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Characteristics of proflavine solubilized in complexes of anionic polymers with a cationic surfactant

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Abstract: Absorption and emission spectra of 3,6-diaminoacridine (proflavine) are reported in mixed solutions of dodecyltrimethylammonium bromide (DTAB) with various polyelectrolytes including the sodium salts of poly(acrylic acid) (PAA). poly(methacrylic acid) (PMA), poly(styrenesulfonic acid) (PSS), poly(garacturonic acid) (pectate), and the alternating copolymers of maleic acid with ethylene (PMA-E) and styrene (PMA-S). The spectral change indicates the association of the dye (blue-shift) on these polyions except on PSS, the easy dissociation of the aggregated form into the monomeric form and the solubilization into the hydrophobic PMA-S/DTAB complex (red-shift), the little dissociation in the PAA/DTAB, PMA/DTAB and PMA-E/DTAB complexes, and the liberation of the bound dye in the case of pectate/DTAB complexes. In the PSS system, the strong interaction of the dye with the styrene groups induces the completely different spectral behavior. These results are discussed with the cooperative binding of the dye and the surfactant ion.

Key words: Polyelectrolyte – cationic surfactant – proflavine – spectroscopy

Introduction

Proflavine (PF, 3,6-diaminoacridine) exists in the dissociated (monomeric) form and the aggregated form in solutions, which depends on the environment and the concentration and appears in the change of the absorption, fluorescence, and circular dichroism spectrum [1–7]. Therefore, the spectral change of PF has been used for the various physico-chemical studies including the cooperative binding of PF by biopolymers [2, 3], the intercalation of PF into DNA [4, 7], the energy transfer between PF and pyrene in the surfactant micelle [5], and the interaction of PF with surfactant premicelle [6]. The spectral change of PF with increasing hydrophobicity of polyion of various structures through cooperative binding of surfactant is of interest. In a previous paper [8], we examined the spectral change of PF and rhodamine 6G (R6G) in the complexes of dodecyltrimethylammonium bromide (DTAB) with sodium salts of dextran sulfate and poly(vinyl sulfate). That study revealed that the bound PF and R6G tend to dissociate in the monomeric form in the polyion/DTAB complexes. We extended the study on R6G with a number of different polymers and revealed three kinds of interaction of R6G with the polyion/DTAB complexes [9]. Here, we examined the spectroscopy of PF with a number of different polymers whose structures are given in Fig. 1 and found somewhat different mode of the interaction between PF and the polyion/surfactant complex. The interaction strongly depends on the polymer structure and then the nature of the polymer-surfactant aggregates.

Experimental

Proflavine hydrochloride (Aldrich, dye content: ca. 95%) was purified by recrystallization from

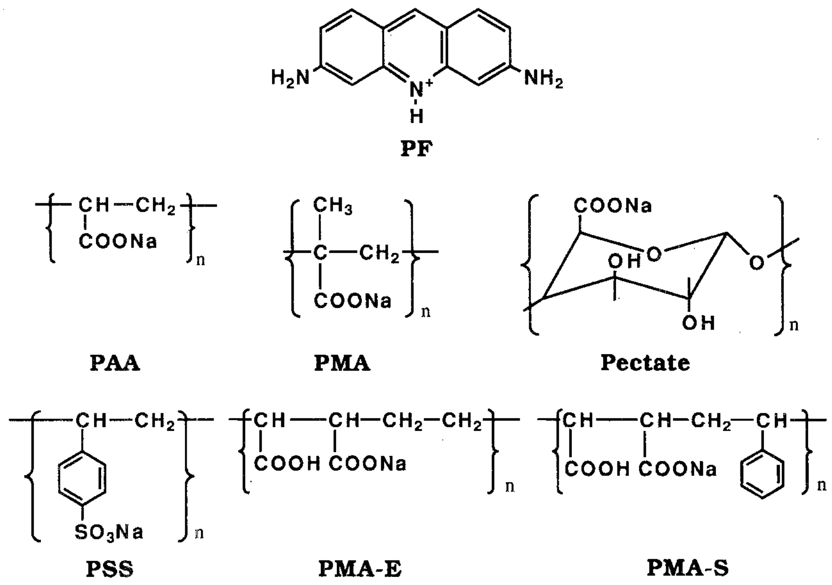

Fig. 1. Structure of proflavine and polyelectrolytes.

