



Photodegradation of 5-methyltetrahydrofolate in the presence of Uroporphyrin

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ABSTRACT

The main form of folate in human plasma is 5-methyltetrahydrofolate (5MTHF). The observation that folate in human serum is photosensitive supports the hypothesis that humans developed dark skin in high ultraviolet fluences areas in order to protect folate in the blood from UV radiation. However, folates alone are quite photostable. Therefore, in this study, we examined for the first time the photodegradation of 5MTHF in the presence of the endogenous photosensitizer uroporphyrin (Uro), which is sometimes present in low concentration in human serum, under UV and near-UV light exposure. We found strong indications that while 5MTHF alone is rather photostable, it is degraded quickly in the presence of Uro. Using deuterium oxide (D₂O) as an enhancer of the lifetime of singlet oxygen and the singlet oxygen sensor green reagent (SOSG) as a scavenger of singlet oxygen, we have found that the photodegradation most likely proceeds via a type II photosensitization. Our results show that singlet oxygen is likely to be the main intermediate in the photodegradation of 5MTHF mediated by Uro. Our findings may be useful for further studies the evolution of human skin colours.

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1. Introduction

Folate is the generic term for a family of chemically similar compounds belonging to the vitamin B family. These compounds are related to many biochemical processes, including the metabolism of amino acids, DNA synthesis, DNA repair, and methylation of DNA [1]. Therefore, folates are vital for all living creatures. Folate is present in the majority of natural foods [2]. Rich sources of folates are green leaf vegetables, liver, yeast, egg yolk, beans, and some fruits. Food folates exist mainly in the forms of 5-methyltetrahydrofolate (5MTHF) and formyltetrahydrofolate (formyl-H₄folate) [3]. Folate act as coenzyme substrates in many reactions of the metabolism of amino acids and nucleotides [4]. Various studies have shown that adequate intake of folate prevents neural tube defects (NTDs), reduces hyperhomocysteinemia, and diminishes the risk of cancers [5].

It has been suggested that folate degradation may relate to evolution of human skin colours [6,7]. A dark skin colour has been proposed to protect folate from photodegradation when exposed to solar radiation [7]. As a first step for evaluating this hypothesis we and others have carried out investigations of photodegradation of folates in simple models [8,9]. Recently, we and others have found that 5MTHF, the predominant form of folates in blood plas-

ma [10], was not sensitive to solar radiation in vitro, but was degraded in the presence of photosensitizers such as Riboflavin and Rose Bengal [8,9,11]. Natural photosensitizers in human tissue and in blood can produce reactive oxygen species (ROS) under solar radiation [12,13]. Uroporphyrin (Uro) is an important photosensitizers in human blood, and can produce ROS when exposed to visible and UV radiation [14]. Formation of ROS is believed to be a key factor for oxidation of 5MTHF, which is considered as a strong antioxidant [15].

The purpose of the present study was to investigate the photodegradation of 5MTHF exposed to light in the absence and presence of Uro in solutions. The mechanism of the Uro-mediated photolytic degradation of 5MTHF was examined.

2. Materials and methods

2.1. Chemicals

(6R,S)-5-methyl-5,6,7,8-tetrahydrofolic acid calcium salt (5-methyltetrahydrofolate, 5MTHF) was purchased from Schircks Laboratories (Jona, Switzerland). Uro, PBS powder and deuterium oxide, 99 atom% D (D₂O) were from Sigma-Aldrich Norway AS (Oslo, Norway). Stock solution of 5MTHF was made in PBS (pH 7.4) at a concentration of 50 μM. It was frozen in liquid nitrogen and stored in 1 ml tubes at −80 °C. Due to the instability of 5MTHF, the tubes containing solutions were always kept on ice before use. Stock solution of Uro was made in PBS at a concentration of 50 μM

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and stored in the fridge at 4 °C. Aluminium foil was used to cover the bottles of Uro in order to protect them from visible light. The same amount of PBS powder was added to H₂O and D₂O. These buffers were used to make solutions of 5MTHF, and/or Uro. The singlet oxygen sensor green reagent (SOSG) was purchased from invitrogen (Eugene, Oregon, USA). It was provided specially packaged in sets of 100 µg were protected from light at ≤20 °C until use. It was dissolved in 33 µl of methanol to make stock solution of 5 mM. Working fresh solutions were made of this reagent immediately before use. Before each experiment, the needed solutions of all compounds were prepared.

