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First steps in the photochemistry of folate in alkaline medium

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Abstract

A laser flash photolysis study is reported on alkaline solutions of folic acid in absence of dioxygen. Spectra of the intermediates and their decays are analysed. A biexponential fit of the experimental decay can be observed in the resolution time window of the technique. Intermediates spectra obtained in laser flash photolysis were compared with those generated in pulse radiolysis experiments performed on these solutions under oxidative and reductive conditions. Conventional flash photolysis were also applied in this studies in absence of O_2. In this case two processes are detected, one in the millisecond time window and a very slow process which can be followed by normal spectrophotometry after flashing solutions. The experimental behaviour of this system is compared with those reported on pterines derivatives, where absorption changes induced by flash photolysis have been attributed to triplet excited state. The evidence presented here shows that folate suffers an early photochemical transformation. Radicals generation as a consequence of the absorption of radiation is postulated in order to give a plausible explanation of the experimental results. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Photochemistry; Folate; Alkaline medium

1. Introduction

Naturally occurring folates and pteridines are widespread in biological systems, but in very small amounts. Folic acid, a vitamin of the B group acts as a coenzyme in reactions concerned with the metabolism of nucleic acids and proteins [1,2]. Folic acid can easily be reduced by a variety of reducing agents to the corresponding hydro derivatives in the pyrazine ring of the molecule.

The photochemistry of folic acid is not completely understood [3]. Some insight about the nature of the first free radical intermediates arises from pulse radiolysis experiments [4], but no information about flash photolysis, as far as we know, has been reported. However, laser flash photolysis and pulse radiolysis studies on pterines are reported in the literature [4–7]. Comparison with this kind of compounds is interesting because a pterine moiety is present in the folic acid molecule. In pulse radiolysis experiments, on pterine and folic acid, cation and neutral radicals in acid-base equilibrium have been reported. On the other side, in laser flash photolysis of pterine solutions, triplet excited state decay instead of photochemical transformation have been postulated. Emission properties (fluorescence and phosphorescence) of the excited states have also been studied.

In the present study, a different behaviour as that reported for pterines can be advanced, i.e., photochemical instead of photophysical processes are observed in alkaline media. Therefore, the generation of an intermediate radical is proposed after the absorption of light on laser flash photolysis experiments.

2. Materials and methods

Folic acid, 98%, was purchased from Merck and other chemicals, from Aldrich, were of the purest commercially available grade and were used without further purification. The pH of solutions was adjusted to 11 using HPO_4^{-2} buffer. Solutions were deareated by bubbling Ar or O_2-free N_2 in gas-tight apparatuses.

UV–visible spectra were taken on a CARY 3 (VARIAN) spectrophotometer, which includes a soft for smoothing and averaging signals. Measurements were performed using quartz cells of 1 cm or 10 cm optical length. Total emission determinations were also performed using this equipment.

The laser flash photolysis equipment for the study of reaction kinetics and the transient absorption or emission spectra

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in a nanosecond to second time domain have been described elsewhere [8]. Ten nanosecond pulses of monochromatic light were respectively generated with a Nd:YAG (Quanta Ray), or a N_2 laser (Laser Photonics, PRA/Model UV-24). In these experiments, traces used to calculate rate constants and to get the transient spectra were at least an average of 30 shots.

The apparatus and procedures used for pulse radiolysis have been described in previous literature reports [9,10]. The investigated reactions were initiated by oxidizing the folic acid with OH radicals in aqueous solutions deareated with streams of N_2O. In the reduction of the folic acid, the reaction was initiated by the e^-(aq.) intercepting the OH radical with 0.1 M 2-propanol in solutions deareated with ultrapure N_2.

In conventional flash photolysis, two FP-8-100C (Xenon) lamps were used as sources of 80-μs polychromatic light pulses. The description of the employed equipment is reported elsewhere [11]. Solutions were irradiated at selected wavelengths by using acetone as cutoff filters ($\lambda > 320$ nm). The solution in the reaction cell was refreshed after each irradiation by using a flow system. The cell was a quartz cylinder of 20 cm length and 1 cm of diameter. The concentration of the photolyte was adjusted for absorbing no more than the 12% of the photolizing light on 0.5 cm of optical path measured from the cell surface to its centre. Under such a conditions any diffusive effect is avoided.

