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Antioxidant effects of reduced water produced by electrolysis of sodium chloride solutions

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Abstract

Antioxidant vitamins and enzymes such as superoxide dismutase, catalase and glutathione peroxidase are considered to function as scavengers against reactive oxygen species and to provide protection against reactive oxygen species, including free radicals. Although antioxidants such as L-ascorbic acid, *d*-catechin and quercetin dehydrate show superoxide dismutation activity, using reduced water produced in the cathode side by electrolysis as a solvent instead of 2 mM NaC1 solution of the same pH level as the reduced water increased the superoxide dismutation activity of these antioxidants. Moreover, neither the reduced water nor its electrolyte solution showed any superoxide dismutation activity by itself. On the other hand, the reduced water was able to decrease hydrogen peroxide levels. It has been found that the behaviour of H_2 in reduced water, which was activated by a platinum electrode, differed from that of H_2 introduced by bubbling of hydrogen gas. The former decreased H_2O_2, whereas the latter did not. These results suggest strongly that the increase in superoxide dismutation activity, with a proton donor such as L-ascorbic acid, is due to an increase in the dissociation activity of water while the scavenging activity for H_2O_2 is due to activated dissolved H_2 in the reduced water.

1. Introduction

A free radical is any molecule, which contains one or more unpaired electrons, and includes superoxide anions ($O_2^{-\cdot}$), singlet oxygen ($^1O_2$) hydroxyl radicals (·OH), hydroperoxyl radicals (·OOH), nitrogen monoxide radicals (NO·) and hydrogen peroxide molecule (H_2O_2). Although it is a reactive oxygen species, the hydrogen peroxide molecule does not contain unpaired electrons and is not a free radical.

Reactive oxygen species or free radicals are extremely reactive and can cause cell injury or death [1–4]. Under certain conditions, these reactive oxygen species play a significant role in the disease development process [5, 6]. On the other hand, antioxidants, including antioxidant enzymes such as superoxide dismutase [7], catalase [8], and glutathione peroxidase [9], as well as vitamins [10–14], protect tissues from the effects of reactive oxygen species or free radicals. A new technology using electrolysis has been studied for clinical improvement of various diseases. A few studies concerning the superoxide dismutation activity of reduced water produced by electrolysis have been reported. Shirahata et al. have reported that reduced water showed superoxide dismutation activity as SOD-like activity and a catalase enzyme-like activity [15]. Their experiments were carried out using ethylenediaminetetraacetic acid (EDTA) with a luminescence method. EDTA dissolved in reduced water will produce higher superoxide dismutation activity by playing the role of a proton donor. As a result, the phenomenon of superoxide dismutation in reduced water seems to occur directly. The reason why this phenomenon occurs has been clarified. In these studies, superoxide dismutation activity and hydrogen peroxide scavenging activity of reduced water produced by electrolysis of electrolyte solutions, and OH^- ions were examined by electron spin resonance, ion chromatography and neutralization titration methods.

2. Experimental details

2.1. *Electrolysis cell*

Two electrolysis half-cells, made of acrylonitrile–butadiene–styrene, were prepared as shown in Figure 1. A non-charged membrane, SGT-010-135-1 (Japan Goatex) with an effective area of 269.36 cm^2 was mounted between the two half-cells. Electrolysis was carried out across the non-charged membrane between these two half cells equipped with platinum-coated titanium electrodes having an effective area of 539 cm^2.

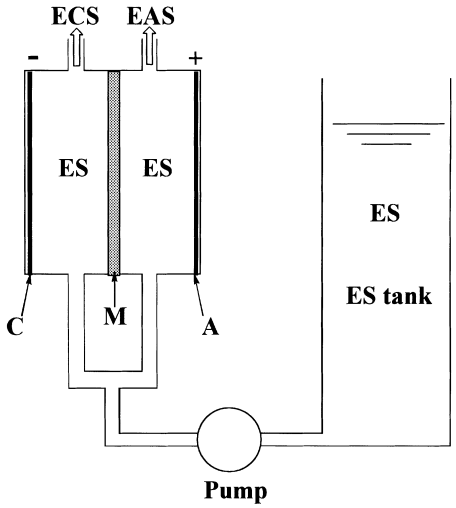

Fig. 1. Electrolysis cells made of an acrylonitrile–butadiene–styrene resin. (A) anode, (ES) 2 mM NaCl solutions, (M) non-charged membrane, (ECS) electrolysed cathode solutions (reduced water), (EAS) electrolysed anode solutions (oxidized water).

