Photoinduced DNA cleavage by formation of ROS from oxygen with a neurotransmitter and aromatic amino acids†

Tomonori Kawashima,[a] Kei Ohkubo[a] and Shunichi Fukuzumi*[a,b]

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UV-B photoirradiation of a neurotransmitter (serotonin) and aromatic amino acids (tryptophan and tyrosine) with oxygen results in DNA cleavage by generation of reactive oxygen species (ROS) as demonstrated by agarose gel electrophoresis with pBR 322 DNA, ESR and laser flash photolysis measurements.

UV irradiation is the most frequent cause of DNA abnormality produced by reactive oxygen species (ROS).[1,2] Photochemistry of aromatic amino acids has widely been studied with regard to generation of ROS such as singlet oxygen ($^1O_2$) and superoxide ($O_2^{\bullet-}$).[3–6] In general, there are two main processes responsible for photosensitized reactions (Scheme 1): (1) the chromophore is excited by light to a triplet state, and undergoes a direct electron or hydrogen exchange with a substrate, creating a free radical (Type I process); (2) energy transfer from the excited chromophore directly to oxygen, resulting in generation of $^1O_2$ (Type II process).[7–11] Formation of $^1O_2$ by UV irradiation of aromatic amino acids such as tryptophan and tyrosine, which absorb the light in the UV-B range (290–320 nm)[12] has been detected by its near IR emission at 1270 nm.[13,14] The $^1O_2$ quantum yields for tyrosine ($\Phi_\Delta = 0.14$) and tryptophan ($\Phi_\Delta = 0.062$) are significantly smaller than those of the corresponding triplet formation ($\Phi_T = 0.50$ and 0.18), respectively.[14] Electron transfer from the triplet excited state to O_2 may contribute to the difference between the triplet quantum yield (Φ_T) and the $^1O_2$ quantum yield (Φ_Δ).[14,15] One of important effects of ROS is abnormality *via* the DNA cleavage by ROS in the biological cell. However, DNA damage by ROS generated by photoirradiation of aromatic amino acid or neurotransmitter with oxygen has yet to be reported.

We report herein the effects of ROS produced by UV-B photoirradiation of a neurotransmitter, serotonin (5-hydroxytryptamine: 5-HT) and aromatic amino acids, tryptophan (Trp) and tyrosine (Tyr), with oxygen on DNA cleavage. The efficiency for the DNA cleavage with ROS produced by photoirradiation of a neurotransmitter and aromatic amino acids in Chart 1 was examined using agarose gel electrophoresis. We have also confirmed formation of superoxide ion by photoirradiation of 5-HT, which was most effective for DNA cleavage, by using electron spin resonance (ESR) and laser flash photolysis.

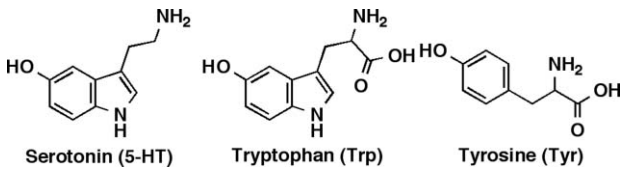
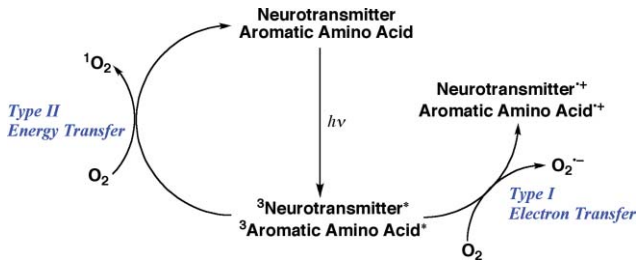


Chart 1 Neurotransmitter and aromatic amino acids used in this study.



Scheme 1 Two possible pathways of generation of active species in the neurotransmitter and amino acid–O_2 system under UV-irradiation.

