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Spectral Behavior of 3,6-Substituted Aminoacridine Dyes with Emphasis on the Perturbation of the Exonuclear Amino Groups

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The optical absorption spectra of several derivatives of Proflavine (PF) and Coriphosphine O (CP) were measured in aqueous solutions at 25 °C. The effect of the perturbation of one or both of the exonuclear amino groups on the spectral behavior was studied in order to explore the relationship between the spectra and the chemical structures of the symmetric 3,6-disubstituted acridine dyes which can bind to various polyelectrolytes, often showing metachromasy. The metachromatic spectra of the complexes between 3,6-diaminoacridine dyes and polyanions were reproduced reasonably well when one of the two amino groups of PF was converted to an acylamino group. A similar result was observed when the amino group of CP was acetylated. In these cases, the spectra show two absorption bands of nearly equal intensity on both sides of the single peak of PF and CP in the visible region. These results are indicative of the close similarity in chemical structures between the monacylamino PF and the PF bound to the electron-rich sites of polyanions.

The symmetric 3,6-diaminoacridine dyes and their derivatives, such as Proflavine (PF), Trypaflavine (TF), and Acridine Orange (AO), show a deceptively simple absorption band in the visible wavelength region. Yet, a recent work has clearly shown that the spectral feature of this band is quite complex, actually consisting of two orthogonally polarized component bands with vibrational structures.[1] The visible spectra of those dyes are all metachromatic, exhibiting large hypo- and hypsochromic changes in solutions in the presence of various polyelectrolytes.[2] Because of this striking metachromatic behavior, the bathochromic change of the spectra has long been ignored, in spite of an early mention of the importance of the long-wavelength absorption component band associated with the dye-polymer complexes.[3] The pure spectra of TF bound to various polymers have indeed shown a long-wavelength band in addition to the short-wavelength band, their intensity being about equal.[4]

In contrast with the above cases, substitution on the 10- and/or 9-position of the acridine nucleus affects the absorption spectrum of acridine itself only slightly.[5] For example, 10-methylacridinium and 9-aminoacridinium both show a visible spectrum in which the short-axis polarized transition (the ^1L_a band) is well separated from the long-axis polarized transition (the ^1L_b band).[1,6,7] These two acridines also bind to some polymers,[8–12] but they show no appreciable metachromatic effect. The bound-dye spectra have revealed that both the short- and long-axis polarized bands shift toward the red slightly with a concomitant decrease in intensity.[11,12] Thus, introduction of amino groups into the 3- and 6-positions of acridine appears to be responsible for the large metachromatic spectra of dye–polymer complexes.

In the course of the study of spin-labeled acridine dyes, attachment of a nitroxide radical to one of the 3,6-amino groups of PF altered the visible spectrum of PF in such a way that the original peak was split into two bands.[13] Interestingly, the spectral profile of this monosubstituted-PF (slPF) resembled the spectra of TF bound to various polyions.[4] This remarkable change of slPF, however, disappeared and a PF-like simple spectrum was restored when both amino groups of PF were coupled with the radicals.[14] These results are suggestive of three possibilities: (1) The nitroxide radical effects the spectral splitting of the mother compound PF. (2) Conjugation of the newly produced acylamino group with the acridine ring perturbs the π-electron system. (3) Lowering of molecular symmetry from a C_{2v} to a less symmetric C_s group increases the separation between the short- and long-axis polarized components of the visible band. The present work was undertaken for the purposes of (1) discriminating these points, and (2) exploring the relation between the spectra of 3,6-substituted diaminoacridine dyes, in which an amino group or groups are perturbed, and the bound-dye spectra of dye-polymer complexes. For this purpose, the absorption spectra of a number of closely related acridine dyes were determined and correlated.

