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Rate constants of quenching of the fluorescence of pterins by the iodide anion in aqueous solution

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ABSTRACT

The fluorescence of various pterins (6-methylpterin, 6-hydroxymethylpterin, 6-formylpterin, and 6-carboxypterin) was studied in the presence of iodide in neutral aerated phosphate buffer. Stern–Volmer plots and the fluorescence lifetimes were used to determine fluorescence quenching rate constants that approach the diffusion controlled limit. Given that pterin derivatives have been detected in human skin exposed to UV radiation, biological implications of the high rate constants are discussed.

1. Introduction

Folic acid, or pteroyl-L-glutamic acid (PteGlu), is an important vitamin that has received increased attention in the past few decades. The electrochemistry of PteGlu and related pterins (Pts) has been well studied, as they participate as co-enzymes in many biological redox reactions (Figure 1) [1–5]. PteGlu is necessary for the biosynthesis of nucleic acids, helps to prevent neural tube birth defects, and has received wide recognition as an effective prenatal vitamin that has resulted in a decreased occurrence of spina bifida [1,6–10]. Pts have been reported to participate in a wide variety of photochemical processes: photosynthesis, the light harvesting antenna, DNA photolyases, and the derivatives are being investigated as a phototherapeutic treatment for some cancers [6,11–20].

The fluorescence quantum yield of PteGlu in aqueous solutions is $\Phi_F < 0.005$ [21]. This low fluorescence indicates strong quenching of an excited singlet state and has been attributed to an electron-transfer reaction between the para-aminobenzoic acid (PABA) moiety and the pterin (Pt) [22]. In contrast, Pts that lack the PABA group, like those produced from the photodegradation of PteGlu, show fluorescence efficiencies between $\Phi_F = 0.07–0.85$ [1,23]. Using DFT calculations, we reported that not only is a photo-induced electron-transfer reaction between PABA and Pt thermodynamically feasible, but this charge-transfer complex may directly result in heterolytic bond cleavage resulting in the observed products of PteGlu photodegradation [24].

Kritsky and co-workers demonstrated that EDTA acts as an electron donor towards Pts resulting in a photoreduction [25]. Iodide anion, another known electron donor, was used to quench both the Pt fluorescence and the EDTA redox photochemistry. The hypothesis was that if the electron transfer between EDTA and Pt was occurring from the excited singlet state, then the concentration of iodide required to quench the fluorescence should be the same as the concentration needed to quench the EDTA redox chemistry. Because the concentration of iodide required to quench half of the Pt fluorescence was nearly triple the concentration required in quenching the EDTA chemistry, it was concluded that the major route of photo-induced electron-transfer chemistry to Pt occurs through the triplet state. This result has been used by other researchers as a basis towards the use of iodide anion as a probe to determine Pt triplet chemistry with other substrates [26,27].

The reduced fluorescence of PteGlu and the theoretical calculations suggest that Pts undergo excited singlet state redox chemistry while the work by Kritsky and others suggest that this photochemistry originates from the triplet state. This result does not necessarily mean that the singlet-state chemistry is either slow or insignificant. It is likely that photo-induced redox chemistry occurs through both excited states. Since Pt excited singlet states have lifetimes (τ) of only a few nanoseconds, efficient singlet-state redox chemistry will be critical in cases of Pt binding with other biological macromolecules, such as DNA. Therefore, more work is needed to determine the absolute reaction rates of the photoreduction of Pts from both excited states. We have determined rate constants of fluorescence quenching of various Pts (6-methylpterin (Mep), 6-hydroxymethylpterin (Hmp), 6-formylpterin (Fop), and 6-carboxyperin (Cap)) by iodide anion under physiological conditions (air equilibrated neutral PBS buffer) using Stern–Volmer quenching studies.

2. Experimental

2.1. General

All pterin and potassium iodide solutions were prepared using neutral phosphate buffer (PBS). The pH was adjusted to 7.3 ± 0.1

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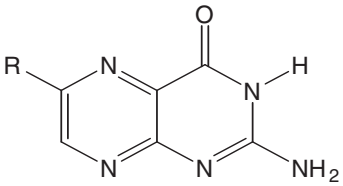

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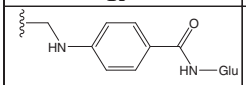	Folic Acid (Pteroyl-L-glutamic acid, PteGlu)
CH_3	6-Methylpterin (Mep)
CH_2OH	6-Hydroxymethylpterin (Hmp)
CHO	6-Formylpterin (Fop)
CO_2H	6-Carboxypterin (Cap)

Figure 1. Chemical structure of various pterins.

by dropwise addition of 1 M HCl. Pterins were purchased from Shircks laboratories and used without any further purification. Complete dissolution of pterins was assisted by gentle heating and sonication.

