J|A|C|S
COMMUNICATIONS

Published on Web 01/25/2007

Design and Synthesis of a BODIPY-α-Tocopherol Adduct for Use as an Off/On Fluorescent Antioxidant Indicator

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Free radical chain autoxidation of polyunsaturated fatty acyl residues disrupts lipid membrane structure and generates a variety of secondary cytotoxic products which altogether account for various pathological effects such as initiation and development of atherosclerosis,[1,2] neurodegenerative diseases,[3] and cell apoptosis.[4]

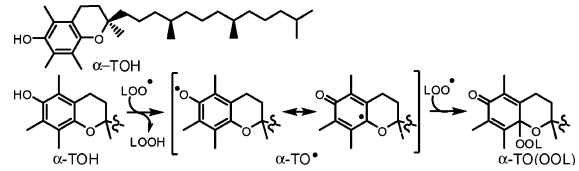
The most active lipid antioxidant present in mammalian tissues is α-tocopherol (α-TOH, Scheme 1).[5] The α-TOH consists of a chromanol head, which is located at the water lipid interface, and a long saturated phytyl chain tail, which is buried within the hydrocarbon chains in the lipid bilayers.[5,6] The lipid antioxidant activity of α-TOH arises from its preferential localization within the lipid, its inherently high chemical reactivity toward radicals, and the stability of the phenoxyl radical intermediate (α-TO•) generated.[6,7] In solution, α-TOH traps two chain propagating peroxyl radicals (LOO•), thus effectively terminating two free radical chain autoxidations.[1,6−8] A first peroxyl radical is scavenged, yielding a hydroperoxide and α-TO•; the latter reacts with a second peroxyl radical to give various chromanones such as 8a-(alkyldioxy)tocopherone (α-TO(OOL), Scheme 1).[9] An induction period is observed in lipid oxidation studies in the presence of α-TOH, the end of which signals the consumption of α-TOH and the onset of the lipid chain autoxidation.[8,10,11]

Lipid peroxidation studies both in solution and within live cell membranes would dramatically benefit from using a real time off/on fluorescent indicator of the antioxidant status, that is, a probe capable of reporting via emission enhancement the depletion of α-TOH and the onset of the lipid chain autoxidation. Such a probe would allow a noninvasive spatial and temporal monitoring of the system oxidative state. We report herein the synthesis and spectroscopic properties of (to our knowledge) the first off/on hydrophobic fluorescent antioxidant indicator.

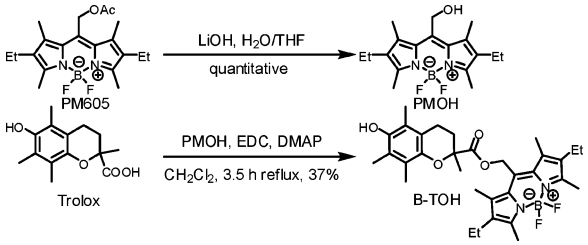
Our strategy involves preparing a two segment receptor−reporter type[12] free radical scavenger-fluorophore that fulfills the following solubility, reactivity, and spectroscopic requirements: (i) The antioxidant indicator partitions in hydrophobic media; (ii) the radical scavenging reactivity of the receptor segment compares to that of α-TOH; (iii) the reporter segment absorbs and emits in the visible region of the spectrum; and (iv) the reporter segment undergoes an emission enhancement upon radicals being scavenged by the receptor segment. In order to preserve the free radical scavenging reactivity characteristic of α-TOH, we chose commercially available Trolox,[6,7] a chromanol-based derivative, for the receptor segment. In order to maintain the solubility within hydrophobic media, we coupled to Trolox a dipyrrometheneboron difluoride (BODIPY) fluorescent probe which is known to be retained in phospholipid bilayers.[13] BODIPY dyes fulfill the reporter segment photophysical requirements: they have narrow absorption and emission bands in the visible region of the spectrum, high absorption coefficient and fluorescence quantum yields, and remarkable photostability.

The coupling of the two segments to obtain the fluorescent antioxidant indicator B-TOH was done as shown in Scheme 2.

Scheme 1. Peroxyl Radical Scavenging by α-TOH and α-TO• (refs 7 and 9)



Scheme 2. Synthesis of B-TOH



We first studied the spectroscopic properties of B-TOH. There are no significant changes in the absorption and emission spectra of B-TOH compared to those of its precursors PM605 and PMOH (see Scheme 2 for abbreviations). The emission quantum yield of B-TOH is, however, 30-, 15-, and 10-fold smaller than that of PM605 in hexanes (Figure 1), acetonitrile, and toluene, respectively. This dramatic decrease in emission quantum yield arises from an intramolecular emission quenching which we speculate is due to photoinduced electron transfer (PET) from the chromanol moiety to the BODIPY excited singlet state. Consistent with the intramolecular quenching observed, free Trolox efficiently quenches the emission of PMOH in solution. From the ratio of PMOH emission recorded without (I^0) and with (I) increasing Trolox concentration versus [Trolox], we obtain a Stern−Volmer quenching constant K_{SV} of 19.3 M^{-1} in acetonitrile. Given the PMOH fluorescence decay lifetime, $\tau^0 = 7.42$ ns, in acetonitrile and the K_{SV} value (where $K_{SV} = k_q\tau^0$), upon applying eq 1, we calculate a bimolecular quenching constant k_q of 2.6×10^9 M^{-1} s^{-1}.

