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Photophysical properties of protoporphyrin IX and thionine covalently attached to macromolecules

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Abstract

Protoporphyrin IX and thionine were covalently linked to the macromolecules poly(acrylic acid) and poly(methylolacrylamide). The absorption and emission properties of the dye molecules bound to the macromolecules were studied. In addition, the fluorescence lifetimes of the bound dyes were measured by time-resolved methods; the decay curves were fitted well to biexponential kinetics, indicating that the chromophore is located in two different environments of the macromolecules. It was inferred from the fluorescence lifetime measurements and emission intensities that the excited state of the porphyrin is quenched by thionine bound to the same macromolecular chain. It is suggested that the quenching process is mediated by the macromolecular random coil which appears to be more efficient when the polymer chain is poly(methylolacrylamide). Stern–Volmer plot analysis of the quenching process suggests that it follows a static mechanism when the polymer chain contains acrylamide groups. © 1997 Elsevier Science S.A.

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1. Introduction

Photoinduced electron or energy transfer processes from donor to acceptor moieties separated by long alkyl chain units are of particular significance in biomimicking systems [1]. Investigations of photoinduced electron transfer reactions aim to determine the factors responsible for enhancing the efficacy of the formation and stability of charge separated species. Many recent studies have focused on the use of organized assemblies, with a view to modifying the chromophores so that the systems mimic effectively biological environments [2–9]. Soluble synthetic high molecular weight polymers create heterogeneous environments for reactions involving donor–acceptor moieties [9]. Numerous attempts have been made to incorporate metal complexes and organic molecules into macromolecular environments to modulate the excited state properties [2–10]. The role of macromolecules, as solid and solution, has been studied by various spectroscopic techniques in order to determine the characteristics of the polymer coil, such as the motion, hydrophobicity, viscosity and other properties in the local environmental domain [2–9]. Photophysical techniques, particularly fluorescence measurements, have an advantage over other methods since very dilute solutions can be used [2]. The luminescent molecule, which functions as a label, is designed in such a way that one or more of its photophysical properties monitors directly the properties of the polymer coil. More recently, concerted efforts have been made to understand the location of the dye molecules in different environments of the polymer coil [9]. It has also been observed that dye molecules located in different environments of the macromolecules show different photophysical properties. However, few studies on the interaction between different dye molecules in the same macromolecular chain have been performed [7]. Hence, in this investigation, we report the preparation and photophysical and photochemical properties of protoporphyrin IX (PPIX) and thionine covalently linked to the macromolecules poly(acrylic acid) (poly(AA)) and poly(methylolacrylamide) (poly(MAAM)) in aqueous medium.

2. Experimental details

2.1. Preparation of samples

PPIX was isolated from natural sources by known methods [11]. Thionine chloride purchased from Fluka was further

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purified by the method of Clark and Eckert [12]. Acrylamide (SD Fine) was purified by recrystallization in hot chloroform. All other solvents and chemicals were of analytical grade and were used as received.

N-Methylolacrylamide (MAAM) was prepared by the reaction of paraformaldehyde with acrylamide according to the procedure of Feuer and Hart [13]. Poly(acrylic acid-co-protoporphyrin IX) (poly(AA-co-PPIX)) was prepared by copolymerizing PPIX (150 mg) with freshly distilled acrylic acid (AA) (30 ml) at 60 °C under a nitrogen atmosphere in dimethylformamide (DMF)–water (2 : 3, v/v) using α,α'-azoisobutyronitrile (AIBN) as initiator. The reaction mixture became more viscous as the polymerization progressed and the absence of monomeric PPIX was confirmed by thin layer chromatography. The completion of the reaction was checked by adding a drop of reaction mixture to methanol which precipitates the polymer. After completion (approximately 24 h), the mixture was slowly poured into excess methanol–acetone (1 : 1, v/v). The polymer was purified by repeated precipitation from water–methanol–acetone. Poly(methylolacrylamide-co-protoporphyrin IX) (poly(MAAM-co-PPIX)) was also prepared by the same procedure using MAAM and PPIX, and purified by repeated precipitation using methanol as a non-solvent.

Thionine was covalently linked to poly(AA-co-PPIX) by the following procedure [14]. Purified thionine was added to an aqueous solution of poly(AA-co-PPIX) in the desired ratio and the mixture was kept at 90–95 °C in a water bath for 6 h. Hydroquinone was added to this mixture to prevent cross-linking. The resulting polymer was precipitated by pouring in a large amount of methanol–acetone (1 : 1, v/v) and purified by repeated precipitation. The uncondensed dye was removed by dialysing the sample (cellulose tubing (Sigma); molecular weight cut-off, approximately 1200 Da) for several days against distilled water. Dialysis was continued until the solution outside the sack showed no absorption for thionine (λ_{max} = 600 nm). Poly(methylolacrylamido-thionine-co-protoporphyrin IX) (poly(TH^+-MAAM-co-PPIX)) was prepared by following the same procedure using poly(MAAM-co-PPIX) and thionine. The resulting polymer was purified by repeated precipitation using methanol as a non-solvent.

2.2. *Quantitative estimation of dyes in the polymer*

The PPIX to thionine ratio in the macromolecular-bound dyes was determined spectrophotometrically assuming that the molar extinction coefficients of PPIX ($\epsilon_{408\ nm}$ = 1.24×10^5 M^{-1} cm^{-1}) and thionine ($\epsilon_{600\ nm}=5.29\times10^4$ M^{-1} cm^{-1}) bound to the polymers are the same as those of the unbound dyes. The ratio of PPIX to MAAM or AA was determined as follows. A polymer–porphyrin solution (5 ml) was evaporated to dryness and the amount of polymer and PPIX present in a given volume was determined. Knowing the amount of PPIX and polymer in a given volume, the number of PPIX units, expressed as the ratio of PPIX to other monomer (MAAM or AA) units, in the polymer chain was calculated.

