J|A|C|S
COMMUNICATIONS

Published on Web 04/19/2007

Fe^{2+} Catalyzes Vitamin E-Induced Fragmentation of Hydroperoxy and Hydroxy Endoperoxides That Generates γ-Hydroxy Alkenals

Xiaodong Gu, Wujuan Zhang, and Robert G. Salomon*

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio

Received December 15, 2006; E-mail: rgs@case.edu

Lipid peroxidation in vivo is involved in many disease processes. Cytotoxic aldehydes are generated through the decomposition of lipid peroxides, especially in the presence of metal ions.[1] Prominent among these aldehydes, are the γ-hydroxyalkenals. They form covalent adducts with biological nucleophiles and have been shown to be physiologically relevant.[2] We previously found that protein adducts of certain γ-hydroxyalkenals are especially abundant in the retinas of individuals with age-related macular degeneration.[3,4] These protein adducts are biologically active. They induce the growth of capillaries into the retina, resulting in destruction of the photoreceptor cells.[5]

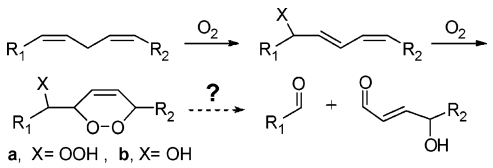
The major primary autoxidation products from polyunsaturated fatty acids—through enzymatic, free radical-induced, or singlet oxygenation pathways—are hydroperoxy dienes (Scheme 1). Enzymes, such as glutathione peroxidase, rapidly reduce hydroperoxides to alcohols in vivo. It seems likely that these conjugated hydroperoxy or hydroxy dienes are further oxidized to give hydroperoxy and hydroxy endoperoxides (**a** or **b**, respectively, in Scheme 1) through singlet oxygenation.[6] For example, in retina, all-trans-retinal can act as a photosensitizer to produce singlet oxygen.[7] We wondered whether hydroperoxy or hydroxy endoperoxides might undergo fragmentation to generate biologically active aldehydes.

We chose hydroperoxy endoperoxide **1** and hydroxy endoperoxide **2** as models to examine the feasibility of such fragmentation reactions (Scheme 2). These model compounds are easily prepared through singlet oxygenation of cyclohepta-1,4-diene[8] and further selective reduction by triphenylphosphane.[9]

It is well-known that hydroperoxides can undergo transition metal ion-mediated decomposition through single electron transfer to form alkoxy radicals.[10,11] Therefore, to promote fragmentation we exposed the endoperoxides to Fe^{2+} in the physiomimetic mixed solvent, acetonitrile and water, at room temperature (Scheme 2 and Table 1). Vitamin (Vit) E alpha (α-tocopherol) is a lipid-soluble chain-breaking antioxidant that can intercept radicals by hydrogen-atom transfer. Therefore, we also examined the effect of Vit E on the Fe^{2+}-promoted decomposition of endoperoxides **1** and **2**. Fragmentation of hydroperoxy endoperoxide **1** generated aldehyde **3**, the lactone of a γ-hydroxyalkenal. With 1 equiv of Fe^{2+}, or with a catalytic amount (0.1 equiv) of Fe^{2+} and 1 equiv of Vit E, the yields of **3** are 43−50%. However, Vit E alone did not promote the fragmentation, and a catalytic amount of Fe^{2+} alone only afforded a low yield (about 5%) of **3**.

A mechanism for the fragmentation of **1** is proposed in Scheme 3. Fe^{2+} initiates the homolysis of hydroperoxy endoperoxide **1** by single-electron transfer to generate hydroxide and alkoxy radical **6**. A subsequent β-scission in conjunction with homolysis of the dialkyl peroxide generates alkoxy radical **7**. This fragmentation is driven by the generation of a carbonyl group. Finally, hydrogen-atom abstraction delivers the γ-hydroxy alkenal **8**. Alternatively, the alkoxy radical **7** might be reduced by electron transfer from a second Fe^{2+} followed by protonation. The γ-hydroxy cis-alkenal **8** readily cyclizes to produce a hemiacetal **9**. In the presence of hydroperoxide, abstraction of an allylic hydrogen from **9** culminates in oxidation to deliver aldehyde **3**.[12] Overall, Fe^{2+} is oxidized to Fe^{3+}. Vit E apparently serves to regenerate Fe^{2+}, and thus promote the homolysis.[13−15] Vit E (α-tocopherol, α-TOH) is concomitantly oxidized to α-tocopheroquinone (α-TQ) which was detected by ^{1}H NMR in the reaction product mixture.