2.2. *Chemicals and reagents*

The reagents used for this study were special-grade NaCl, L-ascorbic acid and H_2O_2 (Wako Pure Chemical Industries Ltd), *d*-catechin (Yoneyama Chemical Industries) and quercetin dehydrate (Kanto Chemicals), and as a solvent, water, which was distilled and deionized with an ion-exchange resin to below 0.03 μS cm^{-1}. To measure the sample superoxide dismutation activity, a sodium phosphate buffer containing 2 mM hypoxanthine (Sigma Chemical Company), sodium phosphate buffer containing 5.5 mM diethylenetriaminepentaacetic acid (DETAPAC), 5,5-dimethyl-1-pyrroline-oxide (DMPO, Labotec), superoxide dismutase (Boehringer Mannheim), and 0.4 unit ml^{-1} xanthine oxidase in sodium phosphate buffer (Merck) were used.

2.3. *Electrolysis of 2 mM NaCl solutions*

Five 10 L sample solutions, each containing 2 mM NaCl, were prepared. These samples were each pumped from the solution container into a pair of electrolysis half cells with a non-charged membrane mounted between them. The sample solutions were subjected to electrolysis under set conditions of 1 to 8 A and 25 °C using a constant electric current source. Electrolysis was carried out with a constant flow rate of 3000 ml min^{-1} in the cathode compartment and, 1000 ml min^{-1} in the anode compartment. The cathode solution produced by electrolysis, which was called reduced water, was used for the experiments in this study.

2.4. *Measurement of superoxide dismutation activity*

1 ml of 10 mM L-ascorbic acid, *d*-catechin, or quercetin dehydrate was added to 50 ml samples of the reduced water and to 50 ml of 2 mM NaCl solution adjusted to the same pH level as the reduced water with 10 mM NaOH. Superoxide dismutation activity was measured using an electron spin resonance (ESR) spectrometer (ES-10, Nikkiso). Each reaction mixture contained 50 mm^3 of 2 mM hypoxanthine in sodium phosphate buffer, 35 mm^3 of 5.5 mM DETAPAC in sodium phosphate buffer, 50 mm^3 of sample, 16 mm^3 of DMPO and 50 mm^3 xanthine oxidase in sodium phosphate buffer. These reaction mixtures were poured into a special flat cell to conduct ESR measurements. This ESR measurement was carried out using 3.7 mW microwave power, 339.1 ± 5.5 mT magnetic field, 100 kHz frequency, 0.1 mT modulation, 0.12 s response time and 1 min sweep time. As the unit for superoxide dismutation activity, we used the same unit as adopted by Fridovich et al. [7]. The superoxide dismutation activity of the samples was estimated by means of interpolation based on a standard curve of 0 to 30 unit per ml of superoxide dismutase.

2.5. *Measurements of pH, oxidation and reduction potential (ORP), and dissolved oxygen (DO)*

Parameters of the reduced water produced by electrolysis of sodium chloride electrolyte solutions were measured as pH, ORP and DO. pH and ORP were measured using a pH meter (Toa Ion Meter IM-4OS) and DO was measured using a DO meter (Horiba OM-12), respectively, at 25 °C.

2.6. *Measurement of OH^- ions*

The concentration of OH^- ions was obtained from the measurement of pH and by a neutralization titration method using an automatic titrator. In this study, pH was measured using a pH meter (Toa Ion Meter lM-40S, Toa Electronics) in samples produced at each of the following currents, OA, 1A, 2A, 3A, 4A, 5A, 6A, 7A and 8A. Neutralization titrations with 0.1 M HCl were carried out using a Comite-550 titrator (Hiranuma Industry) for the reduced water produced at each of the same levels of current as the pH measurements.

2.7. *Measurements of H_2O_2 scavenging activity of reduced water*

Fenton's reaction produces hydroxyl radicals from $FeSO_4$ and H_2O_2. The number of hydroxyl radicals produced through this reaction increases in proportion to the H_2O_2 concentration. Thus, the amount of H_2O_2 consumed by a reducing substance can be estimated from the amount of hydroxyl radicals produced from the remaining H_2O_2.

We prepared a reduced water sample, a reduced water sample boiled for 5 min, a 2 mM NaCl solution of the same pH as the reduced water sample, and a 2 mM NaCl solution of the same pH as the reduced water sample through which hydrogen gas was bubbled. The reactivity of these samples with hydrogen peroxide was measured with an ESR spectrometer using Fenton's

reaction. Mixtures of 50 mm^3 of 1 mM $FeSO_4$, 35 mm^3 of DETAPAC, 16 mm^3 of DMPO and sample were prepared. Measurements of hydroxyl radicals by ESR were carried out 3, 12, 18, 25 and 30 min after the addition of 1 mM H_2O_2 to these mixtures.

