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Direct observation for photophysical and photochemical processes of folic acid in DMSO solution

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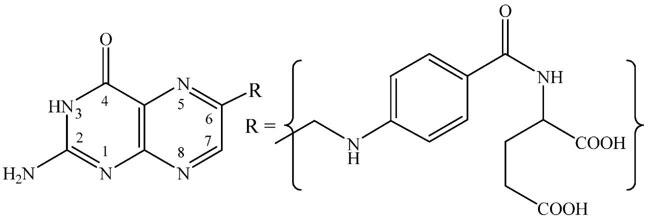
Abstract

Using the fast optical technique of laser flash photolysis by a kinetic absorption detector, the photophysical and photochemical processes of folic acid have been observed. By comparison of transient absorptions from photolysis between folic acid and its components, pteroic acid, pterin and 6-carboxypterin, it has been demonstrated that electron-transfer reactions from 4-aminobenzoyl group to the triplet pterin moiety occur through intra- and inter-molecular processes of folic acid. The intermolecular electron-transfer reaction is suggested to be mediated by two tautomers of triplet states of folic acid, lactim and lactam, in different rate constants, 1.4×10^9 and 1.6×10^8 M^{-1} s^{-1}, respectively.

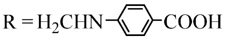
Keywords: Laser flash photolysis; Folic acid; Electron transfer; Excited triplet state; Photochemistry

1. Introduction

Pterins, the 2-amino-4-hydroxy pteridine derivatives, occur in conjugated or unconjugated from in wide range of biological systems, and are involving in photobiological processes, such as acting as photoreceptors in blue-light responses [1–4]. One of conjugated pterins, folic acid, a vitamin of the B group, is an coenzyme in the synthesis of purine and pyrimidine bases [4–6], and it is also converted in vivo through a series of enzymic transformation into a coenzyme form, such as 6-substituted pterin derivatives, 5,10-methenyl tetrahydrofolate, which is a light harvesting chromophore of DNA photolyase in numerous organisms [7]. Recent studies have shown that pterin [8,9] and some derivatives [10] induced sequence-specific DNA damage as photosensitizers under UV-A irradiation. Hence, photophysics and photochemistry of pterins are significant to understand these photobiological processes.



Folic acid

Pterin, R = H
6-CP, R = COOH
Pteroic acid, R =


Photochemistry of folic acid and its photolysis products has been reported. In aerated aqueous solution, folic acid can be photochemically cleavaged to give 6-formylpterin and *p*-aminobenzoyl glutamic acid [11–14], and the former is further oxidized by oxygen molecule to form 6-carboxypterin (6-CP) [11,12,14]. The 2-amino-4-hydroxypterine moiety of pterin derivatives that act as photosensitizers is generally an electron acceptor involving the excited triplet state of pterin rather than the excited singlet state [15].

Using laser flash photolysis technique, Chahidi et al. [16] observed transient absorption spectra of excited triplet states

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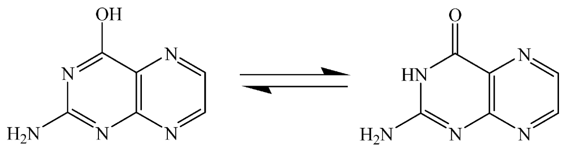
of pterin. On the basis of the observation of two absorption decays plus their phosphorescence data showing two emissions at 77 K for the neutral molecule, Chahidi et al. demonstrated triplet states of pterin with two forms. The source of the two triplet states could be the lactim ↔ lactam tautomers [16]. Furthermore, using methyl derivatives of pterin, 3-methylpterin and 2-amino-4-methoxypteridine, Ledbetter et al. have clearly demonstrated that the two triplet states of pterin derive from the lactim and lactam tautomers [17]. Another similar result, which was an observation of two laser-induced triplet states resulting from the lactim and lactam tautomers of biopterin, was obtained from laser flash photolysis study [18].



Lactam *Lactim*

Because of efficient intramolecular electron transfer from *p*-aminobenzoyl group to the excited pterin moiety in folic acid, both luminescence and transient absorption for excited states of folic acid are very weak, and so the observation for the early photochemical events of folic acid become very difficult. Up to now, the photophysical and photochemical processes of folic acid are very unclear [19].

