

Amphiphilic Copolymers Consisting of Vinylphenanthrene and Cationic Segments. Fluorescence Quenching by Amphiphilic Quenchers in Aqueous Media

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ABSTRACT: Amphiphilic random copolymers were prepared by radical copolymerization of 9-vinylphenanthrene (VPh) and *N*-(*N*',*N*'-dimethylaminopropyl)methacrylamide followed by quaternization with dimethyl sulfate. The related monomer model compound was also synthesized by quaternization of *N*-(*N*',*N*'-dimethylaminoethyl)-3-phenanthrylacetamide with dimethyl sulfate. The copolymers with VPh mole fraction (f_{Ph}) up to about 0.47 were soluble in water. The fluorescence quenching of the copolymers in aqueous solution was compared with that of the model compound by using amphiphilic quenchers, *N*-methyl-*N*-(2-hydroxyethyl)aniline (MHEA) and *N,N*-di(2-hydroxyethyl)aniline (DHEA), and an anionic quencher, fumaric acid (FA). Hydrophobic association of MHEA and DHEA with the copolymers led to far more effective fluorescence quenching than the monomer model system. Electrostatic binding of FA with the copolymer also resulted in severe quenching of the fluorescence, while it was shown that a single charge on the monomer model is not sufficient to bind FA effectively to enhance the fluorescence quenching.

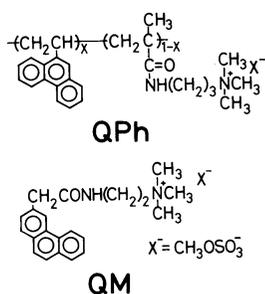
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A prerequisite for the efficient storage of light energy is prevention of the photogenerated ionic intermediates from subsequent rapid thermal back reaction which preferably occurs in homogeneous solutions. Considerable attention has been drawn to microscopically heterogeneous systems such as micelles,¹ microemulsions,² liposomes,³ and surfactant vesicles⁴ as reaction media to achieve high overall quantum efficiency.

The present authors have pointed out that amphiphilic copolymers consisting of electrolyte segments and hydrophobic segments (electron donors or acceptors) are also promising materials in the light-induced electron transfer in aqueous media with following advantages⁵⁻⁷: (i) Uptake of amphiphilic quenchers (electron donors or acceptors) through hydrophobic interaction. (ii) Hence en-

hancement of the electron transfer (forward reaction). (iii) Prevention of back reactions due to electrostatic interaction. (iv) Increase in the reaction cross section of photoexcited polymer chromophores.

As part of a continuing study on the amphiphilic polymers as media for light-induced electron transfer, the present paper is concerned with the random copolymer (QPh) of 9-vinylphenanthrene (VPh) and 3-methacryloylaminopropyltrimethylammonium methylsulfate and its related monomer model (QM). The fluorescence quenching of the copolymer in aqueous solution is compared with that of the model compound, and the hydrophobic and electrostatic effects on the photoprocess are discussed.



EXPERIMENTAL

Copolymerization

VPh was prepared as reported previously.⁶ *N*-(*N*',*N*'-Dimethylaminopropyl)methacrylamide (DMAPMA) was provided through the courtesy of Nitto Chemical Industry Co. and used without further purification.

Copolymerizations of these monomers were carried out as follows: An ampoule containing VPh and DMAPMA in a known ratio (20 mmol in total), 0.25 mol% (on the basis of total monomers) of 2,2'-azobisisobutyronitrile, and 25 ml of tetrahydrofuran (THF) was evacuated by five freeze-pump-thaw cycles and then sealed under vacuum. Polymerizations were carried out at 60°C for 40 h. The ampoule was opened and the contents diluted with THF were poured into a large excess of hexane with vigorous stirring to precipitate the copolymers. The copolymer was further purified by precipitation from a THF solution into an excess of hexane. The total conversions for the copolymerizations with various VPh/DMAPMA ratios fell in the range of 11–20%. The copolymer compositions were determined by the C/N ratio in microanalysis.

