A Pulse Radiolysis Study on the Reactions of Hydroxyl Radical and Sulfate Radical Anion with Guanidine Derivatives in Aqueous Solution

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Abstract

The reactions of hydroxyl radical ($^\bullet$OH) with guanidine, 1,1-dimethylguanidine, and 1-ethylguanidine have been investigated by the method of pulse radiolysis. The characteristic absorption observed in the $^\bullet$OH reaction with guanidine under basic conditions was assigned to a nitrogen-centered radical. On the other hand, reducing carbon-centered radicals were generated in the $^\bullet$OH reaction with 1,1-dimethylguanidine and 1-ethylguanidine over a pH range of 7–13, which may suggest that the primarily formed nitrogen-centered radicals at a high pH are converted into carbon-centered radicals by hydrogen atom shift. Similar transient species were also observed in the reaction of sulfate radical anion with the guanidine derivatives.

1. Introduction

It has been established that free amino acids and amino acid residues in proteins are highly susceptible to oxidation by the reactive oxygen species (ROS), such as hydroxyl radical ($^\bullet$OH), hydrogen peroxide (H2O2), and superoxide radical anion $O_2^{\bullet-}$ which are generated in the process of metabolism or during exposure to high energy radiations [1,2]. In particular, $^\bullet$OH is highly reactive toward amino acids and abstracts a hydrogen atom from their backbones and aliphatic side chains to form carbon-centered (C-centered) radicals as major intermediates. Nitrogen-centered (N-centered) radical species are also found in biological systems exposed to high-energy ionizing radiations. Basic amino acids such as lysine (Lys) and arginine (Arg) are the main components of histone, which tightly bind to DNA in chromatin. Investigations of radiation effects on amino acids have shown that radical intermediates formed by the reaction between $^\bullet$OH and the basic amino acids induce DNA-protein cross-linking [3–9]. It has been reported that an aminium radical is produced in the $^\bullet$OH reaction with glycine (Gly) by one-electron oxidation of the amino group [10]. Therefore, investigations into the formation mechanisms as well as the chemical reactivities of the N-centered radicals are important to understand the chemical events which lead to the damage of amino acids. N-Centered radicals (aminyl radicals) and their protonated counterparts (aminium radical cations) have attracted much attention in the field of organic synthesis, such as in the Hofmann–Loffler–Freytag reaction [11,12]. However, there is little information on the kinetic properties of the N-centered radical species [13,14]. In this study, we investigated $^\bullet$OH reactions with guanidine (Gua), 1,1-dimethylguanidine (DMGua), and 1-ethylguanidine (EGua) to elucidate the radiation effects on Arg. Arg is positively charged at the guanidine moiety under acidic and neutral conditions (pKa = 12.5) [15], and therefore, acid–base equilibrium of the guanidine moiety possibly affects the reactivity toward $^\bullet$OH. So far, only a limited numbers of studies have reported on the radical formation from guanidine derivatives including Arg, and most of the studies focus on the characterization of the radicals by the method of ESR spin-trapping [16–21]. Experimental data obtained from those studies have given evidences for the formation of C-centered radicals as a

consequence of H-abstraction from the methylene groups. On the other hand, the reactivity of $^\bullet$OH toward the guanidino group have not been paid much attention to. Since the three guanidine derivatives have the advantage of simplicity with respect to the structures, it is easy to analyze the characteristic absorption spectra of the transient radical species by the method of pulse radiolysis. As described below, we have for the first time determined kinetic parameters for the reactions of the guanidine derivatives toward oxidizing $^\bullet$OH and sulfate radical anion ($SO_4^{\bullet-}$), and found that C-centered radicals are generated from EGua, on the other hand, at high pH, an N-centered radical is one of the major radical species generated in the one-electron oxidation of Gua.

