

REGENERATION OF VITAMIN E FROM α -CHROMANOXYL RADICAL
BY GLUTATHIONE AND VITAMIN C

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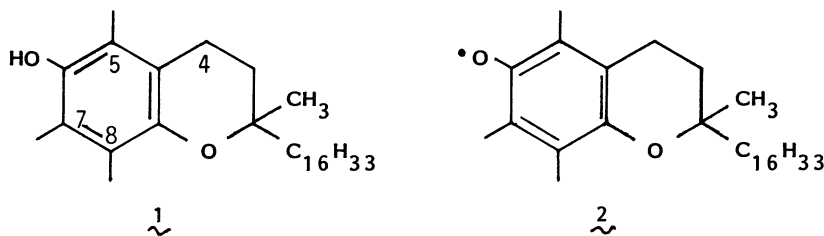
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α -Chromanoxyl radical formed by the interaction of α -tocopherol (vitamin E) with alkoxy radical or DPPH was found by electron spin resonance spectroscopy to react with glutathione and vitamin C to regenerate α -tocopherol.

The formation and behavior of lipid peroxides in biological systems have received much attention recently from not only biological viewpoint but also chemical and medical points of view, especially in relevance to their pathological effects such as aging.¹⁻³⁾ Vitamin E is known to act as an inhibitor for non-enzymatic peroxidation of lipids in vivo.⁴⁾ The primary role of vitamin E is accepted to scavenge the oxygen radicals, the chain carrying species in the peroxidation of lipids. One of the interesting features of vitamin E is its possible regeneration in biological systems.⁵⁻⁷⁾ This report describes the reactions of vitamin E radical and regeneration of vitamin E as studied mainly by electron spin resonance spectroscopy (esr).

α -Tocopherol **1**, the major component of vitamin E, reacts rapidly with alkoxy radical or 2,2-diphenyl-1-picrylhydrazyl (DPPH) to give vitamin E radical (α -chromanoxyl radical) **2**, which is quite stable at room temperature in the absence of oxygen but decays at moderate rate in its presence. When α -tocopherol was added to the benzene solution of DPPH, the spectrum of DPPH at 514 nm decayed rapidly, its esr spectrum disappeared and a new esr spectrum of α -chromanoxyl radical **2** appeared (Fig. 1). The hyperfine splitting constants were determined as $a_{\text{H}}^{4-\text{CH}_2} = 0.148$ mT, $a_{\text{H}}^{5-\text{CH}_3} = 0.602$ mT, $a_{\text{H}}^{7-\text{CH}_3} = 0.458$ mT, and $a_{\text{H}}^{8-\text{CH}_3} = 0.094$ mT.



Since vitamin E is lipophilic and the reducing species such as glutathione (GSH) and vitamin C (ascorbic acid) are hydrophilic, the interactions of α -chromanoxyl radical **2** with glutathione and vitamin C were carried out as follows. α -Tocopherol and DPPH were taken into one of the side arms of the esr tube and

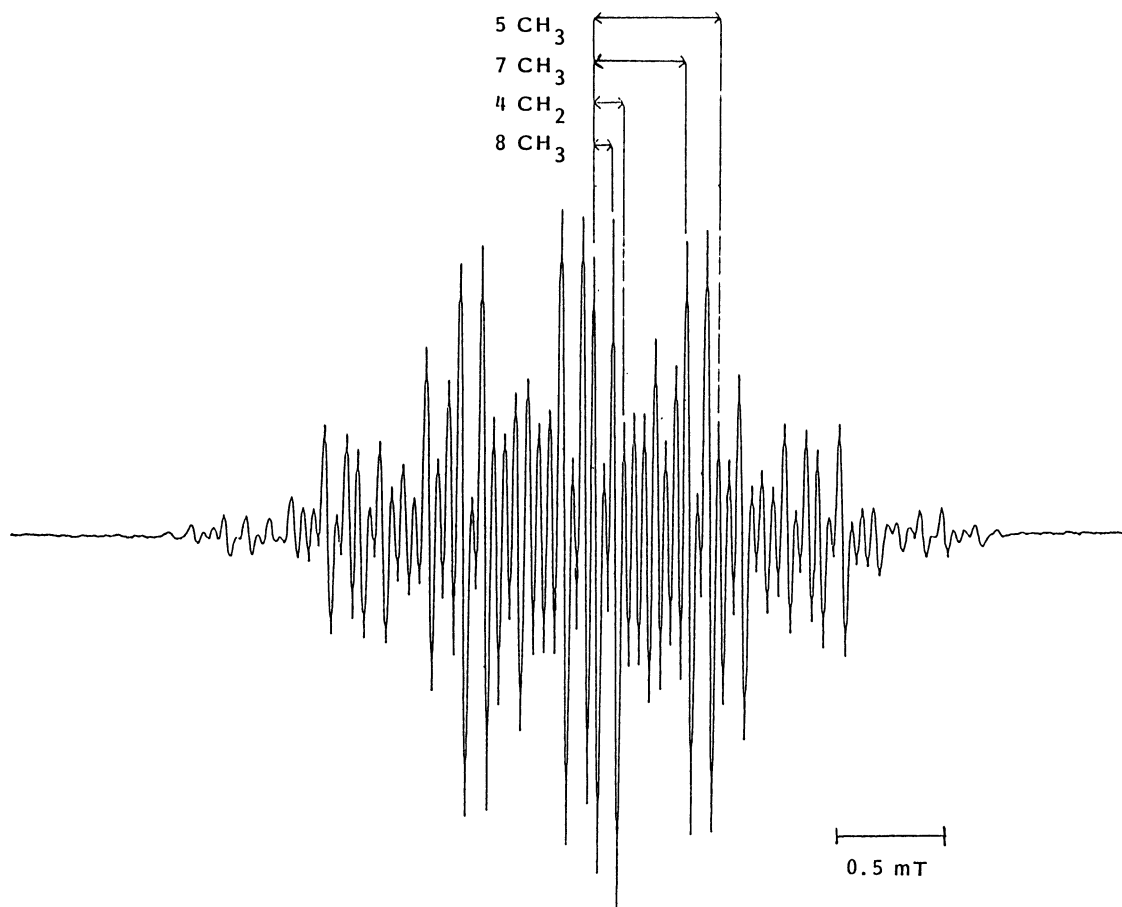
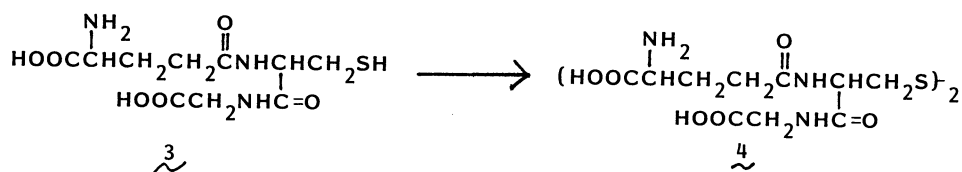