2.3. *Instrumentation*

The absorption and emission spectra of the samples were obtained using Shimadzu 160 UV–visible and Hitachi 650-40 fluorescence spectrophotometers respectively. The lifetimes of the polymer-bound dyes were determined using a picosecond time-correlated single-photon counting system. The instrumental set-up and the deconvolution method have been described previously [15]. The excitation source was a tunable picosecond rhodamine 6G or pyridine 1 dye laser pulse (pulse width, 6–10 ps; cavity-dumped repetition rate, 800 kHz) derived from the frequency-doubled output (532 nm) of a mode-locked continuous wave (CW) Nd-YAG laser (Spectra Physics). The excitation wavelength used for thionine was either 600 or 610 nm and the emission was monitored at 620 or 640 nm. PPIX was excited using a 380 nm light source and the emission was monitored at around 625 nm. Laser flash photolysis experiments were carried out using an excimer laser source (Applied Photophysics). The excimer laser was operated with Kr–F_2 gas mixtures, which produced a laser pulse with a width of approximately 20 ns and an energy of 100–150 mJ per pulse. The excitation wavelength (400 nm) was derived from a PBBO dye laser (supplied by Oriel Scientific Ltd. and the dye dissolved in spectral grade dioxan (1 mM)) pumped by a KrF laser. Conventional flash photolysis experiments were carried out using an Applied Photophysics KN020 model flash kinetic spectrophotometer. The molecular weight distribution of the macromolecules was determined using a Waters 501 gel permeation chromatograph equipped with a Waters 401 differential refractive index detector. Poly(styrene sulphonic acid) standards in water were used to determine the molecular weight distribution of the polymer-bound dyes.

3. Results and discussion

3.1. *Polymer-bound PPIX and thionine dyes*

PPIX is soluble in highly polar organic solvents, whereas all biological processes occur in aqueous medium. In order to prepare water-soluble PPIX and to create a heterogeneous environment, the vinyl substituent was copolymerized with AA or MAAM. Rapid polymerization was obtained in the case of poly(MAAM-co-PPIX) compared with poly(AA-co-PPIX), because of the electron-withdrawing amide functional group in MAAM [16]. In addition, thionine moieties were covalently attached to the above macromolecules by forming an imide linkage at the thionine $-NH_2$ group. The structures of the polymer-bound dyes are shown in Fig. 1.

The amounts of PPIX and thionine present in poly(MAAM) and poly(AA) are presented in Table 1. The PPIX to thionine ratios determined for poly(TH^+-MAAM-

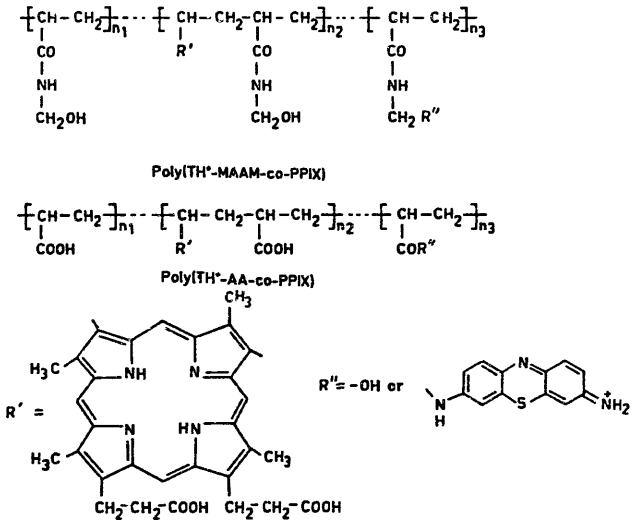

Fig. 1. Structures of polymer-bound dyes.

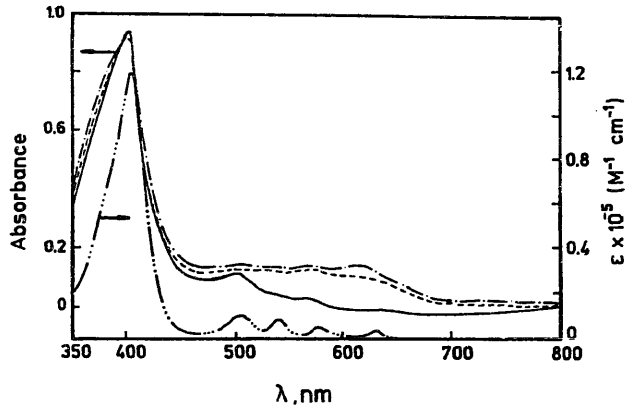

Fig. 2. Absorption spectra of PPIX in DMF (– · · · · –) and of poly(MAAM)-bound dyes in aqueous solution (———, poly(MAAM-co-PPIX); – – –, – · –, poly(TH⁺-MAAM-co-PPIX)).

co-PPIX) with different thionine contents were found to be 13 : 4 and 23 : 6 and for poly(TH⁺-AA-co-PPIX) the values were 10 : 11 and 10 : 13. The average molecular weights determined by gel permeation chromatography (GPC) were 250 kDa and 150 kDa for dye-appended poly(AA) and poly(MAAM) respectively. The molecular weights determined for all the polymers investigated were found to be above 100 kDa and the dyes (PPIX and thionine) were present at very low concentrations in the polymer chain. As the dye concentration is three orders of magnitude less than that of the polymer backbone monomers (Table 1), the interaction between the dyes in dilute solution to form aggregates is negligible, as shown by the absorption spectral studies (see below).

3.2. *Absorption spectral properties of macromolecular-bound PPIX and thionine dyes*

The absorption spectrum of monomeric PPIX in a dilute solution of DMF (Fig. 2) shows four absorption bands in the visible region at 503, 536, 576 and 633 nm (Q bands) and an intense band in the near-UV region at 400 nm (B or Soret band) as reported previously [17]. The absorption spectra of PPIX bound to the macromolecules poly(MAAM) and poly(AA) in aqueous solution are depicted in Figs. 2 and 3 respectively. PPIX in polymer samples (poly(MAAM-co-PPIX) and poly(AA-co-PPIX)) shows the characteristic intense Soret band at 400 nm and Q bands at 504, 536, 568 and 638 nm. As the polymer backbone does not absorb in the visible region, the electronic transitions observed are due to monomeric PPIX. The absence of additional bands reveals that no particular influence is exerted by the macromolecular environment on the electronic structure of PPIX. It is known [18] that PPIX at a concentration above 0.1 mM tends to exist in an aggregated form in aqueous medium, which shifts the absorption bands towards lower energy and splits the Soret band. We did not observe a red shift or split in the Soret band in the absorption spectra of macromolecular-bound PPIX, implying the absence of the aggregation of PPIX monomers.