ethanol. The concentration of the PF stock solution was determined spectrophotometrically in the diluted aqueous solution ($< 10^{-5}$ mol kg^{-1}). The concentration of PF in test solutions was kept at (7–8) × 10^{-6} mol kg^{-1} where no dimer of PF was formed in the aqueous bulk solution [1]. Polyelectrolytes used were sodium salts of poly(acrylic acid) (PAA), poly (methacrylic acid) (PMA), poly(styrenesulfonic acid) (PSS), poly(galacturonic acid) (pectate), and the alternating copolymers of maleic acid, poly(maleic acid-co-ethylene) (PMA-E) and poly(maleic acid-co-styrene) (PMA-S). The characteristics, solution preparation, and concentration determination were described elsewhere [9]. For PAA, PMA, pectate and PSS concentrations as given are for the ionic groups, i.e., as equivalent/L. However, PMA-S and PMA-E were used after they were half neutralized by pH titration, and in this case the concentrations are on a repeated unit basis, i.e., each mol of monomer represents a COO^- and a COOH group.

A commercial DTAB (Sigma) was purified by repeated recrystallization from an acetone-ethanol mixture. All the aqueous solutions were prepared by weight and the concentrations are given as molalities (mol/kg-H_2O).

The absorption and fluorescence spectra were recorded by using a Cary 219 spectrophotometer and a Perkin-Elmer MPF 66 or Aminco Bowman fluorescence instrument. The fluorescence excitation wavelength was carefully chosen at the isosbestic point of the absorption spectra for a series of solutions of different DTAB concentration at a constant polymer and dye concentration when the isosbestic point was available. Otherwise, the emission intensities were given on the basis of equal absorbance at the excitation wavelength, and were corrected for the absorbance of excitation and emission light. The absorbance of the aqueous dye solution was always far from the selected fluorescence excitation wavelength.

Results and discussion

Absorption and fluorescence spectra of PF

The binding of aminoacridines by polymers frequently leads to a shift in the wavelength of maximum absorption of the bound molecules because of the stacking property of the dyes bound to polymer [10]. Figure 2 shows this shift for PF in the presence of various anionic polymers (Aq → O). The absorption maximum at 443 nm in the aqueous solution shifts to a shorter wavelength side (432–436 nm) accompanied with an absorbance reduction in the presence of 0.5 mmol kg^{-1} polyanions except for PSS. The

