ANALYTICAL SCIENCES JULY 2003, VOL. 19

Notes

Signal Enhancement in ESR Spin-trapping for Hydroxyl Radicals

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In an ESR spin-trapping measurement of hydroxyl radicals formed in a Fenton reaction, the trapping efficiency with DMPO increased by about 300 times in a sodium trifluoroacetate solution, whereas it was little changed in a phosphate buffer.

(Received January 20, 2003; Accepted May 2, 2003)

While developing an assay method for hydroxyl radicals, we observed that the ESR-signal intensity of the 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO)–OH· adduct increased remarkably in a trifluoroacetate solution (pH 6.15), whereas it was little or negligibly changed in a phosphate buffer under a similar condition (Fig. 1).

It has been known that a bidentate or tridentate strong ligand, such as bis(picolinate), ethylenediamine-*N*,*N*′-diacetate or nitrilotriacetate, may enhance the Fenton reaction.[1-3] Recently, the effects of a buffer on the auto-oxidation of ferrous ion were reported, where the rate of oxidation of Fe(II) to Fe(III) was small in a tris(hydroxymethyl)aminomethane (Tris) buffer.[4] We examined a Tris buffer for measurements of the hydroxyl radicals formed in a Fenton reaction by the spin-trapping method; however, we could not obtain a sufficient signal intensity at a low concentration of hydrogen peroxide. In Fenton reaction, hydroxyl radical is to be formed at the initial stage:[5,6]

$$Fe(II) + H_2O_2 \longrightarrow HO\cdot + HO^- + Fe(III) \quad (1)$$

$$Fe(II) + HO\cdot \longrightarrow Fe(III) + HO^- \quad (2)$$

$$H_2O_2 + HO\cdot \longrightarrow HOO\cdot + H_2O \quad (3)$$

We speculated that hydrogen radical abstraction from a hydroxyl group, or the oxidation of amino group of Tris might occur, which must result in a minimized formation of the DMPO–OH· adduct radical, because we could not detect the DMPO adduct of C-centered radicals, which should be derived from Tris. Thus, to prevent reactions with a hydroxyl radicals, a monodentate ligand with a less-reactive group to the hydroxyl radicals should be employed.

In this note, we describe the observation of a remarkable signal enhancement of DMPO-trapped hydroxyl radicals in a sodium trifluoroacetate solution by ESR spin-trapping experiments.

Experimental

Chemicals

The water used in this study was prepared by Milli-Q (Millipore Japan Co., Ltd., Tokyo, Japan), which was deaerated under reduced pressure by an aspirator, and then stored under an argon atmosphere by attaching an argon balloon to it.

5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) was obtained from Labotec Co., (Tokyo, Japan), and used by diluting with purified water. Sodium trifluoroacetate was purchased from Wako Pure Chemical Ind. (Osaka, Japan).

ESR measurement

An electron-spin resonance spectrometer, JES-RE1X (JEOL, Tokyo, Japan), was used. The conditions were: field, 336 mT ± 5 mT width; power, 8 mW; field modulation, 0.200 mT; time constant, 0.1; and amplitude, 500. Manganese signal was used for the external standard.

Fenton reaction

Solution A: 5 mmol/l or 50 mmol/l DMPO aqueous solution.

Solution B: Ammonium ferrous sulfate solution (1 mmol/l).

Solution C: Hydrogen peroxide solution (0.2 – 1.5 mmol/l, prepared by diluting 1 mmol/l solution which was titrated with iodine).

Solution D: 200 mmol/l Tris–HCl buffer (pH 7.4), phosphate buffer (pH 7.4 or pH 6.15), sodium acetate buffer (pH 6.15), or sodium trifluoroacetate solution (pH 6.15).

The Fenton reaction was performed by mixing 1.7 ml of solution A, 100 μl of C, 100 μl of D, and finally 100 μl of B. The reaction mixture was measured by ESR 1 min after the addition of solution B. Four different buffers were examined: Tris–HCl (pH 7.4), acetate (pH 6.15), phosphate buffer (pH 7.4, and 6.15), where the final concentration of DMPO was 42.5 mM. In the case of sodium trifluoroacetate (pH 6.15), the DMPO concentration was reduced to 4.25 mM, because the DMPO–OH· adduct signal was too strong.