2.2. Absorption and fluorescence measurements

Absorption spectra were registered with a Perkin–Elmer Lambda 40 UV/VIS spectrophotometer (Norwalk, CT, USA). Fluorescence emission spectra were recorded by means of a Perkin–Elmer LS55B luminescence spectrometer equipped with R-928 photomultiplier (Iwata, Japan). All measurements were performed in standard 10 mm quartz cuvettes.

2.3. Light exposure

Solutions of 5MTHF and Uro were exposed to light in closed quartz cuvettes (1 ml). A bank of fluorescence tubes (Model 3026, Applied Photophysics, London, UK) with the fluence rate at the sample positions was 1.7 mW/cm² (Fig. 1) was used. The emission of this lamp was mainly in the wavelength region 370–450 nm, with a peak at 405 nm. The temperatures of the samples were approximately 25 °C. All experimental work was performed in dim light to avoid external and uncontrolled light exposure.

3. Results

The emission spectrum of the lamp and the absorption spectrum of 5MTHF and Uro are shown in Fig. 1. Uro was not completely stable during light exposure (Fig. 2A). For further experiments, 20 min light exposure was chosen. About 14% of Uro was degraded after the longest exposure time (Fig. 3A). The photodegradation rate of Uro was the same for both concentrations (Fig. 3A and B). 5MTHF alone was not photosensitive (Fig. 2B). However, it was degraded in the presence of Uro (Fig. 2C). By subtracting the spectra of irradiated Uro (Fig. 2A) from the spectra of irradiated 5MTHF in presence of Uro (Fig. 2C), the decomposed spectra can be obtained (Fig. 2D). These spectra show an increase of absorption at 250 nm and a decrease of absorption at 290 nm

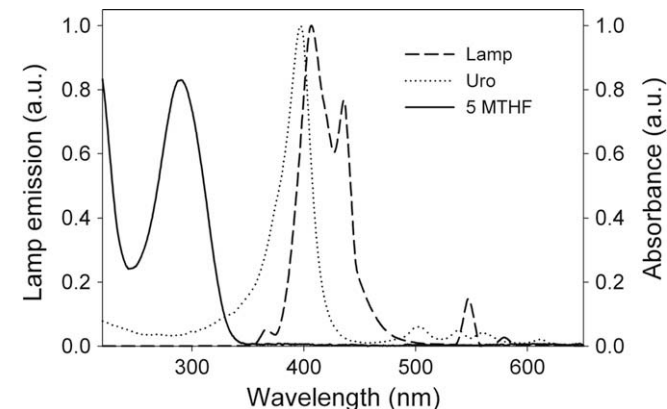


Fig. 1. Absorption spectra of 2.5 µM 5MTHF and 2.5 µM Uro, and fluence spectra of the used lamp.

