Synthesis of a Naphthalene Endoperoxide as a Source of ^{18}O-labeled Singlet Oxygen for Mechanistic Studies

Glaucia R. Martinez, Jean-Luc Ravanat,‡
Marisa H. G. Medeiros, Jean Cadet,‡ and Paolo Di Mascio*

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, CP 26077, CEP 05513−970, São Paulo, SP, Brazil

Received May 13, 2000
Revised Manuscript Received July 28, 2000

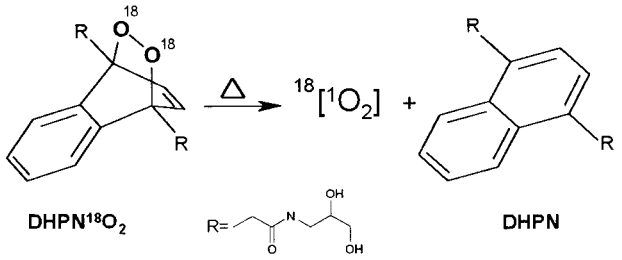
Singlet oxygen ($^1O_2$) exhibits a substantial reactivity toward electron-rich organic molecules, leading to the formation of allylic hydroperoxides, dioxetanes, or endoperoxides.[1] Singlet oxygen has been shown to be generated in biological systems.[2] As possible biological sources of $^1O_2$, we may mention enzymatic processes catalyzed by peroxidases or oxygenases,[3] reactions of hydrogen peroxide with hypochlorite[4] or peroxynitrite,[5] thermodecomposition of dioxetanes,[6] and photosensitization reactions.[7]

The reactions of $^1O_2$ with unsaturated fatty acids, proteins, and DNA have been extensively studied since this activated oxygen species can induce various types of cell damage that are related to aging, cancer, and other cytotoxic effects.[8]

Aqueous sources of $^1O_2$ are required in order to study the reactivity of $^1O_2$ toward biomolecules. In this respect, type II photosensitization reactions have been widely used but this approach may suffer for the presence of the type I competitive reaction.[7] Alternatively, several chemical compounds that are able to convert, in the dark, an oxygen precursor into $^1O_2$ almost quantitatively[1] were designed. However, conditions required by biological media (i.e., an aqueous environment, a neutral pH, a moderate temperature) are not compatible in most cases with the application of the latter sources of $^1O_2$. To overcome these difficulties, alternative methods to generate pure $^1O_2$ under mild conditions that involve the thermolysis of hydrophilic naphthalene endoperoxides have been employed.[9,10] These compounds are chemically inert and, upon heating, regenerate molecular oxygen, partly in the singlet state, and the parent hydrocarbon.

The development of a water-soluble naphthalene endoperoxide acting as a chemical source (Scheme 1) of [^{18}O] isotopically labeled singlet oxygen ($^{18}[^1O_2]$) is of particular interest in order to assess the reactivity of $^1O_2$ toward chemical, biochemical, or biological targets. Oxidation products thus formed will be labeled with, at least, one oxygen atom. Therefore, the oxidation products which contain the labeled oxygen can be detected and quantified using appropriate methods. In this respect, HPLC coupled to mass spectrometry is particularly relevant.

Scheme 1. Release of $^{18}[^1O_2]$ and the Parent Naphthalene Derivative (DHPN) from the Thermodissociation of the Hydrophilic Naphthalene-Labeled Endoperoxide (DHPN$^{18}O_2$)



We report, in the present work, the chemical synthesis of the [^{18}O]-labeled endoperoxide of *N*,*N*′-di(2,3-dihydroxypropyl)-1,4-naphthalenedipropanamide (DHPN$^{18}O_2$). In addition, the ability for DHPN$^{18}O_2$ to release labeled singlet oxygen was checked using the water-soluble disodium salt of anthracene-9,10-diyldiethyl disulfate (EAS) as a chemical trap of $^1O_2$. The analysis of the products of the reaction was achieved by mass spectrometry measurement after HPLC purification.