2.2. Steady-state emission experiments

Measurements were obtained using a Varian CARY Eclipse fluorescence spectrophotometer and absorption measurements were obtained using a Varian CARY Bio-50 UV–VIS spectrophotometer. The excitation wavelength (λ_{exc}) was 350 nm and the emission range was 400–600 nm. The wavelength maximum (λ_{max}) for each emission spectrum (in the absence of iodide) was used as the wavelength for determining the fluorescence intensity of each pterin for the Stern–Volmer calculations. All experiments were performed in triplicate and the average results are reported.

2.3. Time-resolved emission experiments

Fluorescence lifetimes (τ_F) were obtained using time-correlated single-photon counting on a Horiba-Jobin Yvon instrument (FL3 TCSPC-SP). A NanoLED source (maxima at 341 nm) was used for excitation. The emitted photons, after passing through a iHR320 spectrometer (λ_{em} = 450 nm), were detected by a TBX-04 detector and counted by a FluoroHub-B module.

3. Results and discussion

Experimental determination of the energy gap between the ground state and excited state singlets ($S_0 \rightarrow S_1$) can be estimated by examination of the absorption and fluorescence emission spectra. By overlaying the two spectra and scaling the relative intensities to similar heights, an approximation of the energy of the excited singlet state can be obtained by observing the midpoint of these two maxima [24]. In the case of all Pts studied, this region is roughly between 400–410 nm and 69–71 kcal/mol (Figure 2).

The presence of iodide anion was observed to efficiently quench the Pt fluorescence linearly with increasing iodide concentration. The λ_{max} did not change with increasing quencher concentration; therefore, we conclude that there is no aggregation or complexation observed over the concentration range studied between the Pts and iodide anion. The emission spectra of Cap in PBS in the presence of various iodide concentrations is shown in Figure 3. All other Pts studied show similar trends.

The relative intensity of the fluorescence at λ_{max} for each Pt in the absence of iodide anion (I_0) and in the presence of iodide (I) was used to produce a Stern–Volmer plot for each Pt studied Eq. (1).

$$I_0/I = 1 + k_Q \tau_F [Q] \quad (1)$$

where k_Q is the bimolecular quenching rate constant ($L\,mol^{-1}s^{-1}$), τ_F (s) is the fluorescence lifetimes in the absence of quencher and

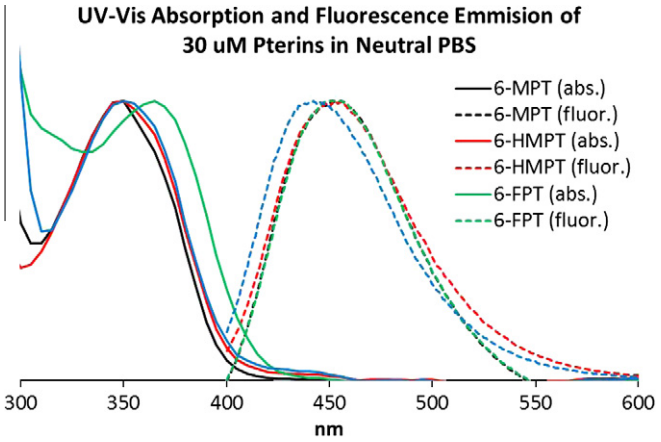

Figure 2. Normalized absorption and emission spectra (λ_{exc} 350 nm) of Mep, Hmp, Fop, and Cap in neutral PBS.

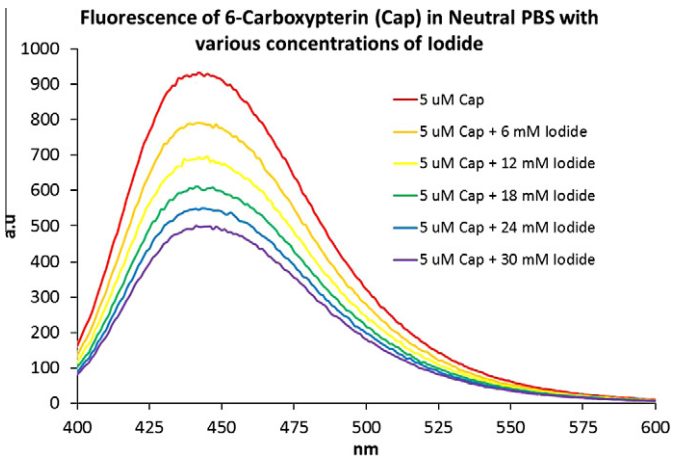

Figure 3. Fluorescence of 5 μM Cap in neutral PBS in the presence of various iodide anion concentrations.