Experimental

Materials. Proflavine (PF), 3,6-diaminoacridinium chloride **1a**, was purified according to Albert.[5] Monoacetylated Proflavine (AcPF), 3-acetamido-6-aminoacridinium chloride **1b**,[15] and diacetylated Proflavine (Ac_2PF), 3,6-diacetamidoacridinium chloride **1c**,[16] were prepared by a standard acetylation procedure. Preparations of monopyrrolidinylated Proflavine (slPF), 6-amino-3-(1-oxy-2,2,5,5-tetramethyl-3-pyrrolidinyl-carbonylamino)acridinium chloride **1d**,[13,17] and dipyrrolidinylated Proflavine (sl_2PF), 3,6-bis(1-oxy-2,2,5,5-tetramethyl-3-pyrrolidinyl-carbonylamino)-acridinium chloride **1e**,[14,17] were described in detail elsewhere. Coriphosphine O (CP), 3-amino-6-dimethylamino-2-methylacridinium chloride **2a**, was purchased from Chroma Gesellschaft, Schmid & Co. and was purified by the silica gel thin layer chromatography, with an eluent system of 1-butanol–acetic acid–water (4:1:5). Acetylated Coriphosphine O (AcCP), 3-acetamido-6-dimethylamino-2-methylacridinium chloride **2b**, was prepared by acetylating CP with an acetic acid–acetic anhydride mixture at 118 °C. Crude AcCP was purified in a manner similar to CP. All chemical structures of dyes **1a** to **2b** are shown in Fig. 1.

Measurements. Absorption spectra were measured on a Hitachi model EPS-3T recording spectrophotometer at 25 °C. Optical titration of each dye was carried out to determine the p*K*-value, usually at the wavelength of maximum absorption in a concentration range of 5×10^{-5} M (1 M=1 mol/l) in the presence of 1.0×10^{-2} M Tris buffer. The pH values were controlled by adding either 0.05 M

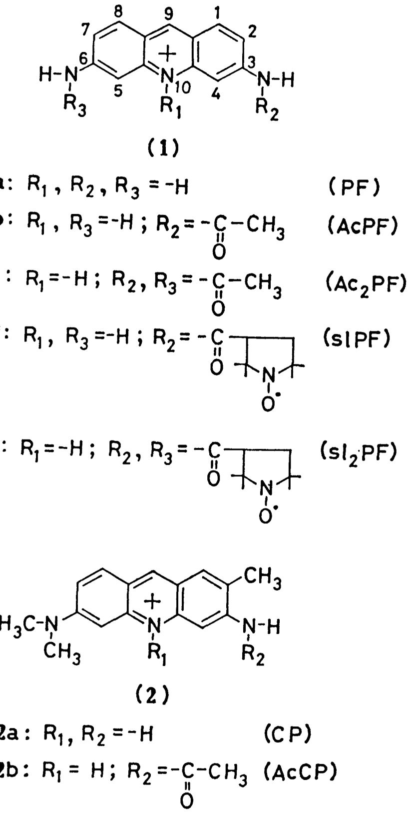

Fig. 1. Chemical formulas of acridine dyes utilized in this work.

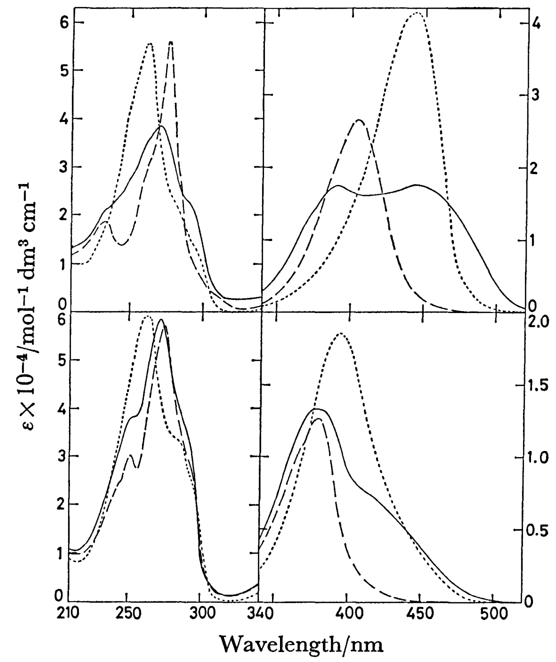

Fig. 2. Absorption spectra of AcPF and Ac_2PF in aqueous solutions.
The protonated forms in a pH range of 4—5 are shown in the upper half and the base forms in a pH range of 10—11 are in the lower half. AcPF (——) and Ac_2PF (— —). PF (······) is also shown for comparison.

HCl or 0.05 M NaOH; no precipitate of dye occurred. The molar absorption coefficient ε was expressed in M^{-1} cm^{-1}. The pH was determined with a Hitachi-Horiba F-7 pH meter.