In the work, we have investigated photophysical and photochemical processes of folic acid and its photolysis products in DMSO solution using 355 nm laser flash photolysis. On the basis of comparing to pterin and 6-CP, two triplet states of folic acid tautomers have been demonstrated through observation of electron transfer (ET) from the 4-aminobenzoyl moiety to triplet pterin moiety. The detailed photophysical and photochemical processes of folic acid have been suggested.

2. Experimental

2.1. Materials

Folic acid (pteroylglutamic acid), pteroic acid, pterin (2-amino-4-hydroxypterine) and 4-aminobenzoic acid (PABA) from Sigma, pterin-6-carboxyl acid (6-CP) from Fluka, were used as received, and other chemicals were of the purest commercially available grade and were used without further purification. The solution samples were prepared in dried dimethylsulfoxide (DMSO) (from Riedel-deHaën, H_2O: $\leq 0.03\%$) and bubbled by argon or high purity oxygen before experiment. Laser flash photolysis experiments were carried out at room temperature.

2.2. Methods

Laser flash photolysis experiments were performed using a Continuum Surelite I Nd:YAG laser provided 355 nm light pulse with a duration of 5 ns. The controlled maximum laser energy was 10 mJ per pulse. The source of analysing light is a 450 W Xenon lamp from Edinburgh Analytical Instruments (EAI) Xe900, and its intensity is increased about 150 times during the detection of transient absorption using an EAI Xp 900 power supply of short pulser. The laser and analysing light beam passes perpendicularly through a quartz cell with an optical path length of 10 mm. The transmitted light enters a monochromator equipped with a R955 photomultiplier. The signals are collected using a 100 MHz Tektronix TDS3012 transient recorder, then processed with a personal computer.

UV–vis spectra were measured on a Hitachi U-3300 spectrophotometer. Phosphorescence emission spectra were determined on a Perkin Elmer Instruments LS55 Luminescence Spectrometer. Redox potentials were determined on cyclic voltammograph using a Ag/AgCl electrode as a reference with tatraethylammonium perchlorate as supporting electrolyte.

3. Results and discussion

3.1. Pterin and 6-CP

Laser flash photolysis of Ar-saturated solutions of 0.2 mM pterin and 6-CP (symbolized as P) in DMSO showed strong transient absorption. The transient absorption spectra recorded after the laser pulse were characterized by two bands with maximum absorption wavelength (λ_{max}) 420 nm and 600 nm, respectively, which is a short-lived component, and isobestic points with ground state absorption at about 330 and 380 nm (Figs. 1 and 2). The spectra are identical to that from photolysis of pterin aqueous solution (pH 9.2), which is assigned to a short-lived component with absorption maximum at 415 and 600 nm [16]. The absorption decay traces at 420 and 600 nm did not fit a single exponential or a first-order process. The traces at 600 nm were fitted with biexponential decay with $k_1 = 5.4 \times 10^5\ \mathrm{s}^{-1}$ and $k_2 = 8.8 \times 10^4\ \mathrm{s}^{-1}$ for pterin, 9.4×10^5 and $k_2 = 1.1 \times 10^5\ \mathrm{s}^{-1}$ for 6-CP. These rate constants are lower than that from aqueous solutions, 3.3×10^6 and $4.3 \times 10^5\ \mathrm{s}^{-1}$ for pterin [16], and 3.6×10^6 and $4.9 \times 10^5\ \mathrm{s}^{-1}$ for biopterin [18]. The two rate constants were assigned to decays for triplet states of pterin tau-