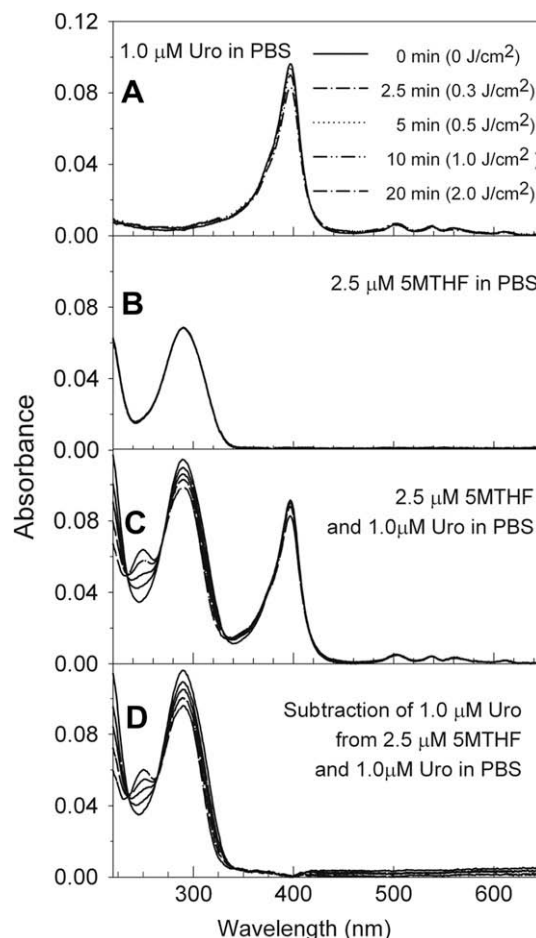


Fig. 2. Absorption spectra of: (A) 1 µM Uro before and after blue light exposure; (B) 2.5 µM 5MTHF before and after blue light exposure; (C) 2.5 µM 5MTHF with 1 µM Uro before and after blue light exposure; and (D) 2.5 µM 5MTHF in the presence of Uro, obtained by subtracting absorption spectra of irradiated of Uro (A) from the absorption spectra irradiated of 5MTHF with Uro (B).

(Fig. 2D). It has similar characteristic with the spectra of photo-product of 5MTHF, 5-methyldihydrofolate (5MDHF) [8,11], which can be used to determine the oxidation of 5MTHF.

The oxidation of 5MTHF during light exposure in presence of Uro in H₂O and D₂O were studied. The photodegradation rates of 2.5 µM 5MTHF in the presence of 1 µM Uro in H₂O and D₂O were 0.1 and 0.6 min^{−1}, respectively (Fig. 4A), and in the presence of 2.5 µM Uro were 0.3 and 0.9 min^{−1} in H₂O and D₂O, respectively (Fig. 4B). Thus, the photodegradation of 5MTHF was dependent on the concentrations of Uro.

The rates of photodegradation of 1 µM Uro in H₂O and D₂O were 0.01 and 0.02 min^{−1}, respectively (Fig. 3A). In the presence of 2.5 µM 5MTHF, the degradation rates of Uro were 0.005 and 0.01 min^{−1} in H₂O and D₂O, respectively. Under the present conditions, the photodegradation of Uro was slower by about factor of two in presence of 5MTHF.

The singlet oxygen sensor green reagent (SOSG) exhibited weak fluorescent (Fig. 5). 5MTHF (2.5 µM) and/or Uro (2.5 µM) had no effect on SOSG fluorescence as long as the solutions were kept in the dark. However, the fluorescent intensities (at an emission wavelength of 527 nm and an excitation wavelength of 429 nm) of SOSG increased during light exposure in presence of Uro (Fig. 5). The fluorescence intensities of SOSG alone or in presence of 5MTHF were unchanged during light exposure (Fig. 5).