3. Results and discussion

3.1. *Characteristics of reduced water produced in the cathode side by electrolysis*

When electrolyte solutions are electrolysed across the membrane using electrodes, reduction occurs on the cathode and oxidation on the anode. Dissociation of H_2O generates H^+ and O_2 at the anode, and OH^- and H_2 at the cathode. Therefore, cathodic alkaline water (reduced water) is abundant in dissolved hydrogen (DH), whereas anodic acidic water (oxidized water) is abundant in DO. Results of measuring pH, ORP and DO corresponding to electric current are shown in Figure 2. pH increased from 10.46 to 11.11 between 0 and 9 A, ORP decreased from −110 mV to −229 mV and DO decreased from 5.85 to 5.28 mg dm^{-3}.

3.2. *Reactivity of reduced water and NaOH with L-ascorbic acid, d-catechin and quercetin dehydrate*

The radical scavenging effects of tannins and flavonoids as polyphenols have been studied by several researchers [16, 17]. We used *d*-catechin, quercetin dehydrate and L-ascorbic acid, as radical scavengers. When DMPO as spin trapping reagent was used for superoxide anion radicals produced by the hypoxanthine–xanthine oxidase system, a typical signal pattern for superoxide anion radicals was observed as shown in Figure 3.

The superoxide dismutation activity of L-ascorbic acid, *d*-catechin and quercetin dehydrate in the reduced water or in 2 mM NaCl solutions adjusted to the same pH level as the reduced water are shown in Figure 4. The results were obtained through ESR measurements conducted using 10 mM L-ascorbic acid, 10 mM *d*-catechin or 10 mM quercetin dehydrate as proton donors. With 2 mM NaCl solutions adjusted to the same pH level as the reduced water, 85, 27.4 and 263.9 units per ml of superoxide dismutation activity were measured for L-ascorbic acid, quercetin dehydrate and *d*-catechin, respectively. Using the reduced water 179.8, 47.7 and 306.9 units per ml were found for these three scavengers, respectively. The reduced water electrolysed using 8 A current and 2 mM NaCl solutions adjusted to the same pH level as the reduced water without any added scavengers had almost no activity. The differences between superoxide dismutation activity of L-ascorbic acid, quercetin dehydrate and *d*-catechin dissolved in

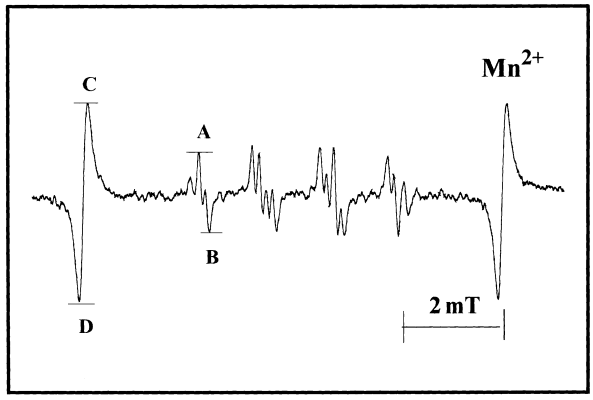

Fig. 3. Typical ESR signals of superoxide anion radicals produced by the hypoxanthine–xanthine oxidase system. Relative signal intensity was obtained as the ratio of the distance between A and B, to the distance between C and D.

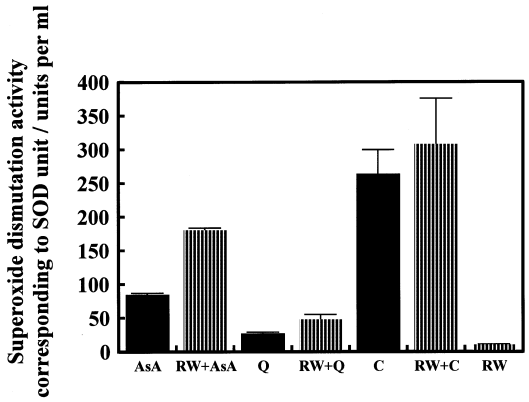

Fig. 4. Superoxide dismutation activity of L-ascorbic acid, *d*-catechin and quercetin dehydrate in reduced water or 2 mM NaCl solutions. AsA: L-ascorbic acid in 2 mM NaCl, RW + AsA: L-ascorbic acid in reduced water, Q: a solution of quercetin dehydrate in 2 mM NaCl, RW + Q: a solution of quercetin dehydrate in reduced water, C: a solution of *d*-catechin in 2 mM NaCl, RW + C: a solution of *d*-catechin in reduced water and RW: reduced water.

