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Carbon- and nitrogen-centered radicals produced from L-lysine by radiation-induced oxidation: A pulse radiolysis study

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ABSTRACT

Radical species generated from the reactions of a basic amino acid, L-lysine (Lys), with hydroxyl radicals (·OH) and sulfate radical anion ($SO_4^{-\cdot}$) have been detected by the method of pulse radiolysis. On the basis of electron transfer reactivities toward tetranitromethane (TNM), it was demonstrated that reducing carbon-centered radicals are generated as a result of hydrogen abstraction from CH_2 of Lys with a *G*-value of 1.9×10^{-7} mol J^{-1}. On the other hand, direct oxidation of L-Lys by $SO_4^{-\cdot}$ formed a transient species with different spectroscopic properties, most likely, the ε-N-centered Lys radical.

1. Introduction

Under aerobic conditions, amino acid residues of proteins are susceptible to attack by reactive oxygen species (ROS), and the damages in proteins and peptides are implicated in a variety of disease states, as well as in the progression of aging [1–4]. Varieties of amino acid radicals produced by ionizing radiations have been characterized by the methods of electron spin resonance (ESR) [5–10] and pulse radiolysis [11–14] in the last few decades.

Hydroxyl radical (·OH) generated under oxidative stress is an oxidizing radical species toward biomolecules, and is considered to induce harmful effects on living things. It has been reported that reaction of such primary radicals with nucleohistone in aqueous solution produces crosslinks between DNA bases and amino acids such as lysine (Lys) and arginine (Arg) [15,16]. Since ·OH induces oxidation of amino acids near the interfaces of proteins and solvent water molecules, radiation-induced footprinting coupled with mass spectrometry has become a powerful technique for mapping the solvent accessible surface of proteins [17].

In the case of simple α-amino acid of glycine (Gly), ·OH abstracts hydrogen from the carbon skeleton at neutral or acidic pH. In the basic pH range, ·OH also directly attacks the nitrogen atom of the deprotonated amino group and initiate decarboxylation leading to the formation of a strongly reducing α-amino carbon-centered radical [13,18,19].

Most spectroscopic studies have focused on the radical reactions of simple α-amino acids induced by ·OH, on the other hand, limited studies have been reported for basic amino acids, such as Arg and Lys [20]. Previous ESR spin trapping studies on ·OH reaction with Lys identified C-centered radical structures generated as a result of ·OH attack at the side chain. C-Centered radicals thus generated form DNA–protein crosslinks via addition to thymine base or radical–radical recombination with thymine radicals [15,16]. Recent product analysis studies on DNA–protein crosslink identified *N*-ε-(guaninyl)-lysine adducts by photosensitized or chemical oxidation of guanine base in the presence of Lys derivatives [21–23]. Burrows and co-workers have demonstrated that attack of the ε-N-centered radical (cation) generated by direct oxidation of Lys toward guanine base is involved in the crosslink formation [22].

Here we describe the dynamic behavior of intermediate radicals produced in the reaction of L-Lys and oxidizing species such as ·OH and sulfate radical anion ($SO_4^{-\cdot}$) as investigated by the method of pulse radiolysis to understand the oxidative protein damage mechanism. Redox reactive radicals generated during the radiolysis were quantitated by the redox titration technique, by which we can detect oxidizing or reducing radicals of amino acids by converting the radicals into easily observable radical cation of *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine ($TMPD^{+\cdot}$) or nitroform anion (NF^-) [23,24].

2. Experimental

2.1. Materials

L-Lysine and *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (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) and L-norleucine were purchased from Aldrich Chemical. All chemicals were used as received.

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2.2. Pulse radiolysis

Amino acids were dissolved in phosphate buffer solution (5.0×10^{-3} mol dm^{-3}) with water purified by a Millipore Milli-Q system. Radiolysis of water produces primary radical species including ·OH, hydrated electron (e^-_{aq}), and hydrogen atom (·H) with G-values [25] of 2.9×10^{-7} mol J^{-1}, 2.9×10^{-7} mol J^{-1}, and 0.6×10^{-7} mol J^{-1}, respectively:

$$H_2O \rightarrow {}^{\cdot}OH,\ e^-_{aq},\ {}^{\cdot}H$$

For the ·OH reaction, the amino acid solutions were saturated with N_2O to scavenge e^-_{aq}:

$$e^-_{aq} + N_2O \rightarrow N_2 + {}^{\bullet}OH + OH^-$$

On the other hand, for the $SO_4^{\cdot-}$ reaction, aqueous solution of L-Lys solution containing $K_2S_2O_8$ (1.0×10^{-2} mol dm^{-3}) and t-BuOH (0.10 mol dm^{-3}) were saturated with Ar prior to the irradiation. Under the conditions, ·OH and e^-_{aq} are scavenged by t-BuOH and $S_2O_8^{2-}$, respectively.

$$^{\cdot}OH + t\text{-BuOH} \rightarrow t\text{-BuOH}(-H^{\cdot}) + H_2O$$

$$e^-_{aq} + S_2O_8^{2-} \rightarrow SO_4^{\cdot-} + SO_4^{2-}$$

The electron beam (7 Gy pulse^{-1}) 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 an aluminum plate 4 mm thick, entered into a quartz cell (light path length = 1.5 cm) filled with the sample solution 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.) was passed the cell perpendicularly 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 optical fibers. Dosimetry was performed with 5.0×10^{-3} mol dm^{-3} KCNS solutions taking molar absorption coefficient of $(CNS)_2^{\cdot-}$ at 480 nm, $\varepsilon[(CNS)_2^{\cdot-}]_{480} = 7600$ dm^3 mol^{-1} cm^{-1} and $G[(CNS)_2^{\cdot-}] = 2.9 \times 10^{-7}$ mol J^{-1}.