Quaternization of the Copolymer

To a solution of 0.3 g of the copolymer in 5 ml of dimethyl sulfoxide (DMSO) was added dimethyl sulfate in a 2.5 times excess of the molar quantity of the tertiary amino group in the copolymer. The reaction mixture was stirred at 30°C for 3 days and then poured into an excess of acetone. The white product was isolated by filtration, washed with acetone, and dried *in vacuo*. In the 100-MHz ¹H NMR spectra of the quaternized copolymer (QPh), a characteristic sharp singlet peak at 3.16 ppm due to $-N^+(CH_3)_3$ appeared in place of a 2.23-ppm peak due to $-N(CH_3)_2$ in poly(VPh-*co*-DMAPMA), in-

dicating quantitative quaternization. From the C/N and C/S ratios in microanalysis, quaternization was confirmed to be quantitative.

Synthesis of Monomer Model (QM)

3-Phenanthreneacetic acid (**I**) was prepared according to the literature.⁸

N-(*N*',*N*'-Dimethylaminoethyl)-3-phenanthrylacetamide (**II**) was prepared as follows: To a refluxing solution of 4.75 g (0.02 mol) of **I** in 50 ml of toluene was added 3.38 g (0.038 mol) of *unsym*-dimethylethylenediamine in 10 ml of toluene. Water was separated as the toluene azeotrope by distillation over a period of 5 h and toluene was finally evaporated under reduced pressure to dryness. The crude product was dissolved in methanol and passed through silica gel column. Evaporation of methanol left 5.47 g of crystalline product: yield 89.0%, mp 93.5–95.0°C; NMR δ 2.13 (s, 6H), 2.36 (t, 2H), 3.31 (m, 2H), 3.75 (s, 2H), 6.5 (s, 1H), 7.36–7.89 (m, 7H), and 8.48–8.60 ppm (m, 2H).

Quaternization of **II** was performed by the following procedures. To a stirred solution of 5.0 g (0.016 mol) of **II** in 50 ml of the mixture of acetone-methanol (4:1, v/v) was added an equimolar (2.06 g) amount of dimethyl sulfate at one time. The reaction was performed at 30°C for 24 h and the solvents were then evaporated under reduced pressure. A 30-ml chloroform solution of the residue was poured into 200 ml of ether to precipitate a solid product which was recrystallized from acetone-ethanol (4:1, v/v) to give 6.52 g of QM as pale yellow flakes; yield 91.6%, mp 163.5–165°C; NMR δ 3.41 (s, 7H), 3.62–4.01 (m, 4H), 4.07 (s, 2H), 4.30 (s, 3H), 7.40–7.95 (m, 7H), and 8.60–8.84 ppm (m, 2H).

Anal. Calcd: C, 61.09%; H, 6.53%; N, 6.48%; S, 7.41%. Found: C, 60.51%; H, 6.49%; N, 6.42%; S, 7.52%.

Fluorescence Quenchers

N-Methyl-*N*-(2-hydroxyethyl)aniline (MHEA) was prepared according to the literature⁹ and purified by fractional distillation under reduced pressure: bp 106.5°C/0.2 mmHg.

N,N-di(2-hydroxyethyl)aniline (DHEA) was synthesized in accordance with the method of Benn *et al.*¹⁰ and purified by recrystallization twice from ethyl acetate: mp 58.0°C.

Fumaric acid (FA) was obtained from Wako

Pure Chemical Ind. Co. and purified by recrystallization from water.

Measurements

Fluorescence spectra were recorded on a Shimadzu RF-502A Spectrofluorophotometer at room temperature. Prior to the measurement sample solutions were deaerated by bubbling with nitrogen for 30 min. The concentrations of the phenanthrene residue of the solutions were reconfirmed by measuring the optical density each time the fluorescence was measured.

The fluorescence lifetime was measured using a small Blumlein-type N₂ gas laser: N₂ pressure, 1 atm; peak output power, ~30 kW; laser pulse width, 0.8–1 ns.¹¹ Prior to measurement, the sample solutions (containing 3.3×10^{-3} M of the phenanthrene residue) were evacuated and sealed in 2-mm ϕ quartz tubes on a vacuum line. The lifetimes were calculated from the decay curves; first-order plots were obtained between about 5 and 80 ns after the pulse.