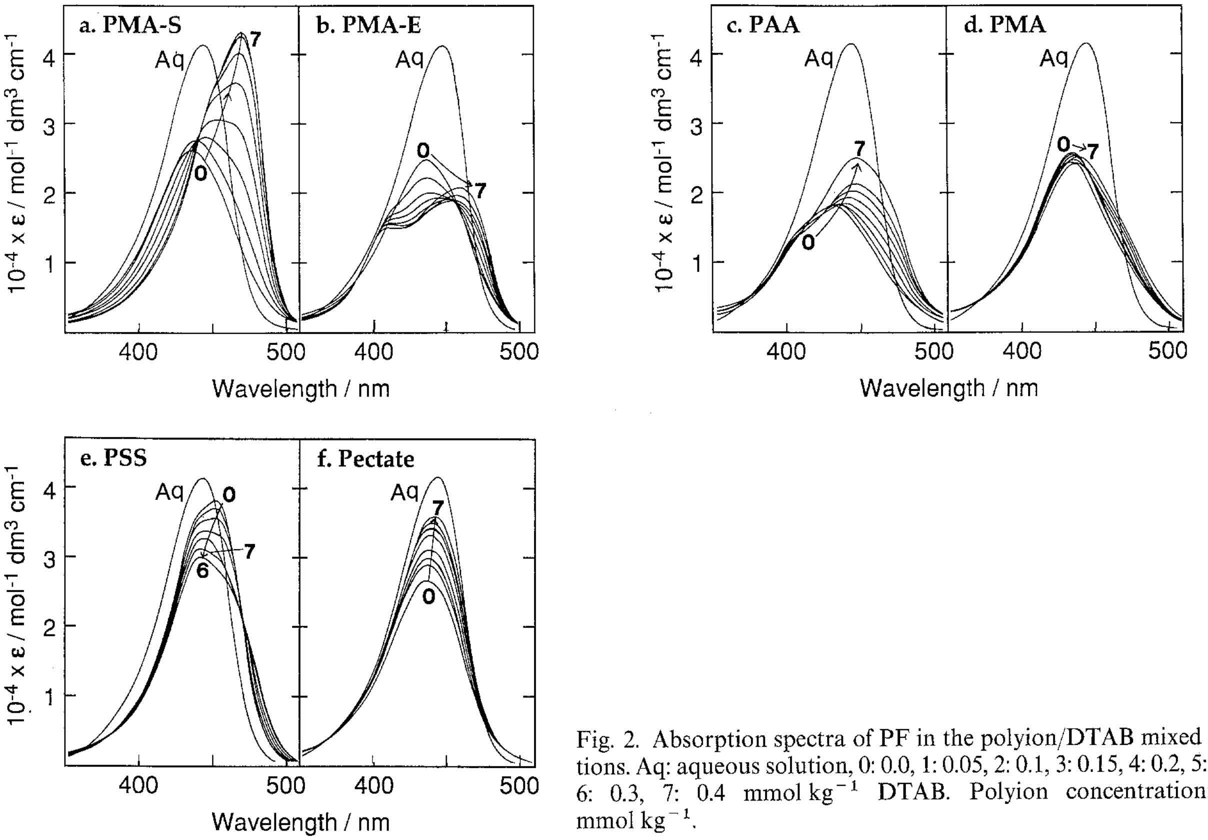

Fig. 2. Absorption spectra of PF in the polyion/DTAB mixed solutions. Aq: aqueous solution, 0: 0.0, 1: 0.05, 2: 0.1, 3: 0.15, 4: 0.2, 5: 0.25, 6: 0.3, 7: 0.4 mmol kg^{-1} DTAB. Polyion concentration: 0.5 mmol kg^{-1}.

blue shift and the absorbance reduction are summarized in Table 1, which lists the wavelength and molar absorbance at the band maximum. The blue shift is attributed to the formation of dimer or a stacked form of PF on the polyion chain due to the cooperative binding [2, 10–13].

The addition of DTAB surfactant induces a shift to a longer wavelength side of the band maximum (O → 7 in Fig. 2) except for PSS (Fig. 2e). The wavelength and the relative absorbance at the band maximum are plotted as a function of DTAB concentration in Figs. 3 and 4, respectively. The red shift arises from dissociation of the stacked PF into the monomeric form, possibly due to releasing into aqueous bulk solution or solubilization in the polyion/surfactant complexes. Since highly cooperative binding of the surfactant cation by polyanion produces hydrophobic domain in the polyion/surfactant complex, the bound dye stacks dissolve into the monomeric form in the complexes. This leads to the red shift of PF spectrum because of the hydrophobic environment [8, 9]. The band maximum of PF appears at a longer wavelength in hydrophobic environment than in aqueous solution.

Figure 5 shows an example of emission spectra of PF in the solutions of 0.5 mmol kg^{-1} PAA including different DTAB quantity. The wavelength (516 nm) at a maximum emission is independent from DTAB concentration, while the wavelength in the absorption spectrum changes with DTAB concentration (Fig. 2c). The band maximum appeared at 512 nm in the pectate/DTAB and PMA-E/DTAB systems, 516 nm in the PAA/DTAB and PMA/DTAB systems, 515 nm in the PMA-S/DTAB system and 499 nm in the PSS/DTAB system, all of which include the uncertainty of ± 3 nm. The emission maximum of PF was observed at 512 nm for the aqueous solution. The emission once strongly quenched in polyion solutions grows quickly upon the DTAB addition. Relative emission intensity at the band maximum is plotted against DTAB concentration in Fig. 6. Since only monomeric PF is responsible

Table 1. Characterization of absorption spectrum of PF in polyelectrolyte-surfactant mixed solutions

Polyelectrolyte	Charge Density ξ	Wavelength nm	Molar absorbance mol^{-1} kg cm^{-1}	Wavelength nm	Molar absorbance mol^{-1} kg cm^{-1}
		(Polyelectrolyte[1])		(Polyelectrolyte/DTAB[2])	
Aqueous		443	41000		
PMA-St	1.4	436	26000	470	42600
PMA-E	1.4	435	24600	459	20700
PAA	2.8	432	17200	447	22800
PMA	2.8	434	24100	439	24800
PSS	2.8	453	37800	442	32500
Pectate	1.6	436	26200	441	35300

