Photoinduced Electron Transfer from Amino Acids and Proteins to 4-Nitroquinoline 1-Oxide in Aqueous Solutions

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The reactions of the triplet state of 4-nitroquinoline 1-oxide (4NQO) with a series of amino acids and some proteins in aqueous solutions have been studied by using a laser flash technique. Only tryptophan (TrpH) and tyrosine (TyrOH) among a series of amino acids quench the triplet 4NQO (^{T}4NQO) at a diffusion-controlled rate. Lysozyme, ribonuclease, and histone, which contain TrpH and/or TyrOH residues, had rate constants comparable to those of TrpH and TyrOH. The formation of the H adduct of 4NQO (4NQOH$^\bullet$), which may be produced by the reaction of $4NQO^-$ with water, was confirmed from the transient absorption spectra for 4NQO solutions containing these quenchers. The transient absorption spectra observed for TrpH and TyrOH solutions elucidated the formation of the deprotonated forms of $TrpH^+$ and $TyrOH^+$ (Trp$^\bullet$ and TyrO$^\bullet$) together with 4NQOH$^\bullet$. The result demonstrates that the electron transfer from TrpH or TyrOH to ^{T}4NQO occurs in the triplet quenching by TrpH or TyrOH. Since the almost same transient spectra as Trp$^\bullet$ and TyrO$^\bullet$ were observed for lysozyme and ribonuclease solutions, respectively, TrpH residues on lysozyme and TyrOH residues on ribonuclease are main quenching sites, where electron transfer and deprotonation occur. The quantum yields of ^{T}4NQO, 4NQOH$^\bullet$, Trp$^\bullet$, and TyrO$^\bullet$ produced by the excitation of the 4NQO solution containing TrpH or TyrOH with a 355-nm light pulse were determined to be 0.46, 0.47, 0.41, and 0.41, respectively. The result shows that the efficiency in electron transfer from TrpH or TyrOH to ^{T}4NQO is ~90%. For the reaction of ^{T}4NQO with methionine, arginine, histidine, lysozyme, or ribonuclease, the efficiency in electron transfer was also estimated to be nearly equal to that for the reaction with TrpH or TyrOH.

Introduction

4-Nitroquinoline 1-oxide (4NQO), one of the typical carcinogenic compounds, has the property to bind DNA bases via charge-transfer interaction in an aqueous solution.[1,2] The property may be closely related to carcinogenicity. The genetic function which DNA possesses emerges by forming chromatin, a complex of DNA and proteins such as histone and nonhistone chromosomal proteins. Such proteins bonded to DNA, as well as DNA and nucleic acid bases, also may participate in the process of carcinogenicity induced by chemicals. It, therefore, is worthwhile in the study of the reaction of 4NQO with chromatin to elucidate the reactions of 4NQO with amino acids and proteins. We have previously studied the photochemical reactions of 4NQO with DNA bases and related compounds as well as DNA in aqueous solutions by using a laser flash photolysis technique.[3] The triplet state of 4NQO (^{T}4NQO) produced by excitation with a 355-nm light pulse has been found to be efficiently quenched by the compounds having lower oxidation potentials. The transient absorption spectra have confirmed the formation of the H adduct radical of 4NQO (4NQOH$^\bullet$) in photoirradiated solutions of 4NQO and the quenchers. These results suggested the participation of charge transfer in the reaction of ^{T}4NQO with the quenchers, because 4NQOH$^\bullet$ is considered to be produced by the reaction of $4NQO^-$ with H_2O.[3] However, evidence for the formation of any quencher cation was not obtained. One of the primary purposes of the present work is to find definitive proof of whether electron transfer occurs in the quenching of ^{T}4NQO and to clarify the quenching mechanism by the determination of the quantum yields of the transients produced in the irradiated solutions. Our attention in the present work is especially focused on tryptophan (TrpH), tyrosine (TyrOH), and the proteins including these amino acids, because TrpH and TyrOH are easily oxidized to their cations or deprotonated forms of the cations, which have the characteristic absorption spectra, by UV-light irradiation[4-6] or the reactions with $Br_2^{\bullet-}$, $(SCN)_2^{\bullet-}$, $SO_4^{\bullet-}$, and $N_3^\bullet$.[7-10]