RESULTS AND DISCUSSION

A copolymer composition curve for VPh (M₁) and DMAPMA (M₂) in THF at 60°C is shown in Figure 1. The monomer reactivity ratios determined from the Fineman–Ross plots are as follows:

$$r_1 = 3.261 \pm 0.253$$

$$r_2 = 0.232 \pm 0.029$$

Quantitative quaternization of poly(VPh-co-

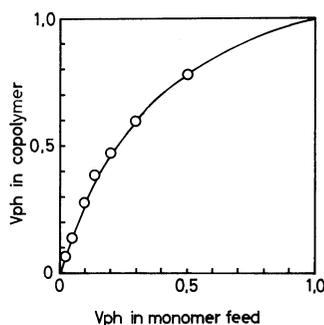


Figure 1. Copolymerization composition curve for 9-vinylphenanthrene (M₁) and DMAPMA (M₂): (M₁) + (M₂), 20 mmol; THF, 25 ml; AIBN, 0.25 mol%/monomers.

DMAPMA) with dimethyl sulfate could be achieved in DMSO in which the reaction proceeded homogeneously from beginning to end. Table I lists the solubility characteristics of QPh with various VPh content in various polar solvents. It is noted that QPh with a VPh mole fraction (f_{Ph}) up to about 0.47 was found to be soluble in water and in methanol. In DMSO, copolymers with a wide range of f_{Ph} were soluble while in DMF those with f_{Ph} lower than 0.14 were insoluble. In the ¹H NMR spectra of the copolymer with $f_{Ph} = 0.47$, peaks due to phenanthryl protons were observed in DMSO-*d*₆ but not at all in D₂O. This seems to be a common observation often encountered for amphiphilic copolymers of this type, whether the electrolyte sequences are cationic or anionic, because of the compact packing of the hydrophobic residues aris-

Table I. Solubility of QPh

Polymer	f_{Ph}^a	Solvent ^b			
		Water	MeOH	DMSO	DMF
QPh-78	0.78	I	I	S	S
QPh-60	0.60	I	I	S	S
QPh-47	0.47	S	S	S	S
QPh-39	0.39	S	S	S	S
QPh-28	0.28	S	S	S	S
QPh-14	0.14	S	S	S	I
QPh-7	0.07	S	S	S	I

^a Mole fraction of 9-vinylphenanthrene in QPh.

^b S, soluble; I, insoluble.

Table II. Relative fluorescence intensity at 500 nm and maximum emission wavelength of ANS in the presence of QPh-28 in aqueous solution^a

[Ph] _{residue} ^b	λ_{max}	I/I_0^c
10^{-3} M	nm	
Nil	528	1
0.2	486	154
0.4	484	188
0.8	480	220
2.0	476	266

^a [ANS] = 5×10^{-5} M; excitation wavelength, 365 nm.

^b Residual concentration of QPh-28.

^c I_0 and I are the emission intensity of ANS in the absence and presence of QPh-28.

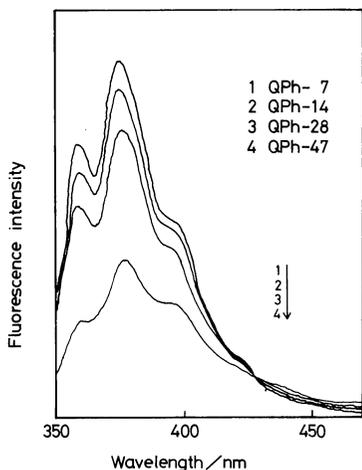


Figure 2. Fluorescence spectra of QPh in aqueous solution: $[VPh]_{\text{residue}}, 1.5 \times 10^{-4} \text{ M}$; excitation wavelength, 337 nm.

ing from an extensive hydrophobic association in an aqueous solution. We previously discussed these phenomena in some detail.⁵ The presence of the hydrophobic microdomain was also suggested by the uptake of a hydrophobic fluorescence probe, sodium 8-anilino-1-naphthalenesulfonate (ANS), by the copolymer. As shown in Table II, an addition of QPh-28 to an aqueous solution of ANS induced drastic enhancement of the fluorescence intensity of the probe with a marked blue shift of the emission maximum.