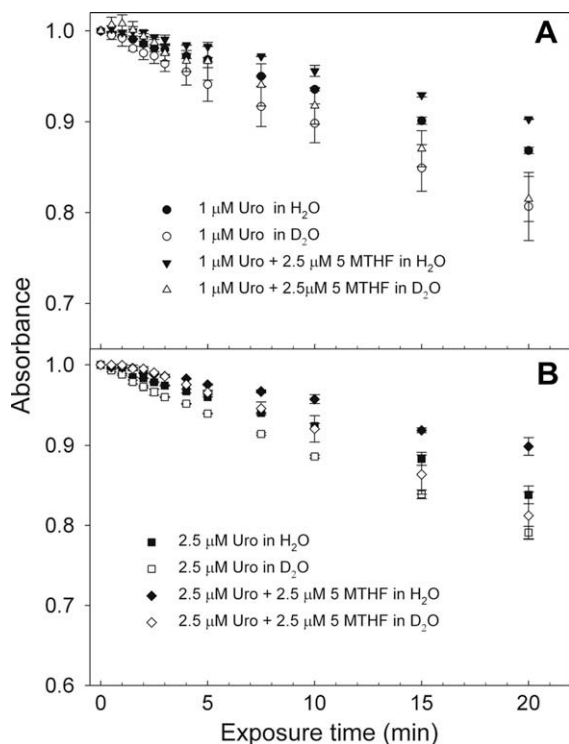


Fig. 3. Absorbance at 397 nm (Uro destruction) for different concentration of Uro: (A) 1 μM Uro in PBS and in D₂O and (B) 2.5 μM Uro in PBS and in D₂O, normalized to 1 at zero light dose exposure.

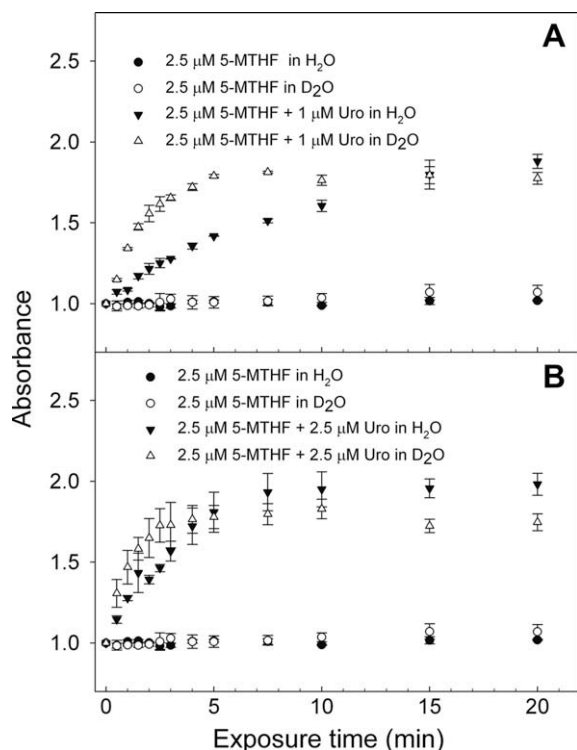


Fig. 4. The degradation of 2.5 μM 5-MTHF by blue light exposure in the presence of different concentrations of: (A) 1 μM Uro and (B) 2.5 μM Uro in PBS and D₂O, measured by the increase of absorbance at 250 nm, normalized to 1 at zero light dose exposure.

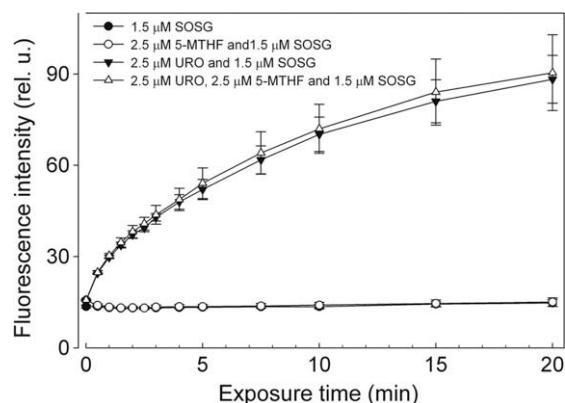


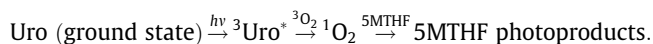
Fig. 5. The fluorescence emission intensity at 527 nm ($\lambda_{\text{ex}} = 429$ nm) of PBS + 1.5 μM SOSG, 2.5 μM 5-MTHF + 1.5 μM SOSG, 2.5 μM Uro + 1.5 μM SOSG, 2.5 μM 5-MTHF + 2.5 μM Uro + 1.5 μM SOSG.

