

Platinum Nanoparticle Catalyst Scavenges Hydrogen Peroxide Generated from Hydroquinone

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Platinum, palladium, gold, rhodium, and silver monometallic nanoparticles protected by poly(*N*-vinyl-2-pyrrolidone), a water-soluble polymer, were prepared using an alcohol reduction method. Platinum/silver bimetallic nanoparticles were prepared by self-organization from the platinum and silver monometallic nanoparticles. Platinum nanoparticles showed the highest catalytic activity for the decomposition of hydrogen peroxide in the monometallic nanoparticles used in this study. Platinum, silver, and platinum/silver nanoparticles effectively catalyzed the decomposition of hydrogen peroxide generated from the autooxidation of hydroquinone, a derivative of carcinogenic benzene. The autooxidation of hydroquinone itself was hardly inhibited by platinum nanoparticles. The platinum nanoparticles in particular showed the highest catalytic activity per unit atom. The activity of a 2 μg platinum nanoparticle was comparable to that of 20 units of catalase. The modification of platinum nanoparticles with silver rather suppressed the activity of hydrogen peroxide decomposition. These results suggest that Pt nanoparticles can be used as antioxidants against oxidative chemical compounds.

The modification of biomacromolecules upon exposure to reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), dioxide(1−) (superoxide $O_2^{\bullet-}$), hydroxyl radical ($HO^{\bullet}$), and singlet oxygen ($^1O_2$), is the likely initial event involved in the induction of the mutagenic and lethal effects of various oxidative stress agents.[1–3] Therefore, the activity of ROS generation by various chemical compounds is closely related to their toxicity, carcinogenicity, or both. For example, hydroquinone, a metabolite of carcinogenic benzene, causes DNA damage via H_2O_2 generation.[4] Many studies have addressed the role of antioxidants, such as vitamins[5,6] and catechins,[7] in protection against cancers and cardiovascular diseases. These antioxidants can scavenge ROS and protect against cancer occurrence. On the other hand, every antioxidant is in fact, a redox agent, protecting against ROS in some circumstances and promoting free radical or secondary ROS generation in others. Indeed, an excess of these antioxidants elevates the incidence of cancer.[8,9] Solovieva et al. reported that antioxidants, ascorbic acid[10] and dithiothreitol,[11] exhibit cytotoxicity via H_2O_2 generation. Relevantly, it has been reported that vitamins A[12] and E[13] and catechins[14] induce DNA oxidation through H_2O_2 generation during their oxidation. H_2O_2 is a long-lived ROS which plays an important role in biomacromolecular damage induced by various chemical compounds.[2,4]

Various studies have demonstrated the catalytic decomposition of H_2O_2 by noble metals such as platinum (Pt),[15–17] palladium (Pd),[16–18] silver (Ag),[19–21] and gold (Au).[18,21] These metals themselves are hardly oxidized by ROS, however, it is difficult to use metal powder or foils as anti-oxidative drugs. Recently, Kajita et al. reported that Pt nanoparticles catalyze the decomposition of ROS.[22] These nanoparticles can be dispersed in water and used as homogenous solution. Because this removal mechanism is catalytic decomposition, no oxidized product is formed through this reaction. Platinum metal is used as a food additive and is not considered to be a toxic material. This result led us to the idea that inorganic materials, in particular noble metals, rather than organic antioxidants, can be used as novel chemopreventive agents against ROS-mediated biomolecules damage. In this study, we examined the removal of H_2O_2 generated from a chemical compound, hydroquione, using water-soluble polymer-protected Pt nanoparticles. In addition, the effect of bimetallization[23] of Pt nanoparticles with Ag was investigated. Silver, a relatively inexpensive noble metal, is also used as a food additive.

