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# Energy Transfer between Singlet ($^1\Delta_g$) and Triplet ($^3\Sigma_g^-$) Molecular Oxygen in Aqueous Solution

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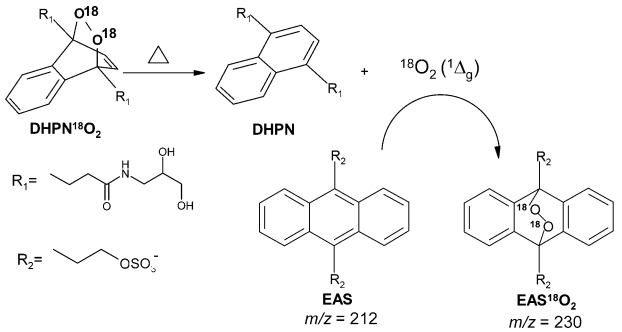
Singlet oxygen in its first excited state, denoted as $O_2$ ($^1\Delta_g$), can be produced by type II photosensitization reaction[1] and chemically in the reaction of hydrogen peroxide with hypochlorite[2] or peroxynitrite,[3] in the self-reaction of peroxyl radicals by the Russell mechanism,[4] and through decomposition of dioxetanes[5] and endoperoxides.[6] $O_2$ ($^1\Delta_g$) is also generated enzymatically by peroxidases and oxidases.[7] The presence of a pair of electrons with opposite spins in the highest occupied molecular orbital confers dienophile features to $O_2$ ($^1\Delta_g$) that explain its substantial reactivity toward electron-rich organic molecules, particularly with those exhibiting conjugated double bonds.[8]

We have recently synthesized the water-soluble $^{18}$O-labeled endoperoxide of *N*,*N*′-di(2,3-dihydroxypropyl)-1,4-naphthalene dipropanamide (DHPN$^{18}O_2$).[9] Thermal decomposition of the endoperoxide DHPN$^{18}O_2$ generates high yields of $^{18}$O-labeled singlet oxygen [$^{18}O_2$ ($^1\Delta_g$)] (Scheme 1). Oxidation products thus formed in the presence of such a generator of $^{18}O_2$ ($^1\Delta_g$) should be labeled with at least one $^{18}$O atom. Therefore, a mass spectrometry-based approach could be used to detect labeled products. Using electrospray ionization tandem mass spectrometry (ESI-MS/MS), we have shown that $O_2$ ($^1\Delta_g$) is able to react directly with cellular DNA to produce [$^{18}$O]-8-oxo-7,8-dihydro-2′-deoxyguanosine ([$^{18}$O]-8-oxodGuo).[10] In addition, the labeled endoperoxide was successfully used to study the reaction of $O_2$ ($^1\Delta_g$) with 8-oxo-7,8-dihydroguanine (8-oxoGua) inserted into oligodeoxynucleotides[11] or as free nucleoside in aqueous solution.[12]

Jones and Bayes[13] had demonstrated that, in the gas phase, $O_2$ ($^1\Delta_g$) is able to transfer energy to $^{18}O_2$ in the ground state ($^3\Sigma_g^-$) with subsequent conversion of the latter species into its singlet excited state ($^1\Delta_g$). For this purpose, $O_2$ ($^1\Delta_g$) was generated by gaseous discharge, and the gas in the flow system was sampled through a Pyrex pinhole into a quadrupole mass spectrometer equipped with an argon resonance lamp as the photoionization device. The argon resonance radiation (11.83, 11.62 eV) ionizes $O_2$ ($^1\Delta_g$) but not $O_2$ ($^3\Sigma_g^-$). Energy transfer to introduced $^{18}O_2$ ($^3\Sigma_g^-$) was found to be an extremely fast process ($\sim 1 \times 10^{10}$ $M^{-1}$ $s^{-1}$) that occurs at least once in every 10 hard sphere collisions. It may be added that this does not involve atomic rearrangement.

We report in the present communication that such an energy transfer reaction may also occur in aqueous solution. This is inferred from the results of incubation experiments involving DHPN$^{18}O_2$ as a chemical generator of $^{18}O_2$ ($^1\Delta_g$) and the water soluble disodium salt of anthracene-9,10-diyldiethyl disulfate (EAS) as a chemical trap of singlet oxygen. The products of the reaction (Scheme 1) were analyzed by ESI-MS measurement at the output of an HPLC column (see Supporting Information).