4. Discussion

5MTHF absorbs mostly in the UVB region, with a peak at 290 nm, (Fig. 2B). It was stable during UVA and near-UV light exposure (Figs. 2B and 5). This result was in agreement with those of Steindal and Offer [8,9,11]. In earlier studies we demonstrated that in the absence of photosensitizers, 5MTHF was not photodegraded by UVA radiation [8,9,11]. The present study shows that in the presence of the water soluble photosensitizer Uro 5MTHF is photodegraded under light exposure (Fig. 2C). The decomposed spectra has two peaks, one at 250 nm and one at 290 nm (Fig. 2D). These spectra have contributions with similar characteristics as those of one of the photoproducts of 5MTHF in photolyzed, 5-methylidihydrofolate (5MDHF) [8,11].

Uro is an endogenous photosensitizer [16,17]. Under light exposure it can produce reactive oxygen species (ROS) such as H₂O₂ and ¹O₂ [14]. Uro is also photodegraded during light exposure (Fig. 2A). The lifetime of ¹O₂ is approximately 15-fold greater in D₂O than in H₂O [18]. Thus, if the lifetime of ¹O₂ is an influencing factor, the rate of the photobleaching will be larger in D₂O than in H₂O. This holds if the photooxidation is mediated by ¹O₂. Our results show that the photodegradation rate of Uro was about two times lower in H₂O than in D₂O (Fig. 3A and B). This means that ¹O₂ is most likely involved in these photodegradation processes. The results were supported by using SOSG, which is a sensitive probe for ¹O₂ and does not react with hydroxyl radicals nor with superoxide [19]. On the basis of these measurements, we concluded that ¹O₂ is a major intermediate in the photooxidation mediated by Uro, both of itself and of other molecules.

Folate and Uro are both localized in blood plasma, their concentrations being about 10–30 nM [10,20] and 0–11 nM [21], respectively. Photosensitizers can under light exposure generate the highly reactive ¹O₂, which may destroy the photosensitizer itself, in a process called photobleaching [22,23]. At the same time it can degrade neighboring molecules. Thus, there exists a possibility for generation of ¹O₂ during light exposure. Formation of ¹O₂ is believed to cause photodegradation of 5MTHF in human serum. The mechanism of photodegradation of 5MTHF in presence of Uro proposed to be:



However, other ROS, such as H₂O₂, may also be involved in this process [14].

If there is an association between folate photosensitivity and evolution of human skin colour, this association is quite complicated. Preliminary studies suggest that dark skin may protect fo-

lates against photodegradation [6,7]. 5MTHF absorbs mainly in the UVB region. UVB radiation can not penetrate the epidermis and reach human blood vessel as efficiently as UVA and visible light can [24]. Therefore, the photodegradation of 5MTHF in healthy humans is probably not a direct reaction, in which the radiation is absorbed by 5MTHF. It is more realistic that 5MTHF in the human body can be photooxidized by ROS generated by endogenous photosensitizers such as riboflavin, and Uro [8]. Based on this fact, people who have low levels of folate, such as pregnant women, old people and alcoholics are advised stay away from exposure to intense UV radiation in order to avoid any indirect photodegradation of folate. Our findings are important for further studies of human skin colour evolution.

We would like to investigate further the influence of endogenous photosensitizers on folate photodegradation. This will be done by investigating the degradation of 5MTHF in the presence of photosensitizers (riboflavin, uroporphyrin, protoporphyrin IX) exposed to visible light in solutions in the absence and presence of proteins and in human serum.

5. Conclusions

5MTHF is stable under UV and near-UV light exposure. However, it is degraded in the presence of low concentrations of Uro. The photosensitized oxidation occurred mostly via a type II mechanism involving $^1\text{O}_2$. These findings should be paid attention to when hypothesis for development of different human skin colours are elaborated.

6. Abbreviations

5MTHF	5-methyltetrahydrofolate
Uro	Uroporphyrin
NTD	neural tube defects
UVA	Ultraviolet A (400–320 nm)
SOSG	singlet oxygen sensor green reagent

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