2. Experimental section

2.1 Materials

Guanidine phosphate, potassium thiocyanate (KCNS), potassium peroxodisulphate ($K_2S_2O_8$), and 2-methyl-2-propanol (*t*-BuOH) were purchased from Nacalai Tesque (Japan). Tetranitromethane (TNM), 1,1-dimethylguanidine (DMGua) and 1-ethylguanidine (EGua) were purchased from Aldrich Chemical. All chemicals were used as received. Aqueous phosphate buffer (5×10^{-3} mol dm^{-3}) solutions of guanidine derivatives were prepared with water purified by a Millipore Milli-Q system.

2.2 Pulse Radiolysis

In the pulse radiolysis of a diluted aqueous solution, water radicals such as $^{\bullet}$OH and hydrated electrons (e^{-}_{aq}) are primarily generated with the *G*-values [1,22] of $G(^{\bullet}OH) = 2.9 \times 10^{-7}$ mol J^{-1}, $G(e^{-}_{aq}) = 2.9 \times 10^{-7}$ mol J^{-1}, and $G(^{\bullet}H) = 0.6 \times 10^{-7}$ mol J^{-1}.

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

For the studies on $^{\bullet}$OH reaction with guanidine derivatives, the sample solutions were saturated with N_2O to scavenge hydrated electrons e^{-}_{aq}. Oxidizing $SO_4^{\bullet-}$ were generated in the radiolysis of Ar-saturated aqueous solution of *t*-BuOH and $K_2S_2O_8$. Under the conditions, $^{\bullet}$OH are scavenged by *t*-BuOH, on the other hand, e^{-}_{aq} are scavenged by $S_2O_8^{2-}$ to produce $SO_4^{\bullet-}$. The pulse radiolysis experiments were carried out with a 10-MeV electron linear accelerator (High Voltage). Sample solutions were irradiated with electron pulses (3~10 Gy pulse^{-1}) of about 1 μs duration. Using a multi-channel spectrometer (USP-500, Unisoku, Japan) equipped with a 300W Xe-lamp (L2479, Hamamatsu Photonics, Japan), transient absorption spectra of the sample solution in a quartz cell (path length = 15 mm) were monitored and analyzed. Dosimetry was performed with 5×10^{-3} mol dm^{-3} KCNS solutions taking ε_{475} (($CNS)_2^{\bullet-}$) = 7600 dm^3 mol^{-1} cm^{-1} and $G((CNS)_2^{\bullet-})$ = 2.9 $\times$ 10^{-7} mol J^{-1}.

3. Results and discussion

3.1 $^\bullet$OH reaction with Guanidine

To the best of our knowledge, transient absorption spectra of the radical species derived from $^\bullet$OH reaction with Gua have not been reported so far. To investigate the reactivity of the guanidino group toward oxidizing $^\bullet$OH, pulse radiolysis of three guanidine derivatives in N_2O-saturated aqueous solution was carried out. Fig. 1 shows typical transient absorption spectra obtained in the pulse radiolysis of Gua. Characteristic absorption of the intermediate radical was not observed in the $^\bullet$OH reaction with protonated Gua at pH 7.0, however, broad absorption spectra having an absorption maximum (λ_{max}) at 300 nm were observed in the higher pH region. The pH dependence of the absorbance at 300 nm exhibited a pKa value of 10.5 as shown in the inset of Fig. 1. Because of the low extinction coefficient, direct spectroscopic measurements of $^\bullet$OH during the reaction was not successful. The rate constant of the $^\bullet$OH reaction with Gua was thus evaluated by the competition method using KSCN. In the pulse radiolysis of N_2O-saturated aqueous solutions of the substrate ($0\sim16 \times 10^{-3}$ mol dm^{-3}) containing KSCN (1×10^{-3} mol dm^{-3}), G-values for the formation of $(SCN)_2^{\bullet-}$ were calculated from the absorbance at 475 nm due to $(SCN)_2^{\bullet-}$. Since the rate constant for the $^\bullet$OH reaction with KSCN has been determined to be $k(SCN^- + {}^\bullet OH) = 1.1 \times 10^{10}$ dm^3 mol^{-1} s^{-1},[23] rate constants for the reaction of $^\bullet$OH with Gua [k(Gua + $^\bullet$OH)] under various pH conditions can be estimated by equation 1.

