Quenching of the Fluorescence of Aromatic Pterins by Deoxynucleotides

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Steady-state and time-resolved studies of the fluorescence of four aromatic unconjugated pterins (pterin (Ptr), 6-(hydroxymethyl)pterin (Hmp), 6-methylpterin (Mep), and 6,7-dimethylpterin (Dmp)) in aqueous solutions in the presence of different nucleotides (2′-deoxyguanosine 5′-monophosphate (dGMP), 2′-deoxyadenosine 5′-monophosphate (dAMP), and 2′-deoxycytosine 5′-monophosphate (dCMP)) have been performed using the single-photon counting technique. The singlet excited states of acid forms of pterins are deactivated by purine nucleotides (dGMP and dAMP) via a combination of dynamic and static processes. The efficiency of the dynamic quenching is high, independently of the nature of the purine base of the nucleotide and of the chemical structure of the substituents linked to the pterin moiety. Analysis of the static quenching indicates that ground-state association between pterins and purine nucleotides takes place, but the formation of the corresponding complexes is significant only at relatively high reactant concentrations. The quenching of the fluorescence of acid forms of pterin derivatives by dCMP, a pyrimidine nucleotide, is slightly less efficient than the quenching by purine nucleotides and is purely dynamic. In alkaline media, the fluorescence quenching is much less efficient than in acidic media, the deactivation by purine nucleotides being purely dynamic, whereas quenching by dCMP is negligible. Possible mechanisms for the quenching of fluorescence of pterin derivatives by the different nucleotides are discussed.

Introduction

Pteridines in their multiple forms are widespread in biological systems and play different roles ranging from pigments to cofactors for numerous redox and one-carbon transfer reactions. Within the pteridine family, pterins are derived from 2-aminopteridin-4(3*H*)-one (pterin). The participation of pterins in (photo)biological processes has been suggested or demonstrated in the past decade, and interest in the photochemistry and photophysics of these compounds has subsequently increased.[1] In addition, some pterin derivatives (e.g., biopterin, 6-formylpterin, 6-carboxypterin) accumulate in the skin of patients affected by vitiligo, a depigmentation disorder, where the protection against UV radiation fails due to the lack of melanin.[2,3] Finally, pterins participate in biologically important photosensitization processes; for example, pterins act as sensitizers in photochemical reactions that induce DNA damage[4,5] and are able to generate singlet molecular oxygen[6] and other reactive oxygen species.[1]

Pterins behave as weak acids in aqueous solutions, where several acid−base equilibria may be present. In general, the dominant equilibrium at pH > 5 involves the lactam group (pyrimidine ring) (Figure 1).[7] The pK_a of this equilibrium is about 8 for several pterin derivatives. Other functional groups of the pterin moiety (e.g., 2-amino group or ring nitrogen atoms) have pK_a values lower than 2. Photophysics and photochemical properties of pterins are dependent on the pH.[1]

The fluorescence emission of aromatic unconjugated pterins, oxidized derivatives that bear substituents of low molecular weight at position 6 (Figure 1), has been known for several decades and used for analytical purposes. Some assays for analyzing the concentration of folic acid, a conjugated pterin, use the emission of 6-carboxypterin obtained by oxidation of the folic acid.[8] Many experimental assays for determining pterin concentrations (e.g., in food) by means of chromatographic methods have been developed on the basis of their fluorescence.[9] In addition, DNA probes containing fluorophores are increasingly used to investigate different aspects of the physicochemical properties of DNA, such as the kinetics of interactions with other biomolecules and changes in structure. Recently some pteridine-based fluorophores that are chemical analogues of the nucleosides of DNA have been developed.[10−12]

We have recently studied in detail the fluorescence of pterins in aqueous solutions.[13,14] The wavelengths of the emission maxima (λ_{max}), the fluorescence quantum yields (Φ_F), and lifetimes (τ_F) for both acid and basic forms of the pterin derivatives used in this work have been previously reported and are listed in Figure 1. The quenching of pterins fluorescence by some anions of analytical importance has also been investigated.[15]

