— **Regular Article** —

In Vitro Physicochemical Properties of Neutral Aqueous Solution Systems (Water Products as Drinks) Containing Hydrogen Gas, 2-Carboxyethyl Germanium Sesquioxide, and Platinum Nanocolloid as Additives

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(Received August 28, 2009; Accepted January 13, 2010; Published online January 29, 2010)

We studied the *in vitro* antioxidant activities of neutral aqueous solution systems (water products marketed as drinks) containing hydrogen gas (H_2), 2-carboxyethyl germanium sesquioxide (Ge-132), and platinum (Pt) nanocolloid as additives. We evaluated the abilities of these aqueous solutions to inhibit the oxidation of biomolecules catalyzed by an enzyme and induced by reactive oxygen species (ROS) and also to scavenge ROS directly using electron spin resonance (ESR) spectrometry. The concentrations of inorganic elements including Ge and Pt were measured by inductively coupled plasma-mass spectrometry (ICP-MS) and inductively coupled plasma-atomic emission spectrometry (ICP-AES). All the water products examined more or less inhibited the oxidation of 3,4-dihydroxyphenylalanine by tyrosinase and that of L-histidine in an L-ascorbic acid/Cu^{2+} reaction system. The results of ICP-MS and ICP-AES analyses revealed that Ge, Pt, and some major minerals existed in the water products at concentrations approximately equivalent to those reported by their manufacturers. The ESR spectra indicated that the dissolved Ge-132 molecules and the supplemented Pt nanocolloid particles reduced hydroxyl and superoxide anion radicals. However, under the conditions employed, aqueous H_2 did not display such a scavenging ability for these ROS. Our results suggest that H_2, Ge-132 and Pt nanocolloid dissolved or supplemented in neutral aqueous media exhibited antioxidant activities *in vitro* due to the direct scavenging of ROS and/or by other mechanisms.

Key words —— hydrogen gas, germanium-132, platinum nanocolloid, antioxidant activity, reactive oxygen species

INTRODUCTION

It is generally accepted that various diseases are caused by the actions of reactive oxygen species (ROS). In recent years, many companies have developed and sold water products as drinks, and some of these drinks are advertised as being able to scavenge the harmful ROS in the human body (e.g., http://www.kangen-water.jp; http://water-institute.org; http://www.hitatenryosui.com, *etc.*). We have been studying the *in vitro* physicochemical properties[1] and the *in vivo* effects[2] of some of these aqueous solution systems. We previously examined the existence of several mildly alkaline aqueous solutions (alkaline ionized waters, AIWs), that were assumed to contain active hydrogen (H) as the effective component.[1] In that study, we confirmed that the *in vitro* antioxidant activities of these AIWs were derived not from H but from the ordinary dissolved hydrogen gas (H_2) and/or originally contained reductive cations, such as vanadium (V) ion(s).[1] We also revealed that ingesting an AIW did not significantly reduce oxidative stress *in vivo*.[2] In this study, we investigated the *in vitro* physicochem-

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ical properties of neutral aqueous solution systems (water products marketed as drinks) containing H_2, 2-carboxyethyl germanium sesquioxide (Ge-132), and platinum (Pt) nanocolloid, as additives expected to exhibit the antioxidant activities.