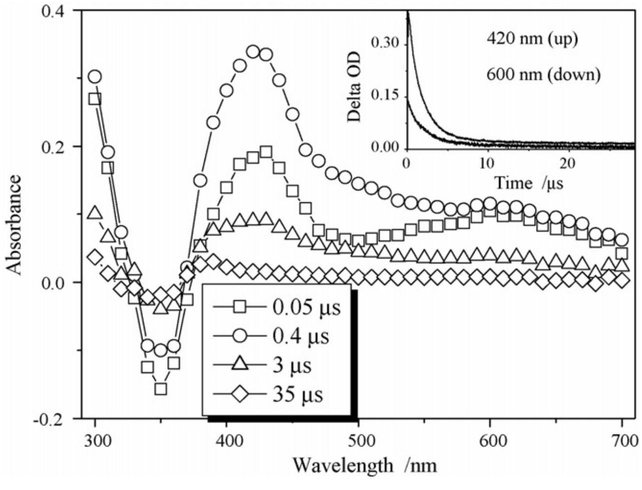


Fig. 1. Transient absorption spectra from laser flash photolysis of 0.2 mM pterin deaerated DMSO solution: 0.05 μs, 0.4 μs, 3 μs, 35 μs. Inset: traces at 420 and 600 nm.

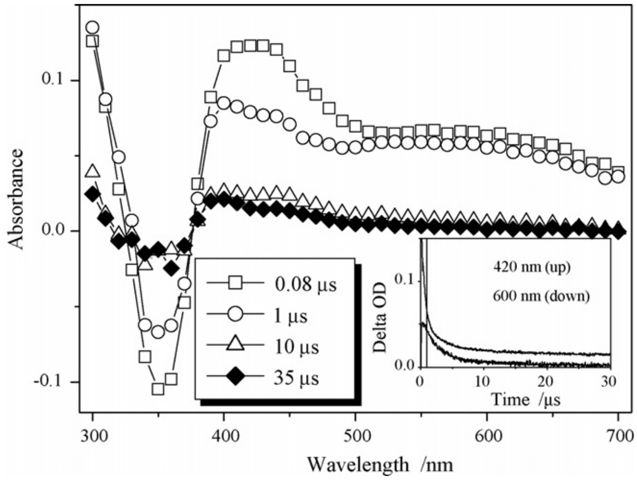


Fig. 2. Transient absorption spectra from laser flash photolysis of 0.2 mM 6-carboxylpterin deaerated DMSO solution: 0.08 μs, 1 μs, 10 μs, 35 μs. Inset: traces at 420 and 600 nm.

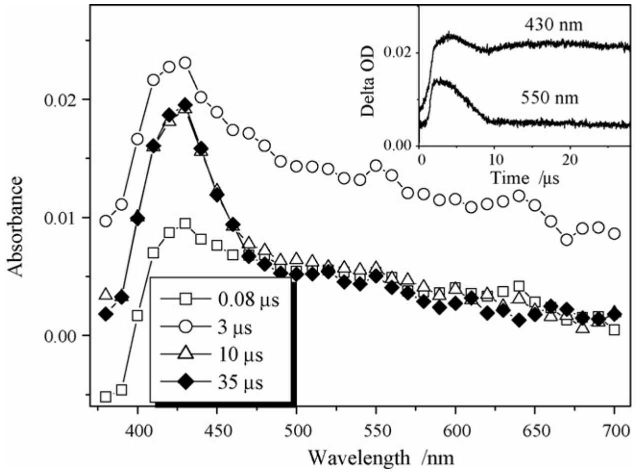


Fig. 3. Transient absorption spectra from laser flash photolysis of 0.2 mM folic acid DMSO deaerated solution: 0.08 μs, 3 μs, 10 μs, 35 μs. Inset: trace at 430 and 550 nm.

tomers, lactim and lactam [16]. Hence, the species with two lifetimes may also be two triplet states of pterin and 6-CP resulting from their two tautomers, lactim and lactam (Eq. (1)), respectively:

$$P \xrightarrow[\text{ISC}]{h\nu} {}^3P^*(\text{lactim}) + {}^3P^*(\text{lactam}) \qquad (1)$$