Experimental

Materials. Dihydrogen hexachloroplatinate(IV) hexahydrate ($H_2PtCl_6 \cdot 6H_2O$), palladium(II) acetate ($Pd(CH_3COO)_2$), rhodium(III) chloride ($RhCl_3$), hydrogen tetrachloroaurate(III) tetrahydrate ($HAuCl_4 \cdot 4H_2O$), silver nitrate ($AgNO_3$), sodium tetrahydroborate ($NaBH_4$), hydroquinone, folic acid, a 30% H_2O_2 solution, ethanol, copper(II) chloride, and nicotinamide adenine dinucleotide disodium salt (NADH) were purchased from Wako Pure Chemical Industries (Osaka Japan). Poly(*N*-vinyl-2-prrolidone) (PVP, K30, MW: 40000) was from Tokyo Kasei Co. (Tokyo, Japan). Sodium phosphate buffer (pH 7.6) was from Nacalai Tesque Co. (Kyoto, Japan). Catalase (45000 units/mg from bovine liver) was from Sigma Chemical Co. (St. Louis, MO, USA).

Colloidal dispersions of PVP-protected Pt, Pd, Rh, and Au nanoparticles were prepared using an alcohol reduction method.[24] 50 mL of water/ethanol (1/1, v/v) solution containing 1 mM metal salts and 40 mM PVP (monomer unit) was refluxed for 2 h, resulting in the formation of typical colored sols of metal

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nanoparticles. The solvent was removed by vacuum evaporation, and the nanoparticles were dispersed into water to prepare 1 mM/atom (atomic concentration) metal colloidal sols. An aqueous solution of PVP-protected Ag nanoparticles[25] was prepared from reduction of 1 mM $AgNO_3$ with $NaBH_4$ in the presence of 40 mM PVP. The obtained Ag colloidal dispersion was purified with an ultra-filter. The Ag/Pt (Ag-atom/Pt-atom, 1/1) bimetallic nanoparticles were prepared using a self-organization method to mix Pt and Ag monometallic nanoparticles according to previous reports.[23,26–28]

Measurements. The absorption spectra of the colloidal sols were measured with a UV–vis spectrophotometer (UV-1650PC, Shimadzu, Kyoto, Japan). The fluorescence intensity of sample solutions was measured with a fluorescence photometer (650-60, Hitachi, Tokyo, Japan). The absorbance of NADH was measured with an absorbance reader (Sunrise, Tecan Group Ltd., Männedorf, Switzerland). Transmission electron microscopy (TEM), a JEM-2100FX electron microscope (JEOL Ltd., Tokyo, Japan), was used to visualize the metal nanoparticles. Samples for TEM measurement were prepared by placing a drop of colloidal dispersion of metal nanoparticles onto a carbon-coated copper microgrid, followed by naturally evaporating the solvent. The average diameter and standard deviation were calculated by counting the diameters of two handled particles with a magnifier (ten times) on the TEM photograph of 200000 magnification.

Measurement of the H_2O_2 Concentration. The generated H_2O_2 was measured by a previously reported method using folic acid.[29] This assay is based on the fluorescence enhancement of less-fluorescent folic acid via oxidative decomposition by H_2O_2 and copper(II) ion into strong-fluorescent 2-amino-4-oxo-3*H*-pterine-6-carboxylic acid. The concentration of H_2O_2 ($[H_2O_2]$) can be determined using a calibration curve. A reaction mixture (1 mL) containing 10 μM folic acid, 20 μM copper(II) chloride, and the H_2O_2 sample (or H_2O_2 generator[4]) with or without the metal nanoparticle in a 10 mM sodium phosphate buffer (pH 7.6) was incubated in a microtube (1.5 mL eppendorf) for 30 min. After incubation at 37 °C, the fluorescence intensity of the reaction mixture at 450 nm was measured using a fluorescence photometer with 350-nm excitation.

Measurement of NADH Consumption. The consumption of NADH during the autooxidation of hydroquinone was measured by a previously reported method.[4] A sample solution containing 100 μM NADH, 50 μM hydroquinone, and 20 μM copper(II) chloride was incubated at 37 °C in the absence or presence of 20 μM/atom Pt nanoparticles. The concentration of NADH was determined by the measurement of absorbance of NADH at 340 nm using a microplate absorbance reader.