Figure 2 shows the fluorescence spectra of QPh with various f_{Ph} in aqueous solution. The fluorescence spectra of the copolymers with high f_{Ph} in aqueous solution are broad with significant tailing in the longer-wavelength region accompanied with a significant decrease in intensity at the emission maximum. As seen in Figure 2, the fluorescence intensity of QPh-47 ($f_{\text{Ph}}=0.47$) was about 40% that of QPh-7 ($f_{\text{Ph}}=0.07$) in an aqueous solution. The fluorescence lifetime was also found to decrease with increasing f_{Ph} (Table II) in aqueous solution; *e.g.*, the lifetime of QPh-47 was about 23% shorter than that of the monomer model, QM. In DMSO the shapes of the fluorescence spectra of the copolymers were identical to each other over a wide range of f_{Ph} and also identical to that of poly(9-vinylphenanthrene) (PVPh). The fluorescence intensity in this solvent also decreased gradually as f_{Ph} increased; *e.g.*, the intensity of QPh-47 was about

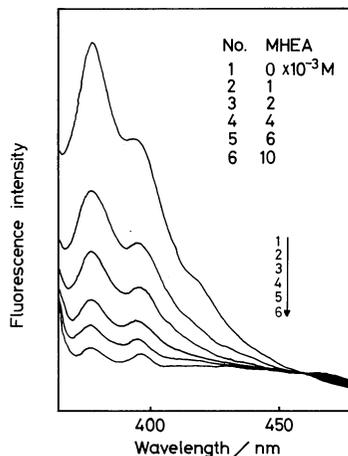


Figure 3. Fluorescence spectra of QPh-47 in aqueous solution in the presence of various concentrations of MHEA: excitation wavelength, 352 nm.

70% that of QPh-7. These observations also seem to be common to this type of amphiphilic copolymers regardless of the sign of the charge on the electrolyte segments.⁶

Our previous articles reported that oxidative electron transfer quenching of the excited phenanthrene residues in various types of amphiphilic copolymers with anionic segments occurred effectively with di(2-hydroxyethyl) terephthalate (BHET), an amphiphilic acceptor, in aqueous media.^{6,7} We demonstrated that these remarkable events originated from hydrophobic binding of the quencher molecules by hydrophobic microdomains consisting of phenanthrene residues.^{6,7} In the present study, we used MHEA and DHEA, amphiphilic electron-donor quenchers to examine the photoinduced electron transfer processes in aqueous media. The half-wave oxidation potentials $E_{0x,1/2}$ for both MHEA and DHEA were determined by cyclic voltammetry to be +0.65 V *vs.* SCE. The lowest-energy absorption band of these compounds shows up at 297 nm, which is a shorter wavelength than that of the phenanthrene group. These compounds are expected to act thermodynamically as electron donors toward excited phenanthrene groups since the $E_{0x,1/2}$ for excited phenanthrene can roughly be estimated as -2.07 V *vs.* SCE by simply subtracting the excitation energy from the oxidation potential for phenanthrene (+1.50 V *vs.* SCE).¹² Figure 3 shows the fluorescence spectra of the $1 \times 10^{-4} \text{ M}$

phenanthrene residue of QPh-47 in pure water in the presence of various amounts of MHEA. Considerable quenching of the fluorescence intensity at the emission maximum was observed, whereas this intensity in the longer wavelength region slightly increased with an isoemissive point appearing at 460 nm as the amount of quencher increased. We have no explanation for this except that the possibility of exciplex formation cannot be precluded. Figure 4 illustrates the dependence of I_0/I on the concentration of the quencher for the copolymers and monomer model in aqueous solution, where I_0 and I are the emission intensity of the excited phenanthrene in the absence and presence of the quencher, respectively. A distinctive feature of the Stern–Volmer plots in Figure 4 is that, qualitatively speaking, the fluorescence of the copolymer with a higher content of phenanthrene groups, QPh-47, is quenched far more effectively by MHEA and

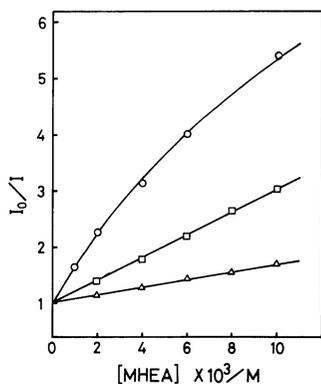


Figure 4. Stern–Volmer plots for QPh–MHEA system in aqueous solution: ○, QPh-47; △, QPh-14; □, QM.