1) 0.5 mmol kg^{-1} polyelectrolyte
2) 0.5 mmol kg^{-1} polyelectrolyte and 0.4 mmol kg^{-1} DTAB

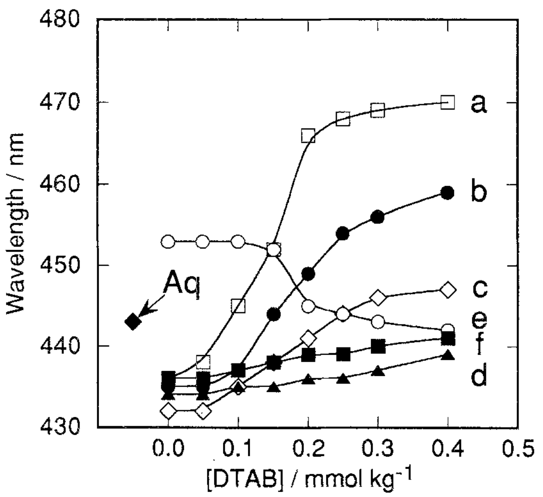

Fig. 3. The wavelength at the maximum adsorption in the PF adsorption spectrum. a: PMA-S, b: PMA-E, c: PAA, d: PMA, e: PSS, and f: Pectate.

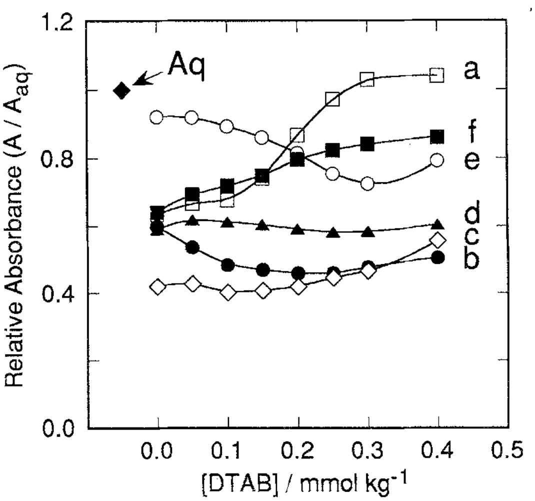

Fig. 4. The absorbance at the maximum adsorption in the PF adsorption spectrum. a–f correspond to those in Fig. 3.

for the fluorescence, we are able to estimate a quantity of monomeric PF from the observed emission intensity.

Thus, the spectrum change of PF in polyion/DTAB complexes generally indicates the stacking of PF in polyion solutions and the dissociation of stacked PF in the hydrophobic polyion/DTAB complexes except for PSS. However, the mode of the change strongly depends on the polyion structure. Each case will be discussed below in detail.

In the PMA-S/DTAB system, DTAB induces a large red-shift to 470 nm and recovery of absorbance of PF spectrum (Figs. 3a and 4a). The wavelength at the band maximum is longer in the presence of 0.4 mmol kg^{-1} DTAB than that in the aqueous solution of PF (443 nm). This fact is similar to those found for PF in the dextran sulfate/DTAB mixed solutions [8]. This is ascribed again to the solubilization of the PF aggregates in the monomeric form in a hydrophobic domain of PMA-S/DTAB complexes. The emission once

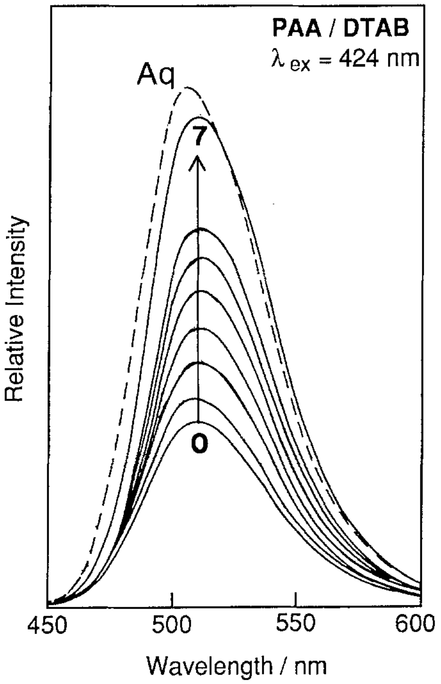

Fig. 5. The fluorescence spectra of PF in the mixed solution of PAA and DTAB. Aq and 0–7 correspond to those in Fig. 2.

