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SPIN-LABELED METACHROMATIC DYES. I. ESR AND SOME OPTICAL PROPERTIES OF SPIN-LABELED PROFLAVINE IN SOLUTION, LIQUID CRYSTAL, AND STRETCHED FILM

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Proflavine was spin-labeled with a stable nitroxyl radical for the study of metachromasy. The magnetic and optical properties of the spin-labeled dye were determined in aqueous solutions (isotropic) and oriented media (anisotropic) by ESR and light absorption. The interaction with the sodium dodecylsulfate micelle was also shown.

The metachromatic change of color of certain cationic dyes, which results from the interaction between dye and biological polyelectrolyte in aqueous solution, has long been of great interest in histo- and cytochemistry. A number of hypotheses have been advanced to elucidate the mechanism for this phenomenon metachromasy.1) The proposed hypotheses are almost solely based on the optical data of the dye-polyelectrolyte and are often difficult to be verified by experiments, because these also employ optical techniques and, as a consequence, arguments inevitably end in a circle. Hence, a new approach, which is based on a nonoptical or combined optical-nonoptical method, is needed to the study of metachromasy. The method should be sensitive to the same extent as the optical one and preferably give information on the local properties of the binding site of dye-polyelectrolyte complex.

We have designed a variety of spin-labeled metachromatic dyes, which can bind to polymers, by chemically attaching a simple and stable nitroxyl radical to a specific position of dye. The use of such dyes should allow the dye-polyelectrolyte interaction to be detected by two independent physical methods of ESR and light absorption. We wish to report a successful preparation of 3-N-(3'-carbonyl-1'-oxyl-2',2',5',5'-tetramethylpyrrolidinyl)-3,6-diaminoacridinium chloride ((**I**) in Fig. 2) or spin-labeled Proflavine (slPF) and its ESR and optical properties in solution, liquid crystal and stretched poly(vinyl alcohol) (PVA) film. The interaction of slPF with aggregates of small ions is exemplified by using the surfactant sodium dodecylsulfate (SDS) above the critical micelle concentration (CMC), since metachromatic dyes have been utilized for such studies.2) In the next paper, the application of slPF to the study of the interaction with polyphosphates will be presented.

Synthesis of slPF. slPF was synthesized by reacting the acid chloride3) of 3-carboxy-2,2,5,5-tetramethylpyrrolidinyl-1-oxy free radical (**II**) (Aldrich Chemical Co.), in the presence of equimolar pyridine, with an excess of pure 3,6-diaminoacridine (Proflavine in the base form) in anhydrous tetrahydrofuran. The crude slPF was purified first by means of silica gel (Merck 60 H) column chromatography (eluent: chloroform 3: ethanol 1) and then by recrystallization from an ethanol solution by dropwise addition of ethylether. Anal. calcd for $C_{22}H_{26}N_4O_2Cl\cdot 3/2H_2O$: C, 59.96; H, 6.58; N, 12.71. Found: C, 59.83; H, 6.34; N, 12.52. The aqueous solution of s1PF was stable in the neutral pH over a month when stored in the dark.

Measurements. ESR and light absorption were measured on a JOEL JES-ME-3X X-band spectrometer at ca. 20°C, the modulation width being 0.5 G, otherwise stated, and on a Hitachi EPS-3T spectrophotometer at 25°C, respectively. Linear dichroic absorption spectra of s1PF in the stretched PVA film were measured on an apparatus already reported.[4)] For the oriented ESR spectra, s1PF (0.1 wt%) was added to a smectic phase liquid crystal of sodium caprylate (25%), *n*-decanol (45%) and water (30%).[5)] The mixture was let into a parallel-plate quartz cell (4x0.8x0.005 cm).