Results and Discussion

Absorption Spectra of Acetylated Proflavines. The absorption spectra of mono- and diacetylated Proflavines, AcPF and Ac_2PF, are shown in Fig. 2, together with the spectrum of PF for comparison. In the upper half, the spectra of the protonated forms of these dyes are given. The spectrum of AcPF shows two absorption bands of nearly equal intensity in the visible wavelength region. Each band clearly consists of some subbands, probably the vibrational structure, which are more pronounced in organic solvents. Close examination indicates that these two bands are separated unevenly with respect to the original single peak at 444 nm of PF, *i.e.*, the longer-wavelength band is almost unshifted but the shorter-wavelength band is shifted by 50 nm toward the blue. It is quite interesting to note that a further acetylation of the remaining amino group of AcPF results in a simple spectral profile in the visible wavelength. This spectrum of Ac_2PF rather resembles that of PF, although their intensity and peak position differ from each other greatly. The UV spectra of these protonated dyes also vary with the degree of acetylation in such a way that the major peak at 262 nm of PF moves toward the long wavelengths.

In the lower half of Fig. 2, the spectra of the deprotonated (neutral or base) forms are shown. Again the effect of the perturbation of the exonuclear amino groups on the spectra is quite remarkable. Just as the removal of the proton from the 10-position gives rise to the hypo- and hypsochromic changes for PF, a very similar effect appears for the base forms of AcPF and Ac_2PF. The visible bands of the base forms are located in the 370—390 nm region regardless of the substituents at the 3- and 6-positions. The spectral behavior of the basic AcPF is most interesting, *i.e.*, the longer-wavelength peak diminishes to be just a shoulder near 410—420 nm, while the shorter-wavelength band remains. The visible and UV spectra of the base forms of PF and Ac_2PF are again similar to each other.

The results in Fig. 2 clearly show three points: The nonsymmetric substitution of either the 3- or 6-position of PF by an acetyl group gives rise to a new spectral feature, *i.e.*, the appearance of two absorption maxima. The symmetric substitution of both the 3- and 6-positions of PF reverts to the original feature of the single peak. Finally, the removal of a proton from the 10-position affects the spectral profiles hypso- and hypochromically.

Absorption Spectra of Spin-labeled Proflavines. The absorption spectra of mono- and dipyrrolidinylated Proflavines, slPF and sl_2PF, are shown in Fig. 3, together with the spectrum of PF for comparison. These protonated PF derivatives (upper half) behave surprisingly similarly to their acetylated counterparts. The visible spectrum of slPF shows two bands of about equal intensity, *i.e.*, the longer-wavelength band is

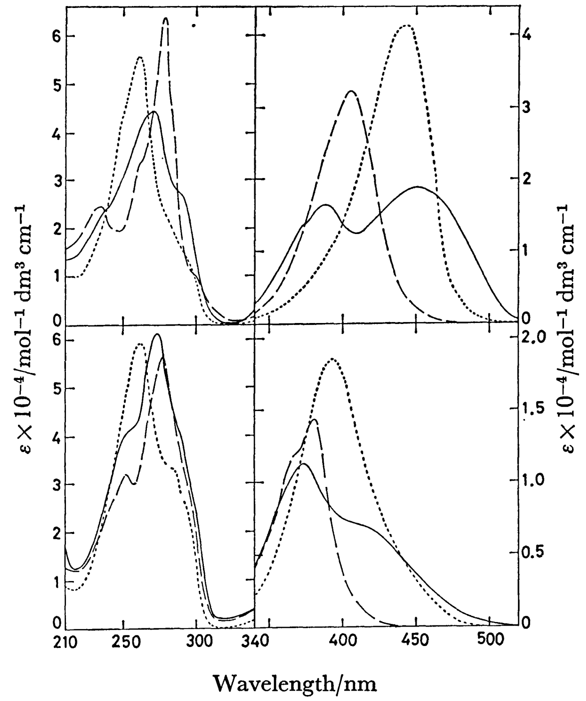

Fig. 3. Absorption spectra of slPF and sl$_2$PF in aqueous solutions.
The protonated forms in pH 4—5 are in the upper half and the base forms in pH 10—11 are in the lower half. slPF (——) and sl$_2$PF (— —). PF (······) is also shown for comparison.