that the plots fail to follow the Stern–Volmer equation. These phenomena were often encountered with various amphiphilic copolymers hitherto investigated in previous studies.^{6,7} We have recently proposed a kinetic model involving both a dynamic and static mechanism occurring concurrently to explain the downward deviation of the Stern–Volmer plot.¹³ It appears that static quenching due to hydrophobic association of the quencher molecules with the microdomains plays an important role. The fluorescence quenching of the copolymer with lower f_{Ph} , QPh-14, and the related monomer model, QM, by MHEA exactly followed the Stern–Volmer equation. It is interesting to note that the quenching for QPh-14 is less effective than that for the monomer model.

The Stern–Volmer quenching constants K_{sv} for the linear plots were calculated by the least-squares method, while the apparent K_{sv} for the nonlinear plots were estimated from their initial slopes. The second-order rate constants for fluorescence quenching, k_q s, were calculated from the K_{sv} s and the fluorescence life time τ . Table III summarizes the results for the copolymers of various phenanthrene content. The k_q s for both MHEA and DHEA for the copolymer with $f_{Ph}=0.47$ were considerably higher than the rate constant predicted from diffusion-controlled kinetics in an aqueous solution. The k_q s for the monomer model were about the same order of magnitude as that of the diffusion-controlled reaction. However, the rate constants for the copolymers with f_{Ph} less than 0.28 were all much lower than those for QPh-47 and even lower than those of the monomer model. These facts lead to the speculation that only in a

Table III. Rate constants for the fluorescence quenching of QPh by MHEA and DHEA in aqueous solution

Sample	f_{Ph}	τ ns	K_{sv} M ⁻¹		$k_q \times 10^{-9}$ M ⁻¹ s ⁻¹	
			MHEA	DHEA	MHEA	DHEA
QPh-47	0.47	34	630 ^a	400 ^a	18	12
QPh-28	0.28	39	153	120	3.9	3.1
QPh-14	0.14	42	73.0	71.3	1.7	1.7
QPh-7	0.07	44	45.4	53.7	1.0	1.2
QM		44	203	184	4.6	4.2

^a Calculated from the initial slope of the Stern–Volmer plot.

Table IV. Rate constants for the fluorescence quenching of QPh by MHEA and DHEA in DMF solution

Sample	f_{Ph}	τ ns	$\frac{K_{sv}}{M^{-1}}$		$\frac{k_q \times 10^{-9}}{M^{-1} s^{-1}}$	
			MHEA	DHEA	MHEA	DHEA
			QPh-47	0.47	40	28.4
QPh-14 ^a	0.14	41	16.3	10.0	0.39	0.24
QM		53	179	144	3.4	2.7
PVPh ^b		22	23.8	11.2	1.1	0.51
Phe ^c		52	210	130	4.0	2.5

^a Measured in DMSO solution.^b Poly(9-vinylphenanthrene) homopolymer.^c Phenanthrene.**Table V.** Rate constants for the fluorescence quenching of QPh by FA in aqueous solution

Sample	f_{Ph}	τ ns	$\frac{K_{sv}}{M^{-1}}$		$\frac{k_q \times 10^{-9}}{M^{-1} s^{-1}}$	
			Pure ^a	Acidic ^b	Pure ^a	Acidic ^b
			QPh-47	0.47	34	20000 ^c
QPh-14	0.14	42	4380	88.4	100	2.1
QM		44	224	181	5.1	4.1

^a Measured in pure water.^b Measured in 0.2 N H₂SO₄ solution.^c Calculated from the initial slope of the Stern–Volmer plot.

