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Influence of Human Serum Albumin on Photodegradation of Folic Acid in Solution

Pavel Vorobey[1,2], Arnfinn Engeset Steindal*[1,3], Morten Kristian Off[1,3], Alexander Vorobey[1,4] and Johan Moan[1]

[1]Department of Radiation Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway
[2]Laboratory of Molecular Markers of Environmental Effect, International Sakharov Environmental University, Minsk, Belarus
[3]Department of Physics, Norwegian University of Science and Technology, Trondheim, Norway
[4]Laboratory of Applied Biophysics and Biochemistry, Institute of Biophysics and Cell Engineering, Minsk, Belarus

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ABSTRACT

It has been proposed that photodegradation of folates may be the reason for the pigmentation of races living under high fluence rates of ultraviolet radiation. The photodegradation of folic acid (FA) induced by ultraviolet-A (UV-A) radiation, in solution and in the presence of human serum albumin (HSA), was studied with absorption and fluorescence spectroscopy. FA photodegradation, with formation of *p*-aminobenzoyl-L-glutamic acid, 6-formylpterin and pterin-6-carboxylic acid, was found to follow an exponential trend. A scheme of FA photodegradation, which involves photosensitization of FA degradation by its photoproducts, was proposed. The rate of FA photodegradation decreased drastically in the presence of HSA, whereas the spectral characteristics of the photoproducts remained constant. The reduction of the FA photodegradation rate by HSA was accompanied by degradation of tryptophan in HSA. Tryptophan, when added to solutions of FA, had a similar effect as HSA. In solutions of FA and HSA the FA photoproducts cause photodamage mainly to HSA rather than to FA itself. The oxygen dependence of FA photodegradation and the inhibition of this process by sodium azide indicate that singlet oxygen may participate in the photosensitizing activity of FA photoproducts.

INTRODUCTION

Folates are composed of a pterin moiety attached to *p*-aminobenzoic acid conjugated with one or more molecules of L-glutamic acid. Folic acid (FA) is a synthetic form of folate, and is the oxidized and most stable form of the folates (Fig. 1). *In vivo* folates exist mainly in reduced forms, for instance as tetrahydrofolate, dihydrofolate and 5-methyltetrahydrofolate. In particular, reduced tetrahydrofolate acts as a cofactor for enzymes in one-carbon transfer reactions. Folate-dependent enzymes are involved in biosynthesis of purine and pyrimidine nucleotides, and also in the metabolism of the amino acids methionine, histidine, serine and glycine (1). Thus, folates are essential for cell division and growth.

*Corresponding author email: a.e.steindal@medisin.uio.no (Arnfinn Engeset Steindal)

Folates are water-soluble B vitamins. Mammals do not synthesize folates and therefore have to get them from food. After intake, folates are rapidly absorbed and transported in blood plasma and in erythrocytes. Organs such as the liver and kidneys store most of the total body folate. The mean serum and erythrocyte concentrations of folates are 7.2 and 196 ng mL^{-1}, respectively, as measured in the U.S. population around 1990 (2). The levels are probably higher now, because food is frequently fortified with folic acid. The main form of folate in human plasma is 5-methyltetrahydrofolate (3,4). It is known (see 1), that 30–40% of plasma folates are associated with low-affinity binding proteins, primarily with albumin ($K_d \sim 1$ m*M*). Plasma also contains a high-affinity folate binding protein ($K_d \sim 1$ n*M*). Erythrocyte folates are largely 5-methyltetrahydrofolate and formyltetrahydrofolate. These folates bind with low affinity to specific deoxyhemoglobin sites (5).

Folate deficiency or impairment of the folate metabolism leads to megaloblastic anemia (6), pregnancy complications (7) and neural tube defects (8). Deficiency may also increase the risk of developing cardiovascular diseases (9) and cancer (10).

It has been proposed that folate deficiency may result from intense solar exposure, and that sun-induced folate degradation may play a key role in the evolution of human skin color (11,12). Folates are sensitive to UV radiation. This might give an advantage to those with dark skin pigmentation. Fair-skinned patients undergoing photochemical therapy for dermatological conditions have lower serum folate levels than healthy individuals (11). However, Gambichler *et al.* (13) further investigated the influence of ultraviolet-A (UV-A) radiation on serum folate *in vivo*. No significant differences in the folate level was found in UV-A exposed and nonexposed persons. Therefore, no definite conclusion about the biological significance of folate photodegradation *in vivo* can be drawn yet.