$$G_0[(SCN)_2^{\bullet-}] / G[(SCN)_2^{\bullet-}] = 1 + k(\text{Gua} + {}^\bullet\text{OH})[\text{Gua}] / k(SCN^- + {}^\bullet\text{OH})[SCN^-] \quad (1)$$

where $G_0[(SCN)_2^{\bullet-}]$ and $G[(SCN)_2^{\bullet-}]$ are G-values for the formation of $(SCN)_2^{\bullet-}$ in the absence and presence of the substrate during the radiolysis, respectively. Considering acid-base equilibrium between $^\bullet$OH and O$^{\bullet-}$ (pKa = 11.9), it is supposed that the O$^{\bullet-}$ reaction with KSCN (1.8×10^9 dm^3 mol^{-1} s^{-1}) occurs at pH 13.0, and the rate constant at pH 13.0 is thus obtained as k(Gua(-H$^+$) + $^\bullet$OH/O$^{\bullet-}$) = 3.0×10^8 dm^3

mol^{-1} s^{-1}. On the other hand, the rate constant at pH 7.0 was too slow to be evaluated by the competition method [k(Gua + $^{\bullet}$OH) <5.0 × 10^5 dm^3 mol^{-1} s^{-1}].

To understand the pH-response of the absorbance at 300 nm (Fig.1, inset), kinetic pKa [24] for the reaction of $^{\bullet}$OH with Gua was estimated as follows. Dissociation factor (α) of Gua at various pH can be given by

$$\alpha = 1 \, / \, [1 + 10^{\,(pKa - pH)}] \quad (2)$$

where pKa is the acid-dissociation constant for Gua (pKa = 13.5). Using the factor α, the reaction ratio for the $^{\bullet}$OH-reaction with protonated Gua and deprotonated Gua(-H$^+$) can be obtained by

$$\text{Reaction ratio} = \alpha \, k(\text{Gua(-H}^+) + {}^{\bullet}\text{OH}) \, / \, [(1 - \alpha)k(\text{Gua} + {}^{\bullet}\text{OH}) + \alpha k(\text{Gua(-H}^+) + {}^{\bullet}\text{OH})] \quad (3)$$

where k(Gua + $^{\bullet}$OH) denote the rate constants in the $^{\bullet}$OH reaction with protonated Gua. If we assume the rate constant at pH 7.0 is k(Gua + $^{\bullet}$OH) = 3 × 10^5 dm^3 mol^{-1} s^{-1}, the kinetic pKa value can be estimated to be pKa = 10.5, which is substantially in good accordance with the pH-dependent behavior of the optical density in the inset of Fig. 1.

Further insight into the $^{\bullet}$OH reaction was gained by the pulse radiolysis in the presence of tetranitromethane (TNM). The formation of reducing radicals can be detected as the increase of characteristic absorption of nitroform anion (NF$^-$) at 350 nm [ε_{350}(NF$^-$) = 15000 dm^3 mol^{-1} cm^{-1}], which is generated as a result of electron transfer between the reducing radicals and TNM.[25-27] However, the pulse radiolysis of N$_2$O-saturated Gua solution in the presence of TMN at pH 7.0 showed no remarkable changes in the absorption spectra, suggesting reducing Gua radicals were not generated during the radiolysis.

