GENERATION OF ¹⁸O-LABELED SINGLET MOLECULAR OXYGEN FROM LIPID HYDROPEROXIDES: MEASUREMENT AND DNA DAMAGE

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Modification of cellular DNA upon exposure to reactive oxygen and nitrogen species is the likely initial event involved in the induction of the mutagenic and lethal effects of various oxidative stress agents. including singlet oxygen (¹O₂). The decomposition of lipid hydroperoxides into peroxyl radicals is a potential source of ${}^{1}O_{2}$ in biological systems. We report herein on evidence of the generation of ${}^{1}O_{2}$ from lipid hydroperoxides involving a cyclic mechanism from a linear tetraoxide intermediate proposed by Russell. Using ¹⁸O-labeled linoleic acid hydroperoxide in the presence of Ce⁴⁺, Fe²⁺ or peroxynitrite, we observed the formation of ${}^{18}[{}^{1}O_{2}]$. The formation of ${}^{1}O_{2}$ was characterized by (i) dimol light emission (λ > 570 nm); (ii) monomol light emission (k=1270 nm); (iii) the enhancing effect on chemiluminescence intensity of deuterium oxide, as well as the guenching effect of sodium azide; and (iv) chemical trapping of ¹O₂ or ¹⁸[¹O₂] with the 9,10-diphenylanthracene and detection of the corresponding endoperoxides by HPLC-ESI/MS/MS. Moreover, the presence of ¹O₂ was unequivocally demonstrated by the direct spectral characterization of the near-IR light emission. Finally, to investigate the effect of the ¹O₂ on DNA, the combined use of the thermolysis of a water-soluble naphthalene endoperoxide as a generator of ¹⁸O labeled ¹O₂ (¹⁸[¹O₂]) and the sensitivity of electrospray ionization-tandem mass spectrometry (ESI-MS/MS) allowed the study of ¹O₂ reactivity toward 2'-deoxyguanosine (dGuo) and 8-oxo-7,8-dihydro-2'deoxyguanosine (8-oxodGuo). The reaction of ¹O₂ with the guanine moiety of DNA produced almost specifically 8-oxodGuo. However, in the presence of free dGuo in solution, additional modified nucleosides were detected upon ¹O₂-mediated oxidation. The gualitative identification of the ¹O₂oxidation products of &oxodGuo in aqueous solution was achieved using HPLC-ESI/MS/MS. Thus, the ¹⁸O-labeled imidazolone, oxazolone, together with the diastereoisomeric spiroiminodihydantoin nucleosides, were detected as the main degradation products. Interestingly, the reaction of ${}^{1}O_{2}$ with 8oxodGua when inserted into oligonucleotides was more specific. These novel observations are fundamental to the elucidation of some proposals of great importance about the mechanisms involved in the generation of ¹O₂ by lipid hydroperoxides and in the reaction with DNA. Supported by FAPESP, CNPg, USP and Guggenheim Foundation.