MATERIALS AND METHODS

Materials —— Neutral H_2-rich water (H_2W), advertised as containing H_2 0.34 mg/l (0.17 mM) in tap water (TW) as the solvent, was purchased from Blue Mercury Co., Ltd. (Tokyo, Japan). A TW solution without H_2, as the control for H_2W ($-H_2W$), was supplied by Dr. Yasutoshi Kiyota of the same company. An aqueous solution containing H_2 at a saturating concentration of 1.5 mg/l (= 0.75 mM) (SH_2W), prepared by dissolving H_2 in deionized water (DW), was supplied by Shigeo Ohta of Nihon Medical University (Kawasaki, Japan). Nanocluster germanium water-400 (GeW-400), -800 (GeW-800), and -1200 (GeW-1200), as well as an authentic sample of Ge-132, were purchased from Organic Germanium Co., Ltd. (Itami, Japan). According to the company, the process for preparing these water products (GeWs) is as follows. Ge-132 400 mg (1.2 mmol), 800 mg (2.4 mmol), and 1200 mg (3.5 mmol) was dissolved in 1 l of a natural water solution containing major minerals, such as Na (11.8 mg/l), K (4.2 mg/l), Ca (9.9 mg/l), and Mg (2.5 mg/l), to yield 171.2, 342.4, and 513.6 mg/l as the Ge concentrations, respectively. Pt-water (PtW) was purchased from Apt Co. Ltd. (Tokyo, Japan), which advertised the product as being prepared by supplementing 4 μg of Pt nanocolloid particles (diameter 3 nm) in 500 ml of a natural water solution with dissolved Na (6.5 mg/l), K (0.55 mg/l), Ca (10.0 mg/l), and Mg (2.3 mg/l). A DW solution (10 ml) supplemented with 3.5 mg of the Pt nanocolloid particles was supplied by Dr. Takeshi Uehara of the same company. Next, standard solutions containing only Ge-132 or the Pt nanocolloid were prepared as follows. The authentic sample of Ge-132 was dissolved in DW to give the concentration of 400 mg/l–10 g/l, while the Pt nanocolloid solution was diluted with DW from 3.5 mg/10 ml (= 350 mg/l) to 50–175 mg/l. The standard solutions of Ge-132 and Pt nanocolloid at various concentrations, as well as SH_2W and $-H_2W$, were examined for their ROS-scavenging abilities using electron spin resonance (ESR) spectrometry, as described below.

Inhibition of Biomolecule Oxidation —— Two reaction systems were used: the oxidative polymerization of 3,4-dihydroxyphenylalanine (dopa) by tyrosinase and the oxidative decomposition of L-histidine (His) by ROS in an L-ascorbic acid (AsA)/Cu^{2+} system. These two reaction systems were employed as to detect the abilities of the aqueous solutions examined to inhibit the oxidation of biomolecules by reducing the reaction products to original substances and by directly scavenging ROS, respectively.

For the first assay, 1.1 ml of 3 mM dopa in 100 mM of phosphate buffer (pH 7.4) was mixed with 1.8 ml of each aqueous solution sample including DW and TW as the controls. The reaction was initiated by the addition of 120 units of mushroom tyrosinase (Sigma Chemical Co., St. Louis, MO, U.S.A.) dissolved in 0.1 ml of DW to the buffer-sample mixtures. As described by Lee,[3] a linear increase in the optical density at 470 nm was initially observed at room temperature. Aqueous oxalic acid (5 and 10 mM), an enzymologic inhibitor of tyrosinase,[4] was also tested in the same manner for comparison.

For the second assay, His and AsA were dissolved in 50 mM phosphate buffer (pH 7.4) at concentrations of 2.5 and 10 mM, respectively. His was selected due to its high sensitivity to oxidation by AsA/Cu^{2+} systems.[5–7] A 0.8-ml aliquot of these prepared buffer solutions was added to 3 ml of the same aqueous solutions as assayed in the first one. The reaction was then initiated by adding 0.2 ml of 0.4 mM aqueous $CuSO_4$. Stoppered vials were filled with these reaction mixtures and left at 37°C for 2 hr, until the reaction was terminated by tetrasodium ethylenediaminetetraacetate at a final concentration of 0.1 mM in each reaction mixture. Finally, the remaining His was quantified with an amino acid analyzer. For the sake of comparison with a known potent oxidation inhibitor for the AsA/Cu^{2+} system,[7] 2000 units of catalase (Wako Pure Chemicals, Tokyo, Japan) in 3 ml of DW was evaluated in the same manner.

Others —— To detect the antioxidant effects of H_2, which could be dissolved in original natural water solutions used in the production of GeWs and PtW, these products were boiled and recooled in an open container and then examined using the aforementioned reaction systems. Moreover, to evaluate the size of the supplemented Pt nanocolloid particles in PtW, the product was centrifuged at 2000 g for 10 min with a Centricon-3 miniconcentrator (Ami-

con Japan, Tokyo, Japan) having a cut-off molecular weight (MW) of 3 kD, and the antioxidant activity of the ultrafiltrate was examined in the same manner.