The hydrophilic naphthalene carrier *N*,*N*′-di(2,3-dihydroxypropyl)-1,4-naphthalenedipropanamide (DHPN) was prepared as the following. In the first step, a double bromination of 1,4-dimethylnaphthalene, in the presence of light, yields 1,4-dibromomethylnaphthalene, which was purified by recrystallization in chloroform. Thereafter, 1,4-naphthalenedipropanoic acid (NDPA) was synthesized by malonic synthesis, hydrolysis, and decarboxylation.[11] NDPA was subsequently used for the synthesis of diethyl-1,4-naphthalenedipropanoate (DENDP) by acid-catalyzed esterification.[12] This was achieved by refluxing during 2 h 21 g (77 mmol) of NDPA and 1 mL of H_2SO_4 (95%) in ethanol. A Dean−Stark trap was settled in the presence of toluene, and the reflux was left for 4 h. The organic phase was washed with 5% aqueous $NaHCO_3$, dried, and evaporated to yield DENDP (22 g, 87%) as an oil. Finally, the amidation[13,14] of the diester DENDP with 3-amino-1,2-propanediol was made by stirring, under reflux, a solution of DENDP (5.1 g, 15 mmol) and 3-amino-1,2-propanediol (9 g, 99 mmol) in 80 mL of MeOH for 24 h. After evaporation of the solvent, the residue was triturated with 100 mL of acetone. The colorless precipitate was filtered by suction, rinsed with acetone, and recrystallized in MeOH, yielding 50% DHPN. The final product, the nonionic carrier DHPN was characterized by mass spectroscopy and ^{1}H NMR analysis.

DHPN$^{18}O_2$ was prepared by photosensitization in the presence of [^{18}O]-labeled molecular oxygen (99%). Typically, in a 50-mL flask, 210 mg of DHPN was suspended in 2.5 mL of deuterated water that contained 0.5 mg of methylene blue. The solution, maintained in the dark, was purged several times with argon and gently heated to dissolve DHPN. Thereafter, the solution maintained under continuous stirring in an atmosphere of $^{18}O_2$ (2 bar) was cooled at 4 °C and irradiated during 6 h with a 500 W tungsten lamp placed at a distance of 30 cm. Then, Chelex 100 cation-exchange resin was added to the blue mixture, which was then stirred for 20 min at 4 °C until complete fixation of the sensitizer onto the insoluble anionic polymer. Using a 5-mL

* Corresponding author. E-mail: pdmascio@iq.usp.br.
‡ Laboratoire "Lésions des Acides Nucléiques" Service de Chimie Inorganique et Biologique, Département de Recherche Fondamentale sur la Matière Condensée, CEA Grenoble, 17 Avenue des Martyrs, F-38054 Grenoble Cedex 9, France.

(1) Frimer, A. A. *Singlet O_2;* CRC Press: Boca Raton, FL, 1985; Vol. I, II.
(2) Steinbeck, M. J.; Khan, A. U.; Karnovsky, M. J. *J. Biol. Chem.* **1993**, *268*, 15649.
(3) Cilento, G. In *Chemical and biological generation of excited states;* Adam, W., Cilento, G., Eds.; Academic Press: New York, 1982.
(4) Held, A. M.; Halko, D. J.; Hurst, J. K. *J. Am. Chem. Soc.* **1978**, *100*, 5732.
(5) Di Mascio, P.; Bechara, E. J. H.; Medeiros, M. H. G.; Briviba, K.; Sies H. *FEBS Lett.* **1994**, *355*, 287.
(6) Briviba, K.; Saha-Möller, C. R.; Adam, W.; Sies, H. *Biochem. Mol. Biol. Int.* **1996**, *38*, 647.
(7) Foote, C. S. *Photochem. Photobiol.* **1991**, *54*, 659.
(8) Halliwell, B. E.; Gutteridge J. M. C. *Free Radicals in Biology and Medicine*, 3rd ed.; Oxford University Press: New York, 1999.
(9) Aubry, J. M. In *Membrane Lipid Oxidation;* Vigo-Pelfrey, C., Ed.; CRC Press: Boca Raton, FL, 1991; Vol. 2, p 65.
(10) Pierlot, C.; Hajjam, S.; Barthélémy, C.; Aubry, J. M. *J. Photochem. Photobiol., B* **1996**, *36*, 31.
(11) Di Mascio, P.; Sies, H. *J. Am. Chem. Soc.* **1989**, *111*, 2909.
(12) Vogel, A. I. In *Vogel's Textbook of Pratical Organic Chemistry*; revised by Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R.; John Wiley & Sons: New York, 1989; p 695.
(13) Dewilde, A.; Pellieux, C.; Pierlot, C.; Wattré, P.; Aubry, J. M. *Biol. Chem.* **1998**, *379*, 1377.
(14) Pierlot, C.; Aubry, J. M.; Briviba, K.; Sies, H.; Di Mascio, P. *Methods Enzymol.* **2000**, *319*, 3.