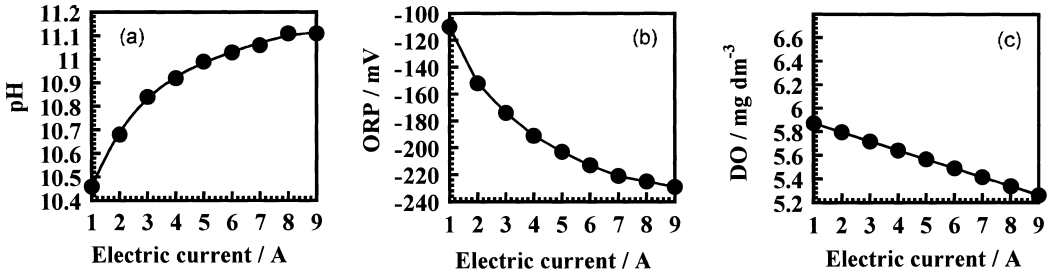

Fig. 2. Relationships of current to pH (a), ORP (b) and DO (c) in the reduced water produced by electrolysis using 2 mM NaCl solutions.

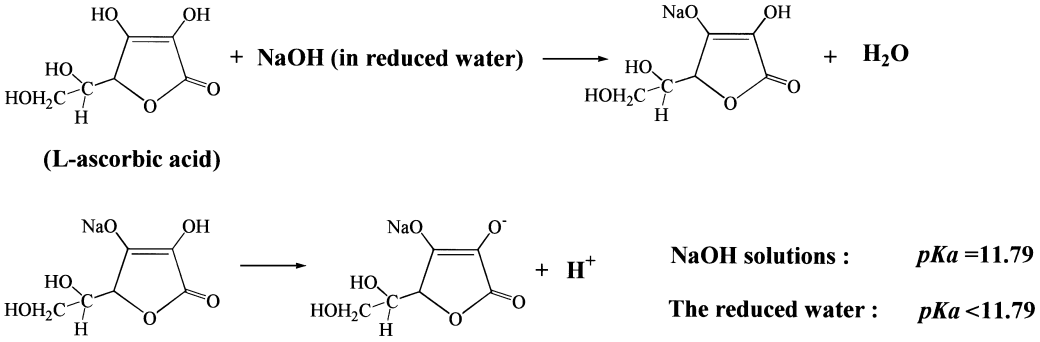

Fig. 5. Differences of pK_a in AsA between NaOH solutions and reduced water. pK_a as dissociation activity of sodium ascorbate is 11.79.

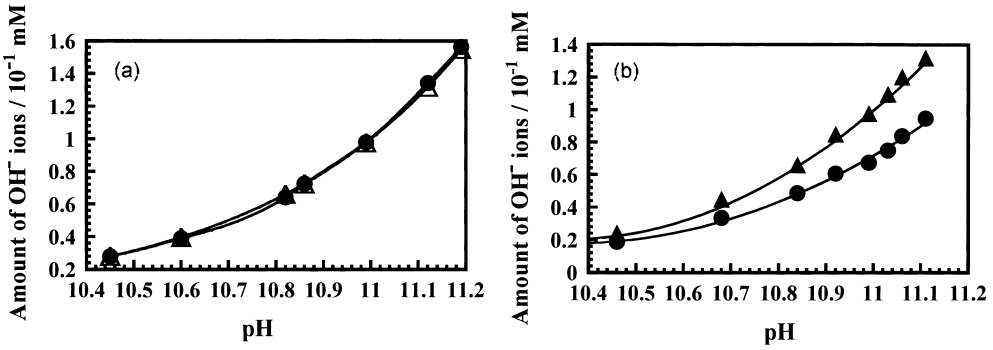

Fig. 6. (a) Concentration of OH^- ions in NaOH solutions, estimated from the pH and the titration method. Key: (●) concentration of OH^- ions estimated from pH, (△) concentration of OH^- ions measured by the titration method. (b) Concentration of OH^- ions in the reduced water, estimated from the pH and the titration method. Key: (●) concentration of OH^- ions estimated from pH, (▲) concentration of OH^- ions measured by the titration method.

reduced water and dissolved in 2 mM NaCl solutions adjusted to the same pH as the reduced water were 94.8, 20.3 and 43 units per ml, respectively. These compounds (called antioxidants) act on superoxide anion radicals as proton donors as shown in Figure 5. These results indicate that reduced water does not show superoxide dismutation activity by itself.