Analyses of Inorganic Elements —— Inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS) were performed, as reported elsewhere,[8] to quantify Ca, Fe, K, Mg, Na, P, Sr, and Si (using ICP-AES) and 63 other elements including Ge and Pt (using ICP-MS) in all the sample solutions.

ESR Spectrometry —— Two ESR systems (I and II) were employed; the former and the latter were used for evaluation of the scavenging ability of the assayed solution samples for hydroxyl radical (·OH) and simultaneous determination of ·OH and superoxide anion radical ($\cdot O_2^-$) in the presence of the antioxidant additives examined, respectively. In both systems I and II, the ratios of signal height of ·OH (and $\cdot O_2^-$) against that of Mn^{2+} as a reference were obtained for all the assayed solutions including the controls (DW and $-H_2W$), and the scavenging ability of each sample for these ROS was obtained as a percentage of the signal height relative to that of the corresponding control. Other conditions for ESR spectrometry were same as those reported in our previous paper,[1] unless explicitly mentioned in this paper.

An aliquot (20 μl) of 1% aqueous dimethyl-1-pyrroline-*N*-oxide (DMPO) was added to a mixture (180 μl in total) of 0.2 mM aqueous $FeSO_4$ (37.5 μl), 0.1 mM aqueous diethylenetriamine-*N*,*N*,*N*′*N*′-pentaacetate (DTPA) (37.5 μl), the aqueous solution samples to be examined (30 μl), and 1 mM aqueous H_2O_2 (75 μl). The purchased water products, SH_2W, and the standard solutions of Ge-132 at 5 and 10 g/l and those of Pt nanocolloid at 100 and 200 mg/l were assayed as sample solutions with DW as the control. For comparison, aqueous AsA, a well-known ·OH scavenger, was also assayed in the same manner at concentrations of 12.5, 25, and 50 mM.

The procedures were the same as those applied in the previous experiments,[1] except for the apparatus JEOL Japan, Akishima, Japan (JEOL JES-FA100), magnetic field (335.3 mT), the operating frequency (9.414 GHz), modulation amplitude (50 mT), and the time at which scanning commenced (3 min after the addition of H_2O_2). That is, 50 μl of dimethyl sulfoxide (DMSO), the same volume of 25 mM NaOH, and the sample solutions to be examined were mixed in a dispensable plastic tube, followed by the addition of 5 μl of DMPO and 50 μl of 30% H_2O_2. It was speculated that in such reaction systems containing H_2O_2, NaOH, and DMSO, several radicals including ·OH and $\cdot O_2^-$ were generated by following step reactions:[9]

H_2O_2+NaOH→ ·OH+$\cdot O_2^{--}$;

·OH+H_2O_2 → $\cdot O_2^-$+H_2O+H^+; and

·OH+DMSO→methane sulfonic acid +·CH_3.

As in previous experiments,[1,9] to remove the influence of time-dependent change of the ($\cdot O_2$)-DMPO adduct into the (·OH)-DMPO adduct, the sample solutions including the controls were added prior to generation of the ROS. It was also confirmed based on ESR spectra obtained when employing such a reaction system that L-AsA and tocopherols exhibited scavenging ability for ·OH and $\cdot O_2$ in a concentration-dependent manner,[9] although it is difficult to determine $\cdot O_2^-$ precisely in the reaction mixtures containing both (·OH)-DMPO and (·OOH)-DMPO adducts. Similarly, the xanthine/xanthine oxidase reaction system, which produces not only (·OOH)-DMPO but also (·OH)-DMPO, is frequently applied to ESR spectrometry in evaluating the $\cdot O_2^-$-scavenging ability of various samples. Moreover, the values for the ·OH and $\cdot O_2^-$ quantities were simultaneously calculated from intensity of the corresponding ESR signals obtained on the basis of step reactions to generate various radicals.[10,11]

In this study, the samples other than the DW control were purchased water products, $-H_2W$, and the standard solutions of Ge-132 at concentrations of 400, 800, and 1200 mg/l and those of Pt nanocolloid at 50, 87.5, 175, and 350 mg/l, respectively. The reaction mixtures were aspirated into the quartz flat cell and set in the ESR apparatus, and then scanning was started at 3 min after the addition of H_2O_2. The signal height was calculated using a radical analyzer program attached to the instrument, and the calculation was done for positive signal height of the (·OH)-DMPO adduct and negative signal height of the ($\cdot O_2^-$)-DMPO adduct in the lowest magnetic field. In this system, the ESR spectra were generally obtained three times for one sample solution. However, in the case of the standard solution of Pt nanocolloid at 50 mg/l, the ESR spectra were recorded six times.