Results

Preparation of Metal Nanoparticles. These PVP-protected metal nanoparticles formed water-soluble sols. The average diameters (d) and standard deviations (σ) of monometallic nanoparticles determined by TEM measurement were as follows: Pt ($d = 2.2$ nm, $\sigma = 1.0$ nm), Pd ($d = 2.0$ nm, $\sigma = 0.9$ nm), Rh ($d = 2.2$ nm, $\sigma = 1.0$ nm), Ag ($d = 10.0$ nm, $\sigma = 1.9$ nm), and Au ($d = 10.2$ nm, $\sigma = 2.0$ nm). The absorption spectrum of the sol of Pt nanoparticles is a flat curve (Figure 1A), indicating the formation of homogenous particles. Ag nanoparticles exhibited a typical yellow color due to surface plasmon absorption around 400 nm. It has been reported that a physical mixture of Ag and Pt nanoparticles spontaneously forms bimetallic nanoparticles, possibly Ag-core/Pt-shell structured particles.[26] The time-course of the absorption spectra of this physical mixture showed the extinction of Ag surface plasmon absorption, and the absorption was completely extinguished within 24 h (Figure 1B), suggesting that the surface of the formed bimetallic nanoparticles is composed of Pt atoms. Typical TEM images showed the formation of relatively small particles of Pt and large particles of Ag (Figures 2A and 2B). TEM photographs showed that the large Ag particles disappeared through interaction with Pt particles, resulting in the formation of bimetallic particles smaller than the parent Ag particles (Figure 2C). A similar result has been observed in the case of Ag/Rh bimetallic nanopaticles.[23] These findings suggest the formation of self-organized Ag/Pt bimetallic nanoparticles. These metal nanoparticles are stable in water for several months.

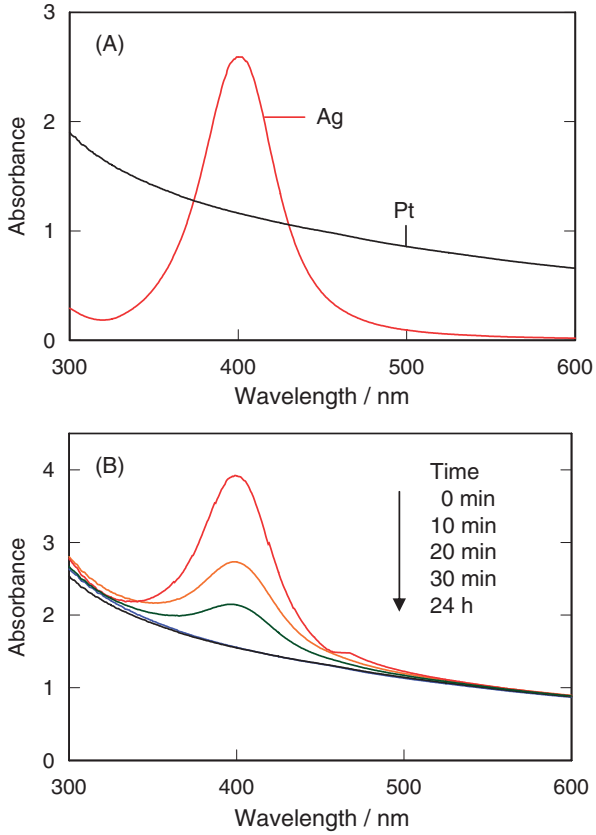


Figure 1. Absorption spectra of PVP-protected metal nanoparticle dispersions. The sample solution contained 0.5 mM/atom Ag or Pt nanoparticles in water (A). Absorption spectral change of the physical mixture of dispersions of Ag and Pt nanoparticles (B). The aqueous solutions of Ag (1 mM/atom, 10 mL) and Pt (1 mM/atom, 10 mL) nanoparticles were mixed and measured at 0, 10, 20, and 30 min, and 24 h after mixing.