$$\frac{I^0}{I} = 1 + k_q\tau^0[\text{Trolox}] \qquad (1)$$

We then investigated the emissive properties of B-TOH in the presence of alkoxyl and peroxyl radicals generated at a constant rate either upon 350 nm photolysis of dicumyl peroxide in hexanes under argon or upon thermolysis of 2,2′-azobis(2-methylpropionitrile) (AIBN) in toluene under air, respectively. A ca. 10-fold increase in the emission of 6 μM B-TOH in 0.1 M dicumyl peroxide solutions is observed following irradiation at 350 nm for a 30 s period (Figure 1). No changes are observed in a nonirradiated control sample. A ca. 9-fold increase in the emission is observed upon incubation of 0.8 mM AIBN and 3.1 μM B-TOH in toluene under air at 37, 65, or 75 °C. Control experiments at 75 °C in the

10.1021/ja066789g CCC: $37.00

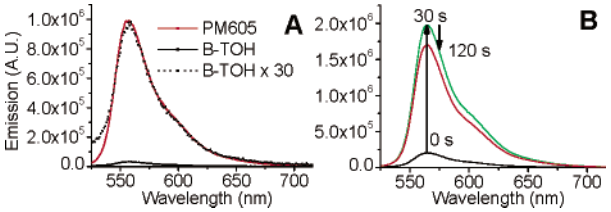


Figure 1. (A) Emission of optically matched hexane solutions of PM605 and B-TOH. (B) Fluorescence enhancement (0−30 s) and decrease (30−120 s) upon 350 nm irradiation of 6 μM B-TOH and 0.1 M dicumyl peroxide hexanes solution under Ar.

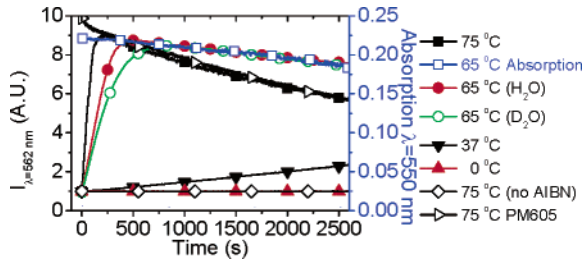


Figure 2. Emission intensity time profiles for B-TOH solutions incubated at 75, 37, and 0 °C with AIBN and at 75 °C without AIBN. Also shown are the B-TOH absorption time profile at 65 °C with AIBN and the emission intensity time profile for PM605 at 75 °C with AIBN. Two runs at 65 °C illustrate the KIE; in these runs, B-TOH toluene solutions were stirred (1 h) in the presence of either 1% v/v D_2O or 1% v/v H_2O. The phases were then separated, and the AIBN was dissolved in the organic phase incubated at 65 °C. Runs were done with 3.1 μM dye and 0.8 mM AIBN in toluene under air. Data points were taken every 5 s.

absence of AIBN reveal no changes in either absorption or emission over time (Figure 2). The rate of emission enhancement increases with increasing temperature, which is consistent with a larger generation rate of peroxyl radicals (Figure 2 and Supporting Information). No emission enhancement is observed upon incubation at 0 °C, a temperature at which the peroxyl radical generation rate from a 0.8 mM AIBN solution is negligible. A ca. 2-fold kinetic isotope effect (KIE) is observed in the rate of emission enhancement (Figure 2 and Supporting Information), which is consistent with a rate-limiting step involving H-atom abstraction from the chromanol head (first step, Scheme 1).[10] The KIE value measured compares to the 4-fold KIE previously reported for α-TOH in styrene at 30 °C.[10] We further observe no changes in the emission and absorption spectrum or absorption intensity in the visible region during the emission enhancement period; that is, there are no changes to the chromophore chemical structure in this period.

We conclude that the intramolecular emission quenching is deactivated, and the emission is restored upon radicals being scavenged by the receptor segment in B-TOH. Two mechanisms could account for the deactivation of the intramolecular quenching. The first one involves a radical-mediated dissociation of PMOH from B-TOH, effectively separating fluorophore from quencher.[14] An alternative explanation is that radical-mediated oxidation of the chromanol head to various chromanones (which may in turn hydrolyze to quinones)[9] increases the receptor segment redox potential, therefore deactivating the PET quenching mechanism.[12] Under experimental conditions similar to those used in our studies, α-TOH scavenges two peroxyl radicals to yield tocopherones.[9] Tocopherones are also formed in the reaction of α-TOH with alkoxyl radicals.[9] Our HPLC results obtained with UV−vis absorption, emission, and mass spectrometry detectors results support the formation of chromanones upon reaction of B-TOH with peroxyl radicals.