As a first step in the elucidation of this problem we have carried out an investigation of the photophysics and photochemistry of folates in simple model systems. Lowry *et al.* (14) have shown that the main products of FA lysis after UV-A irradiation are *p*-aminobenzoyl-L-glutamic acid (PGA), 6-formylpterin (FPT) and pterin-6-carboxylic acid (PCA). The rate of FA photodegradation, and the chemical nature of the photoproducts are strongly affected by the pH of the solution and the presence of oxygen (15–19). No data have been published about the photophysics and photochemistry of folates in the presence of proteins. Thus, the aim of the

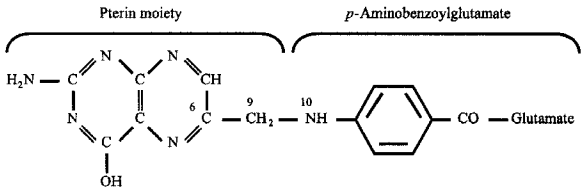


Figure 1. The molecular structure of folic acid.

present investigation was to study the effect of human serum albumin (HSA), the dominant protein in blood plasma, on FA photodegradation in solution.

MATERIALS AND METHODS

Chemicals. FA, HSA, L-tryptophan, sodium azide, L-glucose and glucose oxidase were purchased from Sigma-Aldrich and used as received. The samples were dissolved and diluted in Dulbecco's phosphate-buffered saline (PBS, pH 7.4).

Radiation procedure. The solutions were exposed to UV-A radiation in quartz cuvettes with 4 mm optical path lengths. Before irradiation they were air-equilibrated and the cuvettes were closed to prevent evaporation. The temperature of the samples during the irradiation was 25 ± 2°C. Heating of samples was prevented by forced air-cooling. Control measurements of sample temperatures during the exposure showed that the temperature did not increase by more than 2°C. The source of UV-A radiation was a Phillips TL 20 W/09 lamp (with a peak at 365 nm). The fluence rate at the sample position was 12 W m^{-2}.

Preparation of oxygen-depleted solutions. Oxygen was removed from the FA solutions using an enzymatic glucose–glucose oxidase system. Glucose oxidase (10 μg mL^{-1}) and glucose (1 m*M*) were added 30 min before irradiation, and the cuvettes were hermetically sealed immediately after the addition. Amperometric measurements of oxygen concentration in the cuvettes with Clark electrodes have shown that less than 5 μ*M* of oxygen was present in the solution under the these conditions.

Evaluation of FA binding to HSA. The percentage of FA bound to HSA was determined using the equilibrium dialysis method. The 15 μ*M* HSA solution was extensively dialyzed for 3 days against a 10 μ*M* FA solution in PBS. A membrane with a molecular mass cutoff at 6000–8000 Da was used. The amount of FA bound to HSA was determined from the difference of FA concentrations inside and outside the dialysis bags.

UV/Vis absorption and fluorescence measurements. Absorption spectra were registered on a Perkin-Elmer Lambda40 UV/Vis spectrometer. Fluorescence emission and excitation spectra were recorded by means of a Perkin-Elmer LS50B luminescence spectrometer equipped with a Hamamatsu R-928 photomultiplier (Japan), or a SFL 222 spectrofluorometer (SOLAR, Minsk, Belarus). All measurements were performed in standard 10 mm quartz cuvettes. All experiments were repeated at least three times, and data from one of these independent experiments are shown.

All experimental work was carried out in dim light, in order to avoid external and uncontrolled light exposure.

RESULTS AND DISCUSSION

Photodegradation of FA in solution

Absorption spectra of FA solutions after 0, 28 and 48 min of UV-A exposure are shown in Fig. 2a. A comparison of these spectra with those of individual pterins (FPT and PCA) and PGA shows that the band at 350 nm is because of the pterin moiety, whereas in the region around 280 nm both pterin and PGA absorb (17). The identity chromophore giving the weak shoulder at 300 nm, where UV-A exposure causes the largest change (Fig. 2b), is unknown. Exposure to UV-A causes absorbance changes at both of the major bands. These changes are neither linearly dependent on the fluence nor are they unidirectional. After 28 min of exposure the peak at 350 nm is shifted toward longer wavelengths with a small increase of absorbance. After 48 min of exposure, however, this peak is

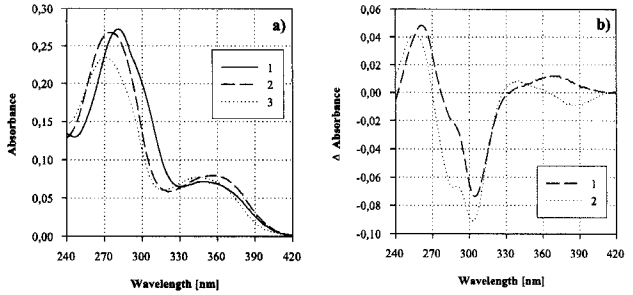


Figure 2. (a) Absorption spectrum of 10 μ*M* FA in PBS (1) and the changes occurring after UV-A exposure for 28 min (2) and 48 min (3). (b) Difference absorption spectra, obtained by subtracting the spectrum of FA solution from the spectra of solutions after UV-A exposure for 28 min (1) and 48 min (2).

shifted back to shorter wavelengths. Differential spectra (Fig. 2b) indicate that first a photoproduct with an absorption peak at about 370 nm is formed. Then this product is converted to a photoproduct with an absorption peak around 340 nm. Changes in the absorption spectra at shorter wavelengths also occur and seem to be nonlinear. The absorbance at 280 nm decreases, the peak shifts to shorter wavelengths and the shoulder at 300 nm almost disappears.

