Electron Paramagnetic Resonance Study on Free Radical Scavenging and/or Generating Activity of Dopamine-4-O-sulfate

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The free radical scavenging and/or generating activity of dopamine-4-O-sulfate was examined and compared with that of dopamine. In humans, dopamine mostly exists in two isomeric forms of sulfate ester conjugates as metabolites; i.e., dopamine-3-O-sulfate and dopamine-4-O-sulfate in the circulation. Dopamine is generally believed to be oxidized by molecular oxygen or another reactive oxygen species under physiological conditions, to form oxidized dopamine derivatives that are cytotoxic. However, it is not known whether dopamine conjugates are generated on interaction with reactive oxygen species or not. In the present study, we measured the susceptibility to oxidation of dopamine-4-O-sulfate by using electron paramagnetic resonance (EPR) spectroscopy and optical absorption spectrometry. Dopamine was easily oxidized and dopamine-derived radicals appeared, whereas dopamine-4-O-sulfate was not oxidized under physiological conditions. Furthermore, dopamine-4-O-sulfate did not react with a strong oxidizing agent, sodium periodate. These results suggest that dopamine-4-O-sulfate has resistance against autoxidation, and seems to be a stable metabolite of dopamine.

Key words dopamine-4-O-sulfate; dopamine; electron paramagnetic resonance; free radical; oxidation

With the increasing understanding of the cytotoxicity of free radicals and other reactive oxygen species as a major cause of human disease and aging, the roles of endogenous antioxidants have drawn attentions. Uric acid, vitamin E, vitamin C, ubiquinone, SH-compounds, bilirubin, and pyruvate are known as endogenous non-enzymatic antioxidants, while catalase, superoxide dismutase and glutathione peroxidase are known to be enzymatic antioxidants.

Dopamine is the immediate metabolic precursor of noradrenaline and epinephrine. Dopamine interacts with dopamine receptors, then exerts its pharmacological effects.

In human and experimental animals, dopamine is present almost entirely as a sulfate conjugated form in the circulation, and very little of the free form is presented in the plasma. The physiological roles of sulfate conjugated dopamine are still unclear. Pharmacologically, conjugated dopamine has convulsive effects, inhibits aldosterone secretion from cultured bovine adrenal cells, and induces a constriction of some vascular beds. One study, however, found that sulfate conjugated dopamine has no physiological activity.

From the viewpoint of free radical chemistry, dopamine can serve electrons via its oxidation pathway (Chart 1). This enables dopamine to act as both an antioxidant and a prooxidant. It acts as an antioxidant by scavenging free radicals. Recently, it was found that dopamine spontaneously react with molecular oxygen to produce reactive oxygen species and semiquinone dopamine radical, which are toxic to catecholamine neurons.

However, there is no evidence that sulfate conjugated dopamine acts as an antioxidant or a prooxidant. Therefore, in the present report, we compared dopamine and dopamine-4-O-sulfate for susceptibility to enzymatic and non-enzymatic oxidization in vitro using alkaline solution, horseradish peroxidase, UV irradiation, hydroxyl radical, superoxide anion radical, and sodium periodate.

Experimental

Chemicals Dopamine, hypoxanthine, xanthine oxidase (from butter milk), and peroxidase (from horseradish) were purchased from Sigma Chemical Co., (MO, U.S.A.), hydrogen peroxide from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and sodium metaperiodate from Acros Organics (NJ, U.S.A.). Dopamine-4-O-sulfate was kindly donated by Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). Other reagents were of the highest quality available from Wako Pure Chemical Industries Ltd.

Free Radical Analysis Using EPR Spectroscopy The free radical metabolites of dopamine and dopamine-4-O-sulfate were examined with an EPR spectrometer (JES-TE 300, JEOL Co., Ltd., Tokyo, Japan) equipped with a cavity and an aqueous quartz flat cell (JEOL Co., Ltd., Tokyo, Japan) at X-band (9.5 GHz). EPR data were analyzed by a computer (HP Apollo 9000 Series 400) with the software, ESPRIT 432 (JEOL Co., Ltd., Tokyo, Japan). Typical operating conditions of the EPR spectrometer were: power, 5 mW; center field, 3360 G; sweep width, 20 G; modulation frequency, 100 kHz; modulation width, 0.1 G; time constant, 0.03 s; sweep time, 1 min; temperature, 20 °C. Hyperfine coupling constants and spectral simulations were obtained with a computer program, Winsim. Because of the short lives of radical species, a fast-flow method was adopted for both Fenton’s and horseradish peroxidase system.

