Radical intermediates generated in the reactions of L-arginine with hydroxyl radical and sulfate radical anion: A pulse radiolysis study

Takeo Ito [a,*], Shota Morimoto [a], Shin-ichi Fujita [b], Sei-ichi Nishimoto [a,*]

[a] *Department of Energy and Hydrocarbon Chemistry, Graduate School of Engineering, Kyoto University, Kyoto 615-8510, Japan*
[b] *Research Institute of Advanced Technology, University of Osaka Prefecture, Osaka 599-8570, Japan*

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ABSTRACT

Reactions of L-arginine (Arg) with hydroxyl radical ($^\bullet$OH) and sulfate radical anion ($SO_4^{-\bullet}$) were kinetically investigated by the pulse radiolysis technique. Hydrogen abstraction from Arg by $^\bullet$OH afforded redox chemically oxidizing, neutral, and reducing carbon-centered Arg radicals. Kinetic properties of the radicals indicated that the reducing species might include the δ-C-centered Arg radical and CO_2 radical anion. Similar transient spectra were observed in the $SO_4^{-\bullet}$ reaction with Arg, suggesting direct oxidation at the guanidino group is less likely.

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1. Introduction

Damage to bio-related molecules may result from exposure to reactive oxygen species (ROS) such as singlet oxygen, super-oxide radical anion, or hydroxyl radical ($^\bullet$OH), generated during cellular metabolism, or following exposure to ionizing radiation or a variety of chemicals. Under aerobic conditions, amino acid residues of proteins are susceptible to attack by ROS, and the damages in proteins and peptides have been implicated in a variety of disease states as well as in the progression of aging (Chandel and Budinger, 2007; Kregel and Zhang, 2007). The radicals produced from amino acids by ionizing radiation have been intensively investigated and identified by the methods of electron spin resonance (ESR) and pulse radiolysis so far (Armstrong and Humphreys, 1967; Taniguchi et al., 1968; Smith et al. 1970; Neta and Fessenden, 1971; Paul and Fischer, 1971; Rustgi et al., 1977; Rustgi and Riesz, 1978). It has been reported that radiolysis of nucleohistone in a diluted aqueous solution induces the formation of cross-linking between DNA bases and amino acids including lysine (Lys) and arginine (Arg) (Smith, 1976; Oleinick et al., 1986; Dizdaroglu and Gajewski, 1989). Radiolysis of water under anoxic conditions produces primary radical species such as hydroxyl radical, hydrated electron (e_{aq}^-),
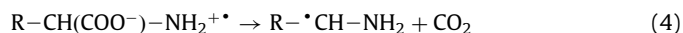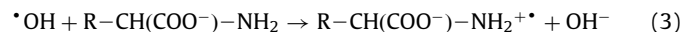
and hydrogen atom ($^\bullet$H) (reaction (1)).

$$H_2O \rightarrow {}^\bullet OH,\ e_{aq}^-,\ {}^\bullet H,\ H_2,\ H_2O_2,\ H_2 \tag{1}$$
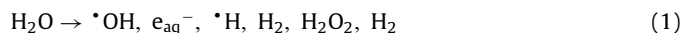
Because $^\bullet$OH induces oxidation of amino acids near the interfaces of proteins and solvent water molecules, the radiation-induced footprinting coupled with mass spectrometry has become a powerful technique for the mapping of solvent-accessible surfaces of proteins (Xu et al., 2003). In the $^\bullet$OH reactions with α-amino acids, hydrogen atom abstraction from their carbon skeletons occurs in the neutral to acidic pH region as follows (reaction (2)):

$$^\bullet OH + HR-CH(COO^-)-NH_3^+ \rightarrow {}^\bullet R-CH(COO^-)-NH_3^+ + H_2O \tag{2}$$
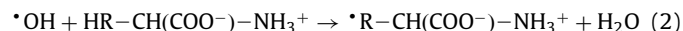
In contrast, in the basic pH region, it has been suggested that $^\bullet$OH also attacks directly at the nitrogen atom in a deprotonated amino group and initiates decarboxylation, leading to the formation of a strongly reducing carbon-centered radical (reactions (3) and (4)) (Armstrong and Humphreys, 1967; Rustgi et al., 1977; Rustgi and Riesz, 1978; Mönig et al., 1985; Bonifačić et al., 1998, 2000; Hug and Fessenden, 2000):

$$^\bullet OH + R-CH(COO^-)-NH_2 \rightarrow R-CH(COO^-)-NH_2^{+\bullet} + OH^- \tag{3}$$

$$R-CH(COO^-)-NH_2^{+\bullet} \rightarrow R-{}^\bullet CH-NH_2 + CO_2 \tag{4}$$

Spectroscopic analyses have mostly focused the mechanisms of $^\bullet$OH reactions with simple α-amino acids, but limited studies have been reported for basic amino acids including Lys and Arg. The basic amino acids consist of an ϵ-amino group or a guanidino group in addition to the α-amino group, and therefore it is