3.3. *Production of OH^- ions and the ionic product of water in the reduced water*

Figure 6 shows the concentration of OH^- ions estimated from the pH value and by the titration method for each level of pH. Figure 6(a) shows results of NaOH solutions estimated from the pH value and by the titration method for each level of pH. Figure 6(b) shows results of electrolyzed reduced water estimated from the pH value and by the titration method for each level of pH. In the case of (a), the concentration of OH^- ions measured by pH of NaOH solutions is the same as that of OH^- ions measured by the titration method. But in the case of (b), the concentration of OH^- ions measured by pH in the reduced water is higher than that of OH^- ions measured by the titration method. Figure 7 shows the ratio between the ionic product of reduced water (K_{RW}) and the ionic product of pure water (K_W). Minimum and maximum values in the ratio of K_{RW} and K_W were 1 and 1.462, respectively. Figure 8 shows the

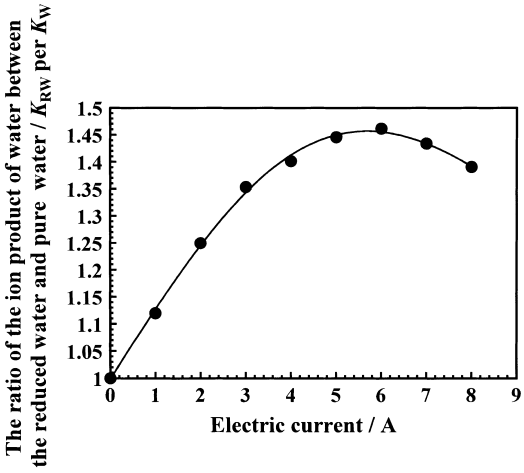

Fig. 7. The ratio (K_{RW}/K_W) of the ionic product of reduced water and pure water used as solvent. K_{RW} is the ionic product of water in reduced water, K_W is the ionic product of water in pure water.

concentration of OH^- ions in NaOH and Na_2CO_3 solutions subjected to increasing electrolysis currents. Although OH^- ions increase in proportion to current from 0 to 8 A in both solutions, the concentration of OH^- ions in the Na_2CO_3 is lower than in NaOH. Figure 9 shows the ratio between the concentration of Na^+ transported to the cathode side and that of Cl^- ions transported to the anode side in the reduced water.

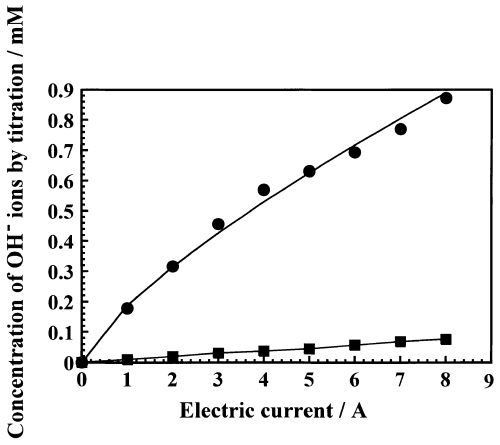

Fig. 8. Concentration of OH^- ions in NaOH and of OH^- ions from the dissociation of Na_2CO_3 generated from NaOH and CO_2 dissolved in the reduced water. Key: (●) concentration of OH^- ions in NaOH, (■) concentration of OH^- ions from the dissociation of Na_2CO_3.

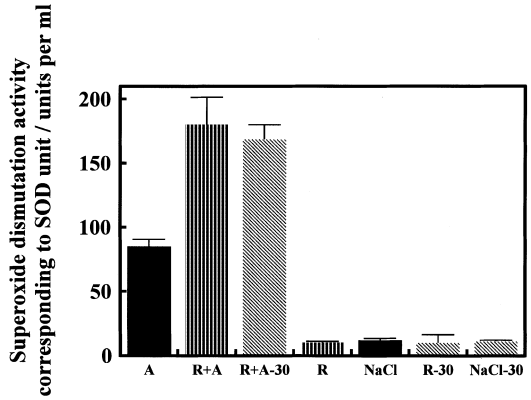

Fig. 10. Superoxide dismutation activity of L-ascorbic acid. A: L-ascorbic acid in 2 mM NaCl solutions, R + A: L-ascorbic acid in reduced water, R + A + 30: L-ascorbic acid in the reduced water 30 days after electrolysis, R: the reduced water, NaCl: 2 mM NaCl solutions, R + 30: reduced water 30 days after electrolysis and NaCl + 30: 2 mM NaCl solutions 30 days after preparation.

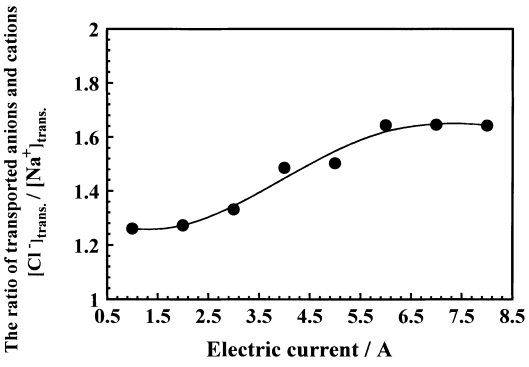

Fig. 9. Ratio of concentration of Cl^- ions transported to the anode side and Na^+ ions transported to the cathode side. Initial concentration of Na^+ ions and Cl^- ions is 2 mM.

