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FURTHER EVIDENCE FOR OH RADICAL PRODUCTION IN FENTON'S REAGENT

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Fenton's reagent[1], a mixture of Fe(II) and H_2O_2, is perhaps the oldest metal ion-peroxide oxidizing agent known to organic chemists. Recently, this reaction has been implicated in initiating NADPH-dependent lipid peroxidation in liver microsomes.[2] It has been suggested that the reactive species in the Fenton reaction (reaction 1) is the hydroxyl radical[3], which is known to be electrophilic in character. This was demonstrated by the ease of hydrogen abstraction from

$$Fe(II) + H_2O_2 \longrightarrow Fe(III) + OH^- + OH\cdot \qquad (1)$$

alcohols, for example, ethanol to yield $CH_2\dot{C}HOH$ radicals as the major product, and $\cdot CH_2CH_2OH$ radicals as the minor product.[4] However, Shiga and his colleagues[5] found that hydrogen abstraction of ethanol by the Fe(II)-H_2O_2 system gives mainly $\cdot CH_2CH_2OH$ and suggested that the reactive species in the Fe(II)-H_2O_2 system may not be the hydroxyl radical but a more nucleophilic species In this communication, we have studied the ease of hydrogen abstraction of alcohols by the Fe(II)-H_2O_2 system using an Electron Spin Resonance (ESR) spin trapping method.

In recent years, spin-trapping techniques have become useful tools in investigating free radical intermediates involved in chemical and biochemical reactions.[6] By using 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as a spin trap, we previously reported[7] that the reactive species in the Fe(II)-H_2O_2 system is the hydroxyl radical. In an attempt to identify alcohol radicals formed resulting from the hydrogen abstraction by the hydroxyl radical, we chose a spin trap, 2-methyl-2-nitroso-propane (MNP) because the nitroxide formed between the reaction of MNP and the radical (reaction 2) exhibits a hyperfine splitting pattern due only to nitrogen and to the radical

$$(CH_3)_3\,C\text{-}N{=}O + R\cdot \longrightarrow (CH_3)_3\,C\text{-}\underset{\displaystyle R}{\underset{|}{N}}\text{-}\dot{O} \qquad (2)$$

attached. When MNP was added to the Fe(II)-H_2O_2 system alone, we obtained an ESR spectrum consisting of a quartet with intensity 1:2:2:1 (Fig. 1). This signal was identified as due to the MNP-OH radical adduct. The unexpectedly large hyperfine splitting constant of the OH hydrogen may be due to hydrogen bonding between the hydrogen atom of the hydroxyl group and the oxygen atom of the nitroxide. However, unlike the DMPO-OH radical adduct which is stable in aqueous solution for an hour or so, the MNP-OH radical adduct disappeared within 10 min. Adding a small amount of ethanol into the Fe(II)-H_2O_2 system with MNP gave the result shown in Fig. 2. The ESR spectrum consists of a doublet (1:1) of triplets and was identified as originating from the MNP-$CH(CH_3)OH$ radical adduct.[8] If the $\cdot CH_2CH_2OH$ radical had been trapped instead of CH_3CHOH, the ESR signal would have been a triplet (1:2:1) of triplets instead of a doublet (1:1) of triplets.