Continuous photolysis were carried out on solutions as described previously. These experiments were performed in either ultrapure dinitrogen or helium atmosphere. Rayonet RPR 3500 Å lamps (Southern N.E. Ultraviolet) were employed in these experiments. Absorption spectra of the irradiated solutions (4.5×10^{-4} M) were taken as a function of irradiation time. Double cells of 1 cm (for irradiation) and 0.2 cm (for absorbance measurements) of optical path were employed. Parallel measurements of the total emission of the starting solutions and the irradiated ones (4.5×10^{-5} M), were also performed. Emission cells of 1 cm optical path were used in these experiments.

3. Results

The UV–visible spectrum of folic acid, at pH = 11, is shown in Fig. 1. At room temperature, on laser experiments, no emission was detected when irradiating on the low energy absorption band. Previously, it has been shown that folic acid phosphorescence can only be seen at 77 K [12,13]. The reported excitation spectrum, between 250 nm and 450 nm, reproduces the absorption one. This fact implies that the quantum efficiency for triplet generation should be higher irradiating at 354 nm than at 337 nm. However, laser excitation at 354 nm (YAG laser) showed no transient. On the other hand, nanosecond flash excitation with 337 nm (N_2 laser) of buffered folic acid solutions showed a transient absorption in the 400 nm region. Fig. 2 shows, for an Ar-saturated 1×10^{-4} M folic acid solution, the transient absorption decay. The curve did not fit a single exponential or a second-order process. It was fitted by a biexponential decay with $k_1 = 8.5\times10^5$ s^{-1} and $k_2 = 1.7\times10^5$ s^{-1}. No emission signal was detected on that time domain in agreement with steady emission experiments that showed no phosphorescence at room temperature.

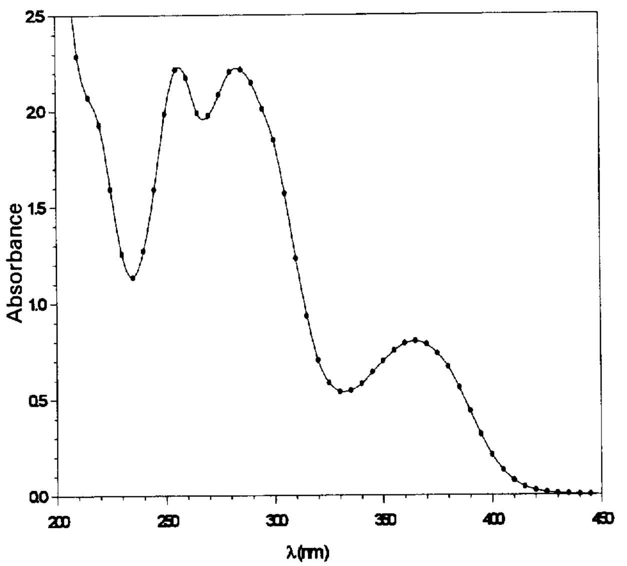

Fig. 1. Spectrum of aqueous solution of folate 1×10^{-4} M at pH 11 and 25°C.

The transient spectra taken immediately after the light pulse can be seen in Fig. 3a. The spectrum of the folic acid oxidized by pulse radiolysis in solutions saturated in N_2O is showed in Fig. 3b. On the other hand, when folic acid 0.1 mM was reduced by pulse radiolysis of solutions containing 2-propanol 0.1 M and saturated in ultrapure N_2, a radical is formed which spectrum can be seen in Fig. 3c.

Regarding conventional flash photolysis experiments, two processes can be observed after the flash. Spectral changes, recorded at different time scales, suggest two consecutive processes. The fast one is observed in the millisecond time

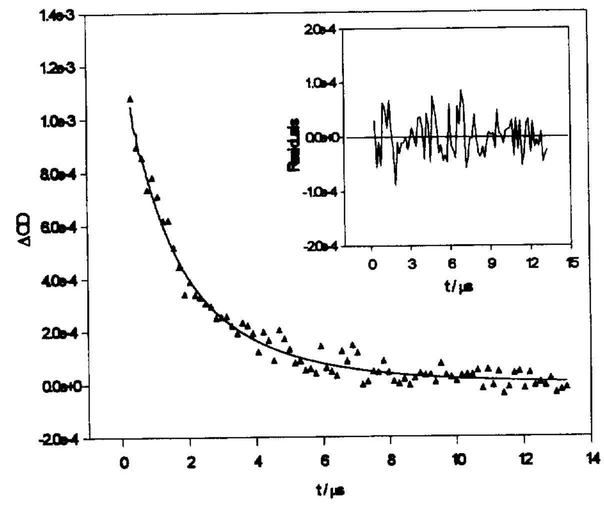

Fig. 2. Typical decay recorded in laser flash photolysis experiments at pH 11. For experimental details see Section 2. Solid line corresponds to the biexponential fit. Inset figure shows the residuals behaviour.