UV Irradiation Experiment Samples were irradiated with UV during passage through the quartz flat cell attached to the EPR cavity. The de-gassed (N2 purge, 15 min) reaction mixture was slowly flowed through the cell (flow rate ca. 1 ml/min) to minimize the depletion of starting materials and buildup of light-absorbing materials. The irradiation source was 300-watt xenon arc light (Ushio, Tokyo, Japan).

Visible-UV Absorption Spectra Visible and UV absorption spectra were measured using a model 330 spectrophotometer (Hitachi Co., Tokyo, Japan). The spectrometer was operated between 250 nm and 600 nm at 25 °C. In the reference cell, 100 mM phosphate buffer (pH 7.4) was placed. Sample solutions consisted of 100 mM phosphate buffer (pH 7.4), 0.1 mM dopamine or dopamine-4-O-sulfate, and 0.2 mM sodium periodate.

Results

Effects of Alkaline on the Formation of Dopamine-Derived Radicals When dopamine (0.1 m) was mixed with a 1 M sodium hydroxide solution under aerobic conditions (pH 12—13), two kinds of EPR signals were detected with time (Figs. 1A, D), i.e., primary and secondary dopamine radical. The hyperfine coupling constants for the primary EPR signal (Fig. 1A: \( a_1^0 = 0.27 \) G, \( a_{10}^0 = 3.73 \) G, \( a_{11}^0 = 0.90 \) G, \( d_0^0 = 3.12 \) G) (Table 1) were very similar to those of reported

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for the autoxidation of dopamine at high pH. Therefore, this radical was identified as a primary o-semiquinone dopamine radical. After 10 min incubation, the secondary o-semiquinone radical (Fig. 1D) appeared with the decrease of primary radical (Fig. 1C). The hyperfine splitting constants of this radical ($a_H^1=0.56 \text{ G}$, $a_H^2=0.84 \text{ G}$, $a_H^3=3.31 \text{ G}$) (Table 1) corresponded to those of 6-0-substituted o-semiquinone dopamine radical obtained on nucleophilic attack of $\text{OH}^-$ of the 6th position of dopamine o-quinone at high pH.

On the other hand, dopamine-4-O-sulfate gave no EPR signal under these experimental conditions (Figs. 1G—l).

Effects of UV Irradiation on the Formation of Dopamine-Derived Radicals When dopamine was UV irradiated under anaerobic conditions, an apparent EPR signal was observed (Fig. 2A). The EPR parameters of this spectrum were $a_H^1=0.43 \text{ G}$, $a_H^2=3.57 \text{ G}$, $a_H^3=0.94 \text{ G}$, and $a_H^b=2.96 \text{ G}$ (Table 1), identical to those of primary o-semiquinone dopamine radical. No EPR signal was observed in the absence of dopamine (Fig. 2C). Dopamine-4-O-sulfate showed a weak EPR signal on UV irradiation under the same conditions (Fig. 2D).

Effects of the Horseradish Peroxidase-Hydrogen Peroxide System on the Formation of Dopamine-Derived Radicals When dopamine was mixed with horseradish peroxidase (40 nm) and hydrogen peroxide (200 $\mu$m) as an enzymatic oxidant, apparent EPR parameters ($a_H^1=0.45 \text{ G}$, $a_H^2=3.61 \text{ G}$, $a_H^3=0.94 \text{ G}$, $a_H^b=2.98 \text{ G}$) (Table 1) were observed in the complete system (Fig. 3A). And these parameters were
Effects of the Hypoxanthine–Xanthine Oxidase System on the Formation of Dopamine-Derived Radicals When dopamine was mixed with hypoxanthine and xanthine oxidase as a superoxide anion radical generating system, an apparent EPR signal ($a^1_d=0.47 G$, $a^2_d=3.57 G$, $a^3_d=0.92 G$, $a^4_d=3.01 G$) was observed (Fig. 4B). This radical was consistent with the primary $o$-semiquinone dopamine radical. However, dopamine-$4-O$-sulfate showed no EPR signal under the experimental conditions (Fig. 4D).