$${}^3P^* + P \rightarrow P^{\bullet -} + P^{\bullet +} \qquad (2)$$

$${}^3P^* + H_2O \rightarrow PH^{\bullet} + {}^{\bullet}OH \qquad (3)$$

$${}^3P^* \xrightarrow{k_0+k_s[P]} P \qquad (4)$$

The transient absorption spectra with λ_{max} 390 nm recorded at 35 μs after laser pulse may be assigned to radicals induced by triplet state of pterin and 6-CP. The absorption spectra of the radicals are similar to that from aqueous solution of pterin [16]. The weak absorption of the radicals in spectra at 35 μs showed that the photochemical reactions are inefficient in DMSO solution. The radicals could result from the reactions of ${}^3P^*$ with P [20] (Eq. (2)) or with a trace of water (Eq. (3)) in the solution. The major deactive pathway in DMSO solution should be unmolecular decay (k_0) and self-quenching (k_s) of ${}^3P^*$ (Eq. (4)).

3.2. Folic acid and pteroic acid

In the case of folic acid and pteroic acid (symbolized as P–A), the chromophore absorbing 355 nm laser light is pterin moiety rather than 4-aminobenzoyl group. So, only pterin moiety of P–A is excited under 355 nm laser light.

The transient absorption spectrum with λ_{max} 430 nm, which recorded 0.08 s after the pulse, was observed from the photolysis of 0.2 mM folic acid DMSO solution, and shown in Fig. 3. The spectra should be assigned to the excited triplet state of folic acid (Eq. (5)). The transient absorption traces from photolysis of folic acid are different with those of pterin and 6-CP. There are two type of traces in the wavelength region detected, a fast decay (∼0.5 μs) before one a fast growth, a fast decay, subsequent one slow growth and a slow decay (at 430 nm), and a fast decay before a fast growth, fast decay and subsequent slow decay at other wavelengths such as at 550 nm shown in inset in Fig. 3. The transient absorption traces for photolysis of pteroic acid show also similar process, and these processes are faster than those of folic acid (Fig. 5A).

$$P\text{–}A \xrightarrow{h\nu(355\,\text{nm})} {}^1P^*\text{–}A \xrightarrow{\text{ISC}} {}^3P^*\text{–}A \qquad (5)$$

For transient absorption from the photolysis of pterin or 6-CP, their traces at all wavelength were simple decay, not like those of folic acid or pteroic acid. Comparing with pterin and 6-CP, both folic acid and pteroic acid possess a 4-aminobenzoyl group, and so the complex processes reflecting in the traces should involve to the group.

According to Rehm–Weller equation [21], we can estimate the free energy change of proposed electron-transfer reaction from 4-aminobenzoyl to triplet pterin moiety:

$$\Delta G\,(\text{eV}) = \left[E_{ox}(\text{D}) - E_{\text{red}}(\text{A}) - \frac{e_0^2}{\varepsilon R_{\text{D}^{\bullet+}\text{A}^{\bullet-}}}\right] - \Delta E_{0,0}$$

where $E_{\text{ox}}(\text{D})$ and $E_{\text{red}}(\text{A})$ are redox potentials of electron donors and acceptors, respectively. The values were measured in DMSO solutions, and similar to values reported [22]. E_{ox} of PABA is +0.88 V versus Ag/AgCl. $\Delta E_{0,0}$ is the lowest triplet energy level of pterins obtained from their phosphoresce spectra. The $-e_0^2/\varepsilon R_{\text{D}^+\text{A}^-}$ is the solvation energy of an ion pairs D^+A^- (the coulomb term). In the case of strong polar solvent, the coulomb term can be ignored. The calculated values of ΔG are listed in Table 1.

The negative values of free energy change in Table 1 show the electron-transfer reaction from 4-aminobenzoyl to triplet pterin moiety can spontaneously occur through an intra- (Eq. (6)) or inter-molecular process (Eq. (7)). The first fast decay (0.12–0.5 μs) is very likely to derive from an intramolecular electron-transfer reaction from 4-aminobenzoyl group to triplet pterin moiety (Eq. (6)), and the electron-transfer reaction via a singlet pathway should occur before the process. The rate constant of intramolecular electron transfer via a triplet pathway

Table 1
Reduction potentials, the lowest triplet energy levels of pterins and calculated free energy changes

Pterins	E_{red}[a] (V)	ΔE_T[b] (eV)	ΔG (eV)
Pterin	−0.77	2.52	−0.87
6-CP	−0.73	2.52	−0.91
Folic acid	−0.80	2.44	−0.75

[a] Ag/AgCl electrode.