Catalytic Decomposition of H_2O_2 by Metal Nanoparticles. Platinum nanoparticles effectively scavenged H_2O_2 in a dose-dependent manner and showed the highest activity among the metal nanoparticles used in this study (Figure 3). A sample solution of 5 μM/atom Pt nanoparticles, among which 1 μg

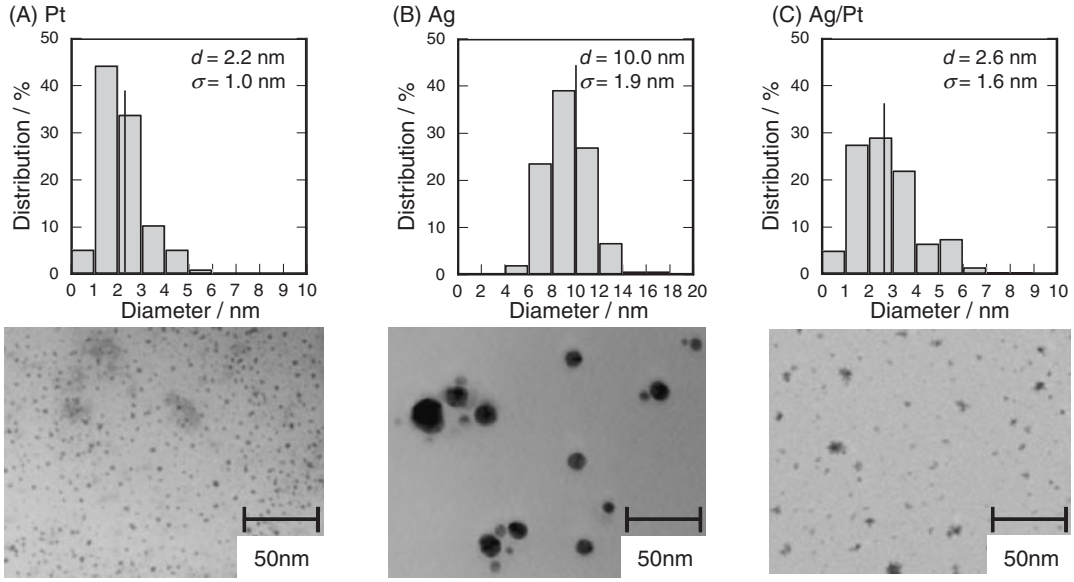


Figure 2. Transmission electron micrographs and particle size distribution histograms of metal nanoparticles. The sample of Ag/Pt nanoparticles was prepared by drying the mixtures of the aqueous solutions of Pt and Ag monometallic nanoparticles 24 h after mixing.

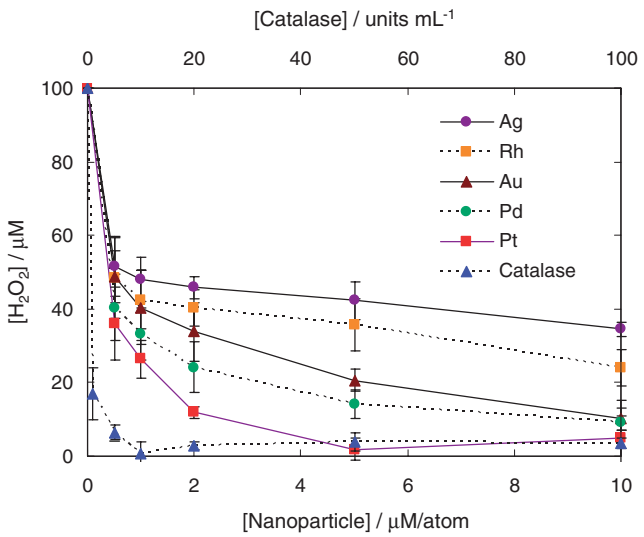
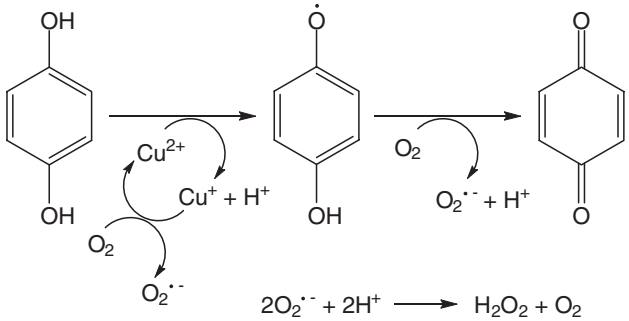


Figure 3. Removal of H_2O_2 by metal nanoparticles and catalase. The 1 mL of sample solution containing 100 μM H_2O_2, 10 μM folic acid, 20 μM copper(II) chloride, and indicated concentration of metal nanoparticles or catalase was incubated for 30 min. The concentration of H_2O_2 was estimated from the fluorescence measurement.

