide bond formation (Craghill et al. 2004).

Glutathione (GSH) is present in high concentrations within the lens and acts as a ROS scavenger. GSH may prevent aggregation by reversing the oxidation of surface thiols, preventing intermolecular disulphide bond formation. Aging is associated with a reduction in GSH levels as the oxidised form GSSG increases alongside an increase in oxidised surface thiols. GSSG may be regenerated to GSH via formation of S-glutahionlyated cysteine, as is reported in γ-S and γ-C crystallins. UV radiation may result in reduction of disulphide contributing to the replenishment of GSH (Zetterberg et al. 2006).

Using serial crystallography (Schulz et al. 2022) and the readily crystallisable R36S mutant of HGD, and DTT as a proxy for GSH, we show that aged crystals of HGD accumulate covalent modifications on surface cysteines. Studies of oxidised crystals, both with and without UV irradiation revealed that UV irradiation disrupted the covalent modification of surface cysteines.

Schulz, E.C., Yorke, B.A., Pearson, A.R. and Mehrabi, P. (2022) Best practices for time-resolved serial synchrotron crystallography. Acta Crystallographica. Section D, Structural Biology 78 (Pt 1), 14–29.

Slingsby, C., Wistow, G.J. and Clark, A.R. (20) Evolution of crystallins for a role in the vertebrate eye lens. Protein Science 22 (4), 367–380.

Zetterberg, M., Zhang, X., Taylor, A., Liu, B., Liang, J.J. and Shang, F. (2006) Glutathiolation enhances the degradation of gammaC-crystallin in lens and reticulocyte lysates, partially via the ubiquitin-proteasome pathway. Investigative Ophthalmology & Visual Science 47 (8), 3467–3473.