Electrochemical Formation of Hydrogen Atom Adduct of 5,5-Dimethyl-1-pyrroline-*N*-oxide and Its Mechanism

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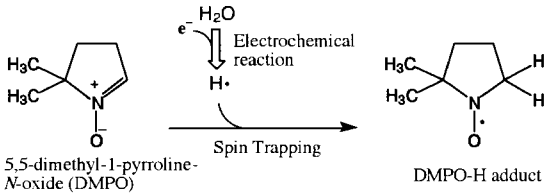
In order to make the method of spin trapping technique with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) more powerful in biological investigation, using electrochemical reaction, we have developed the method to observe an ESR spectrum composed of only a spin adduct of hydrogen atoms (DMPO-H). It has been elucidated that DMPO-H is produced through the hydrogen atom formation, not through the reduction of DMPO.

The technique of spin trapping has been exclusively used to detect and identify short-lived free radicals, particularly those generated in biological systems.[1] 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) has contributed to the mechanistic studies for ageing, inflammation, and so forth in which oxygen-centered free radicals are involved.[2–4] In chemistry, elaborate works have been conducted to design and develop more advanced spin traps which present data explaining naturally occurring events and to figure out the reaction mechanism between the spin traps and oxygen-centered free radicals in biological conditions. On the other hand, however, the methods to detect hydrogen atom has not been developed well although it is known to be produced by biological reactions. This is mainly because a convenient system to yield hydrogen atom has not been established yet so far. Previously reported techniques such as light-illumination of alkyl tin compounds,[5] γ-irradiation of water[6] and so forth, require skills and sophisticated facilities. Sonolytic degradation of water molecules may be the easiest way, but generates concomitant hydroxyl radicals giving rise to rather complex ESR spectra.[7] Only promising method may be electrochemical reaction. In the previous paper, ESR spectrum due to the hydrogen atom adduct of phenyl *t*-butyl nitrone was observed by electrolysis for the aqueous solution with suppressing the hydroxyl radical formation by use of a tungsten counter electrode.[8] In the present study, we investigated in situ electrochemical production of pure hydrogen atom DMPO spin adduct using an electrochemical ESR cell specially designed for in situ ESR electrochemical measurements.[9]



DMPO was purchased from LABOTEC Co. and used without further purification. D_2O was from CEA (99%). Water was purified by Milli-QII (Millipore) immediately before use. CH_3CN of reagent grade was obtained from Nakalai Tesque and purified by double distillation over P_2O_5. Supporting electrolytes, $(n\text{-}Bu)_4NBF_4$ used in CH_3CN and $KClO_4$ in H_2O, were also from Nakalai Tesque. The electrochemical ESR measurements were performed for DMPO solutions sufficiently degassed by the thaw-and-freeze method.

An X-band ESR spectrometer, JEOL-TE300 (JEOL) operated at 100-kHz field modulation, was controlled by WinRad systems (Radical Research Inc.). The magnetic field strength was calibrated using Mn(II) ion doped in MgO as a standard: The peak distance between g = 1.981 and 2.034 is 8.69 mT. An electrochemical ESR cell used was REL-001 of Radical Research Inc. in which the working and counter electrodes are made of gold wire (0.5 mm o.d.). Silver wire is used as the reference electrode. The helical working electrode is placed along with the inner surface of the ESR quartz cell (5 mm i.d.) and the counter electrode wire set in the center. The pitch of the helix is dense and the outer surface of the helix is close to the ESR cell wall, so that the species generated near the counter electrode (inside the working electrode) is ESR-invisible and only the species formed in the outer surface of the working electrode can be observed by ESR. The applied potential on the working electrode was adjusted by a 50-W potentiostat (BAS). The experimental error involved in the applied potential was approximately ±60 mV, since the silver wire reference electrode was insufficient for the evaluation of the accurate potential on the working electrode.