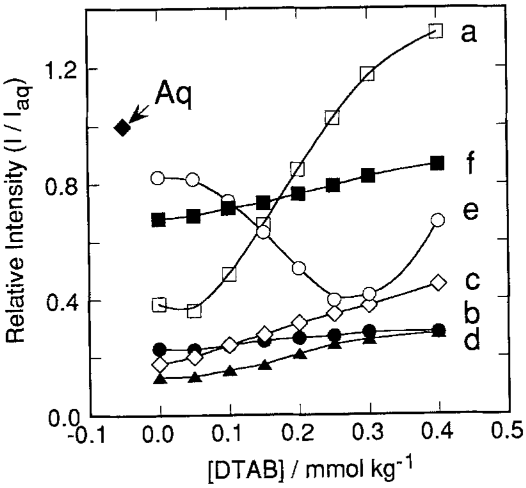

Fig. 6. The relative intensity of emission spectrum of PF at the maximum intensity. a–f correspond to those in Fig. 3.

Table 2. Relative intensity of PF emission in presence of polyion and DTAB

Polyelectrolyte	I/I_{aq}	
	(Polyelectrolyte[1])	(Polyelectrolyte/DTAB[2])
(Aqueous)	(1.0)	
PMA-St	0.385	1.316
PMA-E	0.230	0.285
PAA	0.178	0.451
PMA	0.130	0.281
PSS[3]	0.825	0.669
Pectate	0.683	0.864

1) 0.5 mmol kg^{-1} Polyelectrolyte
2) 0.5 mmol kg^{-1} Polyelectrolyte and 0.4 mmol kg^{-1} DTAB
3) Minimum intensity = 0.397 at 0.25 mmol kg^{-1} DTAB

strongly quenched in the PMA-S solution grows quickly upon the DTAB addition and the intensity becomes even larger at a DTAB concentration above 0.25 mmol kg^{-1} than in the aqueous solution ($I/I_{aq} > 1$). This also points out the solubilization of PF in the monomeric form in the PMA-S/DTAB complex.

The absorption band at 435 nm in the PMA-E solution splits into two bands with the band maximum at 405 and 460 nm upon the DTAB addition (Fig 2b). This indicates that the surfactant binding by PMA-E induces reorganization of bound PF into both directions: 1) the formation of higher order aggregate exposed to water on polyion (blue-shift) and 2) the solubilization of the dissociated PF into the hydrophobic PMA-E/DTAB complexes (red-shift). The absorbance recovery is very little upon the DTAB addition and is less even at 0.4 mmol kg^{-1} DTAB than that in PMA-E solution (Fig 4b). This fact indicates that DTA^+ binding facilitates the PF stacking on PMA-E and provides the hydrophobic environment for PF bound to PMA-E polyion. The PMA-E/DTAB complex has less solubilization power for the bound PF than the PMA-S/DTAB complex. Though the maximum emission appears at 512 nm, the same wavelength of the aqueous PF soltuion, for PMA-E/DTAB system, it is ascribed not to PF liberated in bulk solution, but to PF solubilized in PMA-E/DTAB complex, because of the different absorption spectrum from the aqueous one (Fig. 2b). This strong quenching owes to the PF stacking facilitated by DTA^+ binding.

The red shift of band maximum from 432 nm to 447 nm and the absorbance recovery in the PAA/DTAB mixed solutions (Figs. 3c and 4c) indicate the solubilization of the PF aggregate by the PAA/DTAB complex. A smaller red shift suggests that the PAA/DTAB complex provides less hydrophobic environment than the PMA-E/DTAB complex. The ambiguous shoulder at 470 nm (PAA in Fig. 2c) indicates less monomeric form than dimeric or aggregate form of PF in the hydrophobic domain of the PAA/DTAB complexes.

PMA induces also the aggregation of PF and DTAB addition affects very little the absorption spectrum of bound PF in PMA/DTAB system (d in Figs. 2–4). The slight increase in absorbance at 470 nm indicates that PMA/DTAB complexes induces a little dissociation of PF aggregates into the monomeric form.

