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Effect of pterin impurities on the fluorescence and photochemistry of commercial folic acid

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ABSTRACT

Folic acid, or pteroyl-L-glutamic acid (PteGlu) is a conjugated pterin derivative that is used in dietary supplementation as a source of folates, a group of compounds essential for a variety of physiological functions in humans. Photochemistry of PteGlu is important because folates are not synthesized by mammals, undergo photodegradation and their deficiency is related to many diseases. We have demonstrated that usual commercial PteGlu is unpurified with the unconjugated oxidized pterins 6-formylpterin (Fop) and 6-carboxypterin (Cap). These compounds are in such low amounts that a normal chromatographic control would not detect any pterinic contamination. However, the fluorescence of PteGlu solutions is due to the emission of Fop and Cap and the contribution of the PteGlu emission, much lower, is negligible. This is because the fluorescence quantum yield (Φ_F) of PteGlu is extremely weak compared to the Φ_F of Fop and Cap. Likewise, the PteGlu photodegradation upon UV-A radiation is an oxidation photosensitized by oxidized unconjugated pterins present in the solution, and not a process initiated by the direct absorption of photons by PteGlu. In brief, the fluorescence and photochemical properties of PteGlu solutions, prepared using commercially available solids, are due to their unconjugated pterins impurities and not to PteGlu itself. This fact calls into question many reported studies on fluorescence and photooxidation of this compound.

1. Introduction

Folic acid, or pteroyl-L-glutamic acid (PteGlu) is a conjugated pterin derivative, with a chemical structure composed by three moieties: a 6-methylpterin (Mep) residue, a *p*-aminobenzoic acid (PABA) residue, and a glutamic acid (Glu) residue (Scheme 1a). In living systems, PteGlu is present in multiple forms including molecules attached to several glutamate residues and dihydro and tetrahydro derivatives. Folate is the generic term for this large family of chemically related compounds.

Tetrahydrofolate and its derivatives act as coenzymes in one-carbon transfer reactions required in the biosynthesis of nucleic acids and proteins [1] and, in consequence, these compounds are essential for a variety of physiological functions in humans. Moreover, a deficit of folate leads to several diseases such as megaloblastic anemia [2], coronary heart disease [3], neurological disorders [4], and fertility problems [5–7]. Folate requirements increase in periods of rapid cell division and growth, and, therefore, it is important that pregnant women keep folate concentrations at an appropriate level [8]. Folate deficiency in pregnancy is related to neural tube defects (NTD), such as spina bifida and anencephaly [9,10].

Mammals do not synthesize folates and therefore they have to get them from food. In some situations, like anemia or pregnancy, folate supplementation is needed. PteGlu is inexpensive to produce, more stable than most members of folate's family and efficiently metabolized into biologically active derivatives, such as 5-methyltetrahydrofolic acid. Due to these properties, PteGlu is used in tablet form and in fortified foods for dietary supplementation [1,11].

The correlation between NTD and UV-A (315–400 nm) exposure has been described in amphibian larvae [12] and in women who had used artificial tanning sun beds during the first weeks of pregnancy [13]. In addition, epidemiological data have shown that the prevalence of NTD

Abbreviations: absorbance, A; absorbed photon flux density, $q_{n,\,p}{}^{a,\,V}$; acetonitrile, ACN; ammonium acetate, NH_4Ac; 6-carboxypterin, Cap; emission wavelength, λ_{em}; excitation wavelength, λ_{exc}; folic acid, PteGlu; folic acid radical cation, $PteGlu^{\cdot +}$; formic acid, HCOOH; 6-formylpterin, Fop; fluorescence, FL; fluorescence quantum yields, Φ_F; glutamic acid, Glu; high-performance liquid chromatography, HPLC; hydrogen peroxide, H_2O_2; incident photon flux density, $q_{n,\,p}{}^{0,\,V}$; incident photons per time interval, $q_{n,\,p}{}^{0}$; liquid chromatography/mass spectrometry, LC/MS; 6-methylpterin, Mep; neural tube defects, NTD; *p*-aminobenzoic acid, PABA; *p*-aminobenzoylglutamic acid, PABA-Glu; photosensitizers, Sens; photosensitizer radical anion, $Sens^{\cdot -}$; reference fluorophore, R; retention times, t_r; superoxide anion, $O_2^{\cdot -}$; total fluorescence intensities, I_F; triplet excited state of the sensitizer, $^3Sens^*$; volume, V