Scheme 1



Scheme 2

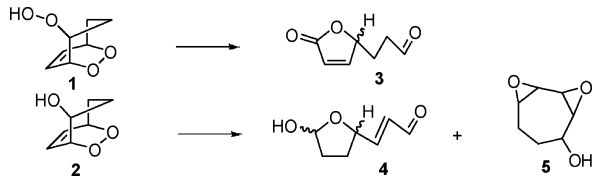


Table 1. Metal-Ion-Promoted Decomposition of **1** and **2**

reagents	reaction time	unreacted (%)	products yields (%) 3	4	5
1, Vit E (1:1)	4 h	100	0	0	0
1, Vit E, Fe^{2+}(1:1: 0.1)	4 h	0	50	0	0
1, Fe^{2+} (1: 0.1)	4 h	90	5	0	0
1, Fe^{2+} (1:1)	10 min	0	43	0	0
2, Fe^{2+} (1:1)	30 min	0	0	30	20
2, Vit E, Fe^{2+} (1:1:0.1)	4 h	90	0	0	0

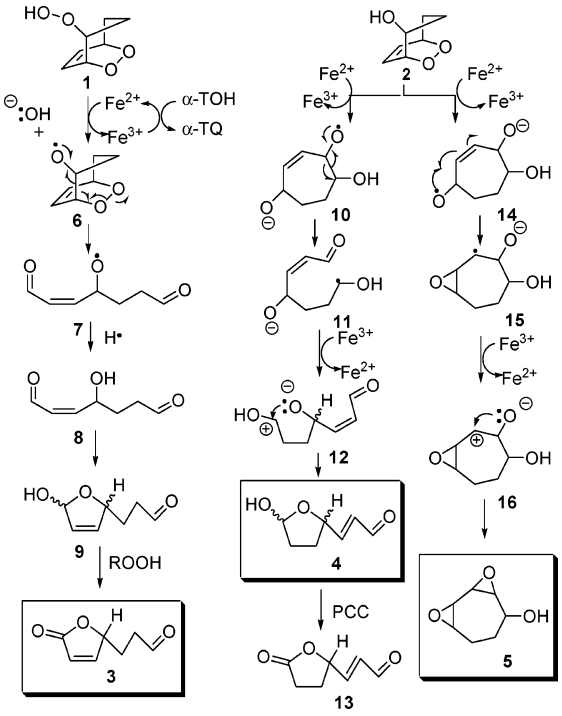
It is noteworthy that fragmentation of **1** is much faster when it is treated with 1 equiv of Fe^{2+} (10 min) compared to treatment with 1 equiv of Vit E and a catalytic amount of Fe^{2+} (4 h). The reductive cleavage of **1** and its subsequent fragmentation is evidently faster than the regeneration of Fe^{2+} from Fe^{3+} by electron transfer from Vit E.

Exposure of hydroxy endoperoxide **2** to 1 equiv of Fe^{2+} results in fragmentation to deliver the hemiacetal **4** of a γ-hydroxyalkenal (30% yield) and a hydroxy diepoxide **5** (20% yield, Scheme 2 and Table 1). Aldehyde **4** has two chiral centers and is a mixture of four isomers. Oxidation of **4** by pyridinium chlorochromate (PCC) to give a known aldehyde **13** (Scheme 3), confirmed the structure assigned to **4**.

A mechanism for the fragmentation of **2** is proposed and contrasted with that for fragmentation of **1** in Scheme 3. Fe^{2+} initiates the homolysis of hydroxy endoperoxide **2** through single electron transfer. This delivers two alkoxy radicals **10** and **14**. Alkoxy radical **10** can undergo β-scission to form an α-hydroxy-

10.1021/ja0689785 CCC: $37.00

Scheme 3

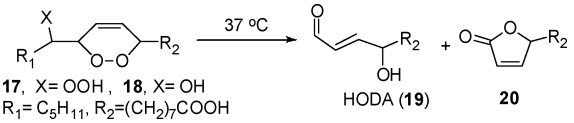


carbinyl radical **11**, that is stabilized by conjugation with a lone pair on the hydroxyl group. Oxidation of **11** by single electron transfer to Fe^{3+} regenerates Fe^{2+} and affords **12**, a resonance stabilized carbocation, that cyclizes to **4**. Alkoxy radical **14**, instead of undergoing the β-scission, cyclizes to generate an epoxycarbinyl radical **15**. Oxidation of **15** by single electron transfer to Fe^{3+} regenerates Fe^{2+} and affords an epoxycarbinyl carbocation **16** that cyclizes to give the hydroxy diepoxide **5**.[16]

Fragmentation of **2**, in contrast with that of **1**, in the presence of 1 equiv of Vit E and 10 mol % of Fe^{2+} was very slow. After 4 h, 90% of **2** remained unreacted, and only traces of fragmentation products were detected. Apparently, reductive homolysis of endoperoxides is more difficult than the corresponding reaction of hydroperoxides. The generation of alternative hemiacetals, **4** and **9**, of the same hydroxydialdehyde is a particularly striking detail of the reactions of endoperoxides **1** and **2** (Scheme 3). Although alternative cyclizations of **12** could produce both **4** and **9**, the latter is expected to be more susceptible to oxidation than the former owing to a preference for abstraction of an allylic hydrogen. Hemiacetal **9** is generated in the presence of hydroperoxide that can promote oxidation to **3**. In the absence of hydroperoxides, as in the Fe^{2+}-promoted fragmentation of **2**, the hemiacetal **4** is isolated.