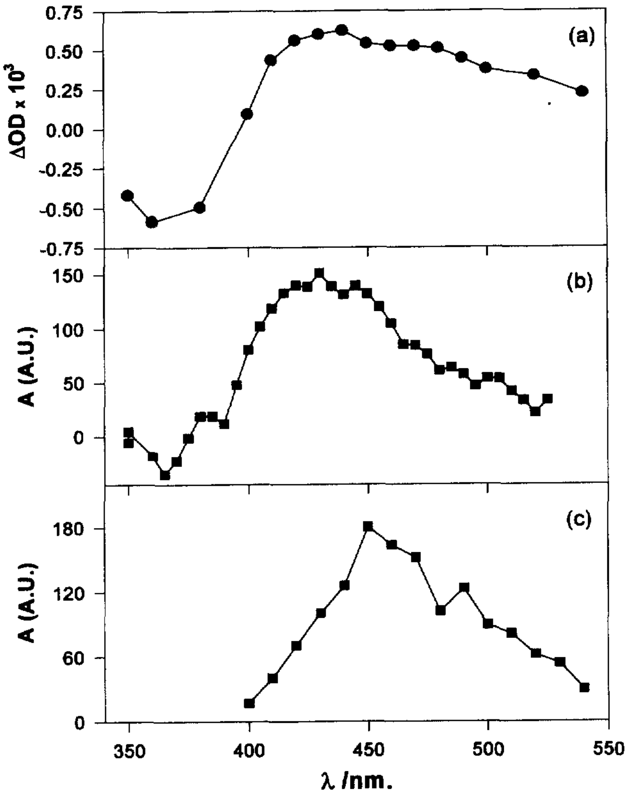

Fig. 3. Spectra of intermediates obtained from (a) laser flash photolysis experiments in the time window between 1 μs and 10 μs, (b) pulse radiolysis experiments in the same time window in one-electro oxidative condition, (c) pulse radiolysis in experiments under reductive conditions. All these spectra were obtained at pH 11.

domain, the second one is slow enough to be followed using a conventional spectrophotometric detection set up. Because these processes are strongly affected by the presence of dioxygen, experiments in atmosphere of oxygen-free dinitrogen and helium were performed.

The spectrum taken one minute after the light pulse presents differences when compared with solutions of folate before irradiation (Fig. 4a). Absorption changes at $\lambda > 390$ nm can be used to follow the kinetics. Both observed processes are first order. The rate constant for the 'faster' process is 13 ± 1 s^{-1}. (inset in Fig. 4). The second process suggests that the intermediates generated after the flash are unstable decaying to more stable products by a slow thermal reaction. The spectrum evolution of a solution after two consecutive flashes is showed in Fig. 4a. The decreasing of the band around 360–370 nm suggests an irreversible photochemistry.

Experiments under continuous irradiation were carried out. The spectrum of the solutions changes steadily with the irradiation time as shown in Fig. 5. The absorbance monitored at 325 nm increases with time (Fig. 5 inset).

Folate is a weak emitter, but pterines derivatives present a strong emission in the same spectral region. Experiments performed in ultrapure nitrogen or helium atmosphere after 260 min of irradiation show an increment of the total emission from 0.8 to 12 in arbitrary units (excitation light at 360 nm).