* Corresponding authors. Fax: +8175 383 2504.
E-mail addresses: takeoit@scl.kyoto-u.ac.jp (T. Ito), nishimot@scl.kyoto-u.ac.jp (S.-. Nishimoto).

expected that pH has a great effect on $^{\bullet}$OH reactivity toward the basic amino acids. It has been postulated that oxidative degradation of Arg under aerobic conditions is initiated by hydrogen abstraction from δ-carbon by $^{\bullet}$OH to generate carbon-centered radicals, which are further oxidized and decarbonated to give guanidines (Xu et al., 2003).

In this study, time-resolved pulse radiolysis method was employed to obtain kinetic parameters for the radiation-induced oxidation of Arg by $^{\bullet}$OH and sulfate radical anion ($SO_4^{-\bullet}$), and to understand the redox properties of intermediate species produced during the reactions (Neta et al. 1970; Marković et al., 1974; Mönig et al., 1985).
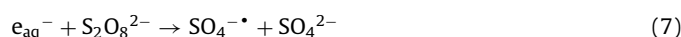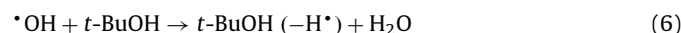
2. Experimental section

2.1. Materials

L-Arginine (L-Arg) and N,N,N′,N′-tetramethyl-p-phenylene-diamine (TMPD) were purchased from Wako Pure Chemical Industries. Potassium peroxodisulfate ($K_2S_2O_8$) and 2-methyl-2-propanol (t-BuOH) were purchased from Nacalai Tesque. Tetranitromethane (TNM) was synthesized as previously reported (Göbel et al., 2006).

2.2. Pulse radiolysis

L-Arg was dissolved in phosphate buffer solution (5.0×10^{-3} mol dm^{-3}) with water purified by a Millipore Milli-Q system. For $^{\bullet}$OH reaction, the amino acid solutions were saturated with N$_2$O to scavenge hydrated electron (e_{aq}^-) (reaction (5))

$$e_{aq}^- + N_2O \rightarrow N_2 + {}^{\bullet}OH + OH^- \qquad (5)$$

For $SO_4^{-\bullet}$ reaction, aqueous solution of L-Arg containing excess amounts of $K_2S_2O_8$ (1.0×10^{-2} mol dm^{-3}) and t-BuOH (0.10 mol dm^{-3}) were saturated with Ar prior to irradiation. Under these conditions, $^{\bullet}$OH and e_{aq}^- are scavenged by t-BuOH and $S_2O_8^{2-}$, respectively (reactions (6) and (7))

$$^{\bullet}OH + t\text{-}BuOH \rightarrow t\text{-}BuOH\,(-H^{\bullet}) + H_2O \qquad (6)$$

$$e_{aq}^- + S_2O_8^{2-} \rightarrow SO_4^{-\bullet} + SO_4^{2-} \qquad (7)$$

The electron beam was produced in a linear accelerator (High Voltage Engineering Co., Ltd.) giving 10 MeV electrons with a variable pulse width up to 5 μs; its peak current being about 0.4 A. The electron beam, spread by a 4-mm-thick aluminum plate, entered the quartz cell filled with the sample solutions previously through a brass slit and fell on a brass collector. The current was monitored as voltage on a condenser with a digital voltmeter having a holding mechanism. The analysis light emitted from a 300-W Xe lamp (L2479; Hamamatsu Photonics Co., Ltd.), supplied with electric power by a DC Power Supply (6268B; Hewlett-Packard Co., Ltd.) was passed to the cell perpendicular to the electron pulses. The absorbance of the radical intermediates formed in the cell was carried to a Multi-channel Spectrometer (USP-500; UNISOKU Co., Ltd.) through the optical fiber, then controlled and analyzed. Dosimetry was performed with 5×10^{-3} mol dm^{-3} KSCN solutions taking a molar absorption coefficient of $(SCN)_2^{-\bullet}$ at 480 nm, $\varepsilon_{480}[(SCN)_2^{-\bullet}] = 7600$ dm^3 mol^{-1} cm^{-1} (Schuchmann et al., 2000) and $G[(SCN)_2^{-\bullet}] = 2.9 \times 10^{-7}$ mol J^{-1}.

