3-Hydroxykynurenine bound to eye lens proteins induces oxidative modifications in crystalline proteins through a type I photosensitizing mechanism

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Photosensitized reactions mediated by endogenous chromophores have been associated with the etiology of age-related cataract disease. Endogenous chromophores such as 3-hydroxykynurenine (3OHKN) can be found in both free form, and bound to crystallin proteins. However, their efficiency in generating photo-induced oxidative modifications on eye lens proteins is not completely understood. In this work, the efficiency and photodynamic activity of 3OHKN bound to both lysine (3OHKN-Lys) and bovine lens proteins (3OHKN-BLP) was assessed and compared with the photosensitizing activity of the major chromophore arising from glucose degradation (GDC). The photosensitizing activity of 3OHKN-Lys, 3OHKN-BLP and GDC was characterized by measurement of singlet oxygen quantum yields, O2 consumption, SDS-PAGE and amino acid analysis of the photo-oxidized proteins. Singlet oxygen quantum yields under 20% O2 atmosphere were 0.02, 0.01, and 0.27 for 3OHKN-Lys, 3OHKN-BLP and GDC, respectively. O2 consumption by photosensitized reactions was more efficient for 3OHKN-BLP, with the extent of O2 consumption being 28% higher than for 3OHKN-Lys and GDC under both 5 and 20% O2. SDS-PAGE showed that protein crosslinking is dependent on the O2 concentration, and more extensive at 5 than 20% O2. GDC and 3OHKN-Lys were the most efficient crosslinkers at 20 and 5% O2, respectively. Amino acid analysis of the irradiated proteins showed consumption of Trp, His, Tyr and Phe, and formation of kynurenine (from Trp), methionine sulfoxide (from Met) and DOPA (from Tyr). Kynurenine formation was dependent on the O2 concentration with higher amounts detected at 5 than 20% O2 for
3OHKN-BLP and 3OHKN-Lys, with 3OHKN-BLP the most efficient sensitizer. Our results suggest that 3OHKN-BLP can elicit photo-oxidative damage mainly by a type I photosensitizing mechanism, with this likely to be the most prevalent pathway at the low physiologic O2 concentrations in the eye lens.