[b] 0.1 mM substrate in water:ethylene glycol = 1:2, pH 7.0.

was estimated to be $\sim 10^7\ s^{-1}$ from transient absorption trace at 430 nm, and the value via a singlet pathway may be calculated, ringing from 10^7 to $10^8\ s^{-1}$ based on fluorescence lifetimes of pterin and folic acid [23]. As very short lifetime (3.5–7.0 ns [23]) of singlet excited state of folic acid, the intermolecular electron transfer is only induced by triplet excited state of folic acid. From structures of pterin moiety and 4-aminobenzoyl group, the transient absorption of the radicals formed $P–A^{\bullet+}$ would be in the region of shorter wavelength than the region recorded of $P^{\bullet-}–A$. As E_{ox} of pterin moiety is higher than that of 4-aminobenzoic acid (PABA), charge transfer within the radical cation $P–A^{\bullet+}$ to form $P^{\bullet+}–A$ would not occur:

$$^3P^*–A \rightarrow P^{\bullet-}–A^{\bullet+} \quad (6)$$

$$^3P^*–A + P–A \rightarrow P^{\bullet-}–A + P–A^{\bullet+} \quad (7)$$

$$^3P^*–A \xrightarrow{k_0} P–A \quad (8)$$

The shape of trace at 430 nm depends on laser intensity and concentration of folic acid. The traces at 430 nm were detected under various laser intensities. The fast growth and subsequent fast decay processes were only observed under higher laser intensities, and were not under low laser intensity (Fig. 4A). Under a higher laser intensity, more molecules of folic acid will be excited, that is, the concentration of $^3P^*–A$ in photolysis system becomes large. Thus, the fast growth process may only appear under high concentration of $^3P^*–A$. Under the same laser intensity, transient absorptions for photolysis of folic acid were detected at various concentrations. The fast growth processes were only observed from photolysis of a higher concentration of folic acid (≥0.2 mM) (Fig. 4B), implying high concentration of folic acid can also lead to the processes. Hence, the fast growth process can occur at high concentrations of triplet excited state ($[^3P^*–A]$) and ground state ([P–A]) of folic acid.

Through enhancing concentrations of ground state and triplet state of folic acid, the same effect on the transient absorption trace can be observed. Obviously, the reaction (7) may be responsible for the phenomenon, appearance of the fast processes. Combining the subsequent slow growth process at all conditions, it is suggested that there are two triplet states of folic acid with different lifetimes, which possess different reactivity, in the reaction (7). The electron transfer mediated by the triplet state with short lifetime is only observed at high concentrations of $^3P^*–A$ and P–A due to the competition between (7) and (8).

Ledbetter et al. demonstrated that the triplet lactim tautomer possessed a shorter lifetime and a greater reactivity than the triplet lactam tautomer in aqueous solution [17,18]. Thus, the fast process, which appears under only high laser power or high concentration of substrate, should be induced by triplet folic acid in lactim form. As competing with unmolecular decay of the excited triplet state of folic acid (Eq. (8)), the fast electron transfer process was only observed under high concentrations of reactants in reaction (7).

Furthermore, the transient absorption from the photolysis of folic acid became very weak in aerated and oxygen-saturated solution, for triplet state of folic acid was quenched by oxygen, to form singlet oxygen ($^1O_2^*$) [24]. Meanwhile, triplet states of folic acid decay by unmolecular process or bimolecular self-quenched process. As triplet state of the lactim form with a high activity for the electron transfer reaction, which can compete with the quenching process by oxygen, the fast process induced by the triplet state of lactam form was still observed in aerated solution and O_2-saturated solution. However, the slow growth process, which is induced by triplet state in lactam form with a low activity, disappeared completely (Fig. 5A). The radical anion ($P^{\bullet-}–A$) formed in the electron-transfer reaction (Eq. (7)) would transfer an electron to O_2 to form $O_2^{\bullet-}$, or abstract a hydrogen atom to form a reductive product.

