

Electrochemical-ESR Detection of Hydrogen Atom Adducts of 5-Membered Ring Nitron Spin Traps

Nobuyuki Endo, Kousuke Higashi,[†] Kenji Kanaori,[†]
Kunihiko Tajima,[†] and Keisuke Makino*

Institute of Advanced Energy, Kyoto University,
Gokasho, Uji, Kyoto 611-0011

[†]Department of Applied Biology,
Kyoto Institute of Technology,
Matsugasaki, Sakyo-ku, Kyoto 606-8585

(Received June 12, 2001)

In order to make the spin trapping technique more useful in biological free radical studies, using an specially designed ESR cell for in situ electrochemical reactions, five 5-membered ring nitrones, which are analogues of 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), one of the most conventionally used spin traps, have been explored for their hydrogen atom ($\bullet$ H) adducts formation. ESR spectra consisting of only $\bullet$ H adducts and the resulting ESR parameters are discussed.

Spin trapping with several nitron analogues, such as DMPO (5,5-dimethyl-1-pyrroline *N*-oxide) and α -phenyl-*t*-butyl nitron (*N*-benzylidene-*t*-butylamine *N*-oxide), is known to be an exclusively useful tool for investigations on the characterization and biological implication of short-lived free radical intermediates arising from biological functions.¹ Several standard methods for the spin trapping of such short-lived radicals as hydroxyl, superoxide anion, and alkyl radicals have already been reported.^{2–4} In the case of the hydrogen atom ($\bullet$ H), which is known to be generated biologically, however, no practical method has so far been established. Although DMPO traps $\bullet$ H, in many cases it is hard to identify $\bullet$ H formation from the ESR spectra because of a very low $\bullet$ H adduct concentration; therefore other major signals disturb the identification.⁵

The aim of the present work, therefore, was to obtain ESR spectra of the $\bullet$ H spin adducts of several potent ring nitron spin traps and their ESR parameters. Our previous study has demonstrated a method to observe an ESR spectrum composed of only DMPO-H, thus allowing us to apply it to other spin trapping systems.⁶ In this work, an electrochemical reaction was conducted in a helical electrochemical ESR cell especially designed for this purpose, and in situ ESR measurements were performed. This technique is useful because, whenever a new spin trap is developed, one can readily obtain the ESR spectrum of its $\bullet$ H spin adduct and its ESR parameters.

Experimental

The used ESR electrochemical cell was a REL-001 of Radical

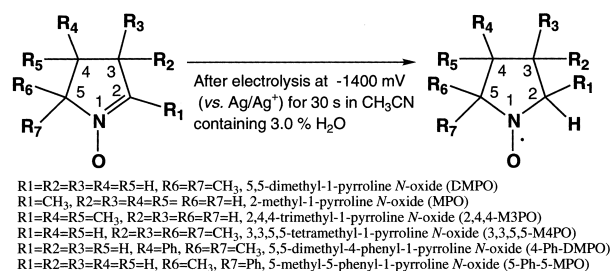


Fig. 1. Chemical structure of 5-membered ring nitron spin traps used in the present study and their spin adducts. The conventional numbering systems and substituted groups are also shown.

Research Inc., in which the working and counter electrodes are made of gold wire (0.5 mm o.d.) and silver wire is used as the reference electrode. The ESR spectrometer used was a JEOL-TE300 (JEOL) operated at 100 kHz field modulation. A magnetic field adjustment was performed using Mn²⁺ in MgO as a reference: The peak distance between $g = 1.981$ and 2.034 was 8.69 mT.

The spin traps used in the present study are summarized in Fig. 1. 2-Methyl-1-pyrroline *N*-oxide (2-MPO),⁷ 2,4,4-trimethyl-1-pyrroline *N*-oxide (2,4,4-M₃PO), and 3,3,5,5-tetramethyl-1-pyrroline *N*-oxide (3,3,5,5-M₄PO)^{8–9} were synthesized according to previously reported methods. 2-MPO and 2,4,4-M₃PO were purified by vacuum distillation (67 °C at 0.5 mmHg and 85 °C at 5 mmHg, respectively) immediately prior to use, and 3,3,5,5-M₄PO by recrystallization from pentane. DMPO (purchased from LABOTEC Co.) as well as 5,5-dimethyl-4-phenyl-1-pyrroline *N*-oxide (4-Ph-DMPO) and 5-methyl-5-phenyl-1-pyrroline *N*-oxide (5-Ph-5-MPO) (purchased from Radical Research Inc.) were used without further purification. Spectroscopy grade CH₃CN was obtained from Nacalai Tesque and purified by double distillation over P₂O₅. Supporting electrolytes, (*n*-Bu)₄NBF₄, used in CH₃CN were also from Nacalai Tesque.