Pt metal is included, exhibits comparable activity for H_2O_2 decomposition to that of 10 units of catalase. One unit of catalase can remove 1.0 μmol H_2O_2 per min in water (pH 7.0, 25 °C). PVP itself did not scavenge H_2O_2 (data not shown). This experiment confirmed that PVP-protected Pt nanoparticles can remove H_2O_2. The mechanism of H_2O_2 removal by Pt nanoparticles can be explained by catalytic decomposition into water and molecular oxygen as follows:

$$H_2O_2 \rightarrow H_2O + \frac{1}{2}O_2 \quad (1)$$



Scheme 1.

The generation of O_2 gas through the H_2O_2 decomposition was confirmed with a gas-burette.[30]

Removal of H_2O_2 Generated from Hydroquinone by Pt and Ag Nanoparticles. The catalytic activity of Pt and its modified particles with Ag (Ag/Pt) on the decomposition of H_2O_2 generated from chemical compounds was evaluated, since Pt showed the highest activity. Hydroquinone, which is a metabolite of carcinogenic benzene, was used as H_2O_2 source. This compound can generate H_2O_2 through autooxidation (Scheme 1).[4] Under these experimental conditions, hydroquinone generated H_2O_2 in a dose-dependent manner (Figure 4). Twenty units/mL catalase effectively removed H_2O_2 generated from this system, and 10 μM/atom (2 μg mL^{-1}) Pt nanoparticles exhibited a comparable activity to that of this catalase. Silver nanoparticles showed apparently weaker activity for H_2O_2 removal than Pt nanoparticles. The bimetallization of Pt with Ag apparently suppressed the catalytic activity per unit atom.

Figure 5 shows the removal activity of H_2O_2 generated from a high concentration of hydroquinone (50 μM) by metal nanoparticles. These metal nanoparticles and catalase scavenged H_2O_2 in a dose-dependent manner. The activity of the 10 μM/atom (2 μg mL^{-1}) Pt nanoparticles was comparable to

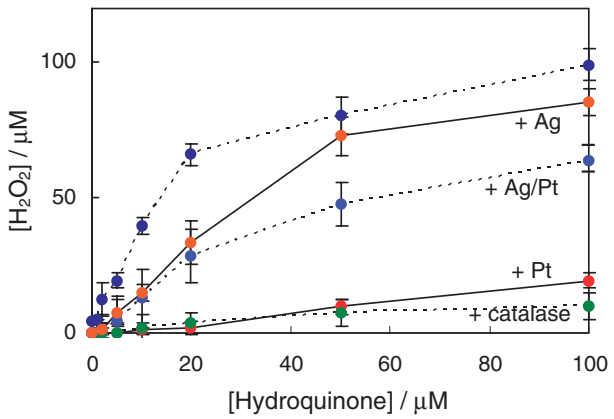


Figure 4. H_2O_2 generation through autooxidation of hydroquinone in the absence or presence of metal nanoparticles and catalase. The 1 mL of sample solution containing 10 μM folic acid, 20 μM copper(II) chloride, and indicated concentration of hydroquinone with or without 10 μM/atom metal nanoparticles or 20 units/mL catalase was incubated for 30 min. The concentration of generated H_2O_2 was estimated from the fluorescence measurement.