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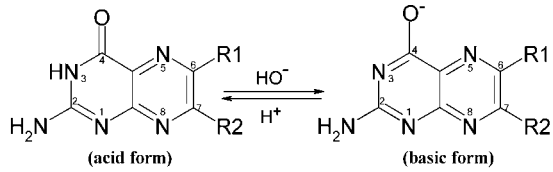

Compound	*R1*	*R2*	acid form			basic form		
			λ_F/nm	Φ_F	τ_F/ns	λ_F/nm	Φ_F	τ_F/ns
Pterin (Ptr)	-H	-H	439	0.33	7.6	456	0.27	5.0
6-(hydroxymethyl)pterin (Hmp)	$-CH_2OH$	-H	449	0.53	11.0	457	0.46	8.4
6-methylpterin (Mep)	$-CH_3$	-H	448	0.61	13.3	460	0.61	11.2
6,7-dimethylpterin (Dmp)	$-CH_3$	$-CH_3$	433	0.85	13.5	445	0.84	11.6

Figure 1. Molecular structures of pterin derivatives and the acid−base equilibrium in aqueous solution. Data from refs 13 and 14: wavelengths of fluorescence maxima (λ_F), fluorescence quantum yields (Φ_F), and fluorescence lifetimes (τ_F) in air-equilibrated aqueous solutions (excitation wavelength, 350 nm).

Fluorescence quenching is a powerful tool for investigating the interaction of singlet excited states of fluorescent compounds with other molecules (quenchers). Different types of processes can lead to fluorescence quenching.[16] The most important process is dynamic quenching due to collisional encounters between the excited fluorophore and a quencher molecule, whereas static quenching results from complex formation between the fluorophore in its ground state and a quencher. Data analysis of steady-state and time-resolved experiments allow to distinguish between the two types of quenching (Experimental Methods).

In the context of our studies on oxidation of nucleotides photoinduced by pterins,[17,18] we detected quenching of fluorescence of pterin by 2′-deoxyguanosine 5′-monophosphate (dGMP). Due to the potential applications of this interaction and the biological implications, we performed a systematic study of the quenching of fluorescence of aromatic unconjugated pterins by nucleotides in aqueous solutions, and the results obtained are presented in this article. Since photophysical and photochemical behaviors of pterins strongly depend on the chemical nature of the 6-substituent,[1] we have chosen for this study a series of four pterin derivatives (pterin (Ptr), 6-(hydroxymethyl)pterin (Hmp), 6-methylpterin (Mep), and 6,7-dimethylpterin (Dmp)) (Figure 1), which exhibit different electronic density distributions on the pyrazine ring of the pterin moiety. Three biomolecules were selected as quenchers: two purine nucleotides (dGMP and 2′-deoxyadenosine 5′-monophosphate (dAMP)) and one pyrimidine nucleotide (2′-deoxycytosine 5′-monophosphate (dCMP)). The experiments were performed under different pH conditions (5.5 and 10.5) in order to evaluate the efficiency of the quenching process by both acid and basic forms of pterins (Figure 1).

Experimental Methods

General. Pterins (Shircks Laboratories, Switzerland) were used without further purification. Nucleotides and other chemicals were used as received (Sigma-Aldrich). The pH measurements were performed using a pH meter CG 843P (Schott, Mainz, Germany) with a pH combination electrode BlueLine 14pH (Schott). The pH of the aqueous solutions was adjusted by adding drops of HCl or NaOH from a micropipette. The concentrations of the acid and base used for this purpose ranged from 0.1 to 2 M. UV−vis spectra were obtained using Varian Cary 5 or Cary 3 spectrophotometers.

Steady-state and time-resolved fluorescence measurements were performed on air-equilibrated aqueous solutions of pterin derivatives using an Edinburgh EAI-FS/FL900 SPC equipment. The quartz cells (1 cm path length) used for the measurements were thermoregulated at 25.0 ± 0.2 °C. Corrected fluorescence spectra obtained by excitation at 350 nm (high-pressure Xe lamp, 419 W) were recorded between 360 and 650 nm, and total fluorescence intensities (I_F) were calculated by integration of the fluorescence band centered at ca. 450 nm.