Experimental Section

4NQO was purchased from Sigma Chemical Co. and recrystallized from acetone solutions. Analytical reagent grade amino acids from Wako Pure Chemical Industry Ltd. and Tokyo Kasei Kogyo Co. were used as received. Ribonuclease A (from bovine pancreas), protamine phosphate (from salmon sperm) and histone H2A (from calf thymus) were supplied by Sigma Chemical Co. Lysozyme (from egg white), crystallized 6 times, was obtained from Seikagaku Kogyo Co. They were used without purification. Bityrosine, used as the standard, was prepared by an enzymatic method and was purified by the Lehrer-Fasman procedure.[11]

Solutions were prepared by using redistilled water and buffered at pH 8 by employing phosphates, except for histone solutions, where sodium hydroxide was used. The concentrations are 1.0-1.4 $\times 10^{-4}$ mol dm^{-3} for 4NQO and 5×10^{-5}-1×10^{-2} mol dm^{-3} for amino acids or proteins. Deaeration was carried out by a freeze-thaw cycle method for amino acid solutions and by blowing argon gas over the surface of the liquid in the cells for protein solutions.

A conventional laser flash photolysis technique was used for the measurements of the absorption spectra and the decay rates of the transients. Excitation light was the third harmonic (355 nm) from a Nd:YAG laser (20-ns pulse duration). The detection system consisted of a 150-W Ushio xenon lamp as an analyzing light source, a monochromator (Ritsu Model MC-20N), a photomultiplier (Hamamatsu R758), and an oscilloscope (Tektronix Model 7904). The optical path lengths of a reaction cell were 10 mm. The details of the procedures for detecting transient absorption have been described previously.[3] The apparatus and procedures used in pulse radiolysis have been also described

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TABLE I: Quenching Rate Constants of ^{T}4NQO and Oxidation Potentials of Quenchers

quencher	k_2/dm^3 mol^{-1} s^{-1} (pH 8)	E_{ox}/eV
tryptophan	3.7×10^9	1.07[a]
tyrosine	3.1×10^9	1.62[a]
methionine	2.9×10^7	1.76[a]
arginine	1.7×10^7	
histidine	1.6×10^7	
proline	1.3×10^7	2.08[a]
phenylalanine	1×10^7	2.45[a]
glutamic acid	1×10^7	
leucine	1×10^7	2.56[b]
lysine	$<3 \times 10^6$	2.54[a]
glycine	$<3 \times 10^6$	3[a]
histone	2.9×10^9	
lysozyme	2.5×10^9	
ribonuclease	2.2×10^9	
protamine	4.0×10^6	

[a] From ref 14. [b] From ref 15.

elsewhere.[12] The 366-nm light from a high-pressure mercury lamp was used for steady-state irradiation. A Hitachi fluorophotometer (MPF-4) was employed for measuring the fluorescence intensity of bityrosine. Sulfuric acid solutions of $K_3Fe(C_2O_4)_3$ were used for the actinometry in steady-light irradiation.

Results and Discussion

Quenching Rate. The irradiation of an aqueous solution of 4NQO with 355-nm laser pulses yields the transient absorption spectrum with maxima at 410 and 590 nm. This spectrum has been assigned to ^{T}4NQO, because T–T transfer to naphthacene occurs in benzene solution containing 4NQO and naphthacene. When quenchers (i.e., amino acids or proteins) are added to aqueous solutions of 4NQO, the absorption due to ^{T}4NQO is quenched, and the long-lived absorption with a peak around 450 nm grows upon the pulse irradiation. The latter spectrum is the same as the spectrum reported previously and assigned to 4NQOH$^\bullet$.[3] The decay of the triplet and the growth of 4NQOH$^\bullet$ obey the first-order kinetics with nearly an identical rate. The plots of decay rates of the triplet against concentrations of the added compounds give straight lines. From the slopes, the rate constants of the reactions of ^{T}4NQO with a number of amino acids and some proteins have been determined in aqueous phosphate buffer solutions at pH 8. The concentration ranges examined in this experiment depend on the reactivity of the additives. Solutions of TrpH, TyrOH, histone, etc., which have high reactivity toward the triplet, were prepared in the range from $\sim 5 \times 10^{-5}$ to $\sim 5 \times 10^{-4}$ mol dm^{-3} and solutions of methionine, arginine, etc., which have moderate or rather low reactivity, were in the range from $\sim 5 \times 10^{-4}$ to $\sim 1 \times 10^{-2}$ mol dm^{-3}. The rate constants are summarized in Table I together with oxidation potentials (in eV) of the compounds estimated from the equation of $E_{ox} = 0.92$ (ionization potential) $- 6.20$.[13] The sources of ionization potentials are the photoelectron spectra of these compounds.[14,15]