The absorption spectra of poly(TH⁺-MAAM-co-PPIX) and poly(TH⁺-AA-co-PPIX) in aqueous solution show an additional intense peak at 610 nm (Figs. 2 and 3), as well as the Soret and Q band transitions due to PPIX, which is characteristic of the thionine moiety. Thionine in aqueous solution shows a sharp intense peak at 600 nm (α band) attributed to the monomeric form and a shoulder at 560 nm (β band) arising from higher aggregates (Fig. 3) [19]. However, in the case of polymer-bound thionines (poly(TH⁺-MAAM-co-PPIX) and poly(TH⁺-AA-co-PPIX)), a red shift of 10

Table 1
Polymer-bound PPIX and thionine dyes

Sample	Average molecular weight (kDa)	Dye content [a]		PPIX to TH⁺ ratio
		PPIX (m/d)	Thionine (m/d)	
Poly(MAAM-co-PPIX)	150	3237 ± 100	–	–
Poly(AA-co-PPIX)	250	8396 ± 150	–	–
Poly(TH⁺-MAAM-co-PPIX)	150	3237 ± 100	8756 ± 50	13 : 4
Poly(TH⁺-MAAM-co-PPIX)	150	3237 ± 100	9346 ± 50	23 : 6
Poly(TH⁺-AA-co-PPIX)	250	8396 ± 150	7633 ± 50	10 : 11
Poly(TH⁺-AA-co-PPIX)	250	8396 ± 150	6459 ± 50	10 : 13

[a] m/d, monomer/dye

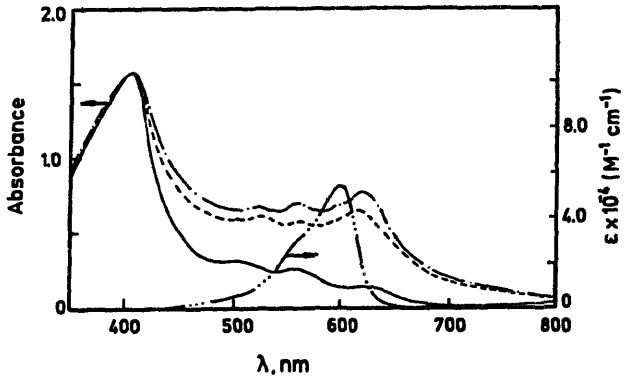

Fig. 3. Absorption spectra of thionine (– · · · · –) and poly(AA)-bound dyes (———, poly(AA-co-PPIX); – – –, – · –, poly(TH⁺-AA-co-PPIX)) in aqueous solution.

nm is observed, which is attributed to the linking of the high molecular weight polymer chain to the dye centre through the electron-releasing $-CH_2-$ group [5–9,20]. As the loading of thionine in the macromolecule increases, the intensity of the 610 nm band also increases. These observations suggest that PPIX and thionine do not interact appreciably in the ground state as there are no additional bands or changes in energy or intensity of the absorption bands corresponding to the dyes in the polymeric samples.

3.3. Emission spectral properties of macromolecular-bound PPIX

The emission spectrum of monomeric PPIX in chloroform on excitation in the Soret band ($\lambda_{ex}=400$ nm) is shown in Fig. 4. The Soret band is chosen for the excitation of PPIX instead of the Q band in emission spectral studies because macromolecular-bound PPIX and thionine have an absorption overlap in the 600 nm region. At 400 nm, the molar extinction coefficients of PPIX and thionine are found to be $\epsilon^{400}_{PPIX}=1.24\times10^5\ M^{-1}\ cm^{-1}$ and $\epsilon^{400}_{Thionine}<100\ M^{-1}\ cm^{-1}$ in DMF and water respectively, which indicates that the absorption of thionine is less than 1% at 400 nm compared with that of PPIX. Therefore the emission spectrum of PPIX can be investigated in macromolecules on excitation with 400 nm light avoiding any interference from the excited state of thionine. The maxima observed for monomeric PPIX at 618 and 676 nm are assigned to the π–π^* emission [21].

The emission spectra of PPIX bound to poly(MAAM) and poly(AA) in aqueous solution are shown in Figs. 4 and 5 respectively. Poly(MAAM-co-PPIX) and poly(AA-co-PPIX) show emission spectra similar to that of monomeric PPIX in chloroform with bands at 585 (sh), 627 and 676 nm. In both cases, the peaks are red shifted by 5–10 nm with the appearance of a shoulder at approximately 580 nm. The observed red shift is attributed to the stabilization of the π^* orbital by the polar solvent water, whereas the shoulder at 580 nm is suggested to be a Stokes shift with respect to the Q band. In the case of poly(TH⁺-MAAM-co-PPIX) and poly(TH⁺-AA-co-PPIX), the emission spectra of PPIX are similar to those observed for poly(MAAM-co-PPIX) and

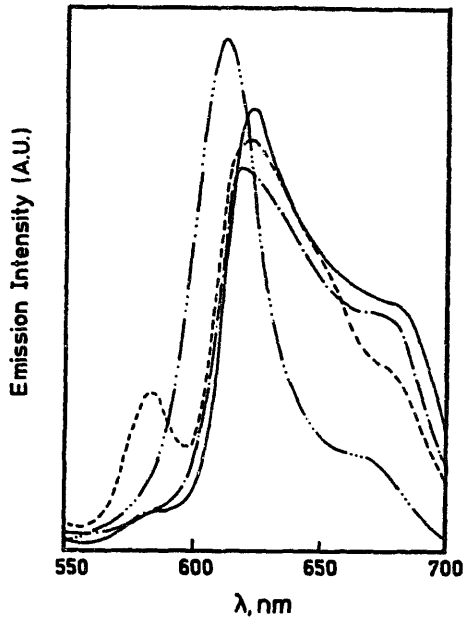

Fig. 4. Emission spectra of PPIX in chloroform (– · · · · –) and of poly(MAAM)-bound dyes in aqueous solution (———, poly(MAAM-co-PPIX); – – –, – · –, poly(TH⁺-MAAM-co-PPIX)); $\lambda_{ex}=400$ nm.

poly(AA-co-PPIX) respectively. However, the fluorescence intensities of the former pair are strongly quenched (by a factor of four) under identical excitation conditions. The observed quenching either involves an energy or electron transfer process by the thionine moieties present in the same macromolecular chain (see below).

