December, 1981] © 1981 The Chemical Society of Japan NOTES Bull. Chem. Soc. Jpn., 54, 3859—3860 (1981) 3859

Metachromatic Absorption Spectra of Spin-labeled Mono- and Diamino-acridine Dyes Bound to Synthetic Polyelectrolytes[1)]

Kiwamu YAMAOKA,* Takumi MATSUDA, and Tsuyoshi MURAKAMI

Faculty of Science, Hiroshima University, Higashisenda-machi, Naka-ku, Hiroshima 730

(Received April 16, 1981)

Synopsis. Interactions between nitroxide spin-labeled dyes (Acridine Orange, Proflavine, and 9-aminoacridinium chloride) and poly(*p*-styrenesulfonate), polyphosphate, and polyacrylate were studied by measuring visible absorption spectra in aqueous solutions. The bound-dye spectra and equilibrium constants were determined by the principal-component-analysis method. The metachromatic behavior of dye–polymer complexes was clarified.

Previous reports have shown that acridine dyes labeled with stable nitroxides can be utilized in the study of dye–polymer interactions by two independent methods of ESR and optical absorption.[2)] Molecular dynamics of spin-labeled dyes bound to various polyelectrolytes were clarified by analyzing ESR signals of spin moieties.[2,3)] The characteristics of absorption spectra of newly synthesized spin-labeled and other related dyes were compared to determine the effect of attaching the labels.[4)] For full utilization of these dyes as ESR-optical double probes, their spectral behavior should be assessed quantitatively in the presence of various polymers under diverse conditions.

In this Note, the metachromatic behavior of three spin-labeled acridine dyes toward three polyelectrolytes with different charged-groups will be reported. By applying the principal-component-analysis (PCA) method to the measured absorption spectra of each dye–polymer combination, the spectrum and the fraction of the bound-dye species were evaluated, together with the apparent equilibrium constant. For each polyelectrolyte, the bound-dye spectra are similar to those of the corresponding unlabeled or mother dyes, indicating that the metachromatic tendency is well preserved.

Experimental

Materials. Spin-labeled dyes were all described in detail previously: monopyrrolidinylated Proflavine (slPF),[2-4)] monopiperidylated Acridine Orange (slAO),[2,3)] and a spin-labeled 9-aminoacridinium chloride (slAA).[2,3,5)] Their structural formulas are shown in Fig. 1. A 3-acetamido derivative of Proflavine (AcPF), for which R_1=–NHCOCH$_3$,[4)] was used as the reference for slPF. Sodium salts of poly(*p*-styrenesulfonate) (NaPSS), polyphosphate (NaPP), and polyacrylate (NaPA) were also described elsewhere.[6,7)]

Measurements and the Procedure for Data Analysis. Absorption spectra were measured at 25 °C as before.[4,6)] A salt-free dye solution was titrated by the dropwise addition of a salt-free polymer solution in an absorption cell with a Gilson micropipet.[1)] The molar absorption coefficients, ε, are defined both for dye–polymer solution and for the bound-dye species in the same manner as before.[6,7)] A series of titration spectra for a given dye–polymer system were analyzed by the extended PCA procedure.[6-8)]

Results and Discussion

Pure Spectra of Bound-dye Species and Equilibrium Constants.

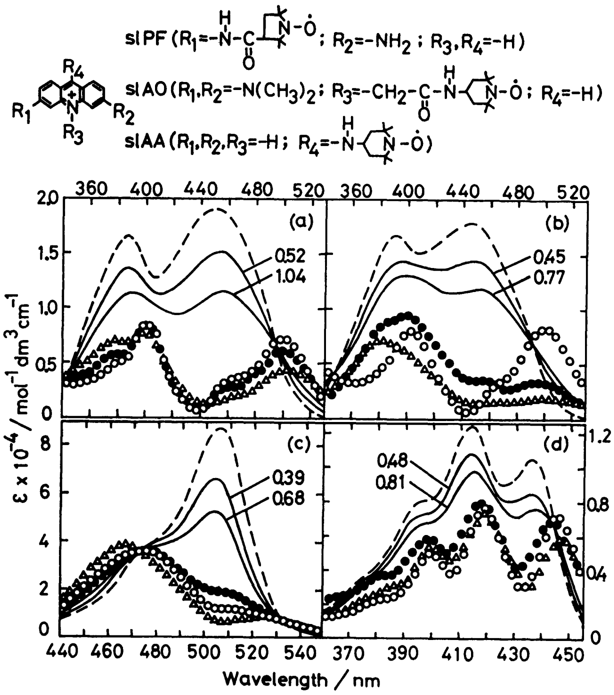

Fig. 1. Structural formulas of dyes and the bound-dye spectra extracted from the series of titration spectra of dye–polymer systems. (a) slPF, (b) AcPF, (c) slAO, and (d) slAA. Polymers bound by each dye are (○) NaPSS, (△) NaPP, and (●) NaPA. Broken lines are the spectra of dyes in the absence of polymers. Solid lines are typical spectra of the respective dye–NaPSS solutions, for which *P*/*D* values are indicated.