Table 1. Inhibition by Aqueous Solutions of Tyrosinase-Catalyzed Oxidative Polymerization of Dopa (A) and ROS-Induced Oxidative Degradation of His in an L-AsA/Cu^{2+} System (B)

Reaction	GeWs			PtW	H_2W	Oxalic acid		Cat[a)]	TW
System	400	800	1200			5 mM	10 mM		
A	93*	81*	66*	71*	78*	63*	54*	—[b)]	103
	(7%)	(19%)	(34%)	(29%)	(22%)	(37%)	(46%)		(−3%)
B	90**	74**	55**	64**	82**	—[b)]	—[b)]	2**	107
	(10%)	(26%)	(45%)	(36%)	(18%)			(98%)	(−7%)

a) 2000 units catalase. *b*) Not assayed. *UV absorbance (at 470 nm) of samples, expressed as a percentage relative to the DW control. **Loss in His content relative to the DW control.

Figures in the parentheses indicate the inhibition rate for the corresponding samples in the reaction systems A (*) and B (**), respectively.

RESULTS AND DISCUSSION

Inhibition by the aqueous solution samples of the oxidation of biomolecules in the employed two reaction systems is summarized in Table 1. In reaction system A, shown in Table 1, all the samples other than the controls (DW and TW) more or less inhibited the tyrosinase-catalyzed oxidative polymerization of dopa. The highest inhibition rate observed among the purchased water product samples (34% for GeW-1200) approximated that for 5 mM oxalic acid as an enzymologic inhibitor (37%). Tyrosinase catalyzes the oxidation of dopa and other orthodiphenols to yield their corresponding orthoquinones, which easily undergo a nonenzymatic transformation into melanin-type pigments.[12, 13)] Using quercetin-3-O-rhamnoglucoside (rutin) as a substrate, we previously elucidated that tyrosinase causes the formation of an orthoquinone from rutin and that this process was significantly suppressed by AIWs containing H_2.[1)] We speculate that the similar inhibition of the enzymatic oxidation of dopa by these neutral aqueous solutions may be due to a reduction by their antioxidant additives of an orthoquinone as the oxidation product to dopa as the original substrate. The inhibition by H_2W may also be caused by a lack of dissolved oxygen (O_2), a part of which spontaneously changes into ROS, since we did not determine O_2 in the reaction mixture. However, as reported previously, the DW extract of metallic Mg powder inhibited the tyrosinase-catalyzed oxidation of rutin, although aqueous $Mg(OH)_2$ did not affect the enzyme reaction.[1)] Hence, we speculate that the enzymatic oxidation of polyphenolics, such as rutin and dopa, is suppressed at least partly by the reducing function of aqueous H_2.

In reaction system B, the concentration of the remaining His in the reaction mixture for the DW control was 238 μM, suggesting that 262 μM of His was oxidatively degraded by reacting with the ROS formed in the AsA/Cu^{2+} system. It is well known that in the L-AsA/Cu^{2+} system, H_2O_2, which is produced by the Cu^{2+}-induced oxidation of AsA, undergoes degradation by Fenton reaction to produce ·OH in the presence of Cu^{1+}, which is formed during the reduction of Cu^{2+} by AsA. Under the conditions employed, as shown in Table 1, the sample for catalase (2000 units) showed the greatest suppression of the oxidative decomposition of His (inhibition rate 98%). All the water product samples significantly reduced the loss of His (10–45%), suggesting that they contain substances, that can scavenge ·OH and/or other ROS derived from ·OH (Table 1).