Similar changes in the absorption spectra of FA solutions under UV-A exposure were described in our previous work were we discussed the nature of the photoproducts (17). Based on this and data from the literature (15,16,18–20) we tentatively concluded that, under the present conditions, FA first undergoes cleavage at the C^9–N^{10} bond with formation of PGA and FPT. Further irradiation leads to photo-oxidation of the 6-formyl group at FPT, and thus formation of PCA.

Figure 3 shows the kinetics as measured at 280 nm (all the participating compounds absorb light in this region), at 370 nm (characteristic for FPT) and at 340 nm (characteristic for PCA). The changes at 300 nm are also shown. The increase at 370 nm indicates that accumulation of FPT continues until 28 min of UV-A exposure. Further irradiation decreases the content of FPT in the sample. This decrease is presumably related to an almost complete degradation of FA at this time. The curve for 340 nm shows that at this time the rate of accumulation of PCA also decreases. The absorbance at 280 nm decreases during the whole UV-A exposure (Fig. 3). When most of the FA is degraded (>28 min of irradiation), a faster decrease of the absorbance at 280 nm begins,

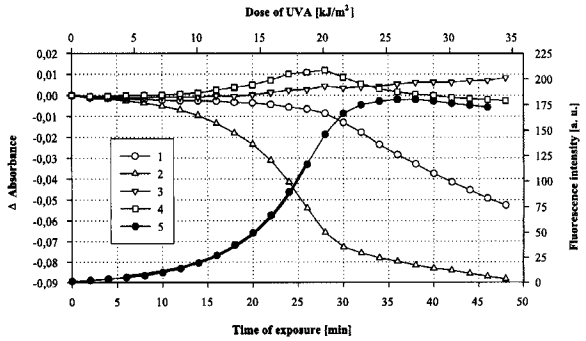
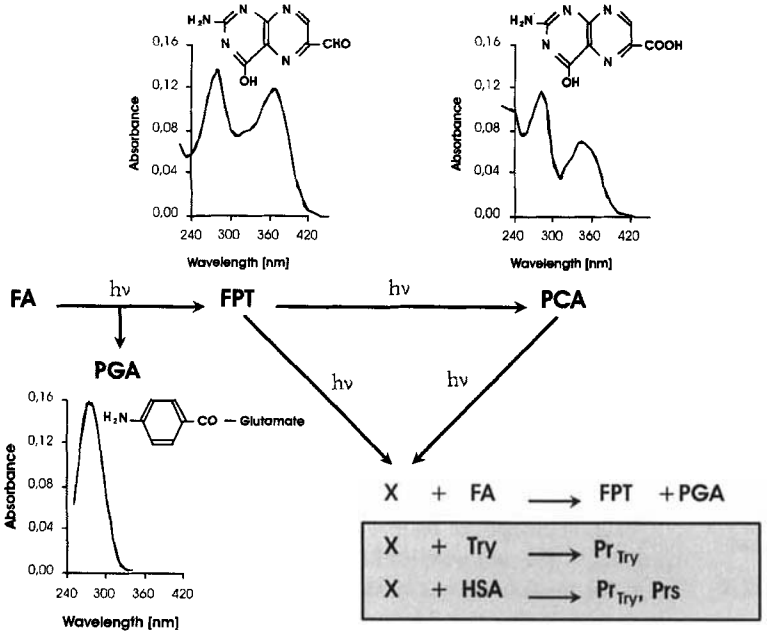


Figure 3. Δ Absorbance at 280 nm (1), 300 nm (2), 340 nm (3) and 370 nm (4) and fluorescence intensity at 450 nm under excitation at 350 nm (5) of a 10 μ*M* FA solution in PBS as a function of exposure time and UV-A dose. An exponential line obtained for curve 5 in the time interval 6–26 min is shown as a bold line.



Scheme 1. A proposed scheme of FA photodegradation, including absorption spectra of the FA photoproducts. Photolysis of FPT and PCA produces a product X that seems to degrade FA. In the presence of tryptophan or HSA, there is a competition between FA degradation and degradation of the indole ring in tryptophan (Try) or amino acid residues in HSA, including tryptophanyles. Product X may be either an excited form of pterin or an active oxygen species.

presumably as a result of photodegradation of the compounds formed. Suárez *et al.* (20) have shown that upon UV-A exposure PCA undergoes degradation through photodecarboxylation and/or photo-oxidation with partial loss of aromaticity. These processes are linearly dependent on exposure and lead to a significant decrease of the absorbance at 280 nm.