Figure 1 shows typical absorption spectra of each of four dyes in the presence and the absence of polyanions. For each dye–polymer system, five to nine titration spectra were measured at a neutral pH over a low *P*/*D* range (0 to 1—2), where *P* and *D* are the concentrations of a polymer in monomer unit and of a dye, respectively.[6)] Only a few observed spectra were drawn here, since the details are the same as those reported previously.[6,7)] The number of light-absorbing, independent species, *i.e.*, *components*, in each dye–polymer solution was concluded to be two, a free and a bound dye species, by comparing the eigenvalues of the correlation matrix.[6-8)] In order to apply the extended PCA procedure to each dye–polymer combination, therefore, use was made of the binding scheme with the apparent equilibrium constant *K* and the adjustable parameter α: $K=[DP^*]/[D][P]^{\alpha}$. The notations are all the same as before.[6-8)] Estimated values of *K* and α are given in Table 1.

The visible spectra of slPF and AcPF show two absorption bands (Figs. 1a and b) as a result of acylamination, differing from those of PF and Trypaflavine (TF).[2,4)] The monosubstituted slPF and AcPF bind

Table 1. The apparent equilibrium constants, K and K', and the parameter α for dye–polymer combinations at 25 °C

		slPF (23.3)[a]	AcPF (20.6)	slAO (5.42)	slAA (37.6)
NaPSS	α	1.3	1.1	1.3	1.2
	K	1.6×10^6	2.1×10^5	5.0×10^7	3.6×10^5
	K'[b]	5.6×10^4	6.8×10^4	9.6×10^5	4.2×10^4
NaPP	α	1.7	1.3	1.5	1.3
	K	2.4×10^8	9.8×10^6	1.2×10^8	3.9×10^5
	K'	8.6×10^4	2.8×10^5	2.1×10^6	1.6×10^4
NaPA	α	1.1	0.9	1.4	0.9
	K	4.1×10^4	9.2×10^6	1.2×10^7	9.0×10^2
	K'	1.4×10^4	2.8×10^4	8.3×10^4	2.5×10^3

a) Values in parentheses are the initial concentrations of the respective dyes in μmol/dm³. b) $K'=K[P]^{\alpha-1}$ calculated at $P/D=1$.

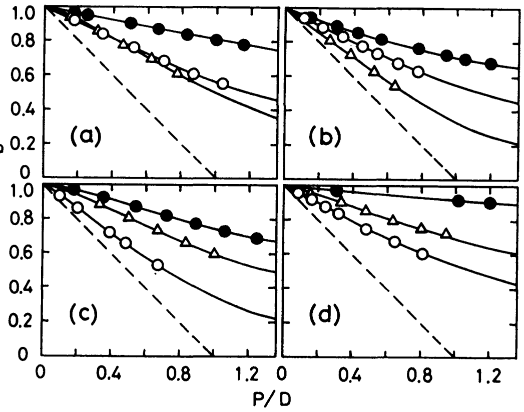

Fig. 2. The P/D dependence of the fraction of free-dye species remaining in the dye–polymer solution, $1-f_b$. (a) slPF, (b) AcPF, (c) slAO, and (d) slAA. The values obtained directly from the t matrix[6–8] are shown by the respective symbols which are the same as in Fig. 1. Solid lines are the binding curves which were calculated with values of K and α in Table 1.[6,7] Dashed lines indicate the relationship: $(1-f_b)=1-(D^b/P)(P/D)$, for which $D^b/P=1$, where D^b is the concentration of a dye species actually bound to the polymer site in solution.

to each of three polyanions, whose functional groups are phosphate, sulfonate, and carboxylate, giving rise to the bound-dye spectra, which resemble each other but differ from those obtained for symmetric TF.[6] Except for the case of AcPF–NaPP, each of the two bands of the bound-slPF or -AcPF spectrum is bathochromic and hypochromic relative to the corresponding band of the spectrum of free slPF or AcPF, showing a possible vibrational structure with energy separations of 1400—1650 cm^{-1};[9] the bound-dye spectra generally show neither hypsochromism nor a new *metachromasy band*, both of which are characteristic of a symmetric 3,6-diaminoacridine dye.[6] The bound-dye spectra of slAO (Fig. 1c) are typically metachromatic, showing the hypochromic effect with new broad bands—one on the longer and the other on the shorter wavelength side of the spectrum of free slAO.[6,10,11] The bound-dye spectra of slAA (Fig. 1d) are associated with a marked hypochromism and a modest bathochromic shift but without any metachromasy band; they are very similar to those of the unlabeled 9-aminoacridinium–polyanion systems reported previously.[7]

Fractions of Bound-dye Species. The fraction of bound-dye species in each solution, f_b, was calculated with values of K and α in Table 1.[6–8] The results are shown in Fig. 2, where the fraction of free-dye species remaining in solution, $1-f_b$, is plotted against P/D. Curvatures of the $(1-f_b)$ *vs.* P/D curves are mostly convex in the initial P/D range, corresponding to values of α larger than unity.[6,7] It is convenient to compare the values of $(1-f_b)$ at $P/D=1$, where equimolar amounts of a dye and a polyanion are present in solution. Spin-labeling lowers the fraction of bound-dye species slightly for slPF but considerably for slAA; the substitution of a label to the amino group may be responsible.[12] Polyacrylate shows a weak affinity toward each spin-labeled dye ($f_b<30\%$ at $P/D=1$) in a way similar to that noted for other dyes;[6,7] this is probably due to the weak acid nature of the side chain.

In conclusion, the spectra of spin-labeled acridine dyes bound to three representative polyanions are very similar to the bound-dye spectra of the corresponding nonlabeled dyes in the low P/D range and, therefore, these labeled dyes can be used for elucidation of metachromasy by the ESR and other magnetic methods.

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