From these observations, it is inferred that the dyes PPIX and thionine attached to macromolecules do not show any ground state interaction, and the emission intensity of PPIX decreases when PPIX and thionine are present (covalent linkage) in the same macromolecular chain. As the emission

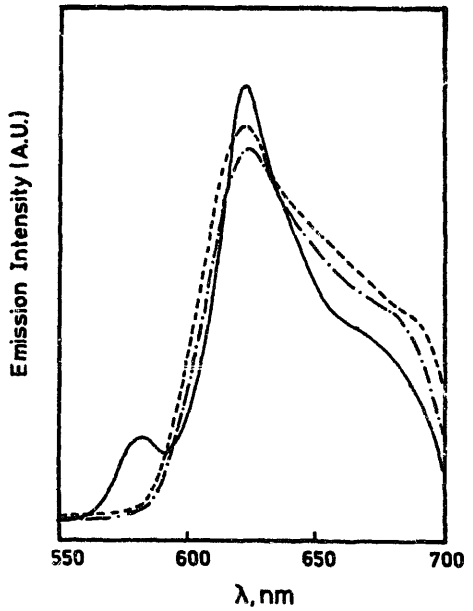

Fig. 5. Emission spectra of poly(AA)-bound dyes in aqueous solution; ———, poly(AA-co-PPIX); – – –, – · –, poly(TH⁺-AA-co-PPIX); $\lambda_{ex}=400$ nm.

bands of PPIX and thionine overlap in the 620–650 nm region, emission spectral studies cannot provide unambiguous information regarding the interaction between the excited states of PPIX and thionine. However, further insight into the nature of the interaction can be obtained by fluorescence lifetime experiments as detailed below.

3.4. Fluorescence lifetimes of macromolecular-bound PPIX

The fluorescence lifetime and dynamics of monomeric PPIX in tetrahydrofuran (THF) have been studied in detail [22], showing a singlet excited state with a lifetime of approximately 12 ns with a rotational correlation time of about 0.17 ns. The fluorescence decay of the polymer samples poly(MAAM-co-PPIX) and poly(AA-co-PPIX) in aqueous solution does not obey a single exponential fit, but a biexponential fit according to Eq. (1)

$$I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \quad (1)$$

where A_1 and A_2 are the pre-exponential factors and τ_1 and τ_2 are the lifetime components of the decay. The biexponential fit for the emission profile of poly(AA-co-PPIX) obtained on excitation of the Soret band (monitored at 627 nm) is shown in Fig. 6. The goodness of fit for the decay process is represented as the weighted residual (top). Acceptable fits

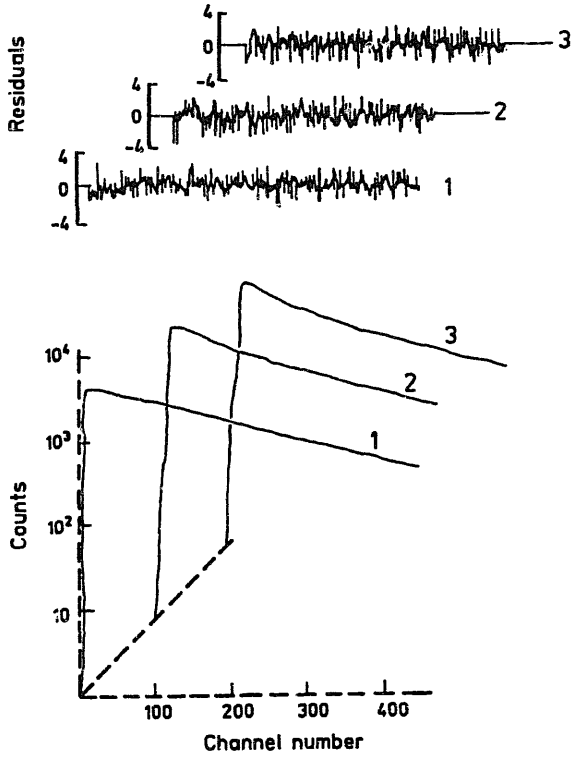

Fig. 6. Biexponential kinetic fit of the emission profiles of PPIX in DMF (1) and poly(AA)-bound PPIX (2) and poly(AA)-bound PPIX and thionine (3) in aqueous solution (pH 7), measured at a resolution of 0.076 ns per channel. $\lambda_{ex} = 378$ nm; $\lambda_{em} \approx 625$ nm. The weighted residuals of the corresponding curves are given at the top.

Table 2
Lifetime[a] (ns) of PPIX bound to macromolecules in aqueous solution; $\lambda_{ex} = 378$ nm and $\lambda_{em} = 627$ nm and 624 nm for samples 1 and 2 respectively

pH	τ_1	A_1	τ_2	A_2	$\langle\tau\rangle$[b]	χ^2
Sample 1[c]						
3	2.62	81.6	11.84	18.4	3.93	1.13
5	2.65	65.1	13.63	34.9	6.49	1.07
7	2.54	45.5	13.56	54.5	8.54	0.90
9	2.43	46.2	13.69	53.8	8.48	1.02
11	2.57	46.8	13.80	53.2	8.54	1.11
Sample 2[d]						
3	2.81	55.2	13.18	44.8	7.45	1.09
5	2.99	48.9	14.29	51.1	8.76	1.04
7	2.88	36.4	13.49	63.6	9.63	1.00
9	3.45	39.7	13.87	60.3	9.73	1.00
11	3.21	38.3	13.63	61.7	9.64	1.09

[a]Lifetime deviations are within 0.04 ns.
[b]$\langle\tau\rangle = (A_1\tau_1 + A_2\tau_2)$.
[c]Poly(MAAM-co-PPIX).
[d]Poly(AA-co-PPIX).

of the fluorescence decay are obtained in all cases ($0.9 < \chi^2 < 1.2$) and the residuals are very close to zero.

The fluorescence lifetimes (τ), pre-exponential factors (A) and average lifetimes ($\langle\tau\rangle$) of PPIX in poly(MAAM-co-PPIX) and poly(AA-co-PPIX) are summarized in Table 2. The observed biexponential fluorescence decay (Fig. 6) shows one lifetime (τ_2) approximately the same as that in THF for monomeric PPIX and the other is significantly shorter (τ_1). Biexponential fluorescence decay is observed when chromophores undergo sufficient self-quenching processes or excimer formation [23]. According to the self-quenching mechanism, the quenching process leads to a short-lived lifetime component and the unquenched dye yields a long-lived component. In the present case, PPIX moieties are randomly distributed in the macromolecules, with a high monomer to dye ratio; the aggregation of PPIX is negligible (see above) which rules out the possibility of self-quenching. Furthermore, excimer formation in macromolecular-bound PPIX is unlikely because a characteristic structureless, broad and red-shifted fluorescence is not observed in the emission spectrum. Neither self-quenching nor excimer formation is responsible for the observed biexponential decay of polymer-bound PPIX and hence the polymer conformation seems to be important.