***Scheme 1.*** Chemical Structure of DHPN and EAS and Related Endoperoxides[a]




[a] Thermal decomposition of DHPN$^{18}O_2$ generates $^{18}O_2$ ($^1\Delta_g$), which is trapped by EAS to form the stable $^{18}$O-labeled anthracene endoperoxide EAS$^{18}O_2$.

Interestingly, both labeled (EAS$^{18}O_2$) and unlabeled (EAS$^{16}O_2$) anthracene endoperoxides were detected when EAS was incubated in the presence of DHPN$^{18}O_2$ (Figure 1). The mass spectrum of the anthracene endoperoxide, in the negative ionization mode, exhibits two ions of similar intensity, one at $m/z = 228$ corresponding to the doubly charged unlabeled EAS$^{16}O_2$ molecule and another one at $m/z = 230$ that corresponds to the doubly charged labeled EAS$^{18}O_2$ molecule (Figure 1A). The presence of high amounts of unlabeled endoperoxide was not expected since the isotopic enrichment of DHPN$^{18}O_2$ was almost 100% (see Supporting Information). Such a result suggests that unlabeled $O_2$ ($^1\Delta_g$) is produced under the latter conditions. The experiments were performed in the dark, ruling out the possibility for the formation of unlabeled $O_2$ ($^1\Delta_g$) by a photosensitization reaction. The ratio EAS$^{18}O_2$/EAS$^{16}O_2$ was found to be dependent on the presence of $O_2$ ($^3\Sigma_g^-$) into the solution. Purging the solution of EAS with $N_2$ prior to DHPN$^{18}O_2$ addition was found to significantly reduce the formation of unlabeled EAS$^{16}O_2$ (Figure 1B). In contrast, saturation of the EAS solution with $O_2$ ($^3\Sigma_g^-$) prior to addition of DHPN$^{18}O_2$ gave rise to an increase in the relative formation of unlabeled endoperoxide EAS$^{16}O_2$ (Figure 1C). The ratios between labeled and unlabeled anthracene endoperoxides EAS$^{18}O_2$/EAS$^{16}O_2$ that were determined in the conditions described above are reported in Figure 2. Furthermore, using the efficient freezing and thawing method to remove $O_2$ from the solution, the amount of EAS$^{18}O_2$ reached a 95% level (see Supporting Information).

Such an observation could be interpreted by an energy transfer process from $^{18}O_2$ ($^1\Delta_g$) to $^{16}O_2$ ($^3\Sigma_g^-$), generating $^{16}O_2$ ($^1\Delta_g$) according to eq 1.

$$^{18}O_2\,(^1\Delta_g) + {}^{16}O_2\,(^3\Sigma_g^-) \leftrightarrow {}^{18}O_2\,(^3\Sigma_g^-) + {}^{16}O_2\,(^1\Delta_g) \quad (1)$$

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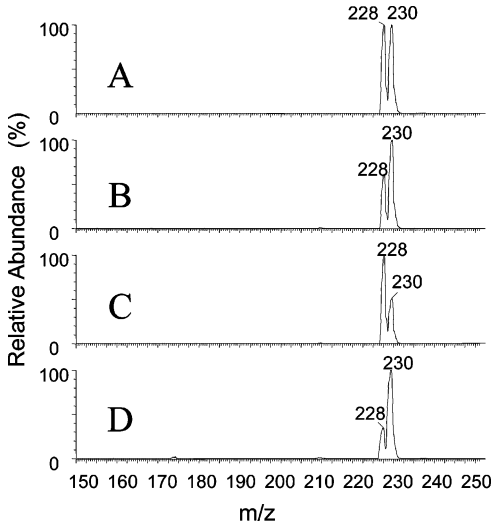


***Figure 1.*** ESI mass spectrometry analysis of EAS$^{16}O_2$ and EAS$^{18}O_2$ under various incubation conditions performed at 37 °C. (A) Reaction involving DHPN$^{18}O_2$ in aqueous solution equilibrated with air. (B) Reaction involving DHPN$^{18}O_2$ after bubbling $N_2$ for 15 min in an ice bath. (C) Reaction involving DHPN$^{18}O_2$ after bubbling $O_2$ for 15 min in an ice bath. (D) Reaction involving DHPN$^{16}O_2$ after saturating the solution with $^{18}O_2$.