The quenching rate constants of TrpH and TyrOH were found to be $3–4 \times 10^9$ dm^3 mol^{-1} s^{-1}. Methionine, arginine, histidine, proline, leucine, glutamic acid, and phenylalanine react with the rate constants of $1–3 \times 10^7$ dm^3 mol^{-1} s^{-1}. The rate constants for lysine and glycine are too small to be determined in the present study. The data in Table I give the obvious correlation that the amino acid with the lower oxidation potential has the larger rate constant. The triplet energies of the amino acids examined here are expected to be larger than that of 4NQO: 4NQO, $\sim$15 000 cm^{-1};[3] TrpH, $\sim$25 000 cm^{-1};[16,17] TyrOH and phenylalanine, $\sim$29 000 cm^{-1};[17,18] etc. These facts suggest that the electron transfer from the amino acids to ^{T}4NQO occurs in the triplet quenching. Protamine, histone, and ribonuclease were chosen as examples of the proteins which are closely correlated with nucleic acid in terms of physiological functions. Protamine has neither TrpH nor TyrOH residue, while ribonuclease and histone have TyrOH residues but no TrpH residue.[19,37] Lysozyme was chosen as an example of the proteins containing both TrpH and TyrOH residues.[19,20,38] The rate constants for the reactions of ^{T}4NQO with lysozyme, ribonuclease, and histone were found to be comparable to those for TrpH and TyrOH. Protamine was found to have a small rate constant of 4×10^6 dm^3 mol^{-1} s^{-1}. These results, therefore, indicate that the residues of TrpH and/or TyrOH on the surfaces of lysozyme, ribonuclease, and histone are the main effective sites for the quenching of ^{T}4NQO.

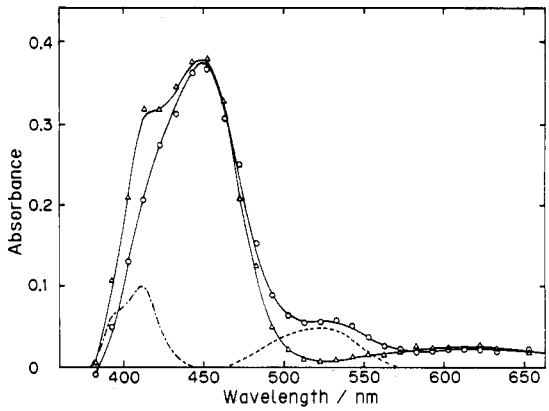

Figure 1. Transient absorption spectra observed at 2 μs after 355-nm laser pulse irradiation for aqueous phosphate buffer solutions (pH 8) containing solutions 1 or 2: (1) 1.2×10^{-3} mol dm^{-3} 4NQO and 1.0×10^{-3} mol dm^{-3} TrpH (○), (2) 1.2×10^{-3} mol dm^{-3} 4NQO and 1.0×10^{-3} mol dm^{-3} TyrOH (△). Spectra represented by (- - -) and (—·—·—) are the differences between the spectra observed for solutions 1 and 2.

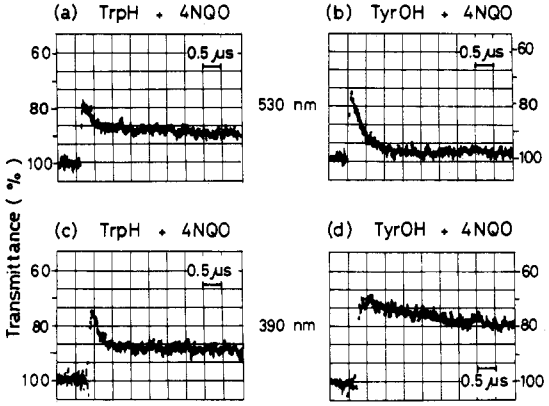

Figure 2. Oscilloscope traces of the changes in transmission at 530 (a, b) and 390 nm (c, d) observed for solutions 1 and 2 which are noted in the legend of Figure 1.