Results and Discussion

Detection of Hydrogen Atom Adducts of Spin Traps.

We have reported that the ESR spectrum due to the hydrogen adduct of DMPO was observed after electrolysis upon supplying -1400 mV (vs Ag/Ag⁺) for an aqueous DMPO solution (10.0 mM) containing KClO₄ (0.1 M). By monitoring the ESR signal intensity of the DMPO-H adduct, the optimum condition was confirmed to be follows: applied potential, -1400 mV; time for electrolysis, 30 s. By the same procedure, ESR spectra ascribable to the $\bullet$ H and $\bullet$ D radical adducts of DMPO derivatives, except for 2-MPO and 2,4,4-M₃PO, were recorded; the obtained hyperfine coupling constants (hfcc's) are listed in Table 1. ESR signals due to the 2-MPO-H and 2,4,4-M₃PO-H radical adducts, however, were never detected, even by prolonging the time for electrolysis (2 min) and the potential (below -1800 mV (vs Ag/Ag⁺)).

Further electrochemical ESR measurements were continued for CH₃CN solutions of DMPO (10 mM) containing 3.0% H₂O in order to obtain the ESR spectra of hydrogen radical adducts. After supplying -1400 mV (vs Ag/Ag⁺) to a solution of DMPO for 30 s, the observed ESR spectrum showed a well resolved hyperfine structure, and the hfcc values of nitrogen and methylene protons at the 2-position were estimated to be $a^N =$

Table 1. Hfcc Values (in mT) of Hydrogen Atom Adducts of DMPO and Its Derivatives

Spin trap	Solvent	H hyperfine coupling constant in mT				
		a^N	$a^{H(2)c)}$	$a^{H(2)c)}$	$a^{H(5)d)}$	$a^{H(5)d)}$
DMPO	H ₂ O	1.66	2.28	2.28	N.R. ^{e)}	N.R. ^{e)}
	D ₂ O	1.66	2.27	0.35 ^{f)}	N.R. ^{e)}	N.R. ^{e)}
	AC/H ^{a)}	1.52	2.01	2.01	N.R. ^{e)}	N.R. ^{e)}
	AC/D ^{b)}	1.52	2.02	0.31 ^{f)}	N.R. ^{e)}	N.R. ^{e)}
2-MPO	H ₂ O	not detected				
	AC/H ^{a)}	1.51	2.11	—	1.84	2.11
	AC/D ^{b)}	1.52	0.33 ^{f)}	—	1.85	2.19
2,4,4-M ₃ PO	H ₂ O	not detected				
	AC/H ^{a)}	1.48	2.22	—	1.48	2.38
	AC/D ^{b)}	1.52	0.35 ^{f)}	—	1.51	2.48
3,3,5,5-M ₄ PO	H ₂ O	1.68	2.20	2.20	N.R. ^{e)}	N.R. ^{e)}
	D ₂ O	1.66	2.18	0.33 ^{f)}	N.R. ^{e)}	N.R. ^{e)}
	AC/H ^{a)}	1.53	1.93	1.93	N.R. ^{e)}	N.R. ^{e)}
	AC/D ^{b)}	1.53	1.94	0.30 ^{f)}	N.R. ^{e)}	N.R. ^{e)}
4-Ph-DMPO	H ₂ O	1.64	1.82	2.68	N.R. ^{e)}	N.R. ^{e)}
	D ₂ O	1.64	1.81	0.41 ^{f)}	N.R. ^{e)}	N.R. ^{e)}
	AC/H ^{a)}	1.51	1.62	2.37	N.R. ^{e)}	N.R. ^{e)}
	AC/D ^{b)}	1.52	1.63	0.36 ^{f)}	N.R. ^{e)}	N.R. ^{e)}
5-Ph-5-MPO	H ₂ O	1.62	2.21	2.28	N.R. ^{e)}	N.R. ^{e)}
	D ₂ O	1.59	2.18	0.34 ^{f)}	N.R. ^{e)}	N.R. ^{e)}
	AC/H ^{a)}	1.46	1.91	2.25	N.R. ^{e)}	N.R. ^{e)}
	AC/D ^{b)}	1.46	1.91	0.31 ^{f)}	N.R. ^{e)}	N.R. ^{e)}

a) CH₃CN containing 3.0% water; b) CH₃CN containing 3.0% D₂O; c) proton hfcc at 2-position; d) proton hfcc at 5-position; e) not resolved; f) deuteron hfcc.

1.52, and $a^H = 2.01$ mT, respectively. When 3.0% H₂O was replaced by D₂O, a triplet splitting due to a deuteron atom at the 2-position was observed, as depicted in Fig. 2a. These results indicate that the electrochemical reduction of H₂O or D₂O gave short-lived •H or •D radicals in CH₃CN. In fact, no detectable amount of hydrogen radical adducts of DMPO derivatives were recorded in the absence of water in CH₃CN.