The emission is strongly quenched due to PF aggregation in PMA, PAA and PMA-E solutions, and is slightly recovered with increasing DTAB concentration. This observation corresponds to the behavior in absorption spectrum. The absorbance around 460 nm due to the monomeric PF in the hydrophobic polyion/DTAB complex is enhanced a little on increasing DTAB concentration for these polyion systems, indicating a little formation of the monomeric PF. A slight enhancement for PAA/DTAB system corresponds to the relatively large increase in absorbance around 460 nm, as seen in Fig. 2c.

In the pectate system, the cationic surfactant affects differently than from the previous polyion/DTAB systems. Pectate induces the association of PF as shown in the blue shift of absorption maximum and lowers the absorbance (Figs. 2f and 3f) as other polyions do. When DTAB is added to the mixture of PF and pectate, the lowered absorbance is recovered (Fig. 4f) and the wavelength at the maximum absorbance tends to converge to that of the aqueous PF solution (Fig. 3f). This suggests liberation of bound PF by the surfactant binding to pectate or competitive binding between PF and DTAB. The emission once quenched upon the pectate addition recovered the intensity with increasing DTAB concentration. This arises from increasing free PF in the aqueous bulk solution, because the band maximum appears at the same wavelength (512 nm) as found in aqueous PF solution.

In the case of PSS, Fig. 2 clearly shows different behavior. This PSS/PF system presents an example of specific polymer/dye interactions. No blue shift, but rather a red shift is observed in PSS solution. This red shift was also found for rhodamine 6G in PSS solution [9] and for acridine derivatives at the very small dye-to-polymer ratio in PSS solution [14]. In the latter case, blue shift of absorption band was observed at the high dye-to-PSS ratio, which was ascribed to dye stacking [10]. The DTAB addition induces the band shift of PF from 453 nm to a shorter wavelength side (442 nm at 0.4 mmol dm^{-3} DTAB). A similar blue shift was found for rhodamine 6G in the PSS/DTAB mixed solution [9]. The spectrum shows an ambiguous shoulder at 442 nm in PSS solution and the shoulder becomes a maximum in the presence of 0.40 mmol kg^{-1} DTAB (Fig. 2e–7). As DTAB increases, the wavelength at the absorbance maximum shifts abruptly to 442 nm at 0.20 mmol kg^{-1} DTAB (Fig. 3e), while the absorbance decreases monotonously below 0.30 mmol kg^{-1} DTAB (Fig. 4e). Figure 2e shows an increase in absorbance at 470–500 nm, which corresponds to the absorption band of PF at 0.4 mmol kg^{-1} DTAB in PMA-S/DTAB mixed solution. These findings suggest that the monomeric PF bound to PSS is reorganized to associate into the dimeric or aggregate form under the hydrophobic environment of the DTAB/PSS complexes upon the DTAB addition. A quite different behavior is also observed in the emission spectra. The weak quenching in PSS solution indicates no stacking of PF in the PSS solution. The lowered intensity with increasing DTAB concentration suggests the stacking of PF in the hydrophobic complexes of PSS with DTAB. The slight enhancement at 0.4 mmol kg^{-1} DTAB corresponds to the increase in absorbance at the concentration (Fig. 4e).

Competitive stacking between PF and DTA$^+$ on polyion

The degree of blue shift and absorbance reduction allow us to estimate the stacking strength of PF bound to polyion or cooperativity of PF binding by polyion. The largest absorbance reduction and band shift to short wavelength side (432 nm) is observed in 0.5 mmol kg^{-1} PAA solution. This indicates a strong stacking of PF in the PAA polyion domain. The spectrum O in Fig. 2c clearly shows a shoulder of higher order aggregate at 410 nm. In this way, we find the order of stacking ability of PF bound to polyions as follows:

$$\text{PAA} \gg \text{PMA} > \text{PMA-E} > \text{PMA-S} \sim \text{Pectate} \quad (1)$$

This order also reflects the cooperativity of PF binding by polyion. According to the Schwarz treatment [11] for a dye stacking on polymer based on the linear lattice model including only the nearest neighbor interaction, the cooperativity of dye binding by polymer is measured by q defined as

$$q = \exp(-\Delta G^\circ/kT), \quad (2)$$

where ΔG° represents the free energy decrease due to the cooperative interaction of nearest neighbor ligands on polymer. ΔG° is equivalent to the exchange energy between interacting sites [15]:

$$\Delta G^\circ(\mathrm{D}) = (E_{\mathrm{DD}} + E_{\mathrm{OO}} - 2E_{\mathrm{DO}})/2, \quad (3)$$

where D and O represent an occupied site by a dye molecule and an empty site, respectively, and E_{DD}, E_{DO}, and E_{OO} represent the pair interaction energies between the corresponding sites. E_{OO} is positive due to the electric repulsive interaction and E_{DD} is supposed to be less than E_{DO} due to the stabilization by stacking between the bound dyes. If this cooperativity parameter q is larger than unity, the aggregation or stacking of bound dyes is favored. The strong stacking means a stable DD pair (low E_{DD}). Therefore, the order in Eq. 1 shows the order of cooperativity of PF binding by polyions.