A quantitative analysis of decay curves is hard to carry out because of simultaneous occurrence of several photochemical processes and overlapping absorption spectra of the involved compounds. However, all kinetic curves show a phase of sharp accelerating changes of absorbance with increasing exposure time in the first region where the majority of FA molecules are degraded. This acceleration is clearly seen in the fluorescence curve of irradiated FA solutions, and is because of formation of fluorescent photoproducts (Fig. 3, curve 5). It is known that, because of intramolecular quenching, FA has an extremely small quantum yield of fluorescence, approximately 0.005 (21). Pterins, formed after cleavage of the C^9–N^{10} bond in the FA molecule, have a marked fluorescence in the 445 nm region when excited at 350 nm. The quantum yield of fluorescence of FPT is nearly 0.1 and that of PCA is about 0.2 (22), so the growth of fluorescence in the region of 445 nm is because of the formation of FPT as a result of the C^9–N^{10} bond cleavage and then because of the photoinduced conversion of FPT to PCA. From the kinetic curves (Fig. 3) it is evident that cleavage of the C^9–N^{10} bond also leads to a disappearance of the shoulder at 300 nm.

The kinetic curves indicate that FA photoproducts induce the degradation of FA itself (Fig. 3). In our earlier work we have shown that both FPT and PCA (and not PGA) may act as sensitizers in the photodegradation of FA (17). In agreement with this we propose that the UV-A induced FA degradation follows Scheme 1.

Photodegradation of FA in the presence of HSA

The photodegradation of FA is strongly dependent on the presence of HSA. Even a low concentration of HSA (3.5 μ*M*) leads to an increase of the half-time of FA photodegradation by a factor of about two. At an HSA concentration 28 μ*M* the half-time of FA photodegradation increased by a factor of about five. However, the characteristic features of the kinetics of FA photodegradation are preserved: a phase of fluorescence growth followed by a phase of photodegradation of products. However, the former phase occurs later and its slope gets smaller when HSA is added (Fig. 4).

The fluorescence curves shown in Fig. 4 may indicate either that HSA prevents the formation of photosensitizing products or reduces their ability to sensitize the degradation of FA (see Scheme 1). It is unlikely that binding of FA or its photoproducts to HSA should influence the effects of HSA on FA degradation. Using equilibrium dialysis methods we have found that for the conditions used in our experiments (an HSA concentration of 15 μ*M* and an FA concentration of 10 μ*M*) less than 15% of the FA molecules are bound to HSA. As can be seen from the fluorescence spectra of the irradiated solutions (Fig. 5), the presence of proteins does not affect the shape of the fluorescence spectra of the photoproducts. In the presence of HSA the small broadening of the spectra and the small blueshift are most likely caused by the formation of the products of photodegradation of tryptophan residues in albumin. One of the major products of photo-oxidation of tryptophan, N-formylkynurenine, has a weak fluorescence around 440 nm (23).

In the 10 μ*M* FA solution with 15 μ*M* HSA, the optical density in the 280 nm region is high because of absorption by aromatic amino acids residues in HSA (Fig. 6a, curve 1). The absorption spectrum is the sum of the spectrum of FA and that of HSA (data not shown). UV-A exposure for 30 min causes only insignificant changes of the absorption spectrum (Fig. 6). As seen from the differential spectrum (Fig. 6b) UV-A exposure causes practically no formation of FPT or PCA. Only an insignificant decrease of the absorbance around 300 nm and some decrease of the absorption at 280 nm are observed. A comparison of the differential spectra of FA without HSA (Fig. 2b) and that with HSA (Fig. 6b) shows that the decrease of the absorption of FA around 280 nm caused by UV-A exposure in the presence of HSA is not because of photo-

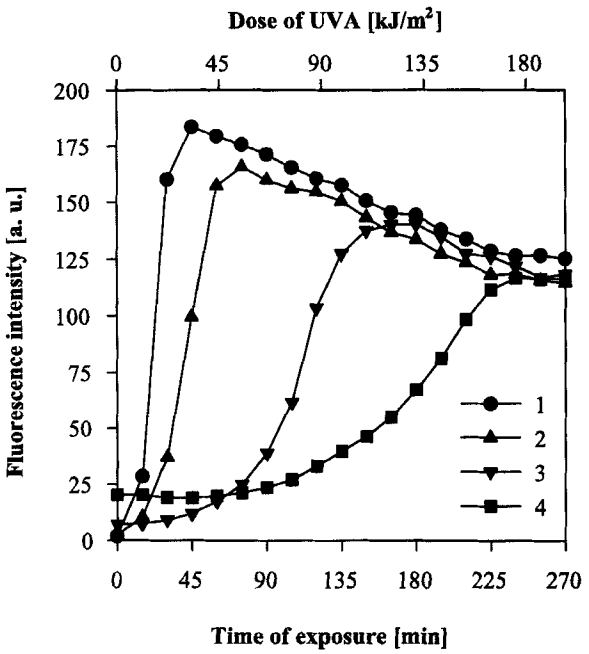


Figure 4. Fluorescence intensity at 445 nm (λ_{exc} = 350 nm) of 10 μM FA solutions in PBS (1) and PBS containing 3.5 μM (2), 28 μM (3) or 77 μM (4) HSA as a function of exposure time and UV-A dose.

degradation of FA, but rather because of photodegradation of the indole ring of tryptophan residues in HSA.