These results suggest that the dissolved Ge-132 and H_2 molecules, as well as the supplemented Pt nanocolloid particles, in the corresponding water products inhibit various oxidative reactions of biomolecules *in vitro*. The mechanisms underlying these phenomena are discussed below, in association with the ESR spectrometry data. The presence of chloride and oxidative transient metals was probably the cause of a slight increase in the oxidation by TW (Table 1).

The inhibitory effects of GeWs and PtW on the biomolecule oxidation using the reaction systems A and B were unaffected by boiling and recooling, and by ultrafiltration in the case of PtW. This indicates that H_2 is not contained in these products and that the size of the supplemented Pt nanocolloid particles was smaller than that of the solutes with a MW of 3 kD.

ICP-MS analyses of inorganic constituents revealed that PtW contained Pt at a concentration of 4.8 μg/l, which is 60% of the value reported on the product labels (4 μg/500 ml = 8 μg/l). The Ge concentrations in GeWs were 156.0 mg/l for GeW-400, 294.3 mg/l for GeW-800, and 520.2 mg/l for GeW-1200. The Ge concentration values were 91%, 86%,

Table 2. ROS-Scavenging Abilities of Antioxidant Additives in Water Products as Detected Using ESR Spectrometry

Antioxidant concentration	System I For ·OH	System II For ·OH	System II For $\cdot O_2^-$
Ge-132			
400 mg/l (= 1.2 mM)	N.D.[a)*]	N.D.[a)]	N.D.[a)]
800 mg/l (= 2.4 mM)	N.D.[a)*]	N.D.[a)]	−9.8 ± 7.1% (mean ± S.D., n = 3)
1200 mg/l (= 3.5 mM)	N.D.[a)*]	−3.1 ± 8.9% (mean ± S.D., n = 3)	−8.4 ± 2.2% (mean ± S.D., n = 3)
5 g/l (= 15 mM)	−19.0%	—[c)]	—[c)]
10 g/l (= 30 mM)	−23.4%	—[c)]	—[c)]
Pt nanocolloid			
8 μg/l (= 0.04 μM)	N.D.[a)*]	N.D.[a)*]	N.D.[a)*]
50 mg/l (= 0.26 mM)	N.D.[1)]	N.D.[1)]	−7.6 ± 5.7% (mean ± S.D., n = 6)
85 mg/l (= 0.44 mM)	—[3)]	N.D.[1)]	−18.6 ± 5.1%[d)] (mean ± S.D., n = 3)
100 mg/l (= 0.51 mM)	−36.6%	—[c)]	—[c)]
175 mg/l (= 0.9 mM)	—[c)]	−14.9 ± 10.3% (mean ± S.D., n = 3)	−31.8 ± 7.2%[d)] (mean ± S.D., n = 3)
200 mg/l (= 1.0 mM)	−59.9%	—[c)]	—[c)]
350 mg/l (= 1.8 mM)	—[c)]	−25.2 ± 6.7%[4)] (mean ± S.D., n = 3)	−67.3 ± 3.0%[d)] (mean ± S.D., n = 3)
H_2			
0.34 mg/l (= 0.17 mM)	N.D.[a)*]	N.D.[b)*]	N.D.[b)*]
1.5 mg/l (= 0.75 mM)	N.D.[a)*]	N.D.[b)]	N.D.[b)]
AsA			
12.5 mM	−71.2%	—[c)]	—[c)]
25 mM	−88.3%	—[3)]	—[3)]
50 mM	−93.8%	—[3)]	—[3)]

a) and *b*) Not detected (calculated value for the ·OH or $\cdot O_2^-$ level is higher than that obtained with DW[a)] or $-H_2W$[b)] as the control, see text); *c*) Not assayed. *d*) Significantly different from the value for the DW control ($p < 0.05$, by Student's t-test), *: Data obtained when using commercially purchased water products. Abbreviations are defined in the text.