The biexponential decay observed in the present systems may be correlated with the heterogeneous behaviour of the polymer chain. When copolymers consisting of a hydrophobic chromophore and hydrophilic environment are allowed to self-assemble in an aqueous medium, the hydrophobic chromophores aggregate to form a micellar core while the hydrophilic environment extends into the solution to form the shell [24,25]. The existence of such a hydrophobic environment in water-soluble polymers has been reported very recently in phenothiazine derivatives bound to macromolecules [9]. Accordingly, the two lifetimes of PPIX are attributable to PPIX residing in two environments, i.e. PPIX buried

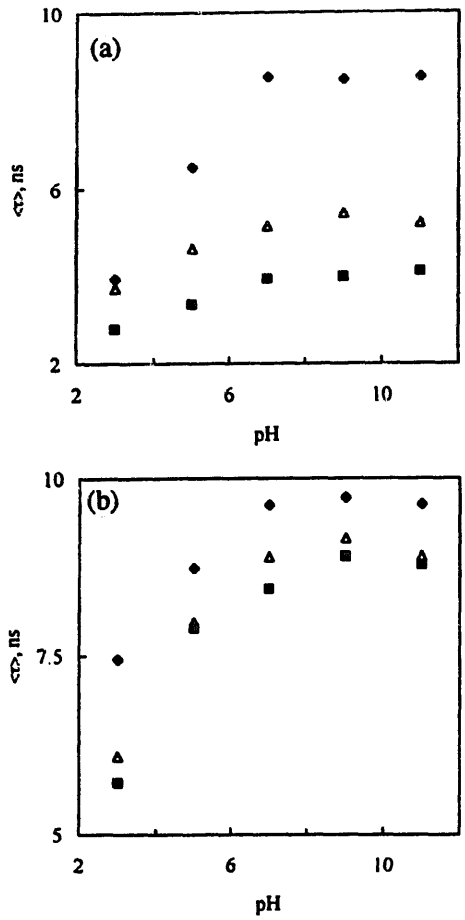

Fig. 7. Plot of the average lifetime $\langle\tau\rangle$ vs. pH for poly(MAAM) (a) and poly(AA) (b) in aqueous solution (◆, PPIX; △, ■, PPIX and thionine).

inside the polymer coil (hydrophobic environment) and PPIX exposed to the aqueous phase (hydrophilic environment). The lifetime of 11–13 ns results from the excited state of PPIX in the hydrophobic environment (as in THF) and the short-lived component (1–3 ns) results from the excited state of PPIX in the hydrophilic environment. Similar results have been reported [9,25,26] with many other chromophores, such as phenothiazine derivatives, provoflavin, acridine orange, rhodamine B and $[Ru(bpy)_3]^{2+}$ ion in different microheterogeneous media. Biexponential fluorescence decay behaviour has also been reported for $[Ru(phen)_3]^{2+}$ ion incorporated into DNA samples [27]. Thus it is concluded that the fluorophore PPIX in polymer samples, poly(MAAM-co-PPIX) and poly(AA-co-PPIX), in aqueous medium exists in hydrophilic and hydrophobic environments resulting in a biexponential decay.

PPIX-bound macromolecules in the present case are soluble in water, which enables the lifetimes of PPIX to be determined at different pH values. A very interesting aspect shown by the lifetime data is that both lifetimes depend on the pH of the solution (Fig. 7). In copolymers, hydrophobically associating chromophores in aqueous solution will form a hydrophobic environment, which allows a certain amount of water molecules to be present within this environment [4]. Hence the change in pH of the solution affects both lifetimes. A plot of the pH vs. $\langle\tau\rangle$ for the polymer samples in aqueous solution is shown in Fig. 7, which indicates that the lifetimes of PPIX in both polymers increase with the pH. This variation can be attributed to conformational changes in the polymer coil or to the proton equilibria of PPIX. As can be seen from Fig. 7, the inflection observed at pH ≅ 4.0 may be due to the deprotonation at the pyrrole ring, which enhances the lifetime of the excited state. Indeed, the protonation equilibria of the first nitrogen in the pyrrole ring, with similar lifetime changes, were reported by Brault and Vever-Bizet [28] for haematoporphyrin (a structural analogue of PPIX) in phosphate buffer.

3.5. Fluorescence lifetimes of macromolecular-bound PPIX: effect of thionine attached to the macromolecules

The emission decay curves of PPIX excited states in poly(TH^+-MAAM-co-PPIX) and poly(TH^+-AA-co-PPIX) in aqueous solution exhibit a biexponential fit (Fig. 6) according to Eq. (1) as in the case of PPIX-bound macromolecules (without thionine). The fluorescence lifetimes (τ), pre-exponential factors (A) and average lifetimes ($\langle\tau\rangle$) of the PPIX singlet state are given in Table 3 and Table 4. The lifetimes of PPIX in poly(TH^+-MAAM-co-PPIX) and poly(TH^+-AA-co-PPIX) are found to be markedly decreased compared with those in poly(MAAM-co-PPIX) and poly(AA-co-PPIX). The decrease in the lifetime is due to the quenching of the singlet excited state of PPIX by the thionine moieties present in the macromolecules. The energy of the singlet excited state of thionine at 1.9 eV is lower than that of the singlet excited state of PPIX (approximately 2 eV). Therefore PPIX* → TH^+ energy transfer will be favourable. If the singlet excited state of thionine forms by an energy transfer process, its fluorescence decay is expected to give a lifetime of approximately 0.3 ns (see below). However, the

Table 3
Lifetime[a] (ns) of PPIX bound to poly(TH^+-MAAM-co-PPIX) in aqueous solution; $\lambda_{ex} = 378$ nm and $\lambda_{em} = 627$ nm

pH	τ_1	A_1	τ_2	A_2	$\langle\tau\rangle^b$	χ^2
Sample 1[c]						
3	1.95	89.0	9.56	11.0	2.79	1.12
5	1.84	84.1	11.30	15.9	3.35	1.24
7	1.77	78.5	11.82	21.5	3.93	1.18
9	1.72	78.1	12.04	21.9	3.98	1.21
11	2.01	78.5	11.76	21.5	4.10	1.40
Sample 2						
3	2.14	83.3	11.53	16.7	3.71	1.21
5	2.45	80.8	13.74	19.2	4.62	1.19
7	2.45	74.1	12.81	25.9	5.14	1.21
9	2.62	73.4	13.17	26.6	5.43	1.24
11	2.48	73.9	12.97	26.1	5.21	1.18

[a]Lifetime deviations are within 0.04 ns.
[b]$\langle\tau\rangle = (A_1\tau_1 + A_2\tau_2)$.
[c]PPIX : TH^+ = 13 : 4.
[d]PPIX : TH^+ = 23 : 6.