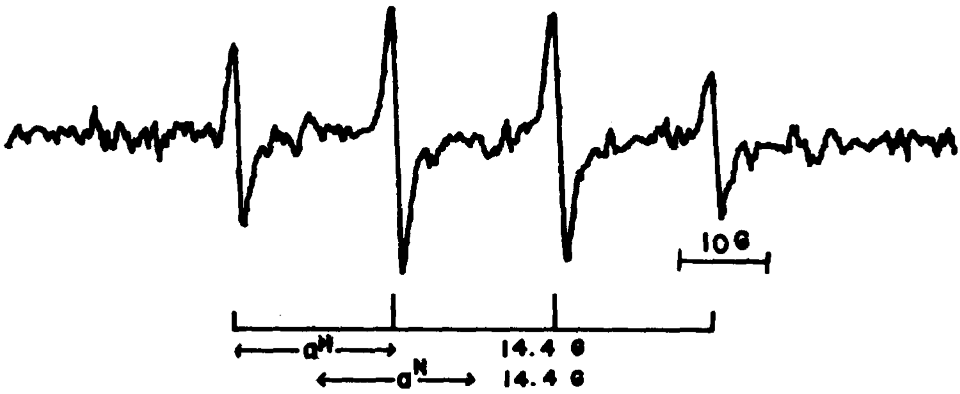

Fig. 1 ESR spectrum of MNP-OH radical adduct.

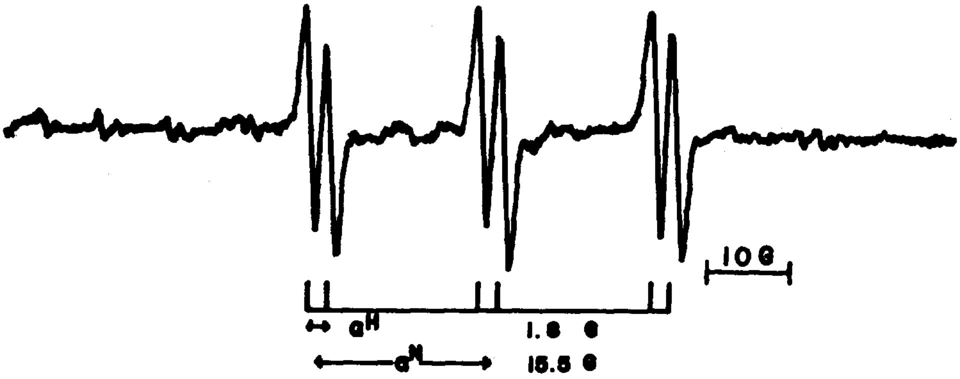

Fig. 2 ESR spectrum of MNP-$CH(CH_3)OH$ radical adduct.

This clearly shows that the $CH_3\dot{C}HOH$ radical is generated and is the major product in the Fe(II)-H_2O_2 system in the presence of ethanol, thus confirming that the hydroxyl radical is the reactive species in the Fenton reaction. It has been shown that $CH_3\dot{C}HOH$ is a more powerful reducing agent for H_2O_2 than is $.CH_2CH_2OH$[9], therefore the former is preferentially destroyed in the presence of excess H_2O_2. This may explain why Shiga and his colleagues[5] failed to detect the production of $CH_3\dot{C}HOH$ in the reaction of Fe(II)-H_2O_2 with ethanol in the presence of excess H_2O_2. Fig. 3 shows that when methanol was added into the Fe(II)-H_2O_2 system with MNP, an ESR spectrum consisting of a triplet of triplets with intensity 1:2:1 was observed and assigned to the MNP-CH_2OH radical adduct.[8] This is consistent with Dixon and Norman's observation.[4]

Fig. 3 ESR spectrum of MNP-CH_2OH radical adduct.

Experimental:

The complete reaction mixtures containing 3.8×10^{-5}M Fe(II), 3.8×10^{-5}M EDTA, 3.2 mM H_2O_2 and 0.78 mg/ml MNP in 1 mM phosphate buffer, pH 7.4 were mixed a) without alcohol (Fig. 1); b) with 0.8% ethanol (Fig. 2); c) with 1.5% methanol (Fig. 3). The reaction was initiated by the addition of H_2O_2 at 25°C and the samples were placed without incubation into the ESR cavity of a Varian Model E-4 Spectrometer equipped with an on-line V-72 mini-computer. The determinations of hyperfine splitting parameters were made by direct comparison with a known standard, tempol (2,2,6,6-tetramethyl-4-hydroxypiperidine-1-oxy), or by direct fieldial measurements.

Aqueous solutions of 2-methyl-2-nitroso-propane (MNP) (1 mg/ml) were prepared in the dark by stirring overnight in the coldroom.

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