

# ESR in vitro assay of singlet oxygen generation in the presence of water-soluble derivatives of C60

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Singlet oxygen, the lowest energy electronically excited state of molecular oxygen, has been implicated in skin aging, inflammation and disease processes as a part of intracellular milieu of reactive oxygen species that arise from cellular processes or exogenous chemical or photochemical agents. Singlet oxygen is also responsible for the therapeutic benefit of photodynamic therapy of cancer (PDT), one of the most promising current trends in selective treatment of neoplastic diseases.

Due to their high triplet quantum yield that is near unity, fullerenes have been found to be excellent singlet oxygen sensitizers. In this study we implemented the ESR technique to determine the yields of singlet oxygen generation in the presence of water-soluble derivatives of C60 in aqueous milieus. Two multifunctionalized, water-soluble derivatives of C60 were studied: (1) with six addends (monomethylethers of polyethylene glycol) attached to the C60 molecule at pseudo-octahedral sites and giving a star-like polymer with the common formula  $C_{60} [ >N-CH_2 CH_2 (OCH_2 CH_2)_2 OCH_3 ]_6$ , and (2) with 20 to 28 hydroxyl (OH) groups that were quasi-symmetrically attached to the C60 molecule. A well-known singlet oxygen scavenger, 2,2,6,6-Tetramethyl-4-piperidinol (TMP-OH), was used to monitor its visible light-induced generation in H<sub>2</sub>O and D<sub>2</sub>O solutions. The concentrations of the substrates were chosen to ensure a pseudo zeroth-order formation kinetics of TEMPO-OH, the ESR active product resulting from the reaction of TMP-OH with singlet oxygen. The total ESR signal intensity of TEMPO-OH was monitored as a function of illumination time. These results were compared to the light-induced generation of singlet oxygen that was mediated by a water-soluble protoporphyrin IX-based photosensitizer (PP IX(Ala)<sub>2</sub>(Arg)<sub>2</sub>), currently clinically tested for PDT applications. Our ESR results confirm the excellent photophysical properties of water-soluble derivatives of fullerenes that conserve their ability to generate singlet oxygen at high yields, even upon functionalization. Additionally, we found that the light-induced oxidation processes of TMP-OH in the presence of the water-soluble derivatives of C60 evolved differently as compared to the one photosensitized by PP IX(Ala)<sub>2</sub>(Arg)<sub>2</sub>. The photo-process of Type II (i.e. the resonant energy transfer from the photosensitizer excited triplet to the ground triplet molecular oxygen) was more pronounced for water-soluble fullerenes, whereas Type I mechanism (i.e. free radical involving photo-process) was predominantly observed for PP IX(Ala)<sub>2</sub>(Arg)<sub>2</sub>, especially at lower oxygen concentrations.