Table 4
Lifetime[a] (ns) of PPIX bound to poly(TH$^+$-AA-co-PPIX) in aqueous solution; λ_{ex} = 378 nm and λ_{em} = 624 nm

pH	τ_1	A_1	τ_2	A_2	$\langle\tau\rangle^b$	χ^2
Sample 1[c]						
3	2.48	60.9	11.71	39.1	6.09	1.25
5	2.43	49.3	13.39	50.7	7.98	1.01
7	3.69	50.4	14.20	49.6	8.91	0.97
9	3.58	44.2	13.60	55.8	9.17	0.96
11	3.06	41.2	13.03	58.8	8.92	0.95
Sample 2[d]						
3	2.42	64.6	11.74	35.4	5.72	1.24
5	2.64	51.5	13.48	48.5	7.89	0.97
7	2.98	46.8	13.28	53.2	8.46	1.05
9	3.52	46.1	13.54	53.9	8.92	1.06
11	3.25	43.9	13.15	56.1	8.80	0.97

[a]Lifetime deviations are within 0.04 ns.
[b]$\langle\tau\rangle = (A_1\tau_1 + A_2\tau_2)$.
[c]PPIX : TH$^+$ = 10 : 11.
[d]PPIX : TH$^+$ = 10 : 13.

lifetime data (Table 3 and Table 4) do not show any short-lived component of less than 1 ns, which suggests that energy transfer from PPIX* to thionine does not take place. Therefore the quenching process is suggested to involve oxidative quenching of PPIX by the thionine moiety. Indeed, such an observation has been reported by Jones et al. [29] for macromolecular-bound metal complexes. However, in homogeneous solution, it is not possible to observe the quenching of the PPIX excited state by thionine, since the maximum concentration of thionine in saturated solution does not exceed 10^{-5} M, and even diffusion-controlled quenching would not take place because of the shorter excited state lifetime of PPIX.

In the case of poly(MAAM-co-PPIX) and its thionine analogue poly(TH$^+$-MAAM-co-PPIX), the quenching of the fluorescent state of PPIX differs to an appreciable extent from that in AA-based polymers. The presence of acrylamide influences the quenching process, as reported in the quenching of tryptophan excited states in the enzyme ferredoxin:NADP$^+$ oxidoreductase [30]. In order to investigate the quenching mechanism, a Stern–Volmer plot was constructed by plotting $\langle\tau\rangle/\langle\tau\rangle_0$ of macromolecular-bound PPIX vs. the concentration of thionine present in the same macromolecular chain (Fig. 8). In the case of poly(AA) macromolecules, the Stern–Volmer plot shows a straight line, indicating that the quenching of the PPIX singlet state in poly(TH$^+$-AA-co-PPIX) by thionine is a "dynamic" process. However, the quenching process in poly(MAAM) shows an upward curvature. This is explained in terms of the increased local concentration of thionine around the hydrophobic environment of PPIX, resulting in a hydrophobic interaction. A similar observation has been made by Stramel et al. [31] for the hydrophobic interaction between a quencher and an aromatic chromophore leading to the formation of a weak complex. Varadaraj et al. [32] have examined a variety of acrylamide-*N*-alkylacrylamide copolymer

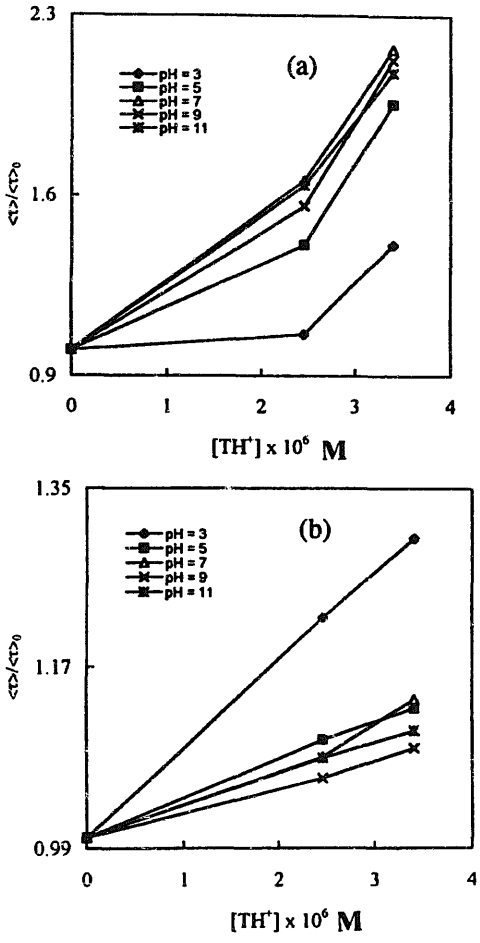

Fig. 8. Stern–Volmer plot of the average lifetimes of macromolecular-bound PPIX ($\langle\tau\rangle_0$) and macromolecular-bound PPIX and thionine ($\langle\tau\rangle$). aA and b represent poly(MAAM) and poly(AA) respectively.

systems and shown that these polymers provide hydrophobic environments for the solubilization of water-insoluble hydrophobic dyes. By analysing the Stern–Volmer plots individually (Fig. 9), the long-lived components show a strong upward curvature, although a smaller number of quenchers are available in the hydrophobic environment. From these observations, it is suggested that the quenching process in poly(TH$^+$-MAAM-co-PPIX) is efficiently mediated by the solvent and acrylamide of the polymer chain by a "static" process.

3.6. Emission spectral and lifetime studies of thionine in poly(TH$^+$-MAAM-co-PPIX)

The steady state emission spectral properties of thionine bound to poly(TH$^+$-MAAM-co-PPIX) were investigated in aqueous solution by exciting thionine at 610 nm. The emission spectra of polymer-bound and monomeric thionine are shown in Fig. 10. The emission spectral maximum of thionine in the polymer occurs around 640 nm. Indeed, the emission maxima of thionine and macromolecular-bound thionine have been reported at 620 nm and around 640 nm respectively [5–9,33]. Hence the presence of PPIX in the same macro-