A N_2 excitation lamp (1.2 bar, 6.3 kV, 40 kHz) was employed for time-resolved studies. The single-photon counting range of the equipment was 500 ps to 500 μs, and the selected counting time window was 0−100 ns for the measurements reported. Emission decays were monitored at 450 nm after excitation at 350 nm. Lifetimes were obtained from the monoexponential decays observed after deconvolution from the lamp background signal, using the Edinburgh Analytical Instruments proprietary software, as previously described in detail.[19,20]

Data Analysis. If a dynamic process is operating, fluorescence quenching may be evaluated by a Stern−Volmer analysis (eq 1):

$$I_F^0/I_F = \tau_F^0/\tau_F = 1 + K_{SV}[Q] = 1 + k_q\tau_F^0[Q] \qquad (1)$$

where I_F^0 and I_F are the integrated fluorescence intensities and τ_F^0 and τ_F (s) the fluorescence lifetimes in the absence and in the presence of quencher, respectively, k_q is the bimolecular quenching rate constant (L mol^{-1} s^{-1}), [Q] is the quencher concentration (mol L^{-1}), and K_{SV} (= $k_q\tau_F^0$) is the Stern−Volmer constant (L mol^{-1}). Therefore, if I_F^0/I_F versus [Q] and τ_F^0/τ_F versus [Q] are linear and have the same slope a purely dynamic process can be assumed.

Static quenching also results in linear relationship between I_F^0/I_F and [Q]. In this case, K_{SV} is equal to the equilibrium constant for ground-state complex formation. The measurement of τ_F at different quencher concentrations is a reliable method for differentiating between static and dynamic quenching, since in the former case $\tau_F^0/\tau_F = 1$.

If both dynamic and static quenching processes are operating, a quadratic plot is observed for I_F^0/I_F versus [Q]. This behavior is mathematically expressed by eq 2

$$I_F^0/I_F = (1 + K_D[Q])(1 + K_S[Q]) \qquad (2)$$

where K_D and K_S are the K_{SV} values for the dynamic and static quenching, respectively, i.e., K_D is equal to $k_q\tau_F^0$ and K_S is the

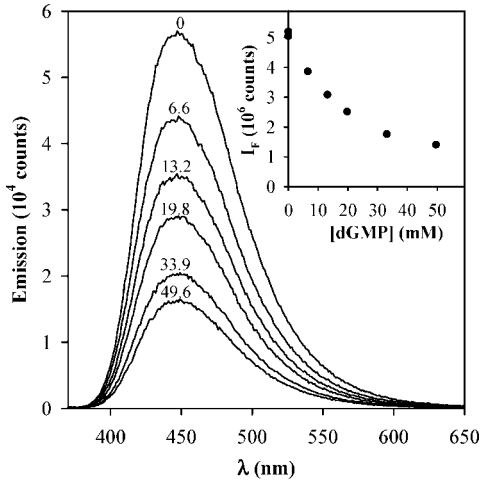

Figure 2. Corrected fluorescence spectra ($\lambda_{exc} = 350$ nm) of a solution of Hmp (2.5×10^{-5} mol L^{-1}, pH = 5.5) in the absence and in the presence of different concentrations of dGMP; the dGMP concentration (mM) appears above each spectrum. Inset: decrease of the total fluorescence intensity (I_F) as a function of the dGMP concentration.

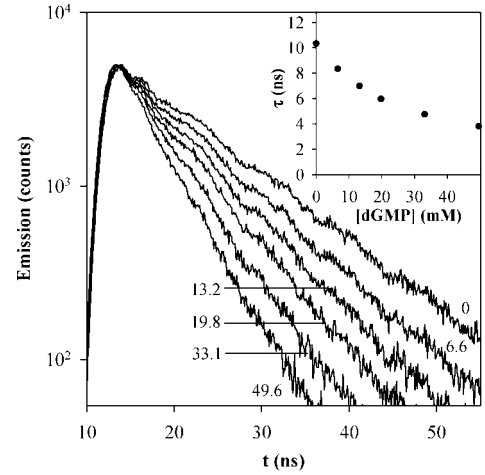

Figure 3. Fluorescence decays of the Hmp emission in aqueous solution (2.5×10^{-5} mol L^{-1}, pH = 5.5) in the absence and in the presence of different concentrations of dGMP ($\lambda_{exc} = 350$ nm, $\lambda_{an} = 450$ nm); the dGMP concentration (mM) is indicated for each trace. Inset: variation of the fluorescence lifetime (τ_F) as a function of the dGMP concentration.