The widely used assay with pBR322 supercoiled DNA[15,16] was employed in order to clarify the active species responsible for the DNA cleavage activity upon UV-B irradiation of 5-HT, Trp and Tyr with O_2. The supercoiled DNA (form I) is efficiently cleaved into form II (nicked DNA) by photoirradiation of an air-saturated phosphate buffer solution (0.10 mol dm^{-3}, pH 7.0) of 5-HT, Trp and Tyr with use of a monochromatized light (λ = 300 nm) from a xenon lamp as shown in Fig. 1 (lanes 5, 8 and 10, respectively). Serotonin exhibits the largest reactivity for the photoinduced DNA cleavage with oxygen. Without oxygen no photoinduced DNA cleavage was observed with 5-HT as shown in Fig. 1 (lane 4). Under dark conditions, no DNA cleavage occurs in the presence of 5-HT, Trp or Tyr as shown in Fig. 1 (lanes 3, 7 and 9, respectively).

To test the possible role of $^1O_2$, the effect of $^1O_2$ stabilizer was tested. When H_2O is replaced by D_2O, which is known to prolong the lifetime of $^1O_2$,[17] the DNA cleavage activity remains the same (ESI,† S1). This indicates that $^1O_2$ does not play a significant role in the DNA-cleaving activity under UV-B irradiation. In contrast to the effect of the $^1O_2$ stabilizer, the DNA cleaving activity was clearly inhibited by the addition of superoxide dismutase (SOD),[18] which quenches $O_2^{\bullet-}$ (lane 6 in Fig. 1). This suggests that formation of $O_2^{\bullet-}$ plays an essential role in the DNA cleavage, although $O_2^{\bullet-}$ is generally regarded as a rather unreactive radical species.[19]

[a]*Department of Material and Life Science, Graduate School of Engineering, Osaka University, SORST, Japan Science and Technology Agency (JST), Yamada-oka 2-1, Suita, Osaka 565-0871, Japan. E-mail: fukuzumi@chem.eng.osaka-u.ac.jp; Fax: (+81)-6-6879-7370; Tel: (+81)-6-6879-7368*
[b]*Department of Bioinspired Science, Ewha Womans University, Seoul, 120-750, Korea*

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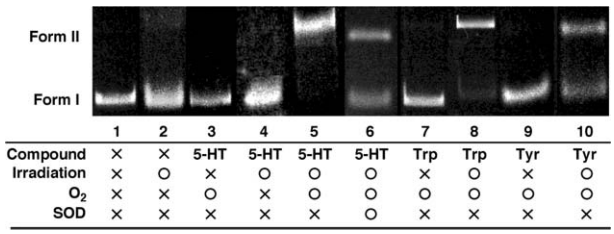

Fig. 1 DNA cleavage after 20 min photoirradiation with monochromatized light ($\lambda = 300$ nm). Lane 1, control; lane 2, photoirradiation; lane 3, no photoirradiation with 5-HT (1.0 mmol dm^{-3}); lane 4, photoirradiation of an Ar-saturated aqueous solution with 5-HT (1.0 mmol dm^{-3}); lane 5, photoirradiation of an O_2-saturated aqueous solution with 5-HT (1.0 mmol dm^{-3}); lane 6, photoirradiation of an O_2-saturated aqueous solution with 5-HT (1.0 mmol dm^{-3}) and SOD (140 μg dm^{-3}); lane 7, no photoirradiation of an O_2-saturated aqueous solution with Trp (1.0 mmol dm^{-3}); lane 8, photoirradiation of an O_2-saturated aqueous solution with Trp (1.0 mmol dm^{-3}); lane 9, no photoirradiation of an O_2-saturated aqueous solution with Tyr (1.0 mmol dm^{-3}); lane 10, photoirradiation of an O_2-saturated aqueous solution with Tyr (1.0 mmol dm^{-3}).

DNA can also be cleaved with 5-HT and Trp under irradiation of the UV-A light ($\lambda = 340$ nm) as shown in Fig. 2, where Tyr exhibits no reactivity because Tyr has no absorption at 340 nm.