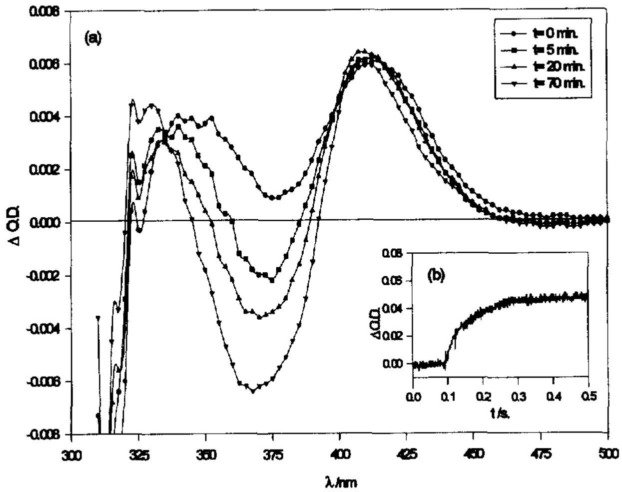

Fig. 4. (a) Time evolution of the difference spectra after two consecutive flashes. (b) Typical trace recorded after the flash. Experiments were performed at pH 11.

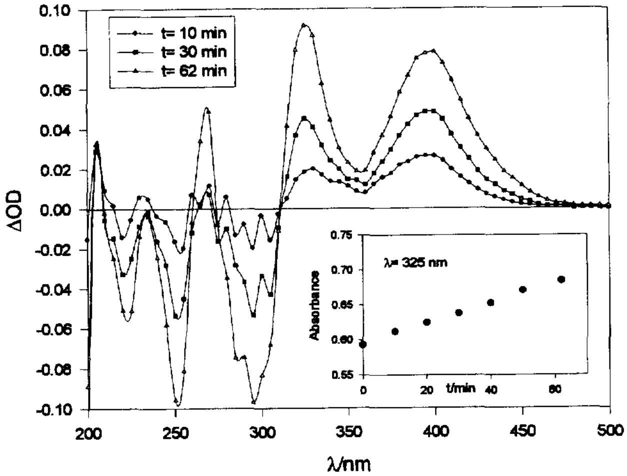

Fig. 5. Difference spectrum after irradiation in oxygen-free solutions. Inset figure shows the absorbance change as a function of time.

4. Discussion

Phosphorescence has not been detected in steady or time resolved experiments performed on oxygen-free solutions at room temperature. Therefore, as emission detection is much more sensitive than the absorption mode, the observed decay can not be attributed to the triplet excited state.

The spectrum shown in Fig. 3a resembles the transient spectrum observed in our pulse radiolysis experiments (Fig. 3b) under the same experimental conditions. On the other hand, the spectrum previously attributed to biopterin triplet excited state [7], is quite alike to the transient observed in our laser flash photolysis experiments. However, laser flash photolysis of biopterine solutions at pH 8 lead to attribute the observed absorption decay to two tautomeric forms of the triplet excited state generated during the lifetime pulse [7], though, pterine in alkaline medium [5] only showed a single exponential decay. As in any case no evidence of triplet emission at room temperature has been found it is plausible to attribute those absorptions changes detected for folate in the microsecond time domain to different species than that of the triplet state as reported previously [5,7].

In former studies [5], the transient spectrum obtained immediately after the light pulse in solutions of 2-amino-4 pteridinone at $pH = 11.8$ showed absorption changes around 670 nm. This absorption has been attributed to triplet excited. Nevertheless, this absorption could be caused by the solvated electrons. However, we were not able to detect transient absorptions beyond 550 nm.

The absence of photolysis at 354 nm, though the pulse intensity is ten times higher than at 337 nm, is an strong support to radical formation. Two ways can be proposed as sources of cation radicals:

$$FOL + H_2O + h\nu \rightarrow FOL^{+} + OH^{-} + H^{\bullet} \qquad (1)$$

or

$$FOL + h\nu \rightarrow FOL^{+} + e^{-}(aq) \qquad (2)$$

However, the last one should be discarded because no absorption changes are detected at $\lambda > 600$ nm under our experimental conditions.[3]

The spectrum obtained 1 h after the flash shown in Fig. 4 is different of the initial spectrum, implying chemical changes. Moreover, the absorbance changes and the increase in the total emission observed in steady photolysis, strongly suggest that folate undergoes irreversible transformations in dioxygen free solutions.

Evidence on the fate of the radical and the nature of the reaction products arise from the increase of the emission with the irradiation time. These changes suggest the formation of pterine derivatives. However, under our experimental conditions, the reaction products could not be isolated by preparative chromatography.

To propose a detailed mechanism further research is underway in our laboratory aimed to thoroughly investigate the photolysis of the folic acid.

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