equilibrium constant for complex formation. As τ_F only depends on dynamic quenching, K_D can be obtained from the plot of τ_F^0/τ_F versus [Q] (eq 3).

$$\tau_F^0/\tau_F = 1 + K_D[Q] = 1 + k_q\tau_F^0[Q] \quad (3)$$

Results and Discussion

Fluorescence Quenching in Acidic Media. Emission spectra of the acid forms of Ptr, Hmp, Mep, and Dmp were recorded in the presence and in the absence of the nucleotides dGMP, dAMP, and dCMP. Groups of experiments were carried out on aqueous solutions of a given pterin derivative (2.5×10^{-5} mol L^{-1}) containing different concentrations of nucleotides (0−0.05 mol L^{-1}) at pH 5.5. In all cases, a strong decrease in the fluorescence intensity was observed; the wavelength of the emission maximum remaining unchanged. The spectra obtained for the quenching of the Hmp fluorescence by dGMP are presented in Figure 2, as a typical example among the 12 cases investigated.

In time-resolved experiments first-order kinetics were observed for all fluorescence decays of the acid forms of pterins (pH = 5.5) in the presence of different concentrations of nucleotides. The corresponding fluorescence lifetimes (τ_F) decreased strongly as a function of the nucleotide concentration. Typical traces obtained for the quenching of florescence of Hmp by dGMP are shown in Figure 3.

For all pterin derivatives studied, the decrease of the integrated fluorescence intensity as a function of the concentration of dGMP and dAMP showed nonlinear Stern−Volmer behavior. In all cases, the curves could be fitted with eq 2, suggesting that the quenching observed is a combination of dynamic and static processes (Experimental Methods). The corresponding K_D and K_S values are listed in Table 1. On the other hand, the dependence of τ_F on the concentration of dGMP or dAMP was linear. The values of the corresponding Stern−Volmer constants are also listed in Table 1. The Stern−Volmer analyses of the results obtained in both steady-state and time-resolved experiments for the quenching of the Dmp fluorescence by dGMP and dAMP are shown in Figure 4 as examples.

For a given pterin derivative and a given quencher (dGMP or dAMP), the K_D values obtained in steady-state and time-resolved experiments are equal within experimental error, which is in agreement with the assumption of a combination of dynamic and static quenching processes. All K_D values obtained for both purine nucleotides are similar, showing that dynamic quenching of the singlet excited state of pterin derivatives is influenced neither by the nature of the purine base of the nucleotide, nor by the chemical structure of the substituents linked to the pterin moiety, for the derivatives investigated. Most probably, the electronic changes resulting from these structural modifications are not large enough to affect the interaction between the excited pterin moiety and the purine base in the collision complex. K_S values (Table 1) are also similar for all the pairs analyzed and rather low. Therefore, the ground-state association between pterin and purine nucleotide takes place, but the formation of the corresponding complexes will be significant only at relatively high reactant concentrations.

TABLE 1: Stern−Volmer Constants for Dynamic Quenching (K_D/M^{-1}) and Static Quenching (K_S/M^{-1}) of the Fluorescence of Pterin Derivatives by Nucleotides[a]

			Ptr	Mep	Dmp	Hmp
dGMP	TR	K_D	30 ± 1	42 ± 1	39 ± 1	34 ± 1
	SS	K_D	38 ± 9	46 ± 6	38 ± 9	35 ± 4
		K_S	5 ± 2	9 ± 4	17 ± 6	10 ± 3
dAMP	TR	K_D	23 ± 1	35 ± 1	33 ± 1	31 ± 1
	SS	K_D	23 ± 1	41 ± 1	37 ± 7	31 ± 1
		K_S	13 ± 1	8 ± 1	19 ± 5	17 ± 1
dCMP	TR	K_D	19 ± 1	27 ± 1	25 ± 1	30 ± 1
	SS	K_D	23 ± 1	30 ± 1	24 ± 1	30 ± 1
		K_S	0	0	0	0

[a] Values obtained in time-resolved (TR) and steady-state (SS) experiments; pH = 5.5, $\lambda_{exc} = 350$ nm.