[Q] is the quencher concentration ($mol\,L^{-1}$). All Pts studied produced a straight line in the Stern–Volmer plot using iodide as the quencher in PBS under aerobic conditions (Figure 4). The fluorescence of Pts substituted with alkyl groups in the 6-position (Mep and Hmp) was shown to be more effectively quenched by iodide anion than the fluorescence of Pts with carbonyl groups (Cap and Fop) at that position.

The τ_F for each Pt was determined under the same conditions as those used in steady-state quenching experiments. A first-order rate law was observed for all the fluorescence decays and τ_F were found to be between 4 and 9 ns using the same conditions as the

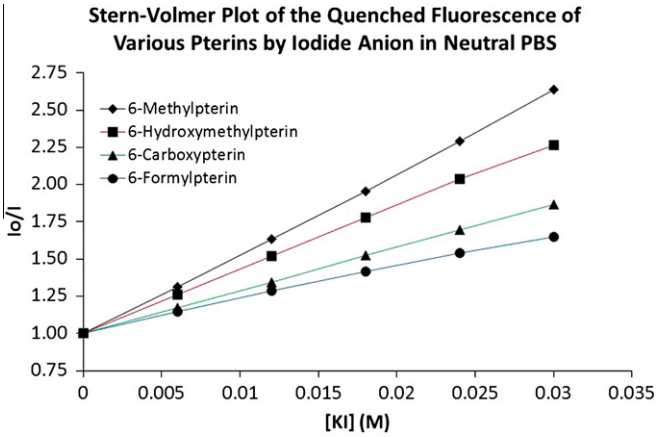

Figure 4. Stern–Volmer plots of the fluorescence of Mep (♦), Hmp (■), Cap (▲), and Fop (●) using iodide anion (0–30 mM) as a quencher in neutral PBS under aerobic conditions. ([Pt] 5 μM).

Table 1
Summary of Stern–Volmer slopes (K_{SV}), experimental fluorescence lifetimes in neutral PBS (τ_F), and the calculated rate constant of fluorescence quenching (k_Q). Literature reported air-equilibrated fluorescence quantum yields (Φ_F) in both the acidic (pH 4.9–5.5) and basic forms (pH 10.0–10.5) are provided for comparison (Fop and Cap, Ref. [21]; Mep and Hmp, Ref. [23]).

	K_{SV} (M^{-1})	τ_F (s)	k_Q (L mol^{-1} s^{-1})	Φ_F (acid)	Φ_F (basic)
Mep	53.66	8.7×10^{-9}	6.2×10^{9}	0.62	0.61
Hmp	42.23	7.4×10^{-9}	5.7×10^{9}	0.53	0.46
Fop	22.68	6.4×10^{-9}	3.5×10^{9}	0.12	0.07
Cap	28.99	4.6×10^{-9}	6.3×10^{9}	0.26	0.18

Stern–Volmer studies (Table 1). When the Stern–Volmer slopes and τ_F are considered, k_Q can be determined Eq. (1). The k_Q values calculated for the Pts studied are all quite similar and very high, near the diffusion controlled limit, Table 1). The rate constant for quenching can also be expressed as a function of the rate constants of fluorescence (k_F), raditionless internal conversion, (k_{IC}), and intersystem crossing (k_{ISC}) to the triplet state Eq. (2).

$$1/k_Q = 1/k_F + 1/k_{IC} + 1/k_{ISC} \quad (2)$$

Since the Φ_F of the pterins under study are very high, it is concluded that $k_F >> k_{ISC}$, which illustrates the importance of pterin fluorescence and the singlet excited state in the photochemistry of these compounds.

Previous studies have demonstrated that excited singlet state of Pts is able to be quenched by anions through proton-transfer [28]. In this mechanism, the anion has to be able to accept a proton transferred from an excited Pt. In agreement with this, anions of weak acids, such as phosphate and acetate, have been reported to be relatively efficient quenchers, whereas anions of strong acids, such as chloride and nitrate, do not quench the emission of Pts. In the case of iodide, HI is stronger than HCl; consequently, proton transfer can be discarded as the predominant mechanism of fluorescence quenching by iodide anion. On the other hand, electron transfer has been proposed to be the main mechanism of quenching of fluorescence of Pts by nucleotides [29]. However, in this case the quenching via proton-transfer cannot be completely discarded because nucleotides bear several groups able to accept protons. The results presented in this work, in connection with previous studies, strongly support the hypothesis that singlet excited states of Pts can be deactivated by both proton- and electron-transfer, the latter being the predominant one for iodide.