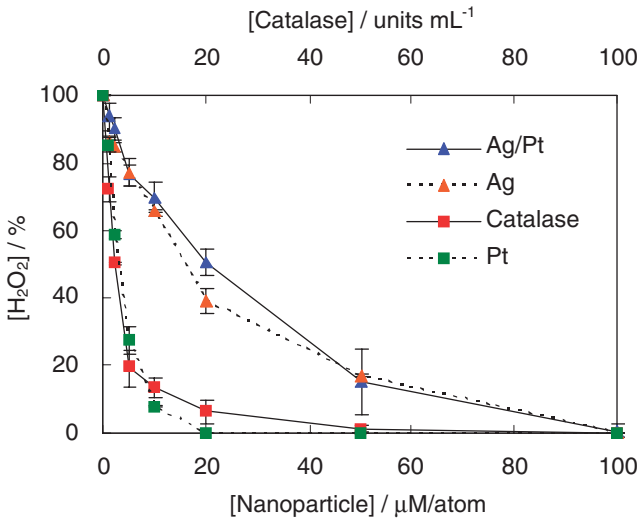


Figure 5. Removal of H_2O_2 generated through the autooxidation of hydroquinone by metal nanoparticles and catalase. The 1 mL of sample solution containing 10 μM folic acid, 20 μM copper(II) chloride, 50 μM hydroquinone, and indicated concentration of metal nanoparticles or catalase was incubated for 30 min. The concentration of H_2O_2 was estimated from the fluorescence measurement.

that of 20 units/mL catalase, and Pt completely scavenged H_2O_2 over 20 μM/atom (4 μg mL^{-1}). The activity per atom of the Ag/Pt bimetallic nanoparticles was almost the same as that of the Ag monometallic nanoparticles.

Effect of Pt Nanoparticles on the Autooxidation of Hydroquinone. To investigate the effect of Pt nanoparticles on H_2O_2 generation through the autooxidation of hydroquinone, NADH consumption during this autooxidation was measured. The oxidized form of hydroquinone can be reduced into the parent hydroquinone by NADH.[4] The concentration of NADH was gradually decreased through the redox of hydroquinone and Pt nanoparticles hardly inhibited NADH consumption (Figure 6). This result indicated that Pt nanoparticles do not inhibit the H_2O_2 generation itself, because H_2O_2 is produced through the autooxidation of hydroquinone.

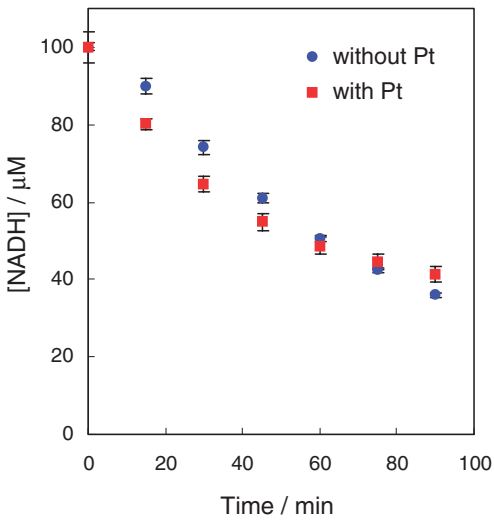


Figure 6. NADH consumption through the autooxidation of hydroquinone in the absence or presence of Pt nanoparticle. The sample solution containing 100 μM NADH, 50 μM hydroquinone, 20 μM copper(II) chloride, and 20 μM/atom Pt nanoparticle was incubated. The concentration of NADH was monitored by absorption measurement at 340 nm.

Discussion

PVP-protected metal nanoparticles, in particular Pt nanoparticles, exhibited a removal effect on H_2O_2 generated through autooxidation of hydroquinone. The H_2O_2 generation was hardly inhibited by Pt nanoparticles. The removal of H_2O_2 by these metal nanoparticles can be explained by a catalytic reaction similar to that by catalase, which decomposes H_2O_2 into H_2O and O_2. The formation of H_2O_2 during autooxidation of hydroquinone is through $O_2^{\bullet-}$, which is generated from a reduction of O_2 by hydroquinone.[4] Because the lifetime of $O_2^{\bullet-}$, which dismutates into H_2O_2 through reaction with H^+, is short (ca. 0.1 ms), the scavenging of $O_2^{\bullet-}$ by a metal nanoparticle can be negligible.[31] The H_2O_2 removal activity per metal atom of these metal nanoparticles occurred in the following order: Pt > Ag ≈ Ag/Pt. The activities of H_2O_2 decomposition per metal atom consisting of these metal nanoparticles (μM-H_2O_2/μM-nanometal) have been estimated from the slope of the initial plots in Figure 5, and the resulting values are 4.2, 12.2, and 3.8 for Ag, Pt, and Ag/Pt, respectively. Further, the activity on the surface area of the Ag/Pt nanoparticles (17 μM-H_2O_2/cm^2-nanometal)[32] was also smaller than that of Pt (49 μM-H_2O_2/cm^2-nanometal). These findings showed that the Pt nanoparticles have the highest catalytic activity for H_2O_2 decomposition of the metal nanoparticles used in this experiment and the activity of Pt nanoparticles is suppressed by modification with Ag.