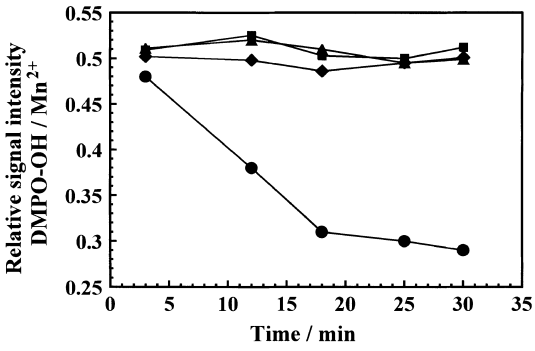

Fig. 11. Hydrogen peroxide scavenging activity. Key: (●) reduced water, (■) 2 mM NaCl solution, (▲) boiled reduced water, (◆) 2 mM NaCl solutions bubbled with hydrogen gas.

Using the non-charged membrane for electrolysis, the number of Cl^- ions transported to the anode side is more than that of Na^+ ions transported to the cathode side.

3.4. *Stability of superoxide dismutation activity of the reduced water*

Measurement of superoxide dismutation activity was carried out in ascorbate solutions in reduced water produced 30 days earlier in order to assess the stability of the enhancement of the superoxide dismutation activity. Figure 10 shows results of superoxide dismutation activity. The reduced water for measurements was kept in a closed glass bottle at 10 °C. As shown in Figure 10, superoxide dismutation activity decreased by about 10% compared to the previous data 30 days before.

3.5. *Scavenging activity of reduced water for H_2O_2*

Figure 11 shows results of measurements of H_2O_2 to examine the H_2O_2 scavenging activity of the reduced water. These were obtained by measuring the number of hydroxyl radicals produced by Fenton's reaction using $FeSO_4$ and H_2O_2. Hydroxyl radicals produced by Fenton's reaction showed the typical ESR signal shown in Figure 12. As shown in Figure 11, hydroxyl radical production was measured in solutions of H_2O_2 in reduced water, 2 mM NaCl adjusted to the same pH level as the reduced water, the reduced water boiled at 100 °C for 5 min, and 2 mM NaCl adjusted to the same pH level as the reduced water, bubbled with hydrogen

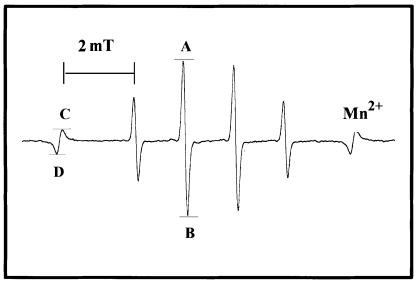

Fig. 12. Typical ESR signal pattern of hydroxyl radicals produced by Fenton's reaction. Relative signal intensity was obtained as the ratio of the distance between A and B, to the distance between C and D.

gas. Hydroxyl radical production decreased steadily to near 0 by 20 min in the reduced water but changed little in the other solutions even after 30 min.

3.6. *Dissociation activity of reduced water*

If reduced water is used as a solvent for these antioxidants the OH group located at the second functional group in L-ascorbic acid as shown in Figure 5 would produce many more protons due to the increase in dissociation activity. As a result, the number of protons which take part in the reaction would increase and superoxide dismutation activity would increase due to the increase in the dissociation activity of, not only L-ascorbic acid, but also other aqueous antioxidants, including *d*-catechin and quercetin dehydrate as proton donors. Therefore, superoxide dismutation activity in reduced water would be higher than in pure water. Although the reduced water increases the superoxide dismutation activity of antioxidants such as L-ascorbic acid, *d*-catechin and quercetin dehydrate, it does not show superoxide dismutation activity itself. It is considered that the superoxide dismutation activity would depend on the dissociation of functional groups in antioxidants and it would be enhanced by the dissociation activity of the solvent used.

EDTA, used as a chelating reagent, itself showed slight superoxide dismutation activity by ESR measurements (data not shown). EDTA with the reduced water would show higher superoxide dismutation activity owing to the higher dissociation activity of the reduced water.

Shirahata et al. reported that reduced water showed superoxide dismutation activity itself using EDTA as a chelating reagent by the chemiluminescence method [15]. As a result, it is considered that they observed superoxide dismutation activity with the reduced water.