On the other hand, in experiments performed with dCMP as a quencher, for all pterin derivatives studied, I_F^0/I_F as a function of the nucleotide concentration was linear (Figure 4). In time-resolved experiments, first-order kinetics were observed for all fluorescence decays and the dependence of τ_F on the dCMP concentration resulted in linear Stern−Volmer plots. Comparison of the values of K_D calculated from steady-state and time-resolved studies reveals that there is no significant difference between both groups of experimental data (Table 1). These values are slightly lower than those corresponding to dGMP

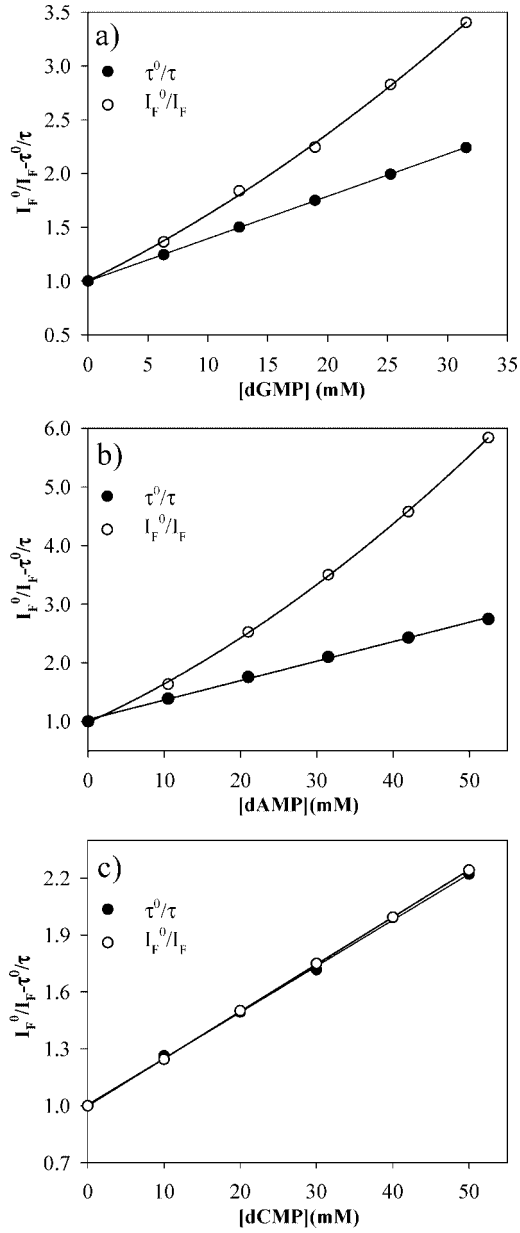

Figure 4. Quenching of the fluorescence of Dmp by nucleotides: (a) dGMP, (b) dAMP, and (c) dCMP. Stern−Volmer plots of the fluorescence intensities (I_F) and the fluorescence lifetimes (τ_F); pH = 5.5, λ_{exc} = 350 nm, λ_{an} = 450 nm.

and dAMP, thus revealing that the quenching of the fluorescence of pterins by dCMP is slightly less efficient than quenching by the purine nucleotides.

Besides, these results show that the quenching of the fluorescence of the pterin derivatives by dCMP is a purely dynamic quenching and no association of dCMP with pterins occurs in the concentration ranges analyzed. It is worth mentioning that the capability of pterins to form complexes with nucleotides strongly depends on the nucleobase chemical nature: under the same experimental conditions, binding of pterins to purine nucleotides was detected, whereas binding to a pyrimidine nucleotide was not. This could be due to the fact that both purines and pterins have bicyclic structures, leading to a stronger electronic interaction than in the case of pyrimidines (monocyclic structure).