Considering that the rate constant for the reaction between $^\bullet$OH and deprotonated Gua($-H^+$) obtained at pH 13.0 is nearly equal to the diffusion-controlled rate, Gua($-H^+$) may undergo one-electron oxidation by $^\bullet$OH (Scheme 1) to form the corresponding iminium radical cation, which could be spontaneously deprotonated into an N-centered radical. Although the pKa value between these N-centered radical species was not determined in the current experiments, it has been reported that pKa values for N-centered radicals of similar amines are generally 2~3 units lower than those of the parent compounds,[28,29] suggesting that the observed characteristic absorption spectra in the $^\bullet$OH reaction with Gua($-H^+$) at pH 11.0 (Fig. 1) can be assigned to the imimium radical cation and/or its deprotonated radical (Scheme 1). Kinetic analysis for the direct oxidation of Gua by the strong oxidizing $SO_4^{-\bullet}$ was not successful since the substrate was decomposed by the oxidation with $K_2S_2O_8$.

3.2 One-electron oxidation of 1-ethylguanidine

According to previous ESR experiments, $^\bullet$OH abstracts a hydrogen atom of Arg mainly from its methylene side-chain to form Arg C-centered radicals.[16,18] 1-Ethylguanidine (EGua) is a simple model compound for further investigation of the $^\bullet$OH reaction with Arg by the method of pulse radiolysis. Fig. 2 shows transient absorption spectra observed in the N_2O-saturated solution of EGua at pH 7.0 and 11.0 after pulse irradiation. Upon raising the pH to 11.0, characteristic absorption with a peak at 330 nm observed in the neutral solution (pH 7.0) was changed into a new band with a peak at 375 nm. The inset of Fig. 2 shows the pH dependence of εG at 330 nm and 375 nm, from which an inflection point at 11.0 was obtained. The rate constants for the reaction at pH 7.0 and 11.0 evaluated by the competition method were 1.1×10^9 and $> 1.0 \times 10^{10}$ dm^3 mol^{-1} s^{-1}, respectively. At pH 13.0, $^\bullet$OH/O$^-$$^\bullet$ presumably attacks EGua($-H^+$), and its rate constant was determined to be $k(^\bullet OH/O^{-\bullet} + EGua(-H^+)) = 2.5 \times 10^9$ dm^3 mol^{-1} s^{-1}.

Redox titration of the $^\bullet$OH reaction with EGua by TNM at pH 7.0 was suggestive the formation of reducing radicals with a G-value of 5.9×10^{-7} mol J^{-1} ($\varepsilon_{330} = 1900$ dm^3 mol^{-1} cm^{-1}). Since H-abstraction from CH_2 is a major reaction in the $^\bullet$OH-induced oxidation of amino acids having an

aliphatic side-chain, the intermediate with absorption λ_{max} at 330 nm can be assigned to a *C*-centered radical [EGua(C$^\bullet$)] produced by H-abstraction from the protonated EGua. In fact, the observed rate constant for the $^\bullet$OH reaction with EGua at pH 7.0 is substantially comparable to that for the reaction between $^\bullet$OH and Arg ($k = 3.5 \times 10^9$ dm^3 mol^{-1} s^{-1})[21], where H-abstraction from the methylene groups of Arg is like to be a major reaction process.[16,17]

The initially formed radical species in the radiolysis of Ar-saturated EGua solution (pH 7.0) containing $K_2S_2O_8$ and *t*-BuOH ($^\bullet$OH scavenger) was oxidizing $SO_4^{-\bullet}$, since the characteristic absorption with λ_{max} at 450 nm was observed immediately after the pulse irradiation (<1 μs). The decay of the absorption at around 450 nm into the secondary transient absorption at around 330 nm was of pseudo-first-order (Fig. 3), and the apparent rate constants (k_{obs}) showed a linear-dependence with the concentration of EGua (Fig. 3, inset). From the slope of the line, the rate constant for the reaction between $SO_4^{-\bullet}$ and EGua was obtained to be $k(SO_4^{-\bullet} + \text{EGua}) = 1.1 \times 10^8$ dm^3 mol^{-1} s^{-1}.