Fig. 1 Esr spectrum of α -chromanoxyl radical 2 in benzene under vacuum

glutathione or vitamin C (and spin trap when necessary) was taken into another side arm. α -Tocopherol and DPPH were first dissolved in benzene to obtain the α -chromanoxyl radical 2, benzene was removed by vacuum transfer, ethanol-water mixture (5:1 by volume) was introduced by vacuum distillation into another side arm to dissolve glutathione or vitamin C, and then this solution was mixed with 2 and analyzed by esr. It was confirmed that the removal of benzene did not affect the stability and esr spectrum of 2.

The esr spectrum of 2 decayed by mixing with glutathione 3. The isotachophoretic analysis⁸⁾ showed the decrease of glutathione, which presumably gave the oxidized glutathione 4. When a spin trap α -(4-pyridyl-N-oxide) N-t-butyl nitron (POBN) was also dissolved, a new esr spectrum appeared in place of 2 as shown in Fig. 2, $a^N = 1.513$ mT and $a_\beta^H = 0.232$ mT. The same esr spectrum was observed when t-butoxyl radical was generated from di-t-butyl diperoxyoxalate in benzene containing glutathione and POBN, $a^N = 1.523$ mT and $a_\beta^H = 0.228$ mT.



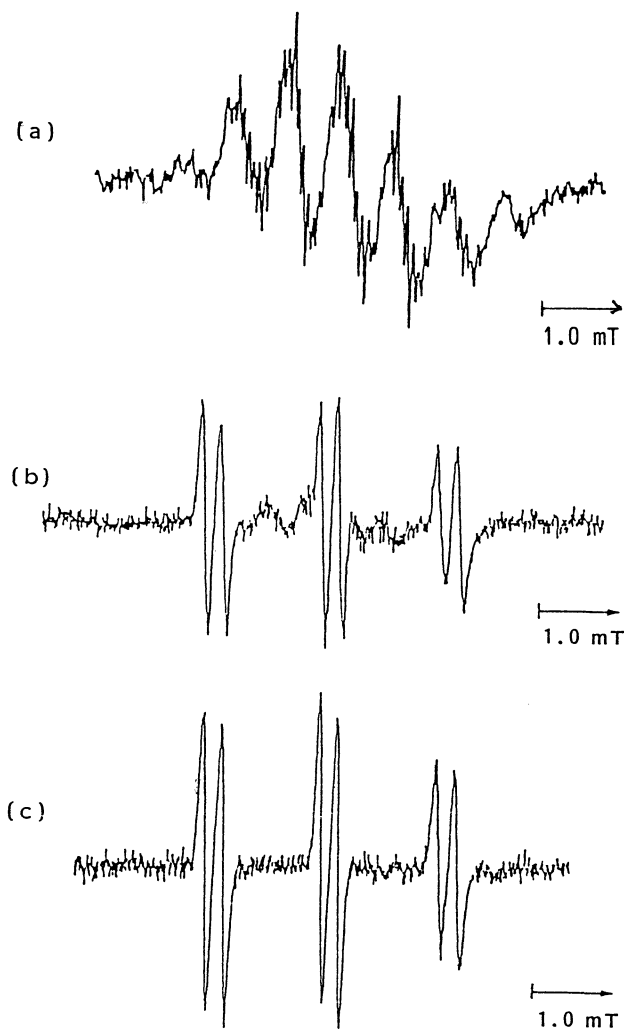


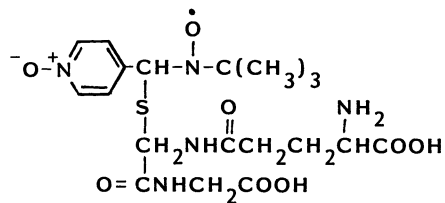
Fig. 2 ESR spectra observed when 2 was dissolved in ethanol-water (5/1 by volume) solution of GSH and POBN at room temperature under vacuum.

(a) 10.1 mM α -tocopherol and 10.0 mM DPPH

(b) 2 hours after mixing (a) with 9.93 mM GSH and 26.1 mM POBN

(c) After standing overnight.

Therefore, these spectra may well be ascribed to the spin adduct of glutathione radical GS• by POBN, 5.



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