H_2O_2 is a long-lived ROS and plays an important role in DNA damage.[2,4] Indeed, various chemical compounds, including carcinogens, generate H_2O_2 during redox.[2,4] Molecular

oxygen is easily reduced by various compounds, leading to the formation of $O_2^{\bullet-}$. Formed $O_2^{\bullet-}$ is rapidly dismutated into H_2O_2. Although H_2O_2 itself is not a strong reactive species, it can generate highly reactive $HO^{\bullet}$ through a Fenton reaction or a Haber–Weiss reaction. Furthermore, H_2O_2 can penetrate a cytoplasm membrane and be incorporated into the cell nucleus. Therefore, H_2O_2 is considered to be one of the most important reactive species or a precursor participating in carcinogenesis. The removal of H_2O_2 is an effective method for cancer chemoprevention. Furthermore, protective agents against H_2O_2 are important to treat acatalasemia, a genetic deficiency of erythrocyte catalase inherited as an autosomal recessive trait. Antioxidants, such as vitamins A and E, are effective protective agents. However, the oxidized products of antioxidants or these molecules themselves promote the formation of secondary H_2O_2.[12,13] Indeed, an excess of these antioxidants elevates the incidence of cancer.[8,9] A catalyst consisting of an inorganic stable material is not oxidized and does not generate secondary ROS. Water-soluble nanoparticles of noble metal may become novel protective agents against ROS.

Conclusion

Pt, Ag, and Ag/Pt nanoparticles effectively scavenge H_2O_2 generated from autooxidation of a highly concentrated hydroquinone. Platinum nanoparticles exhibited the highest catalytic activity among these nanoparticles. Pt is a very stable metal against various chemical compounds and permitted as a food additives. The noble metal nanoparticles may be used as novel chemopreventive agents for cancer or other non-malignant conditions induced by chemical compounds through H_2O_2 generation.

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30 The 10 mL of aqueous solution containing 0.1 M H_2O_2 was treated by 10 μg Pt nanoparticles and generated O_2 gas was measured with a gas-burette. The volume of detected gas coincided with that of the theoretically calculated value of O_2 generation from the decomposition of H_2O_2 in the sample solution.

31 Under these experimental conditions, one Pt nanoparticle (average diameter: 2.2 nm) consists of about 370 Pt atoms, indicating that the sample solution of 10 μM/atom Pt contains 0.027 μM Pt nanoparticles. The diffusion control limit of the collision rate (s^{-1}) in aqueous solution is expressed by $7 \times 10^9 \times$ [Particle concentration], and the reciprocal of this value indicates that the effective reaction between $O_2^{\bullet-}$ and one nanoparticle requires more than 5 ms. Because the lifetime of $O_2^{\bullet-}$ is about 1 ms, the decomposition of $O_2^{\bullet-}$ by Pt nanoparticles may be negligible.

32 The total surface area (S) of nanoparticles in sample solution can be expressed as follows: $S = 6cVA_w/\rho d$ where c, V, A_w, and ρ are atomic concentration of nanoparticles, volume of sample solution (1 mL), atomic weight of metal (Ag: 107.87 $g\,mol^{-1}$, Pt: 195.08 $g\,mol^{-1}$, Ag/Pt: average of those of Ag and Pt, 151.48 $g\,mol^{-1}$), and density of metal (Ag: 10.49 $g\,cm^{-3}$, Pt: 21.45 $g\,cm^{-3}$, Ag/Pt: average of those of Ag and Pt, 15.97 $g\,cm^{-3}$), respectively. When V is 1 μM, the S value indicates the total surface area of 1 μM/atom nanoparticles. The activities on the surface area of metal nanoparticles are computable to divide the slopes of Figure 5 by these S values.