The similarity of the absorption spectra observed in the reactions of EGua with $^\bullet$OH and $SO_4^{-\bullet}$ might be ascribed to the same radical species, although the decay profiles of the transient absorption spectra were quite different from each other; a bimolecular decay process was observed in the $^\bullet$OH reaction, however a pseudo-unimolecular process was involved in the $SO_4^{-\bullet}$ reaction. In view of reducing characteristics of the *β-C*-centered radical, excess $K_2S_2O_8$ might further oxidize the *C*-centered radical following pseudo-first order kinetics, on the other hand, bimolecular decay might be the major process in the $^\bullet$OH reaction.

The results obtained above can be summarized as shown in Scheme 2. Considering the obtained *G*-value for the formation of the *C*-centered radical was quite close to $G(^\bullet OH)$, it seems that $^\bullet$OH attacks the C-H bonds of the methylene group almost exclusively, and that observed inflection point (Fig. 2, inset) corresponds to the p*K*a of the *C*-centered radical.

3.3 One-electron oxidation of 1,1-dimethylguanidine

The transient species generated at short times (<1 μs) in the $^{\bullet}$OH reaction with 1,1-dimethylguanidine (DMGua) at pH 7.0 showed an absorption maximum at λ_{max} = 320 nm (Fig. 4). In addition to the characteristic absorption, another absorption band appeared at around 270 nm in the radiolysis at basic pH. Considering that these absorption spectra are similar to those of the reducing C-centered radical of EGua($-H^+$) and the N-centered radical of Gua($-H^+$) observed above, detectable amounts of the C-centered radical [DMGua($C^{\bullet}$)] and the N-centered radical of DMGua are possibly produced by the $^{\bullet}$OH reaction. pH-Dependence of the optical density at 275 nm showed a sigmoidal shape with an inflection point at pH 12.0 (Fig. 4, inset). The rate constants for the $^{\bullet}$OH reaction at pH 7.0 and 11.0 evaluated by the competition method were 2.2 × 10^9 dm^3 mol^{-1} s^{-1} and >1.0 × 10^{10} dm^3 mol^{-1} s^{-1}, respectively, on the other hand, the rate constant of the O$^{\bullet -}$ reaction at pH 13.0 was estimated to be k = 2.8 × 10^9 dm^3 mol^{-1} s^{-1}. Redox titration of the C-centered reducing radical generated in the $^{\bullet}$OH reaction with DMGua at pH 7.0 gave a G-value for formation of DMGua($C^{\bullet}$) [G(DMGua($C^{\bullet}$)) = 5.2 × 10^{-7} mol J^{-1}], from which molar absorption coefficient of the radical at 320 nm was estimated to be ε_{320} = 1200 dm^3 mol^{-1} cm^{-1}.

Transient absorption band at around 320 nm was also detected in the oxidation with SO$_4^{-\bullet}$ at pH 7.0 (Fig. 5). The rate constant for the decay of SO$_4^{-\bullet}$ monitored at 450 nm was calculated on the basis of a pseudo first-order kinetic model and k(SO$_4^{-\bullet}$ + DMGua) = 4.6 × 10^8 dm^3 mol^{-1} s^{-1} was obtained (Fig. 5, inset). It was noted that DMGua($C^{\bullet}$) decayed slower than EGua($C^{\bullet}$), and that the decay of the former radical seemed to involve a bimolecular process in addition to the pseudo-first order reaction between DMGua($C^{\bullet}$) and S$_2$O$_8^{2-}$, suggesting that DMGua($C^{\bullet}$) is less reducing radical toward S$_2$O$_8^{2-}$ than EGua($C^{\bullet}$).