Additional evidence for the destruction of the indole comes from our experiments with UV-A exposure of FA solutions in the presence of tryptophan. Tryptophan (10 μM) has a similar protective effect as HSA on the photodegradation of FA (Fig. 6). The tryptophan absorbance at 280 nm markedly decreases upon UV-A exposure. Because tryptophan itself does not absorb UV-A radiation, this shows that FA photoproducts sensitize the photodegradation of the indole ring of tryptophan.

When FA solutions containing HSA or tryptophan are UV-A irradiated, two processes seem to occur: proteins and/or amino acids protect FA from photodegradation, and photodegradation of the indole ring of tryptophan residues in the protein and tryptophan in solution occurs. To elucidate the connection between these two processes we studied UV-A–induced changes of the fluorescence emission spectra of 10 μM FA solutions with and without 15 μM HSA or 25 μM tryptophan (Fig. 7). For FA solutions, the UV-A–induced changes of the FA fluorescence in the region of 450 nm (λ_{exc} = 280 nm) were similar to those for λ_{exc} = 350 nm. In both cases, the fluorescence intensity reached approximately 45% of the maximal value after 24 min of UV-A exposure, and the maximum value was observed after 36 min of irradiation (Figs. 3 and 7a). The shape of the spectrum in this region was preserved. As shown earlier (17), the fluorescence of UV-A–exposed FA solutions in the region of 360 nm is because of PGA. UV-A–induced growth of this fluorescence reached a maximum after 24 min of irradiation. This corresponds to the findings reported above, and confirms that most of the FA molecules are photodegraded by this time.

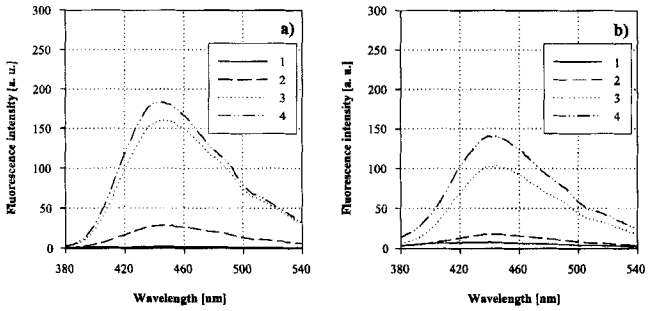


Figure 5. (a) Fluorescence emission spectra (λ_{exc} = 350 nm) of 10 μM FA in PBS (1) and changes observed after UV-A exposure for 15 min (2), 30 min (3) and 45 min (4). (b) Fluorescence emission spectrum (λ_{exc} = 350 nm) of 10 μM FA in PBS containing 28 μM HSA (1) and its changes after UV-A exposure for 60 min (2), 120 min (3) and 180 min (4).

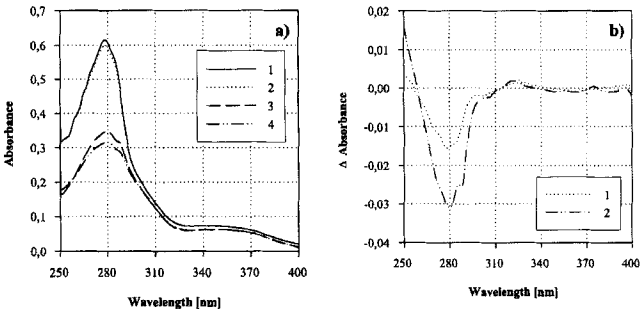


Figure 6. (a) Absorption spectra of 10 μM FA in PBS containing 15 μM HSA (1) or 10 μM tryptophan (3) and their changes after UV-A exposure for 30 min (2 and 4 correspondingly). (b) Difference absorption spectra, obtained by subtracting the spectra of FA solutions in PBS containing 15 μM HSA or 10 μM tryptophan from the spectra of solutions after UV-A exposure for 30 min (1 and 2 correspondingly).