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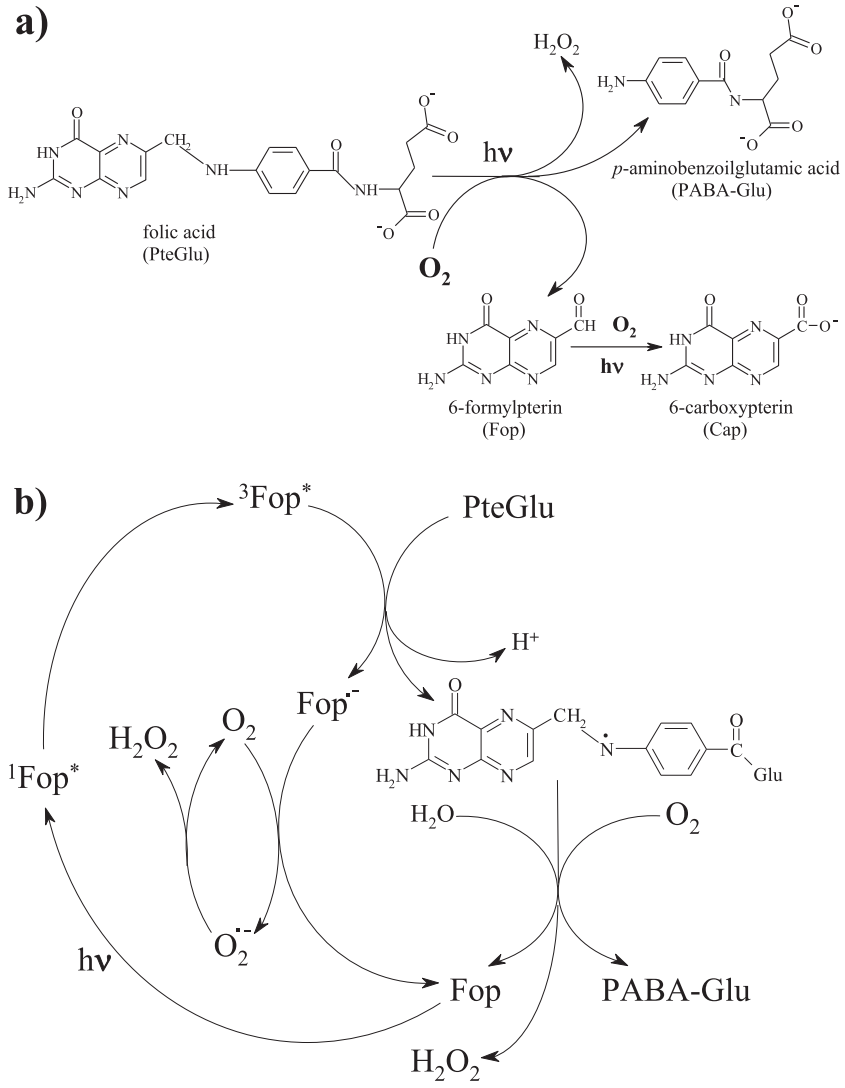

Scheme 1. a) Photooxidation of PteGlu in air-equilibrated aqueous solutions under UV-A irradiation. b) Mechanism of the oxidation of PteGlu photosensitized by Fop. Similar processes take place with other aromatic pterins acting as photosensitizers.

is higher in light-skinned people [14,15]. In 1978, Branda and Eaton proposed that one of the main functions of skin pigmentation is to avoid photolysis of folate [16]. This hypothesis was based on the following facts: i) light-skinned patients undergoing photochemotherapy (*i.e.*, psoralen plus UV-A) showed lower serum folate levels than healthy controls, and ii) a 30–50% loss of folate in human plasma was observed after *in vitro* exposure to simulated sunlight. Recent reports indicate that both *in vitro* and *in vivo* exposure of human blood to UV-A radiation, lead to photodegradation of folate [17,18].

PteGlu can be excited by solar radiation and artificial sources of light since its absorption spectrum shows bands in the UV-B (280–315 nm) and UV-A spectral regions (Fig. S1, Supplementary material). The photodegradation of PteGlu was reported for the first time in the late 1940s [19] and, since then, many studies on the photochemistry of this compound, as a model of folates, have been conducted in aqueous solutions and other media [20–28]. In the absence of oxygen, PteGlu is photostable, but excitation in air-equilibrated solutions leads to cleavage and oxidation of the molecule, yielding 6-formylpterin (Fop) and *p*-aminobenzoylglutamic acid (PABA-Glu) as photoproducts (Scheme 1a). In turn, Fop is transformed into 6-carboxypterin (Cap) upon further photooxidation.

Upon UV-A irradiation the degradation of PteGlu in the presence of oxygen is a sigmoidal function [22,24–26]: after a period of time the rate of PteGlu consumption significantly increases. In 2010, we reported that the photooxidation products of PteGlu are able to photoinduce the degradation of this molecule (Scheme 1b) [29]. That means that an “auto-photo-catalytic” effect is involved, where Fop photosensitizes the oxidation of PteGlu, and that explains the acceleration in the consumption of reactant when its products accumulate in the medium. In general terms, the photosensitization consists in the chemical alteration occurring in one molecular entity as a result of the initial absorption of radiation by another molecular entity called the photosensitizer. This process, in which no excitation of PteGlu is needed, also takes place with other pterins as photosensitizers (Sens), thus revealing a general mechanism. After excitation of the Sens, the first step of the process involves an electron transfer from the PABA unit of PteGlu to the triplet excited state of the Sens ($^3Sens^*$) to form the corresponding radical ions, the photosensitizer radical anion ($Sens^{\cdot-}$) and the PteGlu radical cation ($PteGlu^{\cdot+}$). The electron transfer from $Sens^{\cdot-}$ to O_2 regenerates the Sens and forms superoxide anion ($O_2^{\cdot-}$), which disproportionates to form hydrogen peroxide (H_2O_2). Finally, PteGlu degradation occurs as a result of the trapping of $PteGlu^{\cdot+}$ by oxygen (Scheme 1b).

Although a large amount of reports about photodegradation of PteGlu under UV-A radiation have been published, many questions about the photochemistry of this compound remain without answer.