Effects of Hydroxyl Radical on the Formation of Dopamine-Derived Radicals Because of the short lives of the hydroxyl radicals generated by the Fenton system, we adopted a fast-flow method. As shown in Fig. 5A, a radical from dopamine ($a^1_d=0.45 G$, $a^2_d=3.56 G$, $a^3_d=0.93 G$, $a^4_d=2.98 G$) (Table 1) was detected after dopamine was mixed with hydrogen peroxide and ferrous. This radical was similar to the primary $o$-semiquinone dopamine radical. In the absence of ferrous, no EPR signal was observed (Fig. 5B).
formation of dopaminochrome. However, under the same conditions, dopamine did not show any 30) change in absorption even on addition of NaIO4, whereas dopamine-4-O-sulfate was unchanged at any experimental conditions 2) dopamine-4-O-sulfate was partially hydrolyzed by human arylsulfatase A, and C in vitro, then converted to dopamine. 31) From the physiological study of free and sulfated dopamine during exercise, Yoshizumi et al. found a positive relationship between free dopamine and the conjugated form in plasma, 32) which suggests that dopamine-4-O-sulfate, not dopamine-3-O-sulfate, 33) acts as a reservoir of dopamine.

Dopamine is known to be an antioxidant and to protect neurocytes from oxidative stress 35—17) by scavenging reactive oxygen species. Meanwhile, dopamine also acts as a prooxidant through a reaction with molecular oxygen that produces reactive oxygen species 18,19).

During the autoxidation of dopamine, α-semiquinone dopamine radicals, 30) –OH substituted α-semiquinone dopamine radicals, 31) and 5,6-dihydroxyindole radicals 29) are produced. Photooxidation of dopamine also produced α-semiquinone dopamine radical. 30) These radical species are responsible for melamine formation by cyclization, 19) irreversible protein binding by nucleophilic addition 19) and cytotoxicity of catecholamine neurons, 20—29) which may relate to the progression of Alzheimer disease 40) and Parkinson disease. 41) Plasma soluble melanin formed from dopamine is toxic to human CD4+ lymphoblastic cells. 32) However, it is not known whether sulfoconjugated dopamine has the same redox properties as dopamine or not. Therefore, in the present study, we compared the antioxidant and prooxidant activities of dopamine-4-O-sulfate with those of dopamine. 42)

In the present study, dopamine was subjected to spontaneous oxidation at alkaline pH, reacted with reactive oxygen species such as superoxide anion radical and hydroxyl radical, then converted to radical species (Figs. 1, 4—5). Furthermore, dopamine was subtracted one electron by the horseradish peroxidase/hydrogen peroxide system then formed α-semiquinone dopamine radical (Fig. 3) as previously reported. 43)

Dopamine-4-O-sulfate, by contrast, showed no such formation of radicals in the presence of reactive oxygen species (Figs. 4, 5) or in the horseradish peroxidase-hydrogen peroxide system (Fig. 3) under physiological conditions in vitro (pH 7.4). In addition, dopamine-4-O-sulfate showed no change in absorption even on addition of NaIO4, whereas dopamine did (Fig. 6). 30) Furthermore, the HPLC technique had applied to check the stability of dopamine-4-O-sulfate under these oxidative conditions mentioned above. No additional peaks were observed and the peak area of dopamine-4-O-sulfate was unchanged at any experimental conditions (date not shown). These results indicated that 1) dopamine-4-O-sulfate does not act as either an antioxidant or a prooxidant under our experimental conditions, 2) dopamine-4-O-sulfate itself cannot participate in the reaction to form melanin through the formation of α-semiquinone or α-
quinone compounds.

Generally, intrinsic catechols are degraded to semi-quinone radicals via both enzymatic and non-enzymatic pathways in vivo. And the generation of o-quinone through o-semiquinone radical causes the unfavorable effects of catechol derivatives, such as hepatic injury,44) cardiotoxicity,45) and hemolysis.46) The fact that dopamine-4-quinone compounds.

References