A peak at 357 nm, which is characteristic for tryptophan, was observed in the fluorescence spectrum of nonirradiated FA solution containing 25 μM tryptophan (Fig. 7b). Upon UV-A exposure, the fluorescence of the solution in this region decreased considerably

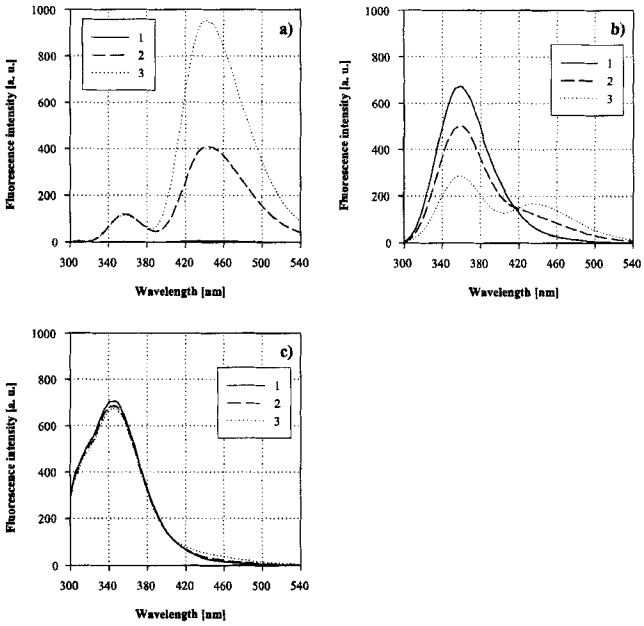


Figure 7. (a) Fluorescence emission spectrum (λ_{exc} = 280 nm) of 10 μM FA in PBS (1) and its changes after UV-A exposure for 24 min (2) and 36 min (3). (b) Fluorescence emission spectrum (λ_{exc} = 280 nm) of 10 μM FA in PBS containing 25 μM tryptophan (1) and its changes after UV-A exposure for 30 min (2) and 60 min (3). (c) Fluorescence emission spectrum (λ_{exc} = 280 nm) of 10 μM FA in PBS containing 15 μM HSA (1) and its changes after UV-A exposure for 30 min (2) and 60 min (3).

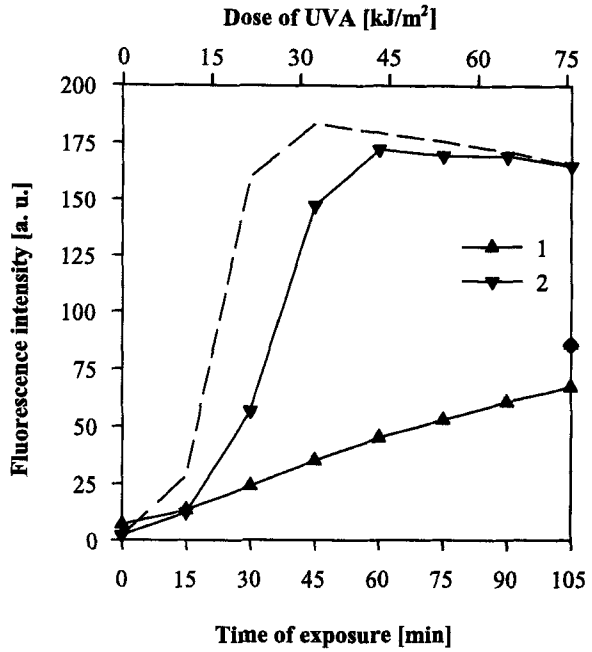


Figure 8. Fluorescence intensity at 445 nm (λ_{exc} = 350 nm) of oxygen-depleted 10 μM FA in PBS (1) and 10 μM FA in PBS containing 1 mM sodium azide (2) as a function of exposure time and UV-A dose. The diamond mark indicates the fluorescence intensity of an oxygen-depleted and UV-A–exposed (105 min) FA solution (10 μM) in PBS after air equilibration. The dashed line corresponds to curve 1 in Fig. 4.

(by 25% and 57% after 30 and 60 min of irradiation, correspondingly). Control experiments showed that FA photoproducts do not quench tryptophan fluorescence, so the observed intensity decrease was because of photodegradation of the indole chromophore. The assumption that FA photoproducts act as sensitizers agrees with the acceleration of the tryptophan degradation with increasing exposure time, and the corresponding increase of the concentration of FA photoproducts in the solution (Fig. 7b).

As estimated from the characteristic fluorescence of pterins in the region of 445 nm, the rate of photodegradation of FA itself markedly decreases in the presence of tryptophan. A comparison of the data shown in Fig. 7a and b indicates that only about 10% of FA molecules in a solution with tryptophan are photodegraded after an exposure degrading all FA in the absence of tryptophan. Thus, tryptophan and FA seem to be photosensitized by FA photoproducts in a competitive manner (see insert on Scheme 1). If this is so, FA photoproducts sensitize mainly the photodegradation of tryptophan. This photodegradation is clearly seen by the drastic decrease of fluorescence in the region of 350 nm, even though one of the FA photoproducts—PGA—has marked fluorescence in this region when excited at 280 nm (Fig. 7b).