Transient Absorption Spectrum. Absorption spectra were measured for the transients produced from the reactions of ^{T}4NQO with TrpH, TyrOH, lysozyme, and ribonuclease. Figure 1 shows the spectra observed at 2 μs after pulse irradiation for the solution containing 1.2×10^{-4} mol dm^{-3} 4NQO and 1.0×10^{-3} mol dm^{-3} TrpH or TyrOH. The time-resolved curves of the absorption changes at 390 and 530 nm for the irradiated solutions are shown in Figure 2. The rapid decay of the absorption immediately after the pulse, as is shown in Figure 2, a–c, corresponds to the decay of ^{T}4NQO. Therefore, it is apparent that ^{T}4NQO should be completely quenched under the condition where the spectra shown in Figure 1 are observed. This is warranted by the half-life of

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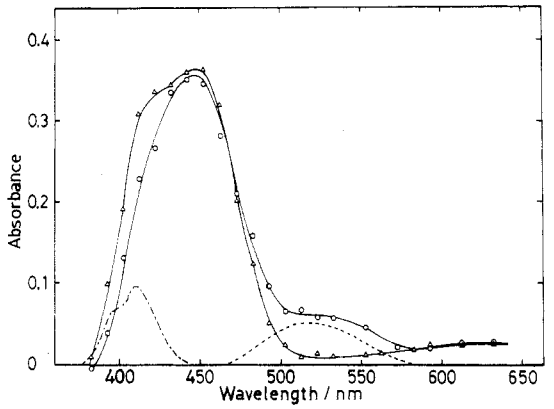

Figure 3. Transient absorption spectra observed at 4 μs after 355-nm laser pulse irradiation for aqueous phosphate buffer solutions (pH 8) containing solutions 1 or 2: (1) 1.2×10^{-3} mol dm^{-3} 4NQO and 5×10^{-4} mol dm^{-3} lysozyme (○), (2) 1.2×10^{-3} mol dm^{-3} 4NQO and 7×10^{-4} mol dm^{-3} ribonuclease (△). Spectra represented by (---) and (-·-·-) are the differences between the spectra observed for solutions 1 and 2.

the triplet decay, $\tau_{1/2} \simeq 0.23$ μs, calculated from $k \simeq 3 \times 10^9$ dm^3 mol^{-1} s^{-1} and [TrpOH] or [TyrOH] = 1×10^{-3} mol dm^{-3}. The strong absorption centered at 450 nm for both solutions of TrpH and TyrOH in Figure 1 is due to 4NQOH• as it is noted in the Quenching Rate section. The remaining absorptions after the decay of the triplet, shown in Figure 2c, are mainly due to this radical. The spectrum obtained from the TrpH solution apparently shows a weak band in the region from 500 to 550 nm, and the spectrum obtained from the TyrOH solution has a shoulder around 410 nm. The difference absorption spectra between the spectra observed for TrpH and TyrOH solutions, also shown in Figure 1, reveal that certain transients other than 4NQOH• are produced by the reactions of ^{T}4NQO with TrpH and TyrOH. The difference spectrum with a maximum at ~510 nm is very similar to the spectrum identified with the deprotonated form radical of TrpH$^+$ (Trp•).[6-9] The other one with a maximum at ~410 nm closely resembles the spectrum assigned to the deprotonated radical form of TyrOH$^+$ (TyrO•).[5,10] Therefore, it is concluded that the electron transfer from TrpH (or TyrOH) to ^{T}4NQO occurs to yield Trp• (or TyrO•) and 4NQOH• in the reaction of ^{T}4NQO with TrpH (or TyrOH). The formation of 4NQOH• also indicates the participation of electron transfer to ^{T}4NQO in the reactions of ^{T}4NQO with other amino acids, i.e., methionine, arginine, histidine, etc., though the transient absorption spectra due to their cations were not observed.

The transient absorption spectra observed at 4 μs after the pulse for the solutions of lysozyme and ribonuclease, as shown in Figure 3, are nearly equal to the spectra obtained from the solutions of TrpH and TyrOH, respectively. This is consistent with a quenching mechanism in which the triplets are quenched at the sites of TyrOH residues on ribonuclease, since the enzyme has neither TrpH residue nor any other amino acid residue more reactive to ^{T}4NQO than TyrOH. In the case of lysozyme-containing TrpH and TyrOH residues,[20,38] on the other hand, both residues can act as possible quenchers. The resemblance of the transient spectra between lysozyme and free TrpH indicates two possible mechanisms for the quenching of ^{T}4NQO by lysozyme: (i) the quenching occurs predominantly at the sites of TrpH residues owing to the effects of steric hindrance for TyrOH residues within the protein; (ii) the oxidized TyrOH residues produced by the attack of ^{T}4NQO undergo radical transformation into TrpH residues (i.e., ···TrpH···TyrO•··· → ···Trp•···TyrOH···) within 4 μs. The following evidences, however, exclude the latter mechanism. An efficient intramolecular transfer of the radical site from TrpH residues to TyrOH residues (i.e., ···Trp•···TyrOH··· → ···TrpH···TyrO•···) has been shown to occur in numerous peptides and several proteins in aqueous solutions.[21-26] The rate