The values of the bimolecular quenching rate constant (k_q), calculated from K_D values ($k_q = K_D/\tau_F^0$, see the Experimental Methods), are listed in Table 2. They vary from 1.8×10^9 to 4.4×10^9 L mol^{-1} s^{-1}. For comparative purposes, previous k_q values reported for the fluorescence quenching by phosphate anions[15,21] (1.2×10^9 to 1.6×10^9 L mol^{-1} s^{-1}) were also included in Table 2. The results obtained show that singlet excited states of acid forms of pterins are efficiently deactivated through a dynamic process, which is even faster than the previously studied deactivation by phosphate and other anions.[15]

TABLE 2: Bimolecular Rate Constants ($k_q/10^9$ L mol^{-1} s^{-1}) for the Quenching of the Fluorescence of Pterin Derivatives by Nucleotides (λ_{exc} = 350 nm)[a]

	pH	Ptr	Mep	Dmp	Hmp
dGMP	5.5	4.4	3.3	2.9	3.1
	10.5	1.8	1.3	1.4	1.7
dAMP	5.5	3.0	2.9	2.6	2.8
	10.5	1.8	0.8	0.3	1.3
dCMP	5.5	2.7	2.1	1.8	2.7
	10.5	<0.2	<0.2	<0.2	<0.2
phosphate[b]	5.5	1.6	1.3	1.2	1.3
	10.5	0.18	0.25	0.18	0.21

[a] Results for phosphate as a quencher are given for comparison. [b] Data from refs 15 and 21.

TABLE 3: Stern−Volmer Constants for Dynamic Quenching (K_D/M^{-1}) of the Fluorescence of Pterin Derivatives by Nucleotides[a]

			Ptr	Mep	Dmp	Hmp
dGMP	TR	K_D	9 ± 1	15 ± 1	16 ± 1	14 ± 1
	SS	K_D	9 ± 1	13 ± 1	16 ± 1	15 ± 1
dAMP	TR	K_D	9 ± 1	7 ± 1	3 ± 1	10 ± 1
	SS	K_D	9 ± 1	10 ± 1	4 ± 1	12 ± 1
dCMP	TR	K_D	1.2 ± 0.5	<0.1	1.2 ± 0.8	0.1 ± 0.1
	SS	K_D	1.6 ± 0.3	<0.2	0.7 ± 0.4	0.8 ± 0.1

[a] Values obtained in time-resolved (TR) and steady-state (SS) experiments; pH = 10.5, λ_{exc} = 350 nm.

Fluorescence Quenching in Alkaline Media. Steady-state experiments performed in alkaline media (pH = 10.5), where the basic form of the pterins is predominant, yielded a reduced efficiency of fluorescence quenching by the three studied nucleotides in comparison with that observed for the acid forms under similar experimental conditions. In all sets of steady-state experiments, the wavelength of the emission maxima remained unchanged, and the variation I_F^0/I_F as a function of the nucleotide concentration followed a linear Stern−Volmer behavior. In time-resolved experiments, first-order kinetics were observed for all fluorescence decays of the basic forms, in the presence of the different nucleotides (dGMP, dAMP, and dCMP). In all the 12 cases investigated, the dependence of τ_F on the nucleotide concentration was linear. The values of the corresponding Stern−Volmer constants are listed in Table 3. As examples, the Stern−Volmer analyses of the results obtained in both steady-state and time-resolved experiments for the quenching of the Dmp fluorescence by the three nucleotides are shown in Figure 5.

Comparison of the values of the Stern−Volmer constants calculated from steady-state and time-resolved fluorescence studies reveals that, either for dGMP or for dAMP, there is no significant difference between both groups of experimental data (Table 3). These results show that the quenching of the fluorescence of the pterin derivatives by purine nucleotides is a purely dynamic quenching. In the case of dCMP the quenching was negligible, even at concentrations higher than 80 mM (Figure 5).