Through changing the concentration of folic acid, the rate constants of the electron transfer reactions concerning

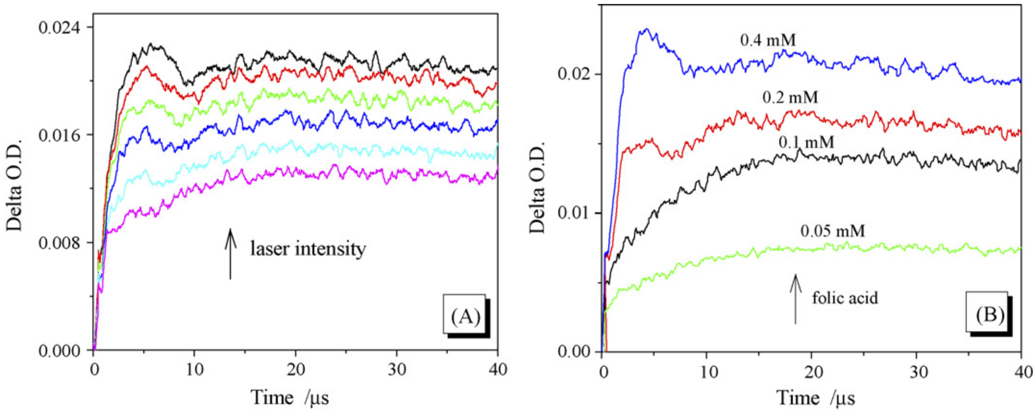


Fig. 4. Transient absorption trace at 430 nm from photolysis of folic acid in deaerated DMSO solution: (A) 0.4 mM folic acid solution under various laser intensities, 1–10 mJ; (B) 0.05–0.4 mM folic acid under the same laser intensity.