Prolonged exposure to peroxyl and alkoxyl radicals leads to a slow decrease in emission (and parallel decrease in absorption) intensity with time (Figure 2). The intensity decrease is consistent with the onset of radical-mediated BODIPY degradation.[11]

As the antioxidant segment in B-TOH is being consumed, the emission increases, and when the antioxidant is depleted (highest emission point), the BODIPY degradation induction period is over and degradation proceeds at the same rate as that in the absence of antioxidant (compare B-TOH and PM605 emission time profiles at 75 °C in Figure 2).[11] The antioxidant depletion and the onset of radical-mediated oxidation can thus be monitored following emission enhancement over time. Applying the induction period method[10,15] using the highest emission time point, we have calculated the generation rate of peroxyl radicals to be *ca.* 3×10^{-8} s^{-1} for 0.8 mM AIBN at 75 °C, which is in close agreement with the expected value (Supporting Information).

Preliminary results reveal a 4-fold increase in emission upon incubating B-TOH intercalated within the lipid bilayer of 100 nm dimyristoyl phosphatidylcholine vesicles in phosphate buffer solution in the presence of water-soluble 2,2′-azobis(2-methylpropionamidine)dihydrochloride.

In conclusion, we report a novel hydrophobic fluorescent antioxidant indicator with optimum off/on ratio properties. Antioxidant depletion and the onset of radical-mediated oxidation can be monitored following emission enhancement over time.

Acknowledgment. We thank Dr. B. Heyne at the University of Ottawa and Prof. K. Auclair at McGill University for assistance with HPLC experiments. Y.I. thanks NSERC for an undergraduate student scholarship. We are grateful to McGill University, NSERC, FQRNT Nouveaux Chercheur, and CFI New Opportunities Fund programs for financial assistance.

Supporting Information Available: Emission growth rate analysis, HPLC chromatograms, and synthesis of B-TOH. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bowry, V. W.; Ingold, K. U. *Acc. Chem. Res.* **1999**, *32*, 27−34.
(2) Steinberg, D. *Lancet* **1995**, *346*, 36−38.
(3) (a) Barnham, K. J.; Masters, C. L.; Bush, A. I. *Nat. Rev. Drug Discovery* **2004**, *3*, 205−214. (b) Montine, T. J.; Neely, M. D.; Quinn, J. F.; Beal, M. F.; Markesbery, W. R.; Roberts, L. J.; Morrow, J. D. *Free Radical Biol. Med.* **2002**, *33*, 620−626.
(4) Kagan, V. E.; Fabisiak, J. P.; Shvedova, A. A.; Tyurina, Y. Y.; Tyurin, V. A.; Schor, N. F.; Kawai, K. *FEBS Lett.* **2000**, *477*, 1−7.
(5) Burton, G. W.; Doba, T.; Gabe, E.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1985**, *107*, 7053−7065.
(6) Niki, E.; Noguchi, N. *Acc. Chem. Res.* **2004**, *37*, 45−51.
(7) Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, *19*, 194−201.
(8) Barclay, L. R. C. *Can. J. Chem.* **1993**, *71*, 1−16.
(9) (a) Liebler, D. C.; Baker, P. F.; Kaysen, K. L. *J. Am. Chem. Soc.* **1990**, *112*, 6995−7000. (b) Liebler, D. C.; Burr, J. A.; Matsumoto, S.; Matsuo, M. *Chem. Res. Toxicol.* **1993**, *6*, 351−355.
(10) Burton, G. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 6472−6477.
(11) (a) Yoshida, Y.; Shimakawa, S.; Itoh, N.; Niki, E. *Free Radical Res.* **2003**, *37*, 861−872. (b) Naguib, Y. M. A. *Anal. Biochem.* **1998**, *265*, 290−298.
(12) (a) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566. (b) Ueno, T.; Urano, Y.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2006**, *128*, 10640−10641. (c) Ueno, T.; Urano, Y.; Setsukinai, K.; Takakusa, H.; Kojima, H.; Kikuchi, K.; Ohkubo, K.; Fukuzumi, S.; Nagano, T. *J. Am. Chem. Soc.* **2004**, *126*, 14079−14085. (d) Gabe, Y.; Urano, Y.; Kikuchi, K.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2004**, *126*, 3357−3367.
(13) Johnson, I. D.; Kang, H. C.; Haugland, R. P. *Anal. Biochem.* **1991**, *198*, 228−237.
(14) Heyne, B.; Maurel, V.; Scaiano, J. C. *Org. Biomol. Chem.* **2006**, *4*, 802−807.
(15) Boozer, C. E.; Hammond, G. S.; Hamilton, C. E.; Sen, J. N. *J. Am. Chem. Soc.* **1955**, *77*, 3233−3237.

JA066789G