TABLE II: Molar Extinction Coefficients (ϵ) and Quantum Yields (ϕ) for the Transients Produced in the Irradiated 4NQO Solutions Containing TrpH or TyrOH

transients	ϵ/dm^3 mol^{-1} cm^{-1} (λ_{max}, nm)	ϕ
^{T}4NQO	7600 (590)	0.46
4NQOH•	13000 (450)	0.47
Trp•	1960 (510)[a]	0.41
TyrO•	3200 (410)[b]	0.41

[a] From ref 9. [b] From ref 10.

of the transformation in lysozyme has been found to be $k = \sim 10^2$ s^{-1} at pH 7.[24-26] The equilibrium constants for the transformation in the following dipeptides were reported to be about 4.3 and 8.7, respectively: TyrOH-Trp• ⇄ TyrO•-TrpH and Trp•-TyrOH ⇄ TrpH-TyrO•.[21] Therefore, TyrO• forms are preferential to Trp• forms in the peptides. The former mechanism is supported by the following evidence. It has been shown that TrpH residues of lysozyme are the main sites which are attacked by OH radicals,[27,28] although the reactions of free TrpH and TyrOH with the radicals have the similar rate constants.[29] We, therefore, conclude that TrpH residues on lysozyme and TyrOH residues on ribonuclease are the main quenching sites where electron transfer to ^{T}4NQO occurs to yield Trp• and TyrO• residues and 4NQOH•, in the reactions of ^{T}4NQO with lysozyme and ribonuclease, respectively.

Quantum Yields and Efficiency of Electron Transfer. The quantum yields of ^{T}4NQO, 4NQOH•, Trp•, and TyrO• formation by 355-nm excitation were determined, using a comparative technique, for the aqueous 4NQO solution containing TrpH or TyrOH. In this technique anthracene in benzene was employed as a standard where the molar extinction coefficient at 430 nm and the quantum yield of the anthracene triplet state were taken to be 45 500 dm^3 mol^{-1} cm^{-1} and 0.67, respectively.[30,31] The number of ^{T}4NQO, 4NQOH•, Trp•, or TyrO• produced by a given number of laser quanta at 355 nm was thus compared with the number of the standard triplets produced by the same number of laser photons. The resulting quantum yields, together with the molar extinction coefficients (ϵ), are given in Table II.

To approach the evaluation of the number of ^{T}4NQO produced in the aqueous solution, $\epsilon(^T\text{4NQO})$ at λ_{max} (540 nm) in benzene was determined by using the energy-transfer method in which ^{T}4NQO is used to sensitize the formation of the naphthacene triplet (^{T}naph). Since the naphthacene concentration (7.5×10^{-5} mol dm^{-3}) was sufficiently large to quench all ^{T}4NQO and the formation rate of ^{T}naph was faster compared to its decay rate under the conditions employed in this work, the following relation holds: $\epsilon(^T\text{4NQO}) = \epsilon(^T\text{naph}) \times \text{OD}(^T\text{4NQO})/\text{OD}(^T\text{naph})$, where OD(^{T}4NQO) and OD(^{T}naph) are the maximum optical densities of ^{T}4NQO and ^{T}naph, respectively. The absorbance of naphthacene was about 5% of the total absorbance of 4NQO and naphthacene at 355 nm in the above solution. OD(^{T}naph) in the above equation was corrected by taking account of ^{T}naph formation due to the direct excitation and its quantum yield in benzene (0.62).[31] When 31 200 dm^3 mol^{-1} cm^{-1} was used for $\epsilon(^T\text{naph})$ at 465 nm,[30] $\epsilon(^T\text{4NQO})$ at 540 nm in benzene was found to be 7600 dm^3 mol^{-1} cm^{-1}. OD(^{T}4NQO)'s at λ_{max} were found to have a nearly equal value in water, ethanol, *tert*-butyl alcohol,

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and benzene, though the wavelength of the absorption maximum changed from 590 to 540 nm in those different solvents. In this experiment, the concentration of 4NQO in each solution was adjusted to give an equal absorbance at the wavelength of the laser irradiation. The absorption spectra of ^{T}4NQO in benzene and water were plotted on a frequency scale, and the areas under the first absorption bands and their widths at half-peak-height were found to be nearly equal in both solutions. Therefore, $\epsilon(^T4NQO)$'s at λ_{max} in benzene and water were concluded to have the same value, on the assumption that the oscillator strength and the triplet yield are independent of solvent.

