Effect of pH on mechanisms and products of photodamage to tryptophan and tyrosine residues in the free state and a model protein

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Cross-linking of proteins in living tissues largely contributes to the development of various diseases and molecular pathways of ageing. This is essentially important in the case of cataract – the progressive opacification of an eye lens – proceeding via extensive protein aggregation. The latter may proceed via different reactions, including cross-linking, which mechanisms are still mostly unclear. Tryptophan (Trp) and tyrosine (Tyr) residues are amino acids with the low redox potentials and known for their participation in photo-induced oxidation and cross-linking of proteins via radical reactions. Recently it has been discovered that Trp/Tyr cross-links are present in human cataract lenses and therefore may directly contribute to the development of this disease.

The oxidative stress is considered as the main cause of cataract, and it may lead to an acidification of a tissue. Thus, the variation of pH during the cataract progression may affect the rate of cross-linking and the accumulation of aggregation products. In this work we have studied the effect of pH on the reaction mechanisms between Trp/Tyr radicals ($Trp^{\bullet}$/$Tyr^{\bullet}$) in the free state and in a model protein, lysozyme.

The photodamage was sensitized by kynurenic acid (KNA), a UV-A chromophore present in the human eye lens. We found that the protonation of $Trp^{\bullet}$ in free state under acidic conditions accelerates the back electron transfer from KNA radical by a factor of two. This leads to more efficient restoration of initial reagents and the reducing of the yield of all products, including Trp-Trp cross-links. However, this protonation effect is absent in the case of $Tyr^{\bullet}$ within the pH range of 3-7 due to its very low pKa (< 0).

Protein globule significantly affects the paths of radical reactions due to steric hindrance and changes in charges on the protein surface with pH variation. We found that the effect of $Trp^{\bullet}$ protonation is significantly reduced within lysozyme globule resulting to a minor variation in the yield of protein degradation within pH 3-7. However, the media acidification results in tremendous differences in the product composition from the domination of Trp-Trp cross-linking at pH 7 to the prevalence of oxygenation (Trp+O) at pH < 5. This drastic change indicates a strong competition between cross-linking and oxygenation for radicals within the protein. Thus, a variation of pH within living tissue may significantly affect the direction of radical reactions and requires further studies under conditions simulating the cell environment.