and 101%, respectively, of those calculated from the values reported on the product labels (400, 800, and 1200 mg/l as Ge-132) and those of the atomic mass for Ge (72.6), C (12.0), H (1.0), and O (16.0), respectively. It may be assumed that the Pt nanocolloid particles are not as adequately introduced into the matrix for ICP-MS as the solute molecules are. It was therefore hypothesized that both Ge-132 and the Pt nanocolloid particles are actually present in GeWs and PtW at concentrations similar to those reported by their manufacturers. In the other aqueous solutions examined, the concentrations of Ge and Pt, as determined by ICP-MS, did not exceed the detection limit (0.1 μg/l), and some other minor metals, such as Fe, Cu, and Zn were barely detected in all samples examined (data not shown). The levels of major minerals (Na, K, Ca, and Mg) in GeWs and PtW, as determined by ICP-AES, roughly approximated those reported on their labels (data not shown).

The direct ROS-scavenging abilities of the antioxidant additives in the water products, as indicated by the ESR spectra, are summarized in Table 2. In the ESR system I, as shown in Table 2, GeWs and PtW exhibited no scavenging ability for ·OH, although the standard solutions of Ge-132 reduced ·OH at concentrations of 5 g/l and 10 g/l in a concentration-dependent manner, as did 12.5–50 mM aqueous AsA. In system II, where the ESR spectra were recorded three times for each sample containing Ge-132 or the Pt nanocolloid, values for the ·OH and $\cdot O_2^-$ quantities varied considerably. However, when the standard solutions of Ge-132 at same concentrations as GeWs (400, 800, and 1200 mg/l) were applied to this system, the values for the ·OH quantity obtained only at the solute concentration of 1200 mg/l tended to be smaller than that at 0 mg/l (DW), and the $\cdot O_2^-$ level also

tended to decrease only at 800 and 1200 mg/l as compared with the DW control (Table 2). The ROS-scavenging effect of PtW was not confirmed in these two ESR systems, but the standard Pt nanocolloid solutions at various concentrations exhibited a concentration-dependent ability to scavenge ·OH (in systems I and II) and $\cdot O_2^-$ (in system II). In system I, the scavenging ability for ·OH of the standard solution at 200 mg/l (reducing ratio 59.9%) was considerably greater than that at 100 mg/l (36.6%), indicating that the reducing ratio at 200 mg/l (which would be 1.0 mM if it were the solute) was only slightly lower than that of an aqueous solution containing AsA at 12.5 mM (71.2%) (Table 2). In system II, ·OH was also significantly scavenged by the solution containing Pt nanocolloid at 350 mg/l ($p < 0.05$) and tended to be scavenged at 175 mg/l, but not at 50 and 80 mg/l (Table 2). $\cdot O_2^-$ was also significantly reduced by the solutions at 85–350 mg/l in a concentration-dependent manner, and tended to be reduced at 50 mg/l (Table 2). It was also noteworthy that lesser variations in the data were clearly associated with higher concentrations of Ge-132 and Pt nanocolloid in the sample solutions, respectively (Table 2). These results indicate that the dissolved Ge-132 molecules and the supplemented Pt nanocolloid particles concentration-dependently exhibit antioxidant activities in neutral aqueous media by directly scavenging ROS. Therefore we speculate that at least part of the antioxidant activity of GeWs and PtW in the reaction system B may be a result of the direct scavenging of ROS by the corresponding additives. These data support the reports that paraquat-induced oxidative stress in mice was alleviated by Ge-132[14] and that Pt nanocolloid is an excellent scavenger of $\cdot O_2^-$ and H_2O_2.[15] The ESR spectra in the system II for the DW control and a sample containing the Pt nanocolloid at 50 mg/l are shown in Fig. 1.

