MEASUREMENT FOR INHIBITION OF ACTIVE OXYGEN SPECIES UNDER CHANGE IN STATE OF WATER BY USE OF ESR

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Abstract - The effect of inhibition against the generation of active oxygen species (O_2^- , OH) by use of water treated with electrolysis or a far infrared ray was examined. The catholyte solution electrolyzed for 5 min by adding 300 ppm calcium ion was observed the most strongest effect of inhibition against generation of O_2^- and OH . Line width of the catholyte solutions by ^{17}O -NMR spectroscopy decreased in 55.0 Hz in the above same condition of electrolyzing from 101.0 Hz in control. In the irradiation of far infrared ray for the water, inhibition of O_2^- increased with increasing in irradiation time. Inhibition of O_2^- at 180 min in irradiation time was 44.8%.

Keywords active oxygen species, electrolysis, water cluster
far infrared ray

Active oxygen species (AOS) such as the superoxide anion (O_2^-), hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and lipid peroxide injure the biological membrane, enzyme and gene in the body, and are attracting much attention as a factor in aging, carcinogenesis and several disease states [1,2]. There is no report in concern with inhibition of the generation of AOS against change in state of water in spite of containing 70% water in the body. The author reports the effect of water treated with electrolysis or a far infrared ray against the generation of AOS.

EXPERIMENTAL

Apparatus A JEOL JES-RE1X X-band spectrometer with a magnetic field modulation of 100 KHz was used for ESR spectra of O_2^- and OH . A TOA Electronics Ltd. CM-11P conductometer was used for conductivity of sample water. A JEOL GX-400 Oxygen (^{17}O) NMR spectroscopy was used for measurement of state of water. Line width of ^{17}O -NMR spectra indicates activation of molecular motion of water [3].

Materials 5,5-dimethyl-1-pyrroline N-oxide (DMPPO) was purchase from Labotec Co., Ltd. Xanthine oxidase was purchased from Sigma Chemical Co.. All other chemicals used were of analytical grade. Deionized water was further purified by a Milli-Q system.

Preparation of Water samples A water added with 100-1000 ppm of calcium chloride was electrolyzed in condition of 20 V, 1.4 mA for 1-30 min, the water obtained from cathode solution was used. Water was kept in the vessel at 50 °C for 10-180 min, which is made of cordierite ceramics with iron oxide, and irradiate a far infrared ray in the range of 2-25 μ m wavelength.

Measurement of inhibition for superoxide O_2^- was obtained by hypoxanthine-xanthine oxidase superoxide generating system. To 50 μ l of the 2mM hypoxanthin, 50 μ l of 0.1M phosphate buffer (pH 7.8), 40 μ l of water sample, 10 μ l of DMPPO and 50 μ l of 0.4 U/ml xanthin oxidase were rapidly added. After shaking of the mixture for 5 s on a Voltex mixer. ESR spectrum was measured. Relative intensity of O_2^- is represented by the ratio of intensity of the first peak in the spectrum of DMPPO- O_2^- spin adduct to intensity of third peak for spectrum of Mn^{2+} .

Measurement of inhibition for hydroxyl radicals OH was obtained by Haber-Weiss reaction between hydrogen peroxide and $Fe(II)$. To 50 μ l of 0.01mM or 1 μ M $Fe(II)$, 50 μ l of 0.1M phosphate buffer (pH 7.8), 50 μ l of water sample, 50 μ l of 0.1mM hydrogen peroxide and 5 μ l of DMPPO were rapidly added. After shaking of the mixture for 5 s, ESR spectrum was measured. Relative intensity of OH is represented by the ratio of intensity of the first peak in the spectrum of DMPPO- OH spin adduct to intensity of Mn^{2+} [4].

RESULTS AND DISCUSSION

Changes in state of water treated with electrolyzing

The cathod solution treated with electrolyzing at 20 V for 1-30 min in the presence of 100-1000 ppm of calcium chloride solution was prepared. pH and conductivity of the cathod solution treated by electrolyzing for 5 min increased with increasing in concentration in the range of 100-1000 ppm calcium ion and in electrolyzing time. However, inhibition of O_2^- wasn't in proportion to decline of the calcium concentration, optimum concentration of calcium against inhibition of O_2^- observed as shown in Table 1. The most strongest inhibition of O_2^- in 37.6% was obtained by electrolyzing for 5 min in addition of 300 ppm calcium ion. A strong inhibition against OH formation also was observed at 5 min of electrolyzing time in addition of 300 ppm of calcium ion (Table 2). Therefore, it was found that optimum condition of reaction between the water and AOS was obtained. Line width of the cathod solution by NMR spectroscopy decreased in 55.0 Hz in condition of electrolyzing for 5 min at 300 ppm of calcium ion from 101.0 Hz in control. There was a correlation between inhibition against O_2^- and OH formation and activity of water. Decrease in line width of water indicates activation of state of water in order to extend the relaxation time by ^{17}O -NMR. Therefore, it is considered that the water treated with electrolyzing is liable to react with the AOS by decreasing of size of cluster of water.

TABLE 1 Changes in pH, conductivity and inhibition of the O_2^- formation on cathodic water obtained by electrolysis.

Electrolysis (min)	pH	Conduct. (mS/cm)	O_2^-	
			O_2^-/Mn^{2+}	inhibition(%)
0	5.23	0.509	2.71	-
1	5.90	0.510	2.17	20.0
5	6.63	0.499	1.69	37.6
10	8.17	0.507	1.85	31.9
20	8.71	0.507	1.98	26.8
30	9.01	0.515	2.01	25.7

TABLE 2 Changes in inhibition of the OH formation and line width in NMR by use of water treated with electrolysis.

Electrolysis (min)	$\cdot OH$		Line width (Hz)
	$\cdot OH/Mn^{2+}$	Inhibition(%)	
0	5.07	-	101.0
1	4.51	11.1	90.0
5	1.85	63.5	55.0
10	2.21	56.4	70.0
20	-	-	83.5
30	-	-	85.0

Changes in state of water treated with irradiation of far infrared ray

A water kept for 10-180 min at 50°C in the ceramic vessel which irradiate far infrared ray was prepared. a small increase in pH and conductivity of water irradiated far infrared ray was observed. Inhibition of O_2^- and OH increased with increasing of irradiation time. The water without calcium ion resulted in a great increase in inhibition of O_2^- as compared with water including calcium ion of 300 ppm. Line width of water treated with the far infrared ray decreased in 70.0 Hz at 30 min irradiation from 101.5 Hz in control. In the water treated with irradiation of far infrared ray, an adequate correlation between inhibition against O_2^- and OH and line width was observed in the same manner as electrolysis.

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