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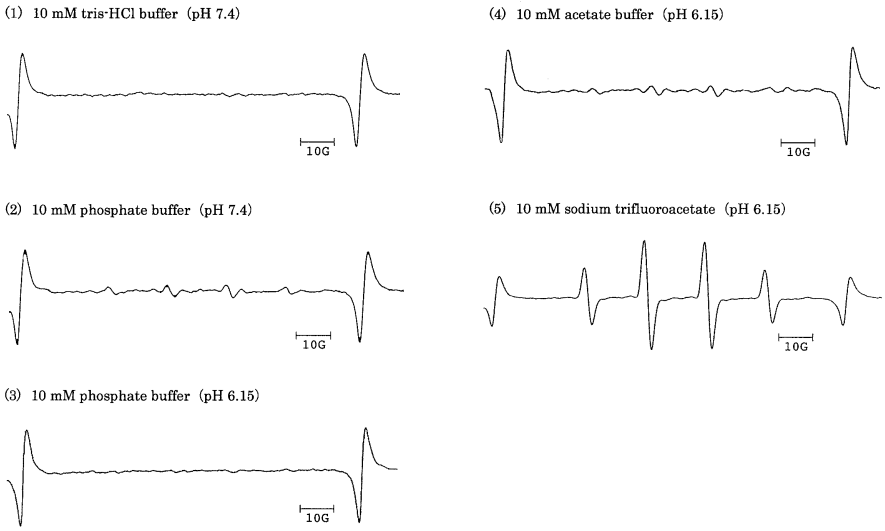

Fig. 1 ESR spectra of the DMPO-OH· adduct. The ESR signal intensity of the DMPO-OH· adduct was remarkably increased in a sodium trifluoroacetate solution. The vertical scale of (5) was set to be half that of (1) – (4), because the signal was over-scaled. The final concentration of DMPO was 42.5 mmol/l for (1) – (4), and 4.25 mmol/l for (5). When the concentration of DMPO was 4.25 mmol/l, no signal was found in a phosphate buffer. The other conditions for ESR measurements were the same.

Results and Discussion

As shown in Fig. 1, the ESR signal of the DMPO-OH· adduct was remarkably increased in a sodium trifluoroacetate solution, while it was quite poor in a Tris-HCl (pH 7.4), sodium acetate (pH 6.15), or phosphate buffer (pH 7.4 or 6.15), even when the concentration of DMPO was ten-times higher. It was about 300-times more sensitive in a sodium trifluoroacetate solution (pH 6.15) compared to the results in a phosphate buffer (pH 7.4). When 4.25 mM of DMPO was used, the signal of the DMPO-OH· adduct could not be found in a phosphate buffer (pH 7.4), where the other experimental conditions were the same.

To simulate a biological system, phosphate buffer was widely adapted as a medium for most reactions in which the pH should be controlled. However, the rate of the auto-oxidation of Fe(II) was found to be fast in a phosphate buffer or in a bicarbonate buffer.[2] Thus, the amount of hydroxyl radicals formed by the Fenton reaction in these media may be underestimated.

In conclusion, the intensity of the ESR signal of hydroxyl radicals trapped with DMPO remarkably increased in a sodium trifluoroacetate solution. We speculated that the oxidation of trifluoroacetic acid should be less than that of acetic acid or phosphoric acid, which resulted in an increase in the DMPO-OH· adduct formation.

Acknowledgements

The authors are grateful for an Araki Grant in the year of Heisei 14th and also for a Grant-in-Aid for Scientific Research for Health (H11-Seikatsu-030) from the Ministry of Health, Labour and Welfare, Japan.

References

1. J. D. Rush and W. H. Koppenol, *J. Am. Chem. Soc*., **1988**, *110*, 4957.
2. C. Sheu, A. Sobkowiak, Z. Li, N. Ozbalik, D. H. R. Barton, and D. T. Sawyer, *J. Am. Chem. Soc*., **1989**, *111*, 8030.
3. D. T. Sawyer, A. Sobkowiak, and T. Matsushita, *Acc. Chem. Res*., **1996**, *29*, 409, and references.
4. K. D. Welch, T. Z. Davis, and S. D. Aust, *Arch. Biochem. Biophys*., **2002**, *397*, 360.
5. C. Walling, *Acc. Chem. Res*., **1975**, *8*, 125.
6. P. A. MacFaul, D. D. M. Wayner, and K. U. Ingold, *Acc. Chem. Res*., **1988**, *31*, 159.