Singlet oxygen prefers to react with an electron-rich double bonds. We observed that the oxidation rate for uric acid with singlet oxygen increased with increasing pH and the oxidation rate dramatically was elevated at around pH 5.4 and 9.8, which are the acidity constants of uric acid, pK_a1 and pK_a2, respectively. Furthermore, we observed that the absorbance near 200 nm and the molar extinction coefficient (ε) increased with increasing pH, similar to the change in oxidation rate. Computer calculations by Chong [Chong, J Theor Comput Sci 2013; 1(1)] revealed that uric acid elongates its C=N conjugated diene structure with increasing pH. This is correlated with an increase in the UV absorbance of C=C double bonds near 200 nm, and may indicate higher electron density in the double bonds. Therefore, we concluded that the increased oxidation rate is due to elongation of the C=N conjugated polyene system at higher pH. On the other hand, the major products were 4-hydroxylallantoin and parabanic acid (hydrolyzed to oxaluric acid at pH 10.7), suggesting that the reaction pathways were the same regardless of pH. Finally, possible reaction schemes are presented.

Materials and Methods

Chemicals. UA and other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Oxidation of UA with \( \text{O}_2 \), produced by photo-irradiation of Rose Bengal. Phosphate buffer solutions (40 mM) adjusted to various pHs (4.0–10.7) were prepared. After UA (200 μM) and Rose Bengal (0.1 μM) were dissolved in each buffer, accurate pH values for these solutions were measured by a pH meter (SevenEasy S20, Sigma-Aldrich, St. Louis, MO). The reaction mixtures were then irradiated by UVA light (1.12 mW/cm²) for approximately 3 h at various pHs until all the UA was consumed. Concentrations of UA and its reaction products were analyzed by an optimized HPLC system as described below.

Measurement of UV absorption spectra. UV absorption spectra of UA at various pHs were measured. UA was dissolved in phosphate buffers of various pHs (4.4–12.0) and the concentration of UA was 50 μM. The solutions at pH higher than 10.0 were diluted 2.5 times with each buffer (UA concentration was 20 μM). UA absorption spectra (190–400 nm) were measured using a quartz cuvette and a UV-1280 spectrophotometer (Shimadzu, Kyoto, Japan) at room temperature.

HPLC analysis. The change in concentration of UA during photooxidation was determined using a reverse phase HPLC system. Aqueous formic acid (pH 3.0) was delivered at 1.0 ml/min as a mobile phase using a Develosil C30-UG column (5 μm, 4.6 mm × 250 mm, Nomura Chemical Co. Ltd, Tokyo, Japan) for separation. Detection was carried out by monitoring the absorption at 210 nm.

Results and Discussion

The concentration of UA decreased during photooxidation in all solutions tested; however its oxidation rate increased with increasing pH (Fig. 2A). Fig. 2B shows a plot of UA oxidation rates determined as the slopes of changes in UA concentration for the first 30 min against pH values of the reaction mixture. The rate increased with increasing pH and was dramatically elevated at around pH 5.4 and 9.8, which are the acidity constants of uric acid, pK_a1 and pK_a2, respectively.

UV spectra of 50 and 20 μM UA at various pHs are shown in Fig. 3. Interestingly, the plot of the UA oxidation rate (Fig. 2B) well matched changes in the absorbance at 200 nm (Fig. 4A) and the molar extinction coefficient (ε) at \( \lambda_{\text{max}} \) (Fig. 4B). These results strongly indicate that the increase in the UA oxidation rate is correlated with both increasing absorbance at 200 nm and increasing molar extinction coefficient at \( \lambda_{\text{max}} \).

Key Words: uric acid, singlet oxygen, conjugated polyene
One reasonable explanation for this is as follows. The absorbance at 200 nm represents the electron density of the double bonds and a red shift of $\lambda_{\text{max}}$ indicates elongation of the double bonds. For example, the $\lambda_{\text{max}}$ values for benzene, naphthalene, and anthracene are 255, 286, and 375 nm, respectively, and their molar extinction coefficients are 180, 360, and 7,100, respectively.\(^6\) This suggests that a pH increase in the UA solution is accompanied by elongation of the conjugated polyene system on the purine ring. The $\lambda_{\text{max}}$ shifted to longer wavelength with increasing pH, suggesting the extension of C=C conjugation with increasing pH.