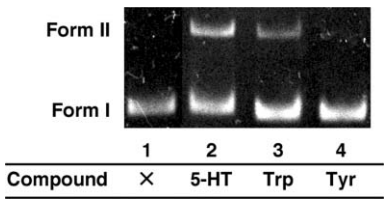

Fig. 2 Agarose gel electrophoresis of cleaved supercoiled pBR322 DNA after UVA-irradiation of aromatic amino acid and neurotransmitter with O_2 in an air-saturated aqueous buffer solution (pH 7.0) at 298 K. Cleavage of DNA after 20 min photoirradiation with monochromatized light ($\lambda = 340$ nm). Lane 1, control (DNA after photoirradiation); lane 2, photoirradiation with 5-HT (4.0 mmol dm^{-3}); lane 3, photoirradiation with Trp (4.2 mmol dm^{-3}); lane 4, photoirradiation with Tyr (4.5 mmol dm^{-3}).

Nanosecond laser flash photolysis measurements were performed to examine the photoinduced electron-transfer dynamics of 5-HT and aromatic amino acids with O_2. Transient absorption spectra of a phosphate buffer solution (0.10 mol dm^{-3}, pH 7.0) containing 5-HT (1.7×10^{-4} mol dm^{-3}) were observed upon the laser excitation at 266 nm (Fig. 3a). The shoulder absorption band at 480 nm is assigned to the triplet excited state of 5-HT (^{3}5-HT*: * denotes the excited state) observed at 0.8 μs after laser excitation.[20] The broad absorption around 700 nm is attributed to hydrated electron (e_{aq}^-),[17] which is produced by photoionization of 5-HT. The absorption band observed at 410 nm at 90 μs after laser excitation is assigned to the neutral 5-indoloxyl radical that is formed by deprotonation of 5-HT$^{\bullet+}$ due to the low pK_a value of –0.7.[17] The decay time profile at 480 nm due to ^{3}5-HT* obeyed first-order kinetics as shown in Fig. 3b.

In the presence of O_2, e_{aq}^- reacts immediately with O_2 by electron transfer to produce $O_2^{\bullet-}$. On the other hand, the first-order decay rate constant of ^{3}5-HT* increases with increasing concentration of O_2. The bimolecular quenching rate constant of ^{3}5-HT* with O_2 is determined to be 9.2×10^8 mol^{-1} dm^3 s^{-1}. This indicates that ^{3}5-HT* is efficiently quenched by energy transfer or/and electron

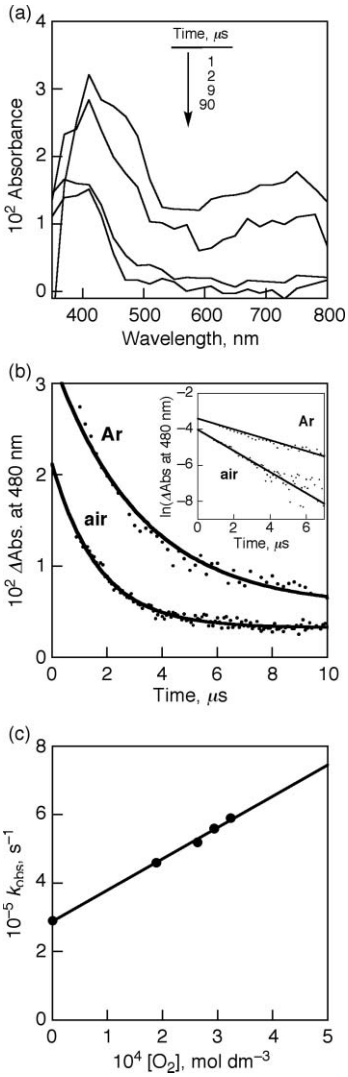

Fig. 3 (a) Transient absorption spectra of 5-HT (2.1×10^{-4} mol dm^{-3}) in a deaerated phosphate buffer solution (0.10 mol dm^{-3}, pH 7.0) after laser excitation at 266 nm. (b) Decay time profiles of ^{3}5-HT* ($\lambda = 480$ nm) in argon- and air-saturated buffer solutions. The solid curves represent the best fit to the exponential decay. Inset: First order plots. (c) Plot of k_{obs} *vs.* [O_2].

transfer from ^{3}5-HT* to O_2. Formation of singlet oxygen by energy transfer form ^{3}5-HT* to O_2 was previously confirmed by near-IR emission spectral measurements at 1270 nm from singlet oxygen.[20]