For the evaluation of ϵ(4NQOH$^\bullet$), argon-flushed aqueous solution of 4NQO (1.4×10^{-3} mol dm^{-3}) containing *tert*-butyl alcohol (0.1 mol dm^{-3}) as a OH radical scavenger and pure water were subjected to pulse radiolysis. In the former solution, all hydrated electrons formed after pulse should react with 4NQO to produce 4NQO$^-$ which is rapidly converted into 4NQOH$^\bullet$ by protonation[3] and any other pathway does not produce 4NQOH$^\bullet$. The maximum concentration of 4NQOH$^\bullet$ formed in the 4NQO solution thus equals that of e_{aq}^- formed in pure water when both samples absorb equal dosage, and ϵ(4NQOH$^\bullet$) was derived from

$$\epsilon(4NQOH^\bullet)(\text{at } 450 \text{ nm}) = \epsilon(e_{aq}^-)(\text{at } 720 \text{ nm}) \times \mathrm{OD}(4NQOH)(\text{at } 450 \text{ nm})/\mathrm{OD}(e_{aq}^-)(\text{at } 720 \text{ nm})$$

This technique is essentially consistent with that used by Land for determination of the extinction coefficient of a benzophenone ketyl radical in water.[32] Consequently, ϵ(4NQOH$^\bullet$) at 450 nm was evaluated to be 1.3×10^4 dm^3 mol^{-1} cm^{-1} by using $\epsilon(e_{aq}^-)$ (at 720 nm) = 1.85×10^4 dm^3 mol^{-1} cm^{-1} [33] and a value of 0.47 was obtained for quantum yield of 4NQOH$^\bullet$.

The quantum yields of Trp$^\bullet$ and TyrO$^\bullet$ formation were estimated from the optical densities at 510 and 410 nm in the difference absorption spectra of Figure 1, which correspond to the difference between the absorptions of Trp$^\bullet$ and TyrO$^\bullet$ at these wavelengths. The following molar extinction coefficients (in dm^3 mol^{-1} cm^{-1}) were employed: ϵ(Trp$^\bullet$)(at 510 nm) = 1960,[9] ϵ(Trp$^\bullet$)(at 410 nm) = 400,[9] ϵ(TyrO$^\bullet$)(at 410 nm) = 3200,[10] and ϵ(TyrO$^\bullet$)(at 510 nm) = 0.[10]

Consequently, a value of 0.41 was obtained for the quantum yield of Trp$^\bullet$ or TyrO$^\bullet$. The yield of 4NQOH$^\bullet$, which is higher than those of Trp$^\bullet$ and TyrO$^\bullet$, can be explained by assuming that 4NQOH$^\bullet$ formation occurs via other pathways, e.g. the reaction of the singlet 4NQO with H_2O, in addition to the reaction of ^{T}4NQO with TrpH or TyrOH. A part of the absorption observed around 450 nm, which corresponds to ~13% of the total absorption, is already formed immediately after laser pulse for a 4NQO solution containing the quencher. A comparable amount is also observed in the solution free from quencher. On the basis of assumption that this portion of the absorption is entirely due to 4NQOH$^\bullet$ formation resulting from the reaction of singlet 4NQO, the quantum yield is estimated to be 0.06. Consequently, the yield of 4NQOH$^\bullet$ formed via ^{T}4NQO is calculated to be 0.41 which equals the yield of Trp$^\bullet$ or TyrO$^\bullet$. Comparison of this value with the yield of ^{T}4NQO, 0.46, leads to the conclusion that about 90% of the ^{T}4NQO quenched by TrpH (or TyrOH) undergoes complete charge transfer to form 4NQOH$^\bullet$ and Trp$^\bullet$ (or TyrO$^\bullet$). The efficiency of charge transfer for the reactions of ^{T}4NQO with other amino acids was estimated from the comparison of the quantum yields of ^{T}4NQO and 4NQOH$^\bullet$. The amino acids which exhibit rate constants less than $\sim 3 \times 10^7$ dm^3 mol^{-1} s^{-1} cannot quench ^{T}4NQO completely, because of solubility limitations. The quantum yields of 4NQOH$^\bullet$ obtained from the reactions of ^{T}4NQO with 1×10^{-2} mol dm^{-3} methionine, arginine, and histidine were found to be 0.35, 0.31, and 0.30, respectively. Therefore, the quantum yields of 4NQOH$^\bullet$ and the efficiency of charge transfer for the reactions with these amino acids at these infinite concentrations were calculated to be nearly equal to those for the reactions with TrpH and TyrOH. For the reaction of ^{T}4NQO with lysozyme or ribonuclease, the efficiency of charge transfer was found to be also nearly equal to that for the reaction with free TrpH or TyrOH from the comparison of the yields of ^{T}4NQO, 4NQOH$^\bullet$, Trp$^\bullet$, and TyrO$^\bullet$, on the assumption that the protein-bound radicals have the same extinction coefficients as the corresponding unbound radicals.