10.1021/ja0016452 CCC: $19.00
Published on Web 09/29/2000

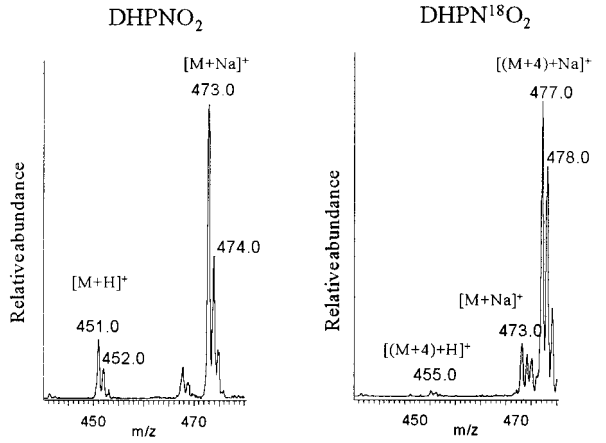


Figure 1. Mass spectrometry analysis of the $DHPNO_2$ and $DHPN^{18}O_2$.

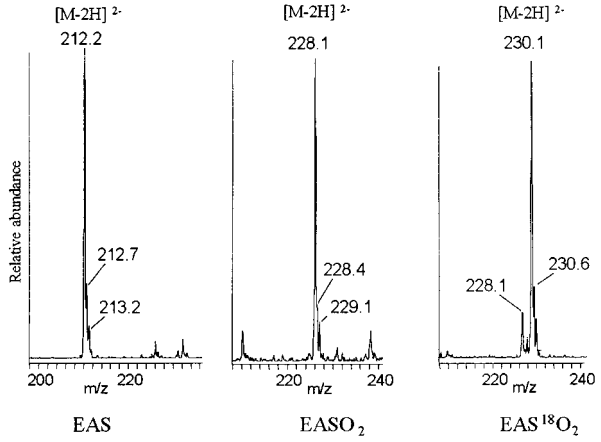
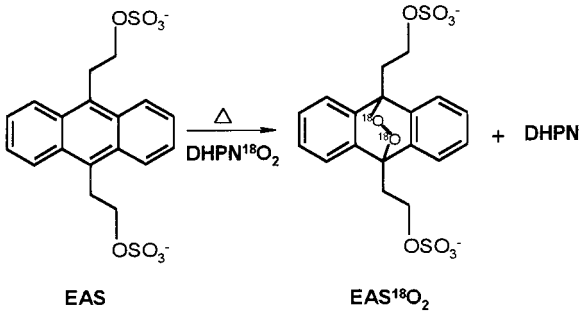


Figure 2. Mass spectrometry analysis of the EAS, $EASO_2$, and $EAS^{18}O_2$.