If PF binds to an anionic site in polyion, the stacking ability may depend on the nearest charge separation of anionic sites of polyion. When the separation is small, PF stacking is stable due to strong intermolecular interaction and E_{DD} is negatively large. The charge separation is estimated by the linear charge density, ξ, of the polyions defined by

$$\xi = e^2/4\pi\varepsilon kTb, \quad (4)$$

where e is the elementary charge, ε is electric permittivity, k is the Boltzman constant, T is temperature in K, and b is the linear charge spacing on polyion. The order of ξ, PAA = PMA $\gg$ Pectate > PMA-E = PMA-S, is not consistent with the order of stacking ability. We used a half neutralized form of PMA-E and PMA-S. The order of ξ for these polymers is estimated for the alternatively protonated form of carboxylic groups. However, the pH of the test solutions was not controlled. It produces a discrepancy from the half neutralized point and uncertainty in ξ. Furthermore, we must take into account that proton is mobile. PF may bind to a carboxyl group next to the site already occupied by PF through ion exchange with proton. In such case, a strong pair stacking may be expected.

Satake and Yang applied the same treatment as Schwarz did to the binding of anionic surfactant by cationic polypeptide, and found highly cooperative binding because of the hydrophobic interaction between the bound surfactant ions [15]. For surfactant binding, the cooperative parameter u is defined as

$$u = \exp[(2E_{\mathrm{SO}} - E_{\mathrm{OO}} - E_{\mathrm{SS}})/2], \quad (5)$$

where the subscript S represents an occupied site by surfactant ion and E_{SS} and E_{SO} represent the interaction energies between corresponding sites. In many polyion/surfactant systems, the strong cooperative binding have been observed due to the large hydrophobic interaction between the bound surfactant ions (E_{SS} is negatively large).

For mixed ligand system in this study, we need to introduce another interaction parameter r as follows:

$$r = \exp[(2E_{\mathrm{SD}} - E_{\mathrm{SS}} - E_{\mathrm{DD}})/2]. \quad (6)$$

When r is larger than unity, the SD pair is hard to form, and the dye stacking is preferred. In the case of $r < 1$, the dye aggregates are dissolved in surfactant aggregates. Since E_{SS} depends principally on hydrophobic interaction between the long surfactant tails, it may be less dependent on the polymer structure. Therefore, we suppose that the complicated spectral behavior in the previous section depends mainly on the different E_{DD} and E_{SD} energies. For PMA-S system, the observed easy break of PF stacking may result from small E_{SD} and $r < 1$, while for the PMA-E, PAA, and PMA systems, the observed stable PF stacking may result from the negatively large E_{DD} and $r > 1$. Since both PF and DTAB favor cooperative binding [2, 16, 17] and SO and OD pairs are hard to form, the reorganization SO + OD = SD + OO can be neglected.

In pectate, the interaction of PF with the polymer is weak and the bound PF is expelled by the competitive binding of surfactant cation and, thus, the spectrum in the presence of DTAB resembles that of aqueous solution. A similar behavior was also observed for rhodamine 6G in pectate/DTAB mixed solution [9].

The case of PSS/PF is an example of specific polymer-dye interactions as discussed for rhodamine 6G [9]. The spectral change indicates that the strong hydrophobic interaction between PSS and PF weakens the stacking interaction between the bound PF cations at low dye/polyion ratios. PMA-S also has the styrene group and shows the regular behavior for the polyanion/cationic dye system as mentioned above. This suggests that the strong interaction of PF with PSS is related to the smaller styrene-styrene distance in PSS, as opposed to PMA-S where the average styrene-styrene distance is twice as large as in PSS. Possibly, the PF cation is sandwiched between adjacent styrene groups in PSS, preventing the stacking of PF.

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