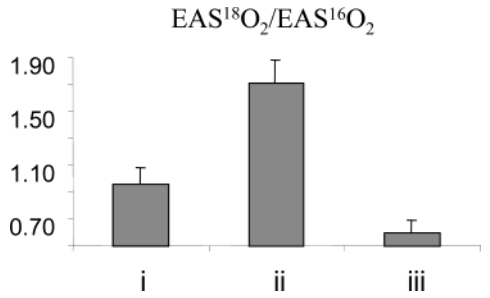


***Figure 2.*** Variations of EAS$^{18}O_2$/EAS$^{16}O_2$ ratios measured upon incubation of EAS with DHPN$^{18}O_2$ under different oxygenation conditions. (i) Solution equilibrated with air, (ii) $N_2$ bubbling before incubation, and (iii) $O_2$ bubbling before incubation.

Another experiment was performed to confirm the occurrence of an energy transfer process. An aqueous solution of EAS was saturated with $^{18}O_2$ ($^3\Sigma_g^-$) prior to incubation with DHPN$^{16}O_2$, a source of unlabeled $^{16}O_2$ ($^1\Delta_g$). The ESI mass spectrum of the anthracene derivative obtained under these conditions is reported in Figure 1D. EAS$^{18}O_2$ was clearly produced, suggesting a transient formation of $^{18}O_2$ ($^1\Delta_g$). It may be also noted that the ratio EAS$^{18}O_2$/EAS$^{16}O_2$ increases when the concentration of DHPN$^{18}O_2$ increases (see Supporting Information). This could be explained by the generation of high amounts of $^{18}O_2$ ($^1\Delta_g$) due to decomposition of the labeled endoperoxide. Therefore, the solution becomes saturated with labeled oxygen and the importance of eq 1 is minimized.

The data presented herein help to explain previously reported results, where the formation of unlabeled products was detected, although the $O_2$ ($^1\Delta_g$) generator was completely labeled. A relevant example[11] concerns the reaction of $O_2$ ($^1\Delta_g$) with 8-oxoGua residue inserted into short oligodeoxynucleotides that has been shown to lead to the almost quantitative formation of oxaluric acid. To unambiguously demonstrate the participation of $O_2$ ($^1\Delta_g$) in the oxidation reaction, DHPN$^{18}O_2$ has been used as a chemical generator of $^{18}O_2$ ($^1\Delta_g$). Interestingly, the oxidation products that were analyzed by HPLC-MS/MS were found to have incorporated one oxygen-18 atom, confirming the addition of $^{18}O_2$ ($^1\Delta_g$) to the 8-oxoGua. However, while the isotopic purity of the DHPN$^{18}O_2$ used in that study was 95%, unlabeled products were significantly formed (up to 50%). The reason for the presence of unlabeled oxaluric acid was unclear, and a possible oxygen exchange between water and a reaction intermediate was proposed to explain the formation of unlabeled oligodeoxynucleotides. The latter results may now be reinterpreted in light of the present findings. Thus, the formation of the unlabeled oxaluric acid derivative is likely to be accounted for the generation of unlabeled $O_2$ ($^1\Delta_g$) through an energy transfer process between $^{18}O_2$ ($^1\Delta_g$) and $^{16}O_2$ ($^3\Sigma_g^-$) (eq 1).

Interestingly, occurrence of eq 1 was not observed in vivo conditions. Thus, incubation of cells in the presence of DHPN$^{18}O_2$ was found to produce almost exclusively [$^{18}$O]-8-oxodGuo.[10,14] This may be explained by the low cellular concentration of molecular oxygen that minimizes the importance of eq 1.

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**Supporting Information Available:** ESI mass spectrum of DHPN$^{18}O_2$ and HPLC-MS conditions of the analysis of EAS$^{18}O_2$ and EAS$^{16}O_2$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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