copolymer with a higher phenanthrene content ($f_{Ph} > 0.47$) the sum of the hydrophobic interaction among the phenanthrene groups along the main chain can prevail over an electrostatic mutual repulsion of polycations to form hydrophobic microdomains. This speculation seems compatible with the fact that the fluorescence intensity of QPh-47 is distinctively lower than those of the other three copolymers as shown in Figure 2, *i.e.*, the self-quenching of the excited phenanthrene is probably facilitated by the compact packing of the phenanthrene groups.⁶ Reference data on fluorescence quenching were obtained in DMF in which the effects of hydrophobic interaction are nil (Table IV). The Stern–Volmer plots for QPh-47 in this solvent gave a linear relationship and the k_q s thus obtained were much lower than those

obtained in aqueous solution. On the other hand, the k_q s for the monomer model observed in DMF were only slightly lower than those in aqueous solution. These results support the forgoing interpretation of the characteristic behavior of fluorescence quenching of QPh-47 in aqueous media. It should be also noted that the quenching rate constants for the polymers were lower than those for the monomer model in both solvents (except for QPh-47 in water). Table IV also includes the rate constants for homopolymer and phenanthrene itself for reference. In this comparison, the k_q s for phenanthrene itself were also found to be four or five times larger than those for the homopolymer. In a homogeneous solution, where both fluorophore and quencher can move freely, the k_q approximates the value of diffusion con-

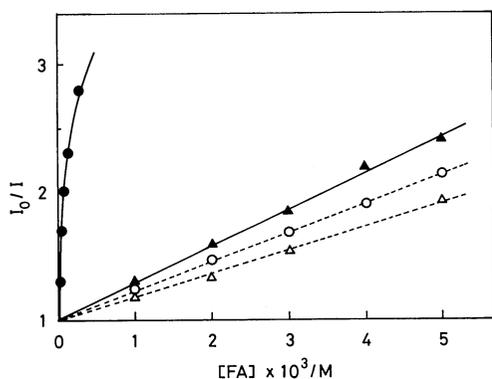


Figure 5. Stern-Volmer plots for the fluorescence quenching of QPh-47 and QM by FA in aqueous solutions: —●— and ---○---, QPh-47 and QM in pure water; —▲— and ---△---, QPh-47 and QM in 0.2N H₂SO₄, respectively.

trolled kinetics when the quencher is sufficiently effective. The difference in the redox potential between the fluorophore and quencher can roughly be a measure of the effectiveness of the quenching agent in a homogeneous solution.¹⁴ In this sense, parallel with the fact that the k_q s for the monomer model with MHEA and DHEA in both solvents are very close to the diffusion controlled limit, both quenching agents are regarded as being effective enough. Thus, we may interpret that the less accessibility of the quencher molecule to the fluorophore surrounded in the polymer matrix is reflected in the lower values of k_q observed for the polymer in both DMF and water.

In order to observe the electrostatic effects on fluorescence quenching, a negatively charged acceptor quencher, FA, was employed in the present study. The fluorescence quenching of the copolymer with this quenching agent was found to occur quite effectively in pure water, in which FA anions are probably electrostatically bound to the cationic sphere of the amphiphilic copolymers. The Stern-Volmer plots strongly deviated downward from the linear relation and the apparent second-order rate constants for the quenching estimated from the initial slope were found to be extremely high; all values fell in the range of $1-6 \times 10^{11} \text{ M}^{-1} \text{ s}^{-1}$ (Table V). However, in acidic solution, the fluorescence was quenched far less effectively than in pure water; the plots obeyed Stern-Volmer relation and the k_q was of the order of $10^9 \text{ M}^{-1} \text{ s}^{-1}$ (Table V).

This drastic difference in the quenching behavior of the copolymer in pure and acidic aqueous solutions is shown in Figure 5. It should be stressed that no such difference was observed with the monomer model shown in Figure 5. These results indicate a dramatic effect of a potential field generated by polyions with a high density of fixed charge along the polymer chain. A single charge combined in the neighborhood of a fluorophore, as in the case of QM, is not sufficiently effective to bind the quencher electrostatically and thus facilitate the quenching process.

The present study demonstrates that the accessibility of the quenching agent to the fluorophore is the prime factor for the effectiveness of fluorescence quenching which is dramatically governed by hydrophobic and electrostatic interactions in amphiphilic polymer systems.

We are currently concerned with the fate of the charged products as an extension of this study, focusing attention on the preventive effects of the high electrostatic potential of the polyelectrolyte on the back electron transfer process.¹⁵

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