The oxidizing or reducing radicals formed in the reactions of L-Lys were titrated by TNM or TMPD, respectively. Pulse radiolysis of the amino acids in the presence of TNM or TMPD forms radical intermediates of the amino acids, which further reduce TNM or oxidize TMPD, and therefore, the yields of intermediate radical species can be quantitated by evaluating the amount of nitroform anion (NF^-) or $TMPD^{+\bullet}$ using $\varepsilon(NF^-)_{350} = 15\,000$ dm^3 mol^{-1} cm^{-1} or $\varepsilon(TMPD^{+\cdot})_{565} = 12\,500$ dm^3 mol^{-1} cm^{-1}.

3. Results and discussion

3.1. Reaction of L-Lys with ·OH

In the pulse radiolysis of N_2O-saturated aqueous solution of L-Lys at pH 7.0, in which ·OH is a primary reactive radical, transient absorption spectra of intermediate species were observed as shown in Fig. 1. A characteristic absorption at 260 nm developed in the first 10 μs and decayed following a second-order kinetics (Fig. 1, inset) with a relatively long lifetime, since it was observed over 100 μs after the pulse. Similar transient absorption spectra were also observed in the pH range between 3 and 8.

Pulse radiolysis of N_2O-saturated solution of L-Lys in the presence of TMPD at pH 7.0 was also investigated, however formation of $TMPD^{+\cdot}$ was not detected, suggesting oxidizing Lys radicals were not involved in the ·OH reaction with Lys under neutral conditions.

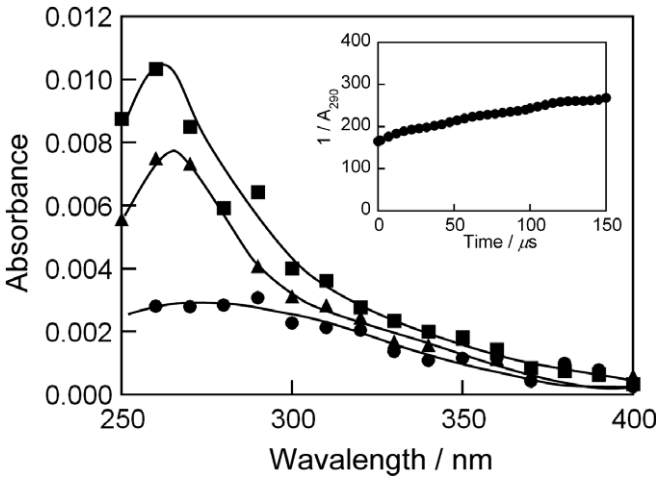

Fig. 1. Transient absorption spectra of the intermediates in N_2O-saturated solution of L-Lys (5.0×10^{-3} mol dm^{-3}) at pH 7, obtained (●) 1, (■) 10, and (▲) 100 μs after the pulse. The inset shows a reciprocal plot of the absorption at 290 nm.

On the other hand, redox titration with TMN as an oxidizing agent showed the formation of NF^- indicating reducing Lys radicals are involved in the ·OH reaction with Lys at pH 7.0. Buildup of absorption at 350 nm assigned to NF^- was a biphasic kinetics (Fig. 2a), and the initial rapid increase in absorption at 350 nm (<20 μs) was followed by the second slower increase which reaches a plateau in 1 ms after the pulse. Kinetic analysis of the two components for the ·OH reaction with Lys under various concentrations of TNM revealed that the observed rate constant for formation of the fast growing component (k_{fast}) increased with increasing the concentration of TNM, and approached to a constant value at high TNM concentrations (Fig. 2b). Similar kinetic behavior have been reported previously in the redox titration of α-(alkylthio)alkyl radicals by TNM, where a TNM-α-(alkylthio)alkyl radical adduct forms as an intermediate [26]. Therefore, it is presumable that this type of an intermediate is also generated in the reaction of Lys radical (Lys·) with TNM, and the formation of NF^- from the Lys–TNM adduct radical becomes a rate-determining step at high concentrations of TNM.

$$Lys^{\cdot} + TNM \rightarrow (Lys{-}TNM)^{\cdot} \rightarrow Lys^+ + NO_2 + NF^-$$

Pseudo-first-order kinetic fitting of the fast buildup component in the low concentration range of TNM gave a rate constant of $k_{fast} = 3.9 \times 10^8$ dm^3 mol^{-1} s^{-1} and a G-value for its formation $G_{fast} = 1.7 \times 10^{-7}$ mol J^{-1}. On the other hand, rate constants for formation of the slow component (k_{slow}) were independent of the concentration of TNM over the same concentration range. G-value for the slow radical (G_{slow}) and the one-electron transfer rate constant (k_{slow}) were estimated to be $G_{slow} = 0.2 \times 10^{-7}$ mol J^{-1} and $k_{slow} = 1.4 \times 10^7$ dm^3 mol^{-1} s^{-1}, respectively.

3.2. Reaction of L-Lys with $SO_4^{\cdot-}$

Pulse irradiation of L-Lys in Ar-saturated buffer solution containing $K_2S_2O_8$ and t-BuOH gave transient species having absorption maxima at around 340 nm and 450 nm as shown in Fig. 3. Transient absorption at around 450 nm decayed in a few μs was assigned to $SO_4^{\cdot-}$ [27]. The absorption spectra with λ_{max} at 340 nm were observed in the pH range between 2.2 and 9.5, which might be assigned to (1) α-N-centered radical (cation) of Lys, (2) ε-N-centered radical (cation), or (3) C-centered radicals. As a separate experiment, we have carried out pulse radiolysis of L-norleucine,