When a 2 mM NaCl solution is electrolysed across a non-charged membrane Cl^- ions are transported to the anode and Na^+ ions are transported to the cathode. The total concentration of sodium ions in the cathode compartment is shown as follows:

$$[Na^+]_{total} = [Na^+]_{NaCl} + [Na^+]_{NaOH} + [Na^+]_{Na_2CO_3} + [Na^+]_x \quad (1)$$

where $[Na^+]_{total}$, $[Na^+]_{NaCl}$, $[Na^+]_{NaOH}$, $[Na^+]_{Na_2CO_3}$ and $[Na^+]_x$ represent the total concentration of Na^+ ions in the reduced water, the concentration of Na^+ in NaCl, the concentration of Na^+ in NaOH, the concentration of Na^+ in Na_2CO_3 and the concentration of other Na^+ ions, respectively. Other sodium ions refers to Na^+ ions in compounds other than hydroxyl compounds. Furthermore, the total concentration of OH^- ions is shown as follows:

$$[OH^-]_{pH} = [OH^-]_{NaOH} + [OH^-]_{Na_2CO_3} + [OH^-]_{increase} \quad (2)$$

where $[OH^-]_{pH}$, $[OH^-]_{NaOH}$, $[OH^-]_{Na_2CO_3}$ and $[OH^-]_{increase}$ represent the total concentration of OH^- ions estimated from pH value, OH^- ions in NaOH, OH^- ions from the dissociation of Na_2CO_3, and OH^- ions from the increase of the ion product of water, respectively. On the other hand, OH^- ions from a neutralization titration with 0.1 M HCl are shown as follows:

$$[OH^-]_{titration} = [OH^-]_{NaOH} + [OH^-]_{Na_2CO_3} \quad (3)$$

The increased concentration of OH^- ions will be estimated from Equation 2 and 3 as follows:

$$[OH^-]_{increase} = [OH^-]_{pH} - [OH^-]_{titration} \quad (4)$$

$[Na^+]_{total}$, as total Na^+ ions and $[Na^+]_{NaCl}$, as Cl^- ions can be obtained by ion chromatography. $[OH^-]_{NaOH}$ and $[OH^-]_{Na_2CO_3}$ can be obtained by the titration method. OH^- ions shown as $[OH^-]_{increase}$ in Equation 4 are not isolated free ions because of the electrical neutrality. The differences between $[OH^-]_{pH}$ and $[OH^-]_{titration}$ are considered as the differences in the ionic product of water between the reduced water and pure water as a solvent. Generally, the ionic product of water is shown as follows:

$$[H^+]\gamma_+[OH^-]\gamma_- = K_W \quad (5)$$

In the case of pure water, the activity coefficients γ_+ and γ_- are unity. Therefore, Equation 5 can be rewritten as follows:

$$[H^+][OH^-] = K_W = 10^{-14} \quad (6)$$

K_{RW} as the ionic product of water in the reduced water is shown in Equation 7:

$$-\log K_{RW} = 14 + \log\{[OH^-]_{titration}/[OH^-]_{pH}\} \quad (7)$$

Using $K_W = 10^{-14}$ at 25 °C and 1 atmosphere as the ionic product of water in pure water, the ratio of K_{RW} and K_W can be obtained as the ratio of the ionic product of water in reduced water and pure water. If the ratio of K_{RW} and K_W is 1, K_{RW} is the same ionic product of water as pure water. $K_{RW}/K_W = 1.462$ indicates that the dissociation activity in the reduced water increases to 1.462 times as high as pure water as shown in Figure 7. These results suggest strongly that when reduced water produced by electrolysis is used as the solvent for antioxidants such as L-ascorbic acid and other polyphenols, these antioxidants show higher superoxide dismutation activity by increasing the dissociation activity of protons in those antioxidants depending on the ionic product of water.

Since the increase in the ion product of water would depend on the dissociation energy of water, the dissociation energy would be increased at the cathode by the electrolysis of water. On the other hand, the number of Cl^- ions transported was higher than that of Na^+ ions

transported in the reduced water as shown in Figure 9. The reason is considered to be that the mobility of Cl^- ions is higher than that of Na^+ ions in aqueous solutions. Differences in ionic mobility between anions and cations in electrolyte solutions may be related to the behaviour of OH^- ions, the concentration of which is determined by the ionic product of water.

When H_2O molecules are reduced on a cathode, OH^- ions are generated as shown in Equation 5,

$$2\,H_2O + 2\,e^- \rightleftharpoons H_2 + 2\,OH^- \quad (8)$$

OH^- ions generated at the cathode diffuse into the bulk solution across the diffusion layer. On the other hand, Na^+ ions as cations are transported to the cathode where they react with the OH^- ions and form NaOH. As a result, the reduced water is alkaline. The differences in mobility between Na^+ ions and $C1^-$ ions depend on the hydration around the ions [18, 19]. As the hydration of Na^+ ions is higher than that of $C1^-$ ions, the rate of migration of $C1^-$ ions is higher than that of Na^+ ions. As seen from Figure 10 the dissociation activity of the reduced water was extremely stable. Thus, superoxide dismutation activity remained even 30 days after electrolysis. It is considered that this stable superoxide dismutation activity is based on the dissociation activity of the reduced water as solvent. As a result, reduced water without proton donors would not show superoxide dismutation activity.