The oxidizing or reducing radicals formed in the reactions of L-Arg were titrated by TNM or TMPD, respectively. Pulse radiolysis of L-Arg forms radicals derived from L-Arg which can oxidize TMPD or reduce TNM, and thus the yields of intermediate radical species can be quantified by evaluating the amount of nitroform anion (NF$^-$) or TMPD radical cation (TMPD$^{+\bullet}$) using ε_{350}(NF$^-$) =

15000 dm^3 mol^{-1} cm^{-1} or ε_{565}(TMPD$^{+\bullet}$) = 12500 dm^3 mol^{-1} cm^{-1} (von Sonntag, 2006).

3. Results and discussion

3.1. Reaction of Arg with $^{\bullet}$OH

The transient species generated in the reaction of L-Arg with $^{\bullet}$OH were studied by the pulse radiolysis of N$_2$O-saturated phosphate buffer solution containing L-Arg (5.0×10^{-2} mol dm^{-3}). Upon pulsed electron-beam irradiation, the optical absorption with an absorption maximum at 320 nm developed in a few microseconds (Fig. 1). The absorbance at 290 nm observed 1 μs after the pulse was dependent on the pH in the range between 7 and 11, and showed a sigmoidal curve with an inflection point at pH ~9 (Fig. 1, inset). Similar absorption spectrum has also been observed in the reaction of $^{\bullet}$OH with 1,1-dimethylguanidine having an N-alkylguanidino group (Morimoto et al., 2008). The transient species observed in the $^{\bullet}$OH reaction with 1,1-dimethyl-guanidine was assigned to a carbon-centered radical generated as a result of hydrogen abstraction from its methyl groups by $^{\bullet}$OH, and therefore the absorption observed in the reaction of L-Arg might be assigned to the C-centered radical [Arg(C$^{\bullet}$)] produced by the hydrogen abstraction from its β-, γ- or δ-position. It has been reported that pKa of the α-amino group in L-Arg is 8.99 (Dawson et al., 1969) and it is thus likely that pKa ~9 obtained above attributes acid–base equilibrium of Arg(C$^{\bullet}$).

Redox titration of reducing or oxidizing Arg radicals produced in reactions of $^{\bullet}$OH with L-Arg was performed using TNM or TMPD as an oxidizing or a reducing agent, respectively. Reducing Arg radicals react with TNM to form NF$^-$ that has well-defined absorption with a maximum at 350 nm (reaction (8)). On the other hand, oxidizing Arg radicals undergo one-electron reduction by TMPD to generate TMPD$^{+\bullet}$ showing characteristic absorption at around 565 nm (reaction (9))

$$Arg^{\bullet} + TNM \rightarrow Arg^+ + NF^- + NO_2 \qquad (8)$$

$$Arg^{\bullet} + TMPD \rightarrow Arg^- + TMPD^{+\bullet} \qquad (9)$$
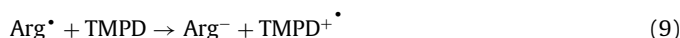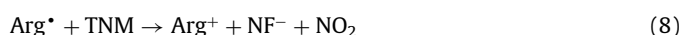
In the pulse radiolysis of L-Arg (5.0×10^{-3} mol dm^{-3}) in N$_2$O saturated solution in the presence of TMPD (5.0×10^{-5} mol dm^{-3}), it is expected that most of the $^{\bullet}$OH react with Arg
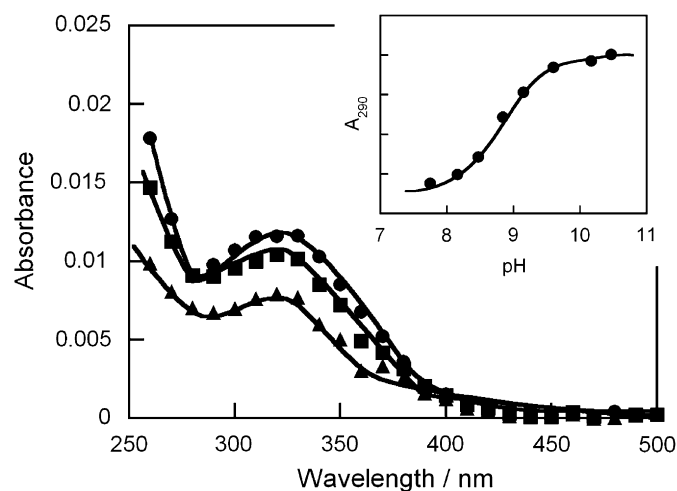

Fig. 1. Optical absorption spectra obtained (●) 1 μs, (■) 50 μs and (▲) 200 μs after the pulse irradiation of N$_2$O-saturated aqueous buffer solution (pH 7.0) containing Arg (5.0×10^{-3} mol dm^{-3}). Inset shows pH dependence of the absorbance at 290 nm.