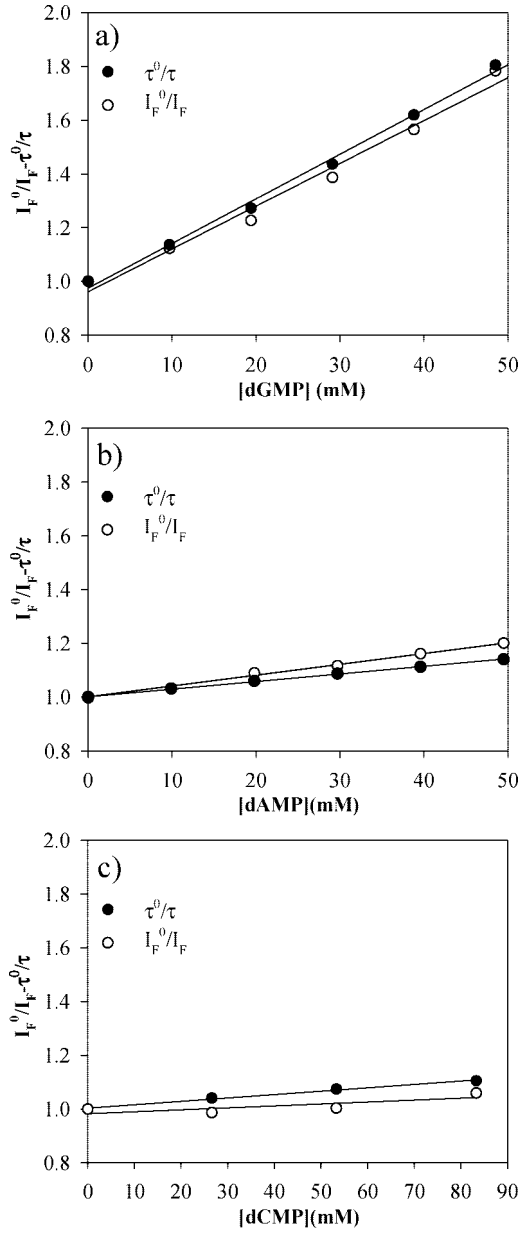

Figure 5. Quenching of the fluorescence of Dmp by nucleotides: (a) dGMP, (b) dAMP, and (c) dCMP. Stern−Volmer plots of the fluorescence intensities (I_F) and the fluorescence lifetimes (τ_F); pH = 10.5, λ_{exc} = 350 nm, λ_{an} = 450 nm.

The values of the corresponding k_q, calculated averaging K_D values obtained from plots of I_F^0/I_F and τ_F^0/τ_F versus nucleotide concentration, are listed in Table 2. These values vary from 0.3×10^8 to 1.8×10^8 L mol^{-1} s^{-1} for fluorescence quenching by dGMP and dAMP. Values of k_q are smaller than 2×10^8 L mol^{-1} s^{-1} for quenching by dCMP, similar to or lower than those observed in the case of fluorescence quenching by phosphate (Table 2).

Comparison of Fluorescence Quenching in Acid and Alkaline Media. Comparison of k_q values obtained from experiments performed under the two different pH conditions shows that the quenching process is much more efficient in acidic than in alkaline media. The different behavior of acid and basic forms of pterins toward quenching by nucleotides may be due to the difference in the charge of the molecules. Acid and alkaline forms of all compounds have charges of 0 and −1, respectively. Coulombic repulsion between alkaline forms of pterins and nucleotides, molecules with negative charges in the phosphate moiety, hinders the interaction necessary to allow the quenching process to proceed. Therefore, this repulsion, which does not exist for acid forms, may prevent the formation of the complexes responsible for the static quenching and diminish the efficiency of the dynamic quenching.