There may be, as suggested for tryptophan, a competition between FA and amino acid residues of HSA (see Scheme 1). This may be the reason for the rapid decrease of the rate of FA photodegradation in the presence of the protein. After 60 min of irradiation in the presence of HSA, less than 5% of FA is degraded (Fig. 7c). The fluorescence of HSA (tryptophanyles mainly) decreases only slightly after such an exposure. FA photoproducts are formed, and, photosensitized by them, degradation of HSA takes place after 90 min of irradiation (data not shown). A comparison of the ratios of the changes of the fluorescence intensity of indole and those of the FA photoproducts upon irradiation of mixtures of FA and HSA or tryptophan, shows that tryptophanyles in the HSA are more stable than tryptophan in solution. We believe that this is related to the "shielding" effect of other amino acid residues in HSA. These may, as tryptophan, participate in processes photosensitized by FA photoproducts. Thus, tryptophanyles in HSA may cause only part of the suppression of FA photodegradation.

It is known that pterins may generate singlet oxygen upon UV-A exposure. Thomas *et al.* (22) have shown that the quantum yields of singlet oxygen production by FPT and PCA in the acid form in air-equilibrated D_2O solutions are 0.45 and 0.27, respectively, and slightly higher for the base form. The quantum yield of singlet oxygen generation by intact FA is very low (less than 0.002), because of the above-mentioned intramolecular quenching. On the other hand, both indole and FA are good quenchers of singlet oxygen. Stern–Volmer rate constants of singlet oxygen quenching by FA and tryptophan in solution are similar, about 3.0×10^7 $M^{-1}s^{-1}$ (22) and 3.2×10^7 $M^{-1}s^{-1}$ (24), respectively. The rate constant of singlet oxygen quenching by albumin (chemical plus physical quenching) is 2×10^8 $M^{-1}s^{-1}$ (25). Because each HSA molecule contains only one tryptophan residue, it is likely that tryptophan residues contribute only partially for the singlet oxygen quenching by HSA.

To check the participation of singlet oxygen in FA photodegradation under the study conditions we investigated the oxygen dependence of this process. Removal of oxygen from the FA solutions before irradiation (see Materials and Methods, *Preparation of oxygen-depleted solutions*) results in a large decrease of the photodegradation rate (Fig. 8, curve 1). Control experiments showed that when glucose and glucose-oxidase were added separately the FA photodegradation rate remained unchanged. Injection of oxygen in oxygen-depleted FA solutions immediately after irradiation resulted in only a small increase of the fluorescence intensity of the FA photoproducts. This means that oxygen participates in the photodegradation of FA. Our experiments with sodium azide, a strong singlet oxygen quencher (26), are in agreement with the assumption that singlet oxygen may play a major role. As seen from Fig. 8, azide suppresses the photodegradation of FA. Thus, the rate of FA photodegradation decreases by about a factor of two in the presence of azide. This is typical for blocking of singlet oxygen dependent processes in aqueous solutions by azide at millimolar concentrations (26).

Under physiological conditions the concentration of proteins is much higher than that of folate. The folate degradation by UV-A is then probably slow and the photoproducts of folates will mainly cause protein damage. Our findings may explain why Gamblicher *et al.* did not find any folate degradation in persons exposed to UV (13). Nevertheless, it is not possible to draw a final conclusion on whether folate is degraded in humans by UV radiation. Further investigations are needed to estimate the physiological role of folate degradation caused by exposure to UV or solar radiation.

CONCLUSIONS

FA in aqueous solutions is degraded by UV-A, and the degradation follows exponential kinetics. FA photoproducts, in particular pterins, contribute substantially to the photosensitized degradation of FA. FA photodegradation is slower in the presence of HSA than in its absence. This is because of competition between HSA and FA products formed from photosensitization of FA photoproducts. The oxygen dependence of the photodegradation and the inhibition

by sodium azide show that singlet oxygen may participate in these photosensitized reactions.