The quenching rate constants (k_q) of the triplet excited states of Trp and Tyr were also determined by the decay of the T-T absorption, which is accelerated by the presence of oxygen (ESI,† S2, S3). The k_q values are listed in Table 1 together with the one-electron oxidation potentials of the ground state (E_{ox}) and the triplet excited states ($^3E_{ox}$*) of Trp, 5-HT and Tyr. The E_{ox} values of ^{3}Trp*, ^{3}5-HT* and ^{3}Tyr* are determined to be –1.84, –1.94 and 2.25 V *vs.* SCE (saturated-calomel-electrode) by subtracting the triplet excitation energies (2.86, 2.82 and 3.18 eV)[16] from the E_{ox} values of the ground states (1.02, 0.88 and 0.93 V *vs.* SCE).[18] The k_q value increases with decreasing the E_{ox} value of the triplet excited state. This suggests that electron transfer from ^{3}Trp*, ^{3}5-HT* and ^{3}Tyr* to O_2 may also play a role in the triplet quenching

Table 1 One-electron oxidation potentials at the ground and triplet excited states of neurotransmitter and amino acids (E_{ox} and $^3E_{ox}$*, respectively), and the rate constants of electron transfer (k_{et}) from the triplet excited states to O_2

Compound	E_{ox} *vs.* SCE[a]/V	$^3E_{ox}$* *vs.* SCE[b]/V	k_{et}[c]/mol^{-1} dm^3 s^{-1}
Trp	1.02	−1.84	8.6×10^8
5-HT	0.88	−1.94	9.2×10^8
Tyr	0.93	−2.25	2.7×10^9

[a] Taken from ref. 17. [b] Determined from phosphorescence reported in ref. 18, 23 and 24. [c] Experimental error is within 5%.

with O_2. This is supported by the large driving force of electron transfer from ^{3}Trp* ($^3E_{ox}$* = −1.84 V *vs.* SCE), ^{3}5-HT* ($^3E_{ox}$* = −1.94 V *vs.* SCE) and ^{3}Tyr* ($^3E_{ox}$* = −2.25 V *vs.* SCE) to O_2 (E_{red} = −0.40 V *vs.* SCE).[21] Thus, photoexcitation of 5-HT, Trp and Tyr with O_2 results in formation of $O_2^{\bullet-}$ *via* photoionization followed by electron transfer from e_{aq}^- to O_2 and also electron transfer from the triplet excited states to O_2.[22] ^{3}Tyr* which has the most negative $^3E_{ox}$* value exhibits the highest reactivity toward O_2 (Table 1). However, no effective DNA cleavage was observed under UVA irradiation (λ = 340 nm) in Fig. 2, because Tyr has no absorption band at 340 nm. The formation of $O_2^{\bullet-}$ upon photoexcitation of 5-HT with O_2 by use of a mercury lamp is confirmed by the ESR spectrum measured at 77 K as shown in Fig. 4, where the anisotropic signals at $g_{\|}$ = 2.080 and $g_{\perp}$ = 2.002 are well assigned to $O_2^{\bullet-}$.[25,26] 5-HT$^{\bullet+}$ is also observed around g = 2.0042,[27] which is overlapped with the $g_{\perp}$ signal of $O_2^{\bullet-}$.

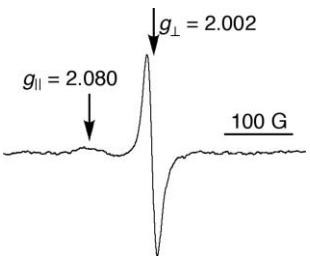

Fig. 4 ESR spectrum of $O_2^{\bullet-}$ generated by photoirradiation of an O_2-saturated aqueous buffer solution (pH 7.0) containing 5-HT (0.47 mol dm^{-3}) with a high-pressure mercury lamp at 298 K and immediately measured at 77 K.

In summary, the photoreactivity of a neurotransmitter (serotonin) for DNA cleavage is significantly larger than aromatic amino acids (tryptophan and tyrosine) at ambient temperature. The photocleavage of DNA is initiated by electron transfer from the triplet excited states of a neurotransmitter and aromatic amino acids to O_2 (Type I) rather than energy transfer to produce $^1O_2$ (Type II).

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