It is worth mentioning that the effect of pH on the dynamic fluorescence quenching by purine nucleotides (dGMP and dAMP) is less pronounced than in the cases of dCMP and phosphate.[15] This fact could be related to the mechanisms involved. A proton-transfer mechanism was proposed for the fluorescence quenching by phosphate and other anions of weak acids, such as acetate.[15] The absence of an acidic hydrogen in the alkaline forms of pterins prevents proton transfer, and the efficiency of the quenching process decreases about 1 order of magnitude (the effect is even higher for acetate). Analysis of several facts suggests that this is not the main mechanism in the case of quenching by nucleotides. Although nucleotides are molecules much larger than phosphate, they are more efficient quenchers than the latter. Fluorescence of the alkaline forms of pterins is quenched by nucleotides with efficiencies comparable to those corresponding to quenching by phosphate in acidic media. Finally, for a given pterin derivative at a given pH, the lower the ionization potential of the nucleobase of the nucleotide,[22] the higher the k_q value. Taking into account these results, a charge-transfer process can be proposed as the main mechanism for the quenching of the singlet excited state of pterins by nucleotides. The free energy change (ΔG_{ET}) for this process, where the pterin derivative (PT) is the electron acceptor and the nucleotide (NMP) the electron donor, can be estimated with eq 4,[23] in the case of strong polar solvents:

$$\Delta G_{ET}\,(\text{eV}) = [E_{(\text{NMP}^{+\bullet}/\text{NMP})} - E_{(\text{PT/PT}^{-\bullet})}] - \Delta E_{0,0} \qquad (4)$$

where $E_{(\text{NMP}^{+\bullet}/\text{NMP})}$ and $E_{(\text{PT/PT}^{-\bullet})}$ are the standard electron potentials of donor and acceptor, respectively. These values have already been reported for the nucleotides[22] and pterins[24,25] used in this work. $\Delta E_{0,0}$ is the energy of the singlet excited state of the pterin derivative and can be estimated from its fluorescence spectrum. The calculated ΔG_{ET} values indicate that electron transfer from nucleotides to the triplet excited state of pterins can spontaneously occur. For example, it was already shown that it is the case for the electron transfer from dAMP to the triplet excited state of Ptr (calculated ΔG_{ET} value of −0.55 eV).[18] The process is even more favorable when the Ptr singlet excited state is involved (ΔG_{ET} of −1.1 eV).

Conclusions

In this work we have shown that the fluorescence of a group of oxidized pterins (Figure 1) in aqueous solutions may be quenched by nucleotides and that this process depends on the pH conditions and on the chemical structure of the quencher.

At pH 5.5, the singlet excited states of acid forms of pterins are deactivated by purine nucleotides (dGMP and dAMP) via a combination of dynamic and static processes. Calculation of Stern−Volmer constants for the dynamic quenching (K_D) showed that the efficiency of the deactivation of singlet excited state is high, independently of the nature of the purine base of the nucleotide and of the chemical structure of the substituents linked to the pterin moiety. Values of Stern−Volmer constants for the static quenching (K_S) indicate that association between pterins and purine nucleotides takes place, but the formation of the corresponding complexes will be significant only at relatively high reactant concentrations. On the other hand, the quenching of the fluorescence of the pterin derivatives by dCMP, a pyrimidine nucleotide, is slightly less efficient than the quench-

ing by purine nucleotides and is purely dynamic, i.e., no association of dCMP with pterins occurs in the concentration range analyzed.

Experiments performed in alkaline media (pH = 10.5), where the basic forms of the pterin derivatives are predominant, showed that the quenching of the pterin fluorescence by purine nucleotides is purely dynamic. In the case of dCMP the quenching was negligible.

Comparison of the k_q values determined under the two different pH conditions showed that the quenching process was much more efficient in acidic than in alkaline media. This difference was attributed to Coulombic repulsion between negatively charged alkaline forms of pterins and nucleotides, diminishing the efficiency of the dynamic quenching and hindering the formation of the ground-state complexes responsible for the static quenching. Nucleotides are more efficient in quenching the fluorescence of pterins than phosphate, and for a given pterin derivative at a given pH, the value of the quenching rate constant k_q increases with the decrease of the ionization potential of the nucleobase of the nucleotide. These facts suggest that the mechanism of quenching of the singlet excited state of pterins by nucleotides most probably involves a charge-transfer process.

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