A pilot study showed that fragmentation of the linoleate-derived hydroperoxy endoperoxide **17** at 37 °C produces 9-hydroxy-12-oxododec-10-enoic acid (HODA, **19**) and butenolide **20**, and Vit E promotes the formation of **19** (Scheme 4). In contrast, hydroxy endoperoxide **18** is relatively unreactive, even in the presence of Vit E. Details will be reported in due course.

Scheme 4



Previously, the formation of γ-hydroxyalkenals in vivo has been generally viewed as the consequence of free radical-induced oxidation of polyunsaturated fatty acyls. Vit E would be expected to inhibit such autoxidation. Clinically, however, treatment with Vit E provides little or no benefit in ameliorating age related macular degeneration.[17−19] The present study suggests two factors that may help to explain this paradox. Hydroperoxy and hydroxy endoperoxides (**a**, **b** in Scheme 1) can be generated through an entirely non-free-radical pathway through singlet oxygenation of polyunsaturated fatty acyls in retina. Subsequent fragmentation to γ-hydroxyalkenals could ensue through the mechanisms of Scheme 3. Vit E could contribute to, as opposed to preventing, their formation by converting redox-active metal ions into their reduced forms that promote the rapid fragmentation of hydroperoxy endoperoxides.

Acknowledgment. We thank the National Institutes of Health for support of this research by Grants EY016813, GM21249, and HL53315.

Supporting Information Available: Spectroscopic and analytical data for new compounds and selected experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Carmen Vigo-Pelfrey, E. *Membrane Lipid Oxidation;* CRC Press: Boca Raton, FL, 1990; Vol 1.
(2) Esterbauer, H. *Aldehydic products of lipid peroxidation*; Academic Press: London, 1982.
(3) Crabb, J. W.; Miyagi, M.; Gu, X.; Shadrach, K.; West, K. A.; Sakaguchi, H.; Kamei, M.; Hasan, A.; Yan, L.; Rayborn, M. E.; Salomon, R. G.; Hollyfield, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14682.
(4) Gu, X.; Meer, S. G.; Miyagi, M.; Rayborn, M. E.; Hollyfield, J. G.; Crabb, J. W.; Salomon, R. G. *J. Biol. Chem.* **2003**, *278*, 42027.
(5) Ebrahem, Q.; Renganathan, K.; Sears, J.; Vasanji, A.; Gu, X.; Lu, L.; Salomon, R. G.; Crabb, J. W.; Anand-Apte, B. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13480−4.
(6) Neff, W. E.; Frankel, E. N.; Selke, E.; Weisleder, D. *Lipids* **1983**, *18*, 868.
(7) Bensasson, R. V.; Land, E. J.; Truscott, T. G. *Excited States and Free Radicals in Biology and Medicine*; Oxford University Press: New York, 1993.
(8) Salamci, E.; Secen, H.; Sutbeyaz, Y.; Balci, M. *J. Org. Chem.* **1997**, *62*, 2453.
(9) Carless, H. A. J.; Batten, R. J. *Chem. Soc., Perkin Trans. 1* **1987**, 1999.
(10) Lee, S. H.; Blair, I. A. *Chem. Res. Toxicol.* **2000**, *13*, 698.
(11) Posner, G.; O'Neill, P. M. *Acc. Chem. Res.* **2004**, *37*, 397.
(12) Roedig, A.; Ganns, E. M. *Liebigs Ann. Chem.* **1982**, 406.
(13) Yamamoto, K.; Niki, E. *Biochim. Biophys. Acta* **1988**, *958*, 19.
(14) Igarashi, O.; Matsukawa, H.; Inagaki, C. *J. Nutr. Sci. Vitaminol*. **1976**, *22*, 267.
(15) Fukuzawa, K.; Kishikawa, K.; Tadokoro, T.; Tokumura, A.; Tsukatani, H.; Gebicki, J. *Arch. Biochem. Biophys.* **1988**, *260*, 153.
(16) Suzuki, M.; Ohtake, H.; Kameye, Y.; Hamanaka, N.; Noyori, R. *J. Org. Chem.* **1989**, *54*, 5292.
(17) Jampol, L. M.; Ferris, F. L., III. *J. Am. Med. Assoc.* **2001**, *286*, 2466−8.
(18) Kuzniarz, M.; Mitchell, P.; Flood, V. M.; Wang, J. J. *Ophthalmic Epidemiol.* **2002**, *9*, 283−95.
(19) Taylor, H. R.; Tikellis, G.; Robman, L. D.; McCarty, C. A.; McNeil, J. J. *Br. Med. J.* **2002**, *325*, 11.

JA0689785