Scheme 2. Chemical Trapping of $^{18}[^1O_2]$ by the Water-Soluble Anthracene-9,10-diyldiethyl Disulfate (EAS) Yielding the Labeled Endoperoxide $EAS^{18}O_2$



syringe, the solution was passed through a polymeric membrane (porosity, 0.45 μm), and the filtrate was stored at −80 °C until use. The residual blue resin was washed with 1 mL of deuterated water to remove the remaining endoperoxide product. The concentration (130 mM) and the yield of formation (close to 95%) of the endoperoxide $DHPN^{18}O_2$ was determined using UV spectroscopy.[14]

In addition, the isotopic purity of $DHPN^{18}O_2$ was determined by electrospray mass spectrometry (Figure 1). The mass spectrum of $DHPNO_2$ exhibits, in the positive mode, a pseudomolecular ion at $m/z = 451$ corresponding to the expected molecular formula $C_{22}H_{30}N_2O_8$. The main intense ion is observed for the sodium adduct $[M + Na]^+$ at $m/z = 473$. The mass spectrum of $DHPN^{18}O_2$ exhibits an intense ion at $m/z = 477$ corresponding to the sodium adduct $[(M + 4) + Na]^+$ of the labeled endoperoxide. The sodium adduct of unlabeled $DHPNO_2$ is also detectable ($m/z = 473$), indicating that the isotopic purity of $DHPN^{18}O_2$ is close to 85% (Figure 1).

The generation of $^{18}[^1O_2]$ by thermal decomposition of $DHPN^{18}O_2$ was monitored using the water-soluble EAS, which can react with $^1O_2$, yielding the anthracene-9,10-diyldiethyl disulfate endoperoxide ($EASO_2$) as the specific oxidation product (Scheme 2). For this purpose, EAS was incubated at 37 °C in the presence of $DHPN^{18}O_2$. The resulting endoperoxide was purified by reversed-phase HPLC and analyzed by electrospray ionization mass spectrometry (Figure 2). The mass spectrum of EAS, recorded in the negative mode, exhibits a major ion at $m/z = 212$. This corresponds to the doubly charged molecule $[M - 2H]^{2-}$ which has a molecular weight of 426. As expected, the corresponding endoperoxide $EASO_2$ (MW = 458), produced by methylene blue photosensitization, exhibits a $[M - 2H]^{2-}$ ion at $m/z = 228$. The $EAS^{18}O_2$ endoperoxide formed in the presence of $DHPN^{18}O_2$ shows an intense $[M - 2H]^{2-}$ ion at $m/z = 230$, corresponding to a molecular weight of 462. This is strongly indicative of the incorporation of two labeled oxygen atoms into the molecule. The ion corresponding to the unlabeled endoperoxide at $m/z = 228$ was also detected with a relative abundance of about 15% compared with that of the labeled molecule. This reaction demonstrated that $DHPN^{18}O_2$ is able to produce $[^{18}O]$-labeled singlet oxygen with an isotopic enrichment of 85%.

The endoperoxide $DHPN^{18}O_2$ is able to produce $^{18}[^1O_2]$ as a clean source and in a high yield (60%).[13] In addition, the naphthalene derivative is soluble in aqueous media and has been shown to penetrate into cells.[15] Therefore, $DHPN^{18}O_2$, in association with a mass spectrometric detection method, will allow study of the mechanistic features of the reaction of $^1O_2$ with cellular targets. All these characteristics contribute to make this new model an excellent tool for mechanistic studies of the reaction of singlet oxygen in biological media.

Acknowledgment. Supported by the "Fundação de Amparo à Pesquisa do Estado de São Paulo", FAPESP (Brazil), the "Conselho Nacional para o Desenvolvimento Científico e Tecnológico", CNPq (Brazil), and the "Programa de Apoio aos Núcleos de Excelência", PRONEX/FINEP (Brazil), "Universidade de São Paulo"/"Comité Français d'Evaluation de la Coopération Universitaire avec le Brésil" (USP-COFECUB). We gratefully thank Colette Lebrun and Calogero Tornabene for their contribution to mass spectrometry analyses. G.R.M. is a recipient of FAPESP fellowship.

Supporting Information Available: ^{1}H-RMN data of DHPN, HPLC purification of $EASO_2$,and the synthetic scheme for $DHPN^{18}O_2$ (PDF) are presented. This material is available free of charge via the Internet at http://pubs.acs.org. See any current masthead page for ordering information and Web access instructions.

JA0016452

(15) Klotz, L. O.; Pellieux, C.; Briviba, K.; Pierlot, C.; Aubry, J. M.; Sies, H. *Eur. J. Biochem.* **1999**, *260*, 917.