The reactivity of $^1\text{O}_2$ to olefins depends on their conjugation length and increases with increasing elongation.\(^7,8\) Dam et al. showed that the rate of photodegradation of phenylenevinylene oligomers became high with a greater extent of conjugation.\(^9\) Therefore, regardless of whether the conjugation was linear or cyclic, these significant increases in reaction rate for $^1\text{O}_2$ and UA at approximately pH 6 and pH 10 are thought to be due to UA ionization resulting in the conjugation related extension of a monoene to a conjugated diene and of a conjugated diene to a conjugated triene, respectively, and their fixation on the purine ring. On the other hand, it was demonstrated that addition of electron-withdrawing groups on the vinyl double bonds reduces the rate of singlet oxygen addition.\(^10\) Considering the electrophilic nature of $^1\text{O}_2$, the electron density on the purine ring of UA is thought to become higher with increasing UA ionization. This seems to be the reason why the dianion (UA\(^{2-}\)) and monoanion (UA\(^-\)) showed greater reactivity toward $^1\text{O}_2$ than UA and UA, respectively. However, further confirmation is needed.

Figure 5 shows HPLC chromatograms obtained following oxidation of UA for 60 min at different pHs. As described above, it was shown that the oxidation of UA proceeded faster at higher pH. However, the products were the same regardless of pH (PA is hydrolyzed to OUA at pH 10.7).

Based on these results, possible reaction schemes are presented in Fig. 6. At pH 7.4, higher than the first acidity constant of UA ($pK_{a1}$ = 5.4), UA is ionized to UA\(^-\) which has a conjugated diene on its purine ring. The $^1\text{O}_2$ molecule reacts with a double bond in UA\(^-\) to yield DIAA via a 5-OH derivative, because the C5–C6 bond has a double bond-like nature. In fact, computer calculations by Chong showed that the C5–C6 bond length became shorter with increasing ionization,\(^5\) indicating the double bond-like
nature of the C5–C6 bond. DIAA is sequentially decomposed into DHAL and 4-HAL by decarboxylation and subsequent hydrolysis. The 4-HAL is a direct precursor for PA production. At pH 10.7, which is higher than the second acidity constant $pK_{a2} (= 9.8)$, UA$^-$ is further ionized to UA$^{2-}$ having a conjugated triene.

Both of the reactions of UA$^{2-}$ and UA$^-$ commonly produce 5-OOH derivatives that are subsequently converted into DIAA because both C5–C6 double bonds are identical. This scheme explains why the reactivity increases with increasing pH and is elevated drastically at around pH 6 and 10 with identical product formation regardless of pH.

A referee pointed out that the oxidation potential should be changed with pH similar to oxidation rate and the molar extinction coefficient, however, we didn’t measure it in this study. We are planning to measure the oxidation potential of UA in future study. We previously proposed that PA and OUA can be adequate

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**Fig. 3.** UV spectra of aqueous 50 µM UA solutions at different pHs. Inset shows UV spectra of 20 µM UA solutions at different pHs (10.0–12.0). See color figures in the on-line version.

**Fig. 4.** Plot of UV absorbance of UA at 200 nm against pH (A) and plot of $\varepsilon$ at $\lambda_{max}$ against pH (B) (Inserted numbers show $\lambda_{max}$).

**Fig. 5.** HPLC chromatograms of reaction solutions after 60 min oxidation with $^1$O$_2$ at pH 4.4, 7.4, and 10.7.
indicators for \( ^{1}O_2 \) formation in vivo because UA is a good scavenger of \( ^{1}O_2 \). In this study, we revealed that the reaction products PA and OUA were formed in common at any pH, whereas the reactivity of UA and \( ^{1}O_2 \) depended on pH. These results suggest that PA and OUA are formed under physiological conditions in the case of \( ^{1}O_2 \) generation in vivo. We expect the analysis of PA and OUA can elucidate the pathological role of \( ^{1}O_2 \) in oxidative-stress diseases.

**Abbreviations**

- DHAL: dehydroallantoin
- DIAA: (2,5-dioxoimidazolidin-4-ylidene)aminocarbonyl-carbamic acid
- 4-HAL: 4-hydroxylallantoin
- \( ^{1}O_2 \): singlet oxygen
- OUA: oxaluric acid
- PA: parabanic acid
- UA: uric acid
- UA\(^{-}\): uric acid monoanion
- UA\(^{-2}\): uric acid dianion

**Conflicts of Interest**

No potential conflicts of interest were disclosed.

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