It was proposed that the decreased fluorescence of PteGlu was due to an intramolecular electron-transfer reaction with the excited singlet state, which resulted in a reduced Pts [22]. The rate of electron transfer to the excited singlet state ($k_{ET(s1)}$) was estimated to be approximately 1×109^{-10} s^{-1}, which is in excellent agreement with the experimentally determined values in this study. Although it has been shown that the triplet state of Pts is more active towards reduction than the excited singlet state, the shorter-lived singlet is still very reactive, as indicated by the rate constants approaching that of the diffusion controlled limit. Since the amount of iodide required to quench half the Pt fluorescence is three times the concentration required to quench half the Pt redox chemistry (presumably from the triplet state), it can be assumed that these two reactions occur with rates within one order of magnitude of each other. The τ_F of the Pts under study were measured to be $\sim 10^{-9}$ s and triplet Pt lifetimes (τ_T) are typically $\sim 10^{-6}$ s.

When the longer τ_T are considered, it can be estimated that the rate constant of photo-induced electron-transfer with the triplet state, $k_{ET(T1)}$, is approximately three orders of magnitude times less than the rate constant of fluorescence quenching, $k_{ET(s1)}$, or $k_{ET(T1)} = 10^{6-7}$ s^{-1}. We believe that the observations of electron transfer occurring predominately from the triplet state is likely due to the increased τ_T; however, the rate constant of electron transfer from the singlet state is quite high and occurs with a high efficiency. The singlet pathway will be increasingly significant in situations when the electron donor is pre-associated with the Pt, as shown in the example of PteGlu where the electron donor (PABA) is covalently linked. Additionally, triplet states of Pts are known to be efficiently quenched by O_2 [30]. We measured the τ_F in both the absence and in the presence of dissolved O_2 and determined no significant effect. Therefore, under physiological conditions, the excited singlet state of Pts might be a significant source of electron-transfer chemistry.

One possible example of the implications of this electron-transfer chemistry is demonstrated in the link between the observation of PteGlu photoproducts and birth defects known to be reduced by PteGlu rich diets. Women who were exposed to an increased amount of UV radiation during the first weeks of pregnancy (i.e. tanning beds) showed an increased rate of babies with neural tube defects [31]. This has been attributed to the photo-degradation of PteGlu. Pt photoproducts have been spectroscopically detected in the skin, specifically in patients with pigment-lacking portions of the skin caused by vitiligo [19]. These photoproducts are fairly insoluble in aqueous media compared to PteGlu as their maximum concentrations in PBS are only a few hundred micromolar. Pt products produced from the photodegradation of PteGlu may bind to hydrophobic pockets of biological macromolecules in the human body. When these complexes are further exposed to UV or visible radiation that further photo-excite these Pt photoproducts, oxidative damage originating from the singlet excited state is possible. Currently, our laboratory is investigating the binding constants of these Pts with various biological macromolecules, including DNA and poly-nucleotides. Preliminary results indicate that the binding constants are low, however strong binding is not required for significant damage to occur from efficient redox chemistry. Electron-transfer oxidation of DNA, through a reaction with an electron acceptor like the Pt excited singlet state, will result in a DNA radical-cation, which may lead to DNA damage.

Based upon these results, we propose that the increased dietary intake of PteGlu might result in an increased amount of Pt photoproducts in the skin upon exposure to UV radiation. These molecules may then bind to hydrophobic pockets, including DNA. Further exposure to UV radiation may result in a very rapid electron-transfer reaction ($k_{ET} = 1 \times 109^{-10}$ s^{-1}) resulting in DNA damage, for example 8-oxo-G, which has been detected and reported

over a decade ago [15]. This damage may result in an increased risk of cancer and contribute to DNA damage beyond T< >T dimers. Unfortunately, the repair mechanism of DNA damage resulting from 8-oxo-G lesions is less efficient than that for T< >T lesions [32]. This hypothesis is consistent with a report indicating that diets containing excess PteGlu may result in an increased risk of cancer [33]. It should be noted that the authors of this work are not suggesting that PteGlu dietary supplementation is unhealthy, merely that the present experimental results and hypotheses are consistent with other reports that suggest a possible link between the intake of a large excess of PteGlu and an increased risk of skin cancer, especially when combined with high levels of UV radiation.

Conclusions

Using Stern–Volmer fluorescence studies, we determined the rate constant of Pts quenching by iodide anion in PBS under aerobic conditions to be near that of diffusion control ($k = 1 \times 10^{9-10}\ s^{-1}$). These values are consistent with earlier estimations of the rate of photo-induced electron-transfer reactions with Pts. Although other literature indicates that the triplet state of Pts is the dominant source of Pt redox chemistry, our results clearly indicate that the excited singlet state possesses a much higher rate constant and might therefore play a significant role in aerobic systems and where Pt binding is significant. These results could have strong implications on the oxidation of biomolecules photosensitized by Pts produced in the skin of humans through different processes, such as the photodegradation of PteGlu.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cplett.2012.06.013.

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