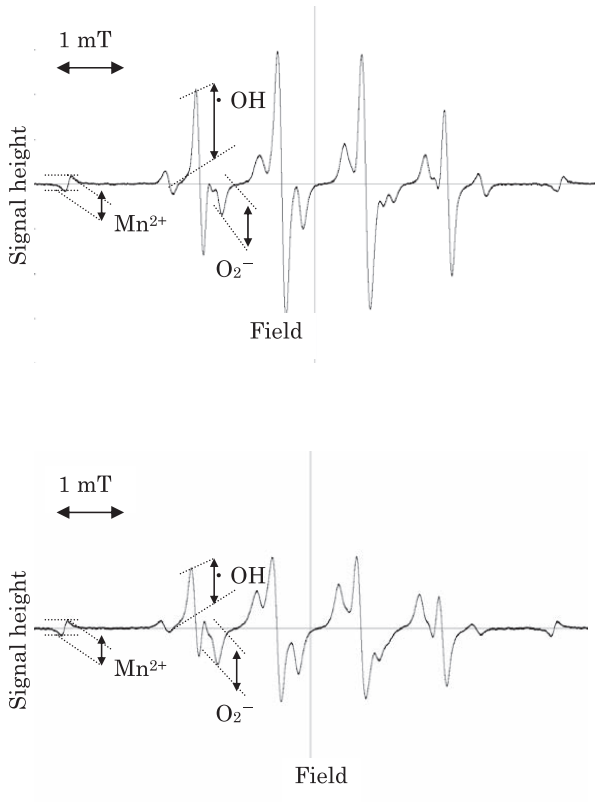

Fig. 1. ESR Spectra of the DW Control (Upper Panel) and Sample Solution Containing Pt Nanocolloid 50 mg/l (Lower Panel) in the H_2O_2/NaOH/DMPO System (System II)

As shown by these spectra, several radicals other than ·OH and $\cdot O_2^-$ are generated, although it is assumed that they have virtually no effect on simultaneous measurement of the scavenging ability for ·OH and $\cdot O_2^-$ of sample solutions examined (see text).

It has been demonstrated that SH_2W scavenges only ·OH among the ROS.[16] However, under the conditions employed in this study, the scavenging ability of aqueous H_2 of ·OH was not displayed (Table 2), possibly due to the lower sensitivity of our ESR systems. The data suggesting that H_2 exhibited no directly scavenging ability of $\cdot O_2^-$ in systems I and II (Table 2) also is in contrast to our previous results obtained using ESR that the $\cdot O_2^-$ level was markedly reduced by adding AIWs containing H_2.[1] This discrepancy might have arisen because of the following reasons: in this study, $-H_2W$ instead DW was used as the control in the system II; and under the alkaline conditions employed in the previous experiments[1] and the system II in this study, $\cdot O_2^-$ is unstable and easily reacts with the sample solution components other than H_2. It is noteworthy that in the oxidation of biomolecules, the inhibitory effect of H_2W relative to that of GeWs and PtW appeared to be greater in reaction system A catalyzed by an enzyme than in B induced by ROS (Table 1). Therefore we speculate that aqueous H_2 functions mainly as an agent that may reduce other substances without directly scavenging ROS. Concerning the *in vivo* antioxidant activities of H_2, it was reported that H_2 significantly reduced various types of oxidative stress-induced damage in rats and mice.[17–21] The above-described results of our *in vitro* experiments suggest that such *in vivo* effects of H_2 may be caused not only by the direct scavenging of ·OH[16] but also by other mechanisms.

In conclusion, the neutral aqueous solution systems examined (water products, as drinks) contain-

ing H_2, Ge-132, and Pt nanocolloid exhibit antioxidant activities *in vitro*, and in some cases, in *in vivo* animal experiments, due to the properties of the dissolved or supplemented additives. However, the actual *in vivo* effects in humans of these additives when ingested orally and absorbed from the intestine remain to be elucidated. A study on the *in vivo* effects of aqueous H_2 in humans is now in progress.

The toxic effects, especially those of Ge-132 and Pt nanocolloid, should also be studied. In animal experiments, Ge-132 acted not only as a compound expressing antioxidant,[14] immunostimulating,[22] and antitumor[23] activities but also as an agent for hepatic and renal dysfunction.[24] Moreover, we have recently found that the ingestion of PtW led to an acute hepatic disturbance in healthy humans.[25] The details will be reported in a subsequent paper.

Acknowledgements We express our gratitude to Dr. Takeshi Uehara of Apt Co. Ltd., Dr. Yasutoshi Kiyota of Blue Mercury, Co. Ltd., and Prof. Shigeo Ohta of Nihon Medical University for supplying the standard solutions of Pt nanocolloid (3.5 mg/10 ml), $-H_2W$, and SH_2W, respectively.

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