Under the same condition, ESR measurements were performed for CH₃CN solutions of DMPO derivatives in the presence of 3.0% H₂O and D₂O. An ESR spectrum ascribed to the •H adducts of 2-MPO was obtained, as shown in Fig. 2b; the estimated hfcc's are listed in Table 1. By a comparison of the hfcc of •H and •D adducts of 2-MPO (Table 1), hfcc's of $a^H = 1.84$, and $a^H = 2.11$ mT were assigned to the non-equivalent methylene protons at the 5-position. A similar ESR spectrum was also observed for 2,4,4-M₃PO, as represented in Fig. 2c. The present results suggest that the lifetime of the hydrogen adducts 2-MPO and 2,4,4-M₃PO may be prolonged in less polar solvents. By the same procedure, ESR spectra were observed for the •H adducts of 3,3,5,5-M₄PO, 4-Ph-DMPO and 5-Ph-MPO; the evaluated hfcc's are listed in Table 1.

Conclusively, in situ electrochemical ESR spectroscopy allowed us to observe the ESR spectra of •H spin adducts various 5-membered nitron spin traps. We report here for the first

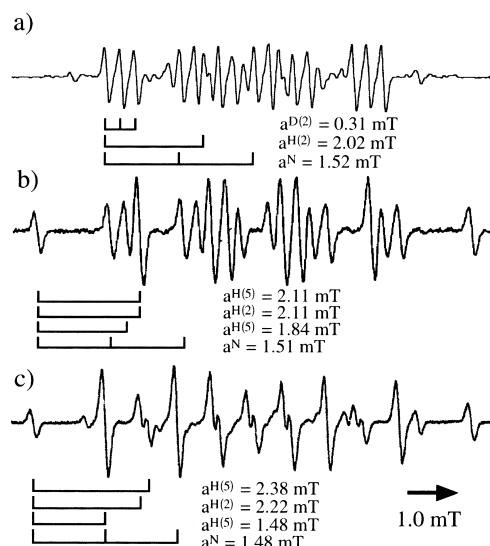


Fig. 2. ESR spectra obtained for DMPO and DMPO derivatives in CH₃CN containing 3.0% H₂O or D₂O and (*n*-Bu)₄NBF₄ (0.1 M), by the in situ electrochemical ESR method at 25 °C. (a) was observed for CH₃CN solution DMPO (10.0 mM), containing D₂O (3.0%), and (b), and (c) were observed for 2-MPO and 2,4,4-M₃PO, in the presence of 3.0% H₂O. Electrochemical reduction was performed at $-1,400$ mV (vs Ag/Ag⁺) for 30 s and the spectra were obtained after turning it off. The stick diagrams illustrated below the ESR spectrum correspond to the estimated hfcc values.

time on the ESR spectra of the •H spin adducts of 2,4,4-M₃PO, 2-MPO, 4-Ph-DMPO, and 5-Ph-5-MPO. This method allows us to detect the hydrogen adducts of newly developed nitron spin traps. In addition, the present procedure is suitable for investigating of biologically important hydrogen atom transfer reaction processes, such as auto-oxidation of unsaturated fatty acids. Further investigations on the molecular structure of short-lived 5-membered nitroxide radicals are in progress.

References

- 1 "Free Radicals: Biology and detection by spin trapping," ed by G. M. Rosen, B. E. Britigan, H. J. Halpern, and S. Pou, Oxford University Press, New York (1999), Chap. 10, p. 377.
- 2 E. G. Janzen, C. A. Evans, and J. I-P Liu, *J. Magn. Reson.*, **9**, 510 (1973).
- 3 E. G. Janzen, R. V. Shetty, and M. Kunanec, *Can. J. Chem.*, **59**, 756 (1981).
- 4 D. L. Haire, U. M. Oehler, P. H. Krygsmann, and E. G. Janzen, *J. Org. Chem.*, **53**, 4535 (1988).
- 5 K. Makino, M. M. Mossoba, and P. Riesz, *J. Phys. Chem.*, **87**, 1369 (1983).
- 6 N. Endo, K. Higashi, K. Tajima, and K. Makino, *Chem. Lett.*, **2001**, 548.
- 7 H. A. Brandman and R. T. Conley, *J. Org. Chem.*, **1947**, **38**, 2236 (1973).
- 8 M. C. Kloetzel, *J. Chem. Soc.*, **1947**, 2271.
- 9 R. Bonnett, R. F. C. Brown, V. M. Clark, I. O. Sutherland, and Sir A. Todd, *J. Chem. Soc.*, **1959**, 2094.