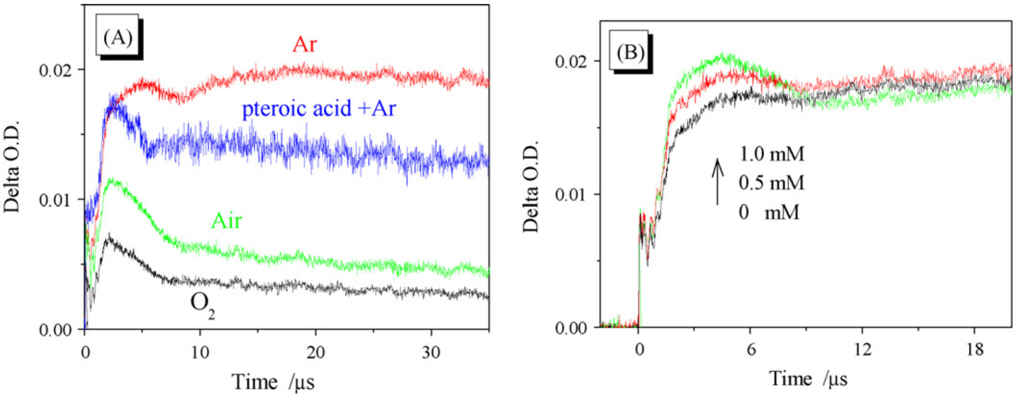


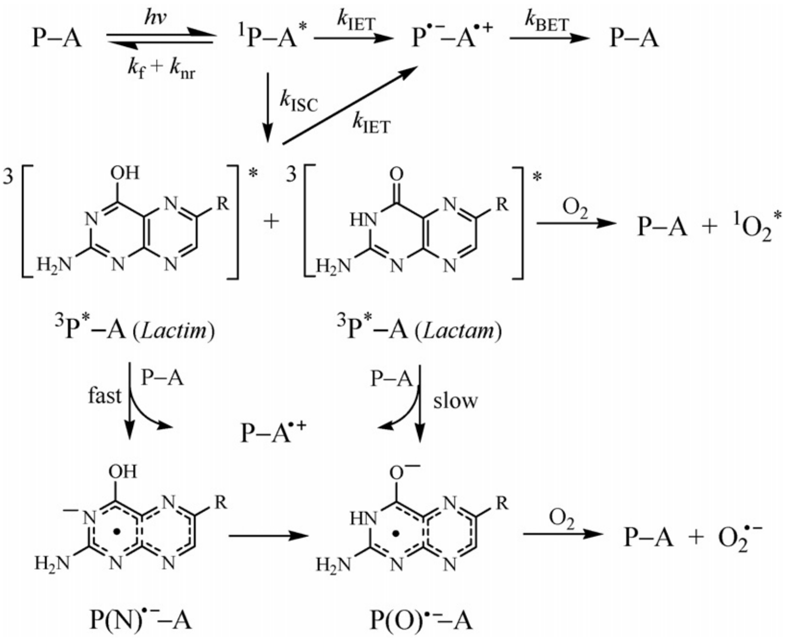
Fig. 5. Transient absorption trace from photolysis of 0.4 mM folic acid in DMSO solution at 430 nm: (A) Ar-saturated (red line), aerated (green line) and O_2-saturated solution (black line), 0.2 mM pteric acid Ar-saturated solution; (B) 0.4 mM folic acid solution with 0, 0.5 and 1 mM PABA, respectively.

two triplet states of folic acid were determined, 1.4×10^9 and $1.6 \times 10^8\,M^{-1}\,s^{-1}$. The quenching rate constants of two triplet states of pterin by PABA were obtained from photolysis of 0.2 mM pterin solution in the presence of PABA, $k_{q1} = 1.9 \times 10^9\,M^{-1}\,s^{-1}$ and $k_{q2} = 5.1 \times 10^8\,M^{-1}\,s^{-1}$. The rate constants for pterin system are rather higher than that for folic acid system.

The fast decay may involve an intramolecular proton transfer of $P^{\bullet -}$–A. The triplet state in lactim form abstracts an electron to form a pyrazine radical anion, $P(N)^{\bullet -}$–A, and a ketyl radical anion, $P(O)^{\bullet -}$–A was formed from the triplet state in lactam form. Moorthy and Hayon have investigated the protonation of anion radicals for pterins in detail, and the pK_a for protonation of the pterin radical anions are 8.0 for pyrazine radical anion, and 6.5 for ketyl radical anion, of folic acid [25]. It can be expected that the transformation of $P(N)^{\bullet -}$–A to $P(O)^{\bullet -}$–A occurs via an intramolecular proton transfer.

To further identify the process, PABA was added to the solutions, we observed that the fast process was remarkably enhanced (Fig. 5B). After PABA was added to solution, another electron-transfer reaction between $^3P^*$–A (lactim) and PABA would occur to result in more $P(N)^{\bullet -}$–A to form. This may well explain the phenomenon observed. The suggested photophysical and photochemical processes of folic acid were showed in Scheme 1.

In summary, early events for photo-degeneration of folic acid were directly observed using laser flash photolysis. The early events involve photophysical and photochemical processes such as fluorescence emission, intersystem crossing from singlet excited state to triplet, the triplet state quenched by O_2 in aerated solution and electron-transfer reactions (including intra- and inter-molecular) from the excited pterin moiety to 4-aminobenzyl group. Among these processes, two intermolecular electron-transfer processes from 4-aminobenzyl group, which



Scheme 1. Photophysical and photochemical processes of folic acid in DMSO solution.

may be mediated by the excited triplet states of folic acid tautomers, lactim and lactam, and give radical anions, $P(N)^{\bullet-}$–A and $P(O)^{\bullet-}$–A, respectively, have clearly been observed from transient absorption traces at 430 nm. The fast growth trace was assigned to electron transfer induced by the excited state of lactim form with a high activity, and the slow growth trace for the excited state of the lactam form with a low activity. An intramolecular proton-transfer, which was thought to lead the fast decay, can occur to achieve a transformation from $P(N)^{\bullet-}$–A from triplet state of lactim form to $P(O)^{\bullet-}$–A formed from triplet state of the lactam form. The latter would transfer one electron to O_2 to give $O_2^{\bullet-}$ in aerated solution. These findings are helpful to understand photochemistry of folic acid and its derivatives.

Acknowledgments

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