It is presumable that oxidation of DMGua by $^{\bullet}$OH proceeds via a similar mechanism to that of EGua. Under neutral conditions, H-abstraction from the methyl group of DMGua generating DMGua($C^{\bullet}$) is the main reaction, since the obtained G-value for DMGua($C^{\bullet}$) formation (5.2 × 10^{-7} mol J^{-1}) was close to G($^{\bullet}$OH) = 5.8 × 10^{-7} mol J^{-1}. Under basic conditions, $^{\bullet}$OH also reacts with the deprotonated form of DMGua [pKa(DMGua) = 11.0] and one-electron oxidation of its nitrogen atom forms the corresponding iminium radical cation, which might further undergo transformation into the N-centered radical. On the

other hand, the N-centered radical (cation) was not detected during the $SO_4^{-\bullet}$-induced oxidation of DMGua at pH 7.0. However, we cannot rule out the possibility that the corresponding iminium radical cation is produced primarily via one-electron oxidation of imino-nitrogen by strongly oxidizing $SO_4^{-\bullet}$. It is well known that aminium radical cations of amino acids often undergo H-shift from the neighboring C-H and generate the corresponding reducing C-centered radicals.[12,30-32] Similar conversion of the N-centered radical into the C-centered radical of DMGua might be involved as a possible reaction channel, although such a minor process could not be observed with our pulse radiolysis system.[31] Similar reaction mechanisms might be applicable to the oxidation of EGua by $^{\bullet}OH$ and $SO_4^{-\bullet}$.

4. Conclusions

In this study, we have investigated $^{\bullet}$OH- and $SO_4^{\bullet-}$-induced oxidation of three guanidine derivatives under various pH conditions. Previous ESR studies on the $^{\bullet}$OH reaction with Arg have suggested that one of the major radical products is the corresponding *C*-centered radical which is generated as a result of H-abstraction from the side-chain of Arg. Our pulse radiolysis data supports these findings since the *C*-centered radicals were observed also in the $^{\bullet}$OH-induced oxidation of structurally similar DMGua and EGua in a pH range between 7 and 13. It is noteworthy that another type of transient absorption bands were detected in the radiolysis of Gua and DMGua under basic pH conditions, which might be ascribed to the formation of the *N*-centered radicals. The difference in the reactivities of $^{\bullet}$OH toward EGua and DMGua might be attributed to the ease of H-abstraction from their alkyl groups. It is not clear yet whether intra/inter-molecular H-shift coupled with deprotonation induces conversion of the *N*-centered radicals into the *C*-centered radicals, because such process is likely to be a minor one. Further investigation on the H-shift reaction in the guanidine derivatives including Arg by the methods of pulse radiolysis and product analysis is now in progress.

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Figure Captions

Fig. 1. Absorption spectra of the intermediates in N_2O-saturated solution of guanidine at ($\times$) pH 7.0 and ($\bullet$) 11.0, obtained 1 μs after the pulse. The inset shows dependence of optical density at 300 nm on the pH of the solution.

Fig. 2. (a) Absorption spectra of the intermediates in N_2O-saturated solution of 1-ethylguanidine at ($\times$) pH 7.0 and ($\bullet$) 11.0, obtained 1 μs after the pulse. The inset shows dependence of optical densities at (+) 330 and ($\blacktriangle$) 375 nm on the pH of the solution. (b) Absorption spectra of the intermediates in Ar-saturated solution of 1-ethylguanidine containing $K_2S_2O_8$ and t-BuOH at pH 7.0, obtained ($\times$) 1, ($\bullet$) 10, and ($\blacktriangle$) 100 μs after the pulse. The inset shows dependence of the observed pseudo-first order rate constants for the decay of absorption at 450 nm on the concentration of 1-ethylguanidine.

Fig. 3. (a) Absorption spectra of the intermediates in N_2O-saturated solution of 1,1-dimethylguanidine at ($\times$) pH 7 and ($\bullet$) 11, obtained 1 μs after the pulse. The inset shows dependence of optical density at 275 nm on the pH of the solution. (b) Absorption spectra of the intermediates in Ar-saturated solution of 1,1-dimethylguanidine containing $K_2S_2O_8$ and t-BuOH at pH 7, obtained ($\times$) 1, ($\bullet$) 10, and ($\blacktriangle$) 100 μs after the pulse. The inset shows dependence of the observed pseudo-first order rate constants for the decay of absorption at 450 nm on the concentration of 1,1-dimethylguanidine.