It has been reported that dimeric products are produced via the combination of phenoxy-type radicals in the photoirradiated aqueous solutions of phenol, cresol, and tyrosine.[34-36] Therefore we attempted to measure the quantum yield of bityrosine produced by the stationary-light (366-nm) irradiation of aqueous solutions of TyrOH and 4NQO. The concentration of bityrosine in the irradiated solutions was determined by measuring the fluorescence intensity at 405 nm upon excitation with 320-nm light,[11] using the calibration curve prepared by the plots of the fluorescence intensities vs. the concentrations of standard bityrosine solutions. The bityrosine formation curve with respect to the absorbed photon number is gradually saturated with an increase of the photon number. The quantum yield of bityrosine was estimated to be 0.0023 from the initial slope. This low yield of bityrosine suggests that in our reaction system a large portion of TyrO$^\bullet$ disappears by the reaction of the radical with 4NQOH$^\bullet$ without dimerizing to form bityrosine.

Concluding Remarks

Laser photolysis studies carried out for the aqueous solutions of 4NQO containing a series of amino acids or some proteins confirmed the following:

(1) Tryptophan, tyrosine, histone, lysozyme, and ribonuclease react with ^{T}4NQO at a diffusion-controlled rate; the rate is nearly equal to the rate obtained previously for the reaction of guanosine with ^{T}4NQO[3] (the quenching rate constants of a series of amino acids and four proteins are listed in Table I).

(2) Electron transfer from tryptophan, tyrosine, and the residue of tyrosine (or tryptophan) in the proteins toward ^{T}4NQO occurs in the triplet quenching.

(3) Electron transfer efficiency is estimated as high as about 90% (in the process of this estimation, the quantum yields of ^{T}4NQO, 4NQOH$^\bullet$, Trp$^\bullet$, and TyrO$^\bullet$ formation as well as the extinction coefficients of ^{T}4NQO and 4NQOH$^\bullet$ were determined to be as listed in Table II).

These results suggest that both the guanosine moiety in DNA and the tyrosine (or tryptophan) residue in DNA-bound proteins such as histones are initial reaction sites where electron transfer occurs in the reaction of ^{T}4NQO with chromatin, i.e., complexes of DNA and the proteins.

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Registry No. 4NQO, 56-57-5; 4NQOH$^\bullet$, 104350-55-2; TrpH, 73-22-3; Trp$^\bullet$, 104419-69-4; TyrOH, 60-18-4; TyrO$^\bullet$, 16978-66-8; methionine, 63-68-3; arginine, 74-79-3; histidine, 71-00-1; proline, 147-85-3; phenylalanine, 63-91-2; glutamic acid, 56-86-0; leucine, 61-90-5; lysine, 56-87-1; glycine, 56-40-6; lysozyme, 9001-63-2; ribonuclease, 9001-99-4.

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(37) Six tyrosine residues (TyrOH-25, -73, -76, -92, -97, -115) are included in bovine pancreas ribonuclease-A. Three tyrosine residues (TyrOH-39, -50, -57) are included in calf thymus histone-H2A.
(38) Hen egg-white lysozyme has three tyrosine residues (TyrOH-20, -23, -53) and six tryptophan residues (TrpH-28, -62, -63, -108, -111, -123). Three of these tryptophan residues, TrpH-62, -63, -123, are distributed over the molecular surface.