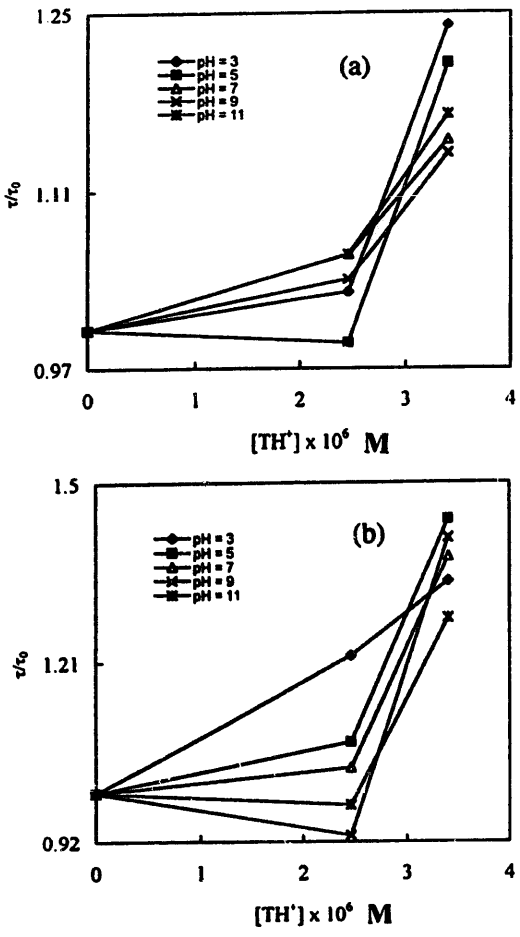

Fig. 9. Stern–Volmer plot of the long-lived (a) and short-lived (b) lifetimes of the dyes bound to poly(MAAM).

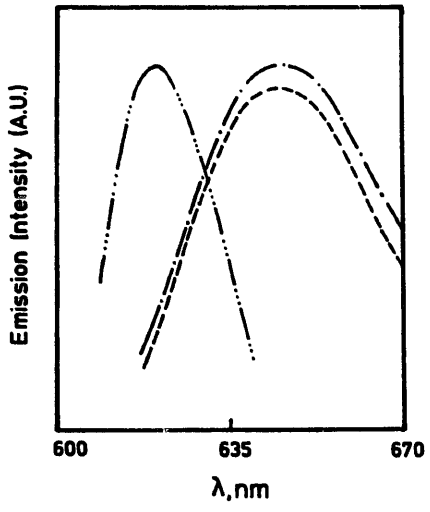

Fig. 10. Emission spectra of monomeric thionine (— · · · —) (λ_{ex}=600 nm) and poly(TH$^+$-MAAM-co-PPIX) (– – – and — · —) (λ_{ex}=610 nm) in aqueous solution.

molecule apparently does not influence the emission spectrum of thionine.

The fluorescence decay of monomeric thionine in water at 620 nm follows a single exponential with a lifetime of 0.302 ns [34]. The emission decay curves of thionine present in

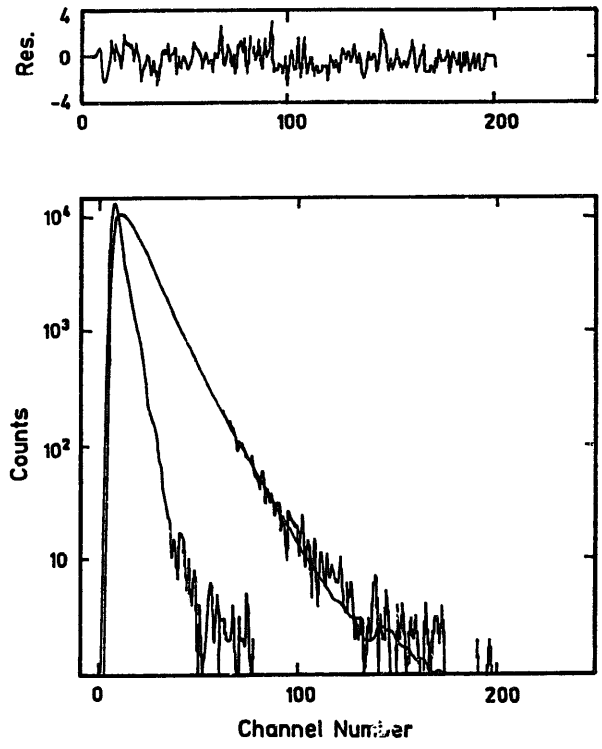

Fig. 11. A typical oscilloscope diagram and calculated biexponential fit, together with instrumental response, for poly(TH$^+$-MAAM-co-PPIX) in aqueous solution (measured at a resolution of 0.038 ns); λ_{ex}=610 nm; λ_{em}=645 nm. Residuals of the decay profiles are presented at the top.

poly(TH$^+$-MAAM-co-PPIX) in aqueous solution follow a biexponential fit according to Eq. (1), as in the case of macromolecular-bound PPIX. A typical oscilloscope decay curve for polymer-bound thionine, together with the instrumental response and biexponential kinetics, is shown in Fig. 11. The fluorescence lifetimes of monomeric thionine and polymer-bound thionine are listed in Table 5. Two different lifetimes are observed for polymer-bound thionine, i.e. one similar to that observed for monomeric thionine (short-lived component) and a longer one. The lifetimes observed are consistent with an earlier report [9] for polymer-bound thionines, which showed that the short-lived component is due to the dye molecule present in a predominantly aqueous environment similar to monomeric thionine, whereas the long-lived component can be attributed to thionine buried inside the macromolecular coil. In contrast with the earlier report (without PPIX), the present investigation shows a 10% increase in the amplitude of the long-lived component, indicating that the macromolecule tends to be more coiled in the presence of the hydrophobic PPIX group. A slight decrease observed in the lifetime of the long-lived component suggests that there is a hydrophobic interaction between PPIX and the thionine moiety.

3.7. Flash photolysis studies of macromolecular-bound dyes

Nanosecond flash photolysis studies of poly(AA-co-PPIX), poly(MAAM-co-PPIX) and poly(TH$^+$-MAAM-co-

Table 5
Lifetimes[a] (ns) of monomeric thionine and polymer-bound thionine in aqueous solution; $\lambda_{ex}=610$ nm and $\lambda_{em}=640$ nm

Sample	PPIX : thionine	τ_1	A_1	τ_2	A_2	$\langle\tau\rangle^b$	χ^2
Monomeric thionine	–	0.302	100	–	–	–	1.11
Poly(TH^+-MAAM-co-PPIX)	13 : 4	0.305	82.5	0.663	17.5	0.368	1.07
Poly(TH^+-MAAM-co-PPIX)	23 : 6	0.319	84.6	0.629	15.4	0.367	1.18

[a]Lifetime deviations are within 0.02 ns.
[b]$\langle\tau\rangle=(A_1\tau_1+A_2\tau_2)$.