In contrast, as shown in Figure 11, hydroxyl radical production was markedly inhibited in H_2O_2 solutions made with reduced water after 10 to 15 min, indicating that scavenging of H_2O_2 by the reduced water may be due to the hydrogen gas (which is activated by the platinum on the surface of the cathode, having a catalytic action on the surface of the cathode during electrolysis) that is generated [20]. In the field of industry, hydrogen is produced by the same method as mentioned above. Hydrogenation of organic compounds on the electrode is based upon the catalytic action of activated hydrogen molecules [21–24]. Thus activated hydrogen molecules in reduced water, which are indicated by the lower reduction oxidation potential, will produce phenomena such as catalase-like activity. As shown by Equation 9, hydrogen peroxide molecules are reduced to H_2O:

$$H_2O_2 + H_2(\text{activated}) \rightarrow 2H_2O \quad (9)$$

4. Conclusions

(i) The inhibitory effect of reduced water (which is produced on the cathode side by electrolysis) on the accumulation of superoxide anion radicals is due to an increase in the dissociation activity of the reduced water.

(ii) Antioxidants dissolved in the reduced water showed higher dismutation activity for superoxide anion radicals than those in pure water.

(iii) The reduced water itself did not show superoxide dismutation activity.

(iv) The scavenging activity of the reduced water relative to hydrogen peroxide probably depends on the concentration of activated hydrogen molecules produced by electrolysis in the reduced water.

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References

1. Y. Yamamoto, E. Niki, J. Eguchi, Y. Kamiya and H. Shimazaki, *Biochemica et Biophysica Acta* **819** (1985) 29.
2. S.V. Jovanovic and M.G. Simic, *J. Am. Chem. Soc.* **108** (1986) 5968.
3. K. Sato, E. Niki and H. Shimazaki, *Biochem. Biophys.* **279** (1990) 402.
4. E.R Stadtman, *Science* **257** (1992) 1220.
5. L. Packer and J.J. Fuchs, 'Vitamin C in Health and Disease' (Marcel Dekker, New York, 1997).
6. R.S. Sohal and R. Weindurch, *Science* **273** (1996) 59.
7. J.M. McCord and I. Friedovich, *J. Biol. Chem.* **244** (1969) 6049.
8. R. Nicholls and G.P. Schonbaum, 'The Enzyme' (Academic Press, New York, 1963).
9. P. Amstad, R. Monet and P. Cerutti, *J. Biol. Chem.* **269** (1994) 1606.
10. M. Takahashi, J. Tsuchiya, E. Niki and S. Urano, *J. Nutr. Sci. Vitaminol.* **34** (1988) 25.
11. P. Palozza and N.I. Krinsky, *Biochem. Biophys.* **297** (1992) 184.
12. B. Frei, L. England and B.N. Ames, *Proc. Natl. Acad Sci. USA* **86** (1989) 6377.
13. E.J. Nanni, Jr, M.D. Stallings and D.T. Sawyer, *J. Am. Chem. Soc.* **102** (1980) 4481.
14. G.S. Omenn, G.E. Goodman and M.D. Thornquist, *N. Engl. J. Med.* **334** (1996) 1150.
15. S. Shirahata, S. Kabayama, M. Nakano, T. Miura, K. Kusumoto, M. Gotoh, H. Hayashi, K. Otsubo, S. Morisawa and Y. Katakura, *Biochem. Biophys. Res. Commun.* **234** (1997) 269.
16. T. Yoshida, K. Mori, T. Hatano, T. Hatano, T. Okumura, I. Uehara, K. Komagoe, Y. Fujita and T. Okuda, *Chem. Pharm. Bull.* **37**(7) (1989) 1919.
17. S.V. Jovanović, S. Steenken, M. Tosci, M. Tosic, B. Marjanovic and M.G. Simic, *J. Am. Chem. Soc.* **116** (1994) 4846.
18. B.R. Breslau and I.F. Miller, *Ind. Eng. Chem. Fundam.* **10** (1971) 554.
19. K. Hanaoka, R. Kiyono and M. Tasaka, *J. Membrane Sci.* **82** (1993) 255.
20. R.L. leRoy, *J. Electrochem. Soc.* **130** (1983) 2158.
21. Z. Takehara, *Electrochim. Acta* **15** (1970) 999.
22. K. Fujikawa, A. Katayama and H. Kita, *J. Chem. Soc. Faraday Trans.* **175** (1973) 1.
23. K. Fujikawa and H. Kita, *J. Chem. Soc. Faraday Trans.* **175** (1979) 2638.
24. K. Fujikawa, H. Kita and S. Sato, *J. Chem. Soc. Faraday Trans.* **177** (1981) 3055.