DMPO (10 mM) aqueous solutions containing $KClO_4$ (0.1 M) were subjected to electrochemical reaction in the cell and in situ ESR measurements carried out. Immediately after applying the potential of –1400 mV (vs Ag/Ag^+), ESR spectrum shown in Figure 1b which is equivalent to the base line was obtained: Figure 1a shows the spectrum of the intact sample solution. When the electrolysis was terminated, a 9-line splitting depicted in Figure 1c arose and increased with time, as given in Figures 1c and 1d. Based on the results of the similar electrochemical ESR measurements performed in the range of applied potential from –400 to –2400 mV (vs Ag/Ag^+), we found that –800 mV (vs Ag/Ag^+) was the highest potential necessary for formation of DMPO-H radical. To our present knowledge, –1400mV is most suitable potential for detection of well resolved ESR spectra of a DMPO-H or -D adduct. The hyperfine splitting constants (hfsc) are a^N = 1.66 mT (1:1:1 triplet) and a^H = 2.25 mT (1:2:1 triplet). Based on the coincidence of these values with the reported ones,[10] the spectrum was assigned to a DMPO-H adduct. Analogous experiments were carried out in D_2O. The spectrum obtained is composed of 18 lines as explained in Figure 1e by the stick diagram, which is due to the triplet splitting by D (I = 1; a^D = 0.35 mT). The computer simulation spectrum obtained using the hfsc values from Figure 1e is also shown in Figure 1f.

In order to see if the hydrogen atom is generated from water molecules or by some other mechanisms, analogous experiments were conducted in CH_3CN. In CH_3CN containing 10

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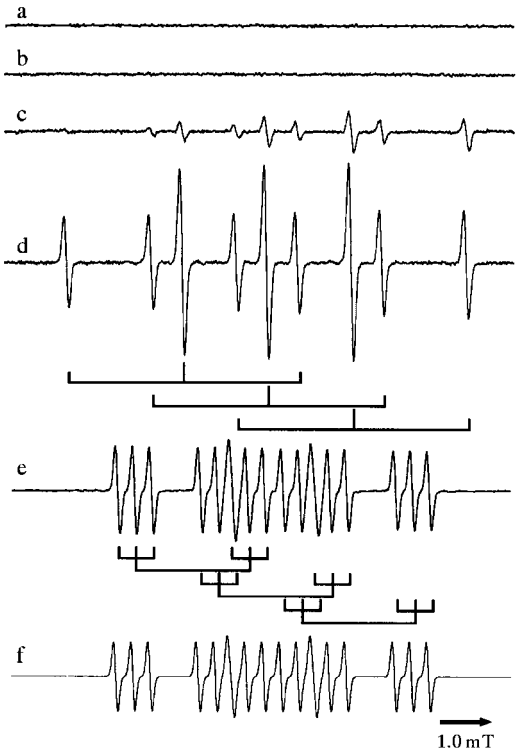

Figure 1. Electrochemical ESR spectra obtained for DMPO aqueous solutions at room temperature (a) before electrolysis, (b) during electrolysis on supplying –1400 mV (vs Ag/Ag^+), (c) immediately after electrolysis for 30 s, (d) 2 min after electrolysis, and (e) 2 min after electrolysis in D_2O at –1400 mV (vs Ag/Ag^+). (f) is a computer simulation obtained using the hfsc values for (e). Sample: Aqueous solution of DMPO (0.01 M) containing $KClO_4$ (0.1 M). ESR settings: center magnetic field, 336.5 mT; sweep width of magnetic field, ±5 mT; microwave power, 2.0 mW at 9.4628 GHz; modulation amplitude, 0.079 mT; time constant, 0.03 s; sweep time 2 min.