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REFERENCES

1. Lucock, M. (2000) Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. *Mol. Genet. Metab.* **71**, 121–138.
2. Wright, J. D., K. Bialostosky, E. W. Gunter, M. D. Carroll, M. F. Najjar, B. A. Bowman and C. L. Johnson (1998) Blood folate and vitamin B12: United States, 1988–94. *Vital Health Stat.* **11**, 1–78.
3. Herbert, V., A. R. Larrabee and J. M. Buchanan (1962) Studies on the identification of a folate compound of human serum. *J. Clin. Invest* **41**, 1134–1138.
4. Thien, K. R., J. A. Blair, R. J. Leeming, W. T. Cooke and V. Melikian (1977) Serum folates in man. *J. Clin. Pathol.* **30**, 438–448.
5. Benesch, R., S. Waxman, R. Benesch and C. Baugh (1982) The binding of folyl polyglutamates by hemoglobin. *Biochem. Biophys. Res. Commun.* **106**, 1359–1363.
6. Blakely, R. L. (1969) *The Biochemistry of Folic Acid and Related Pteridines*. North-Holland, Amsterdam, Netherlands.
7. Butterworth, C. E. Jr. and A. Bendich (1996) Folic acid and the prevention of birth defects. *Annu. Rev. Nutr.* **16**, 73–97.
8. Botto, L. D., C. A. Moore, M. J. Khoury and J. D. Erickson (1999) Neural-tube defects. *N. Engl. J. Med.* **341**, 1509–1519.
9. Refsum, M. D., M. D. Ueland, M. D. Nygard and M. D. Vollset (1998) Homocysteine and cardiovascular disease. *Annu. Rev. Med.* **49**, 31–62.
10. Choi, S. W. and J. B. Mason (2000) Folate and carcinogenesis: an integrated scheme. *J. Nutr.* **130**, 129–132.
11. Branda, R. F. and J. W. Eaton (1978) Skin color and nutrient photolysis: an evolutionary hypothesis. *Science* **201**, 625–626.
12. Jablonski, N. G. and G. Chaplin (2000) The evolution of human skin coloration. *J. Hum. Evol.* **39**, 57–106.
13. Gambichler, T., A. Bader, K. Sauermann, P. Altmeyer and K. Hoffmann (2001) Serum folate levels after UVA exposure: a two-group parallel randomised controlled trial. *BMC Dermatol.* **1**, 8.
14. Lowry, O. H., O. A. Bessey and E. J. Crawford (1949) Photolytic and enzymatic transformations of pteroylglutamic acid. *J. Biol. Chem.* **180**, 389–398.
15. Akhtar, M. J., M. A. Khan and I. Ahmad (1999) Photodegradation of folic acid in aqueous solution. *J. Pharm. Biomed. Anal.* **19**, 269–275.
16. Akhtar, M. J., M. A. Khan and I. Ahmad (2003) Identification of photoproducts of folic acid and its degradation pathways in aqueous solution. *J. Pharm. Biomed. Anal.* **31**, 579–588.
17. Off, M. K., A. E. Steindal, A. C. Porojnicu, A. Juzeniene, A. Vorobey, A. Johnsson and J. Moan (2005) Ultraviolet photodegradation of folic acid. *J. Photochem. Photobiol. B: Biol.* **80**, 47–55.
18. Thomas, A. H., G. Suarez, F. M. Cabrerizo, R. Martino and A. L. Capparelli (2000) Study of the photolysis of folic acid and 6-formylpterin in acid aqueous solutions. *J. Photochem. Photobiol. A: Chem.* **135**, 147–154.
19. Thomas, A. H., G. Suárez, F. M. Cabrerizo, F. S. G. Einschlag, R. Martino, C. Baiocchi, E. Pramauro and A. L. Capparelli (2002) Photochemical behavior of folic acid in alkaline aqueous solutions and evolution of its photoproducts. *Helv. Chim. Acta* **85**, 2300–2315.
20. Suárez, G., F. M. Cabrerizo, C. Lorente, A. H. Thomas and A. L. Capparelli (2000) Study of the photolysis of 6-carboxypterin in acid and alkaline aqueous solutions. *J. Photochem. Photobiol. A: Chem.* **132**, 53–57.
21. Thomas, A. H., C. Lorente, A. L. Capparelli, M. R. Pokhrel, A. M. Braun and E. Oliveros (2002) Fluorescence of pterin, 6-formylpterin, 6-carboxypterin and folic acid in aqueous solution: pH effects. *Photochem. Photobiol. Sci.* **1**, 421–426.
22. Thomas, A. H., C. Lorente, A. L. Capparelli, C. G. Martinez, A. M. Braun and E. Oliveros (2003) Singlet oxygen (1deltag) production by pterin derivatives in aqueous solutions. *Photochem. Photobiol. Sci.* **2**, 245–250.
23. Creed, D. (1984) The photophysics and photochemistry of the near-UV absorbing amino acids. I. Tryptophan and its simple derivatives. *Photochem. Photobiol.* **59**, 284–289.
24. Michaeli, A. and J. Feitelson (1994) Reactivity of singlet oxygen toward amino acids and peptides. *Photochem. Photobiol.* **59**, 284–289.
25. Egorov, S. Yu. and A. A. Krasnovsky Jr. (1986) Quenching of singlet molecular oxygen by component of media used for isolating chloroplasts and studying photosynthetic. *Sov. Plant Physiol.* **33**, 5–8.
26. Linding, B. A. and M. A. J. Rodgers (1981) Rate parameters for the quenching of singlet oxygen by water-soluble and lipid-soluble substrates in aqueous and micellar systems. *Photochem. Photobiol.* **33**, 627–634.