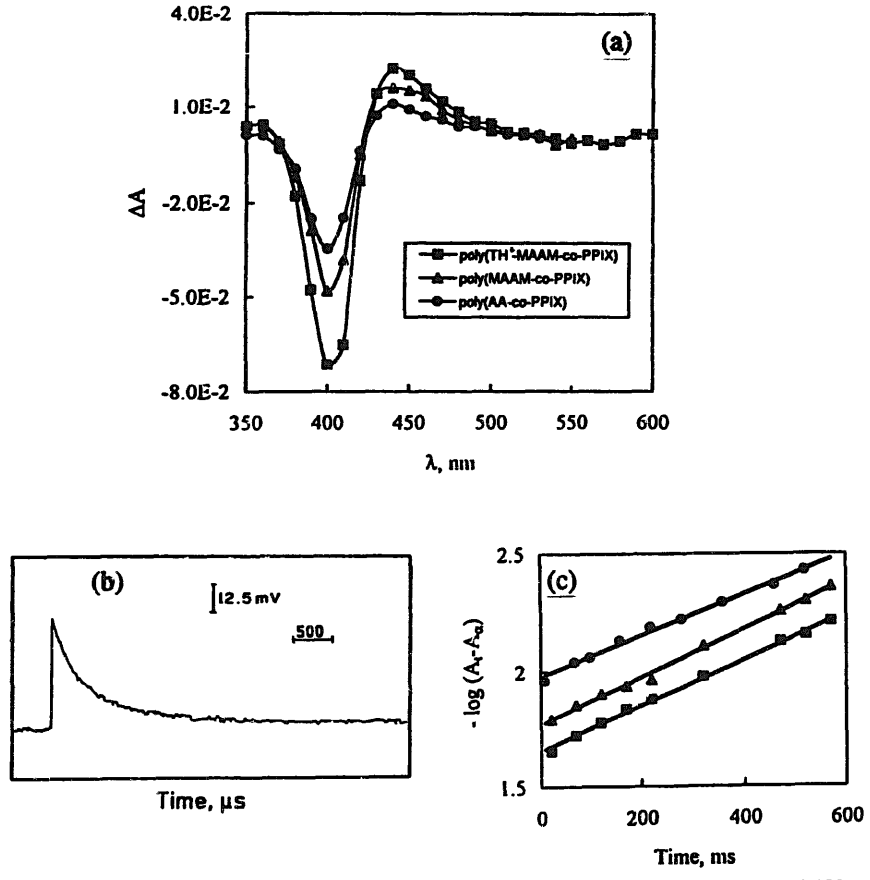

Fig. 12. Triplet–triplet absorption spectra of macromolecular-bound PPIX in aqueous solution: a, absorbance recorded 100 μs after the flash; b, transient absorption decay monitored at 450 nm following laser flash excitation of poly(MAAM-co-PPIX); c, kinetic analysis of the transient decay curves.

Table 6
First-order decay rate constant (k) for triplet state of PPIX

Sample	PPIX : thionine	$k\times10^{-3}\ s^{-1}$
Poly(AA-co-PPIX)	–	1.45
Poly(MAAM-co-PPIX)	–	1.22
Poly(TH^+-MAAM-co-PPIX)	13 : 4	2.20
Poly(TH^+-MAAM-co-PPIX)	23 : 6	2.04

PPIX) were carried out with 400 nm light in deaerated aqueous solution, and the nature of the transient produced is shown in Fig. 12. The first-order decay constants are given in Table 6. The transient absorption spectra observed on flash photolysis of poly(AA-co-PPIX) and poly(MAAM-co-PPIX) show absorption maxima around 450 nm, similar to the well-known triplet–triplet (T–T) absorption spectrum of PPIX [35]. The first-order decay rate constant ($k\cong1.3\times10^3\ s^{-1}$) obtained for the decay of the transient is in agreement with the reported decay constant of triplet PPIX [36]. The similarity of the transient spectra and the comparable rate constant for polymer-bound PPIX and monomeric PPIX indicate that the excited state electronic and thermodynamic properties of macromolecular-bound PPIX are unaltered. Laser excitation of poly(TH^+-MAAM-co-PPIX) results in a T–T absorption spectrum with a rate constant of decay of $k\cong2.0\times10^3\ s^{-1}$. The decay rate constant shows a marginal increase from that observed for poly(MAAM-co-PPIX). However, the energy transfer process $PPIX^*(T_1)\rightarrow TH^+(T_1)$ cannot account for this because the triplet state energy of PPIX (150 kJ mol^{-1}) [36] is lower than that of thionine (163.4 kJ mol^{-1}). Therefore the faster deactivation process may be suggested to be due to the interaction of the

thionine moiety in the hydrophobic environment and/or to singlet excited state electron transfer from PPIX to thionine (see above).

Conventional flash photolysis studies of poly(TH^+-MAAM-co-PPIX) were carried out in aqueous solution; the light for excitation was passed through a thionine filter to eliminate any absorption by thionine in the polymer in order to investigate the formation of a long-lived porphyrin cation and/or the formation of semithionine due to the photochemical reaction between the singlet excited state of PPIX and thionine. No characteristic transient due to the porphyrin cation or semithionine radical was observed. The absence of this intermediate suggests that back electron transfer between reduced thionine and oxidized PPIX in the macromolecules takes place in the solvent cage or in the contact pair to yield the initial dyes within the duration of the laser pulse (approximately 20 ns).

4. Conclusions

The well-known light-absorbing dyes PPIX and thionine were covalently attached to the macromolecules poly(MAAM) and poly(AA). The absorption and emission properties of the polymer–dye systems do not differ significantly in their ground states relative to the unbound dyes. The average molecular weight and electronic spectral properties of the polymer-bound dyes suggest a random distribution of dye molecules without aggregation. The polymer-bound dyes PPIX and thionine exhibit a biexponential fluorescence decay, which is consistent with the existence of a microheterogeneous environment. The fluorescence lifetime measurements and emission intensities of poly(TH^+-MAAM-co-PPIX) and poly(TH^+-AA-co-PPIX) suggest that the excited state of PPIX is quenched by thionine bound to the same macromolecular chain. Stern–Volmer plots of the quenching processes demonstrate that the quenching is efficient and proceeds via a "static" process when the dyes are linked to poly(MAAM). Conventional flash photolysis and cyclic voltammetric studies of macromolecular-bound thionine in poly(TH^+-MAAM-co-PPIX) confirm the presence of an interaction between PPIX and thionine [37]. However, nanosecond and conventional flash photolysis measurements suggest that back electron transfer in the charge separated species is faster than approximately 20 ns.

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