mM DMPO, 3% H_2O and 0.1 M $(n\text{-}Bu)_4NBF_4$, at the same applied potential, similar results were obtained: a^N = 1.52 mT 1:1:1 triplet) and a^H = 2.02 mT (1:2:1 triplet). The decreased hfsc values are due to the decrease in the spin density on the nitrogen atom in the less polar solvent CH_3CN. It should be noted that detectable amount of DMPO-H radical was never produced in the absence of H_2O, suggesting that H_2O is indispensable for the DMPO-H formation. There are two possible pathways. One is that hydrogen atom is generated electrochemically and spin-trapped. The other is that one-electron reduction of DMPO first takes place, and the subsequent protonation converts the resulting DMPO anion radical to DMPO-H. No detectable amount of paramagnetic species ascribable to the DMPO anion radical was observed during the electrolysis carried out in the potential range from –1400 to –2400 mV for both CH_3CN and aqueous DMPO solutions. These observations supported that the electrochemically generated anion radical of DMPO can be ruled out from the candidate of possible intermediate species in the process of DMPO-H radical formation. That is, DMPO-H should be formed by the reaction between electrochemically generated hydrogen atoms and DMPO.

The next question is why an ESR signal was not observed when –1400 mV (vs Ag/Ag^+) was on, while the signal was observed when it was off. The answer may be that DMPO-H generated was immediately reduced at the potential to some unknown ESR-silent species that is convertible to DMPO-H, possible by reaction with hydrogen atom after the electrolysis. A piece of evidence for this was obtained by the following experiments using 3-carboxy-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (PCA), a very stable nitroxide (the half life being years). As depicted in Figure 2, when various potentials were applied to an aqueous PCA (5×10^{-5} M) solution containing 0.1 M $KClO_4$, the ESR signal intensity of the triplet (a^N = 1.62 mT) decreased with time. When the electrolysis was terminated, the signals started being recovered. At –1400 mV (vs Ag/Ag^+), for instance, the signal height reached the 80% height of the original in 300 s. This type of ESR signal recovery has been observed previously for nitroxide radicals, for example, those produced from 2-metyl-2-nitorosopropane by post-radiolysis signal growth.[11] Above results support our mechanism.

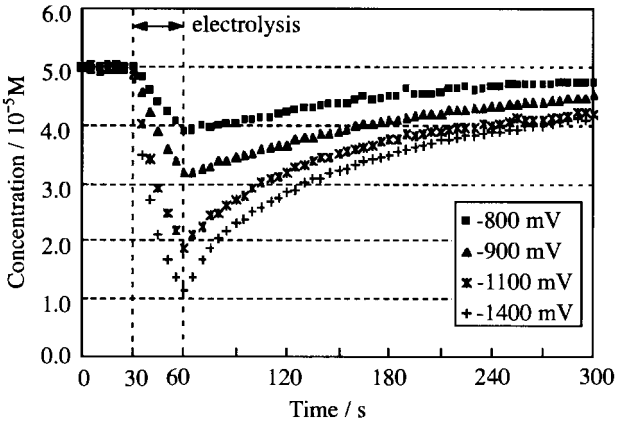

Figure 2. Plot of PCA concentration against time. Electrochemical reduction was performed for 30 s at various potentials in the range from –800 to –1400 mV (vs Ag/Ag^+), and ESR spectra were recorded at 25 °C with 10 s interval. The signal intensity was calibrated using Mn(II) as an external standard. Sample: 5×10^{-5} M aqueous PCA solution containing $KClO_4$ (0.1 M). ESR settings: center magnetic field, 336.5 mT; sweep width of magnetic field, ±0.5 mT; microwave power, 2.0 mW at 9.4628 GHz; modulation amplitude, 0.079 mT; time constant, rapid; sweep time 5 s.

Consequently, we have developed the method to observe an ESR spectrum consisting of only DMPO-H. This technique allows us to measure the system-dependent change in hfsc of the hydrogen atoms spin adducts of various spin traps and, therefore, is useful to investigate complex biological hydrogen atom generation systems by spin trapping.

References and Notes

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