ESR and Absorption Spectra of s1PF in Aqueous Solutions. Both ESR (upper) and optical absorption (lower) of s1PF are shown in Fig. 1. The ESR spectrum of s1PF is almost the same as that of the nitroxyl radical (**II**) in dilute aqueous solution. The isotropic nitrogen hyperfine coupling constant and rotational correlation time[6a)] τ_c were found to be 16.0 G and 0.08 nsec for s1PF (16.0 G and 0.03 nsec for the radical), respectively. The absorption spectrum of s1PF is different from that of Proflavine (PF) which shows a broad single peak at 444 nm with a molar absorption coefficient ε of 4.20×10^4. The peak of PF split in s1PF into two bands at 452 nm and 389 nm with ε of 1.97×10^4 and 1.70×10^4, respectively. Beer's law was obeyed at concentrations lower than 0.1 mM, where the deviation was less than 5%. With inreasing the concentration, the intensity of the absorption spectrum of s1PF gradu-

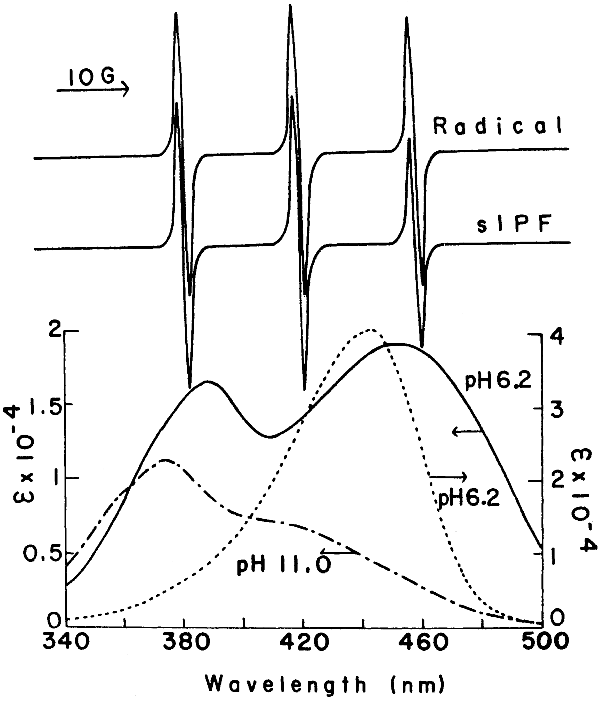

Fig. 1. The ESR spectra of s1PF and the nitroxyl radical (**II**) (——) and the absorption spectra of s1PF (——) and PF (------) together with the neutral s1PF (— - - —) in aqueous solutions.

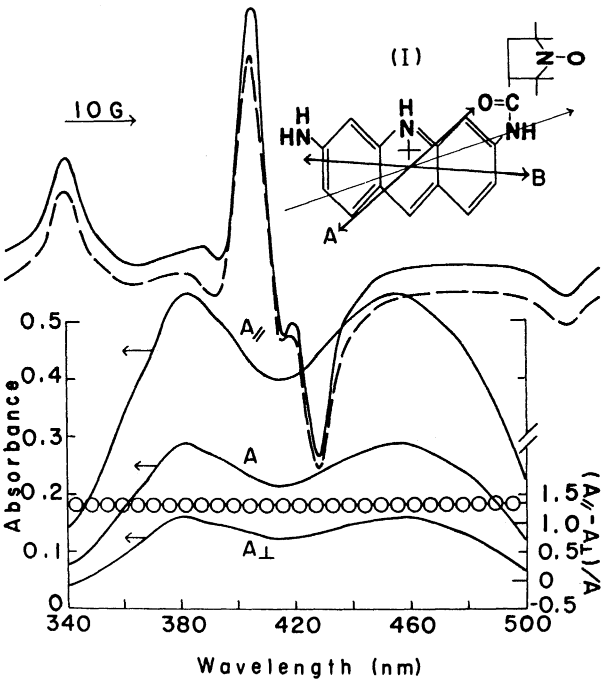

Fig. 2. The parallel (——) and perpendicular (— —) ESR and the isotropic, parallel, perpendicular absorption and reduced dichroic (-O-O-) spectra of s1PF in the stretched PVA film.

ally decreased, whereas the shape of the corresponding ESR spectrum did not change. slPF, which is cationic in the neutral pH, undergoes deprotonation at alkaline pH (pK=7.8±0.1). This deprotonated or neutral slPF shows a different absorption spectrum (Fig. 1). The radical portion of slPF contains an asymmetric carbon; however, this radical-coupled dye is probably a racemic mixture, because no Cotton effect was observed in