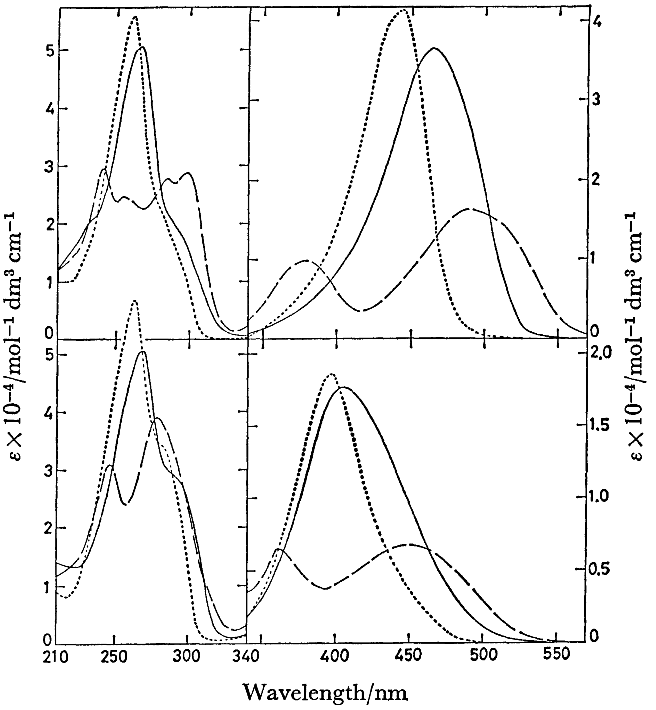

Fig. 4. Absorption spectra of CP and AcCP in aqueous solutions.
The protonated forms in the pH range of 4—5 are in the upper half and the base forms in the pH range of 10—11 are in the lower half. CP (——) and AcCP (— —). PF (······) is also shown for comparison.

at 450 nm, but the shorter-wavelength band is further apart by 60 nm from the peak of PF. The spectrum of sl$_2$PF, which is obtained by substitution of the remaining amino group of slPF, reverts to the simple spectrum of PF. The UV spectra of slPF and sl$_2$PF behave just like those of AcPF and Ac$_2$PF. It should be noted that the free nitroxide radical absorbs light very weakly, showing the maximum absorption at 398 nm ($\varepsilon=8.6$, absorbance$=0.18$ at 21 mM in aqueous solution). The results in Figs. 2 and 3 exclude the contribution of the radical to the observed spectra of slPF and sl$_2$PF in the visible wavelength region.

The spectra of the deprotonated forms of PF, slPF, and sl$_2$PF are shown in the lower half of Fig. 3. The removal of a proton from the 10-position affects each spectrum in the very same way as in the case of the acetylated counterparts. Comparison of the data in Fig. 3 with those in Fig. 2 clearly indicates that the simultaneous substitution of two amino groups on the 3,6-positions by either acetamido or pyrrolidinyl radical groups results in the spectral feature closely related to that of PF itself. This is conceivable in the sense that the substitution at both positions retains the molecular C$_{2v}$ symmetry for all PF, Ac$_2$PF, and sl$_2$PF. Yet, it is surprising to see that the conversion of each exonuclear amino group to an acylamino group restores the original band structure.

Absorption Spectra of Coriphosphines. Now, a question arises: Is the appearance of two bands of nearly equal intensity in the visible region due to the perturbation of one of the two exonuclear amino groups? Alternatively, does the breakdown of molecular symmetry from the C$_{2v}$ to the C$_s$ point group result in two bands instead of one? The answer is given in Fig. 4, where the absorption spectra of CP and its acetylated derivative, AcCP, are shown. The structure of CP differs from PF in that a methyl group is attached at the 2-position of the acridine nucleus and one of the amino groups is substituted by the dimethylamino group (Fig. 1). Hence, the molecular symmetry of CP is lower than the C$_{2v}$ and may be considered to be C$_s$. Yet, the absorption spectrum of CP is close to that of PF, showing a single band at 465 nm in the visible region.

The spectrum of AcCP clearly shows two absorption bands at 490 nm and 381 nm, just as the cases of AcPF (Fig. 2) and slPF (Fig. 3). Results in Fig. 4 at once ascertain that the lowering of the symmetry of PF as such does not cause the splitting of the single peak into two peaks of nearly equal intensity. This view is further supported by the acetylation of the amino groups of CP. The base forms of CP and AcCP behave similarly to the deprotonated PF dyes, as is shown in the lower half of Fig. 4. A noticeable difference is that the longer-wavelength peak of AcCP remains as a distinct peak even when the proton is removed from the 10-position, In conclusion, it is most probably the acylation of one, but not both, of two amino groups on the 3,6-positions that results in the new spectral feature, *i.e.*, the development of two bands.

*Comparison of Optical Properties of Dyes **1a**—**2b**.* Since systematic studies of the effect of the substitution of particular groups on the spectra of the 3,6-disub-

TABLE 1. SPECTRAL PROPERTIES AND pK-VALUES OF DYES **1a**—**2b**

Dyes	pK	$\varepsilon \times 10^{-4}$/mol^{-1} dm^3 cm^{-1} ($\lambda_{max}$)$^{a)}$ in water	
		Cationic form	Base form
PF	9.11$^{b)}$	4.15(444)	1.86(395)
AcPF	8.20±0.09	1.75(446), 1.74(392)	0.65(420), 1.33(377)
Ac$_2$PF	6.25±0.03	2.67(405)	1.27(381), 0.99(365)
slPF	7.98±0.03	1.88(452), 1.64(389)	0.67(420), 1.13(375)
sl$_2$PF	5.88±0.04	3.23(406)	1.44(381), 1.14(365)
CP	9.85±0.05	3.64(465)	1.76(405)
AcCP	8.38±0.02	1.61(490), 0.99(381)	0.67(450), 0.65(362)

a) The value in the parentheses is the peak or shoulder wavelength in nm. b) This value is taken from Ref. 19.

stituted acridine dyes are few, it is appropriate to compare their spectral properties. The data are summarized in Table 1. In the concentration range covered in this work, the Beer-Lambert law holds for each dye in aqueous and ethanolic solutions. Thus, the possibility that the changes in all spectra result from dye-aggregation can be excluded. The pK-values were determined for dyes **1a**—**2b** by optical titration. It is clear from Table 1 that conversion of amino groups to acylamino groups decreases the pK-value in the order of unsubstituted>monosubstituted>disubstituted. This is in conformity with other cases,5) and the electron-donating tendency of an amido group may be responsible for it. The pK-value of 6.25 for AcPF is in good agreement with a previously reported value of 6.2.18)

The symmetric 3,6-disubstituted Ac$_2$PF and sl$_2$PF should belong to the C_{2v} group as PF and, consequently, their apparently simple visible band should contain the long- and short-axis polarized bands.1) It would be helpful to identify the directions of the polarization of the two clearly visible absorption bands for AcPF and slPF. However, these transition moments are no longer polarized along the short- and long-axes in a molecular plane under symmetry considerations. Individual transition moments may be determined by the film dichroism method.1)

All dyes examined in this work bind to sodium polyphosphate, exhibiting the hypso- and hypochromic effects. It is worth noting that the pure spectra of TF bound to various polyelectrolytes are very close to the spectra of AcPF and slPF, each of which contains an exonuclear acylamino group. This apparent similarity suggests that one of the two amino groups of PF or TF in the dye-polymer complexes may be electronically affected by the electron-rich polymer sites such as $-COO^-$, $-SO_3^-$, and $-PO_2^-$- by still unclarified reasons. In this connection, the interaction of AcPF and other dyes described in this work with polyions should be studied to obtain the pure spectra of the bound dyes.19) Molecular orbital calculations capable of taking into account the substitution effect on the exonuclear groups should also be carried out to elucidate these profound spectral changes on a more quantitative and systematic basis.

Conclusion

The absorption spectra of the symmetric 3,6-disubstituted acridine dyes show a single band in the visible wavelength region regardless of the bulkiness of the substituents (PF, Ac$_2$PF, and sl$_2$PF). Conversion of an amino group to an acylamino substituent gives rise to the spectrum which shows two bands of nearly equal intensity. This spectrum resembles the pure spectrum of a 3,6-diaminoacridine bound to various polyelectrolytes. The mere decrease of the molecular symmetry from C_{2v} to C_s cannot result in such a drastic spectral change. The π-electron system of the acylamino group at either the 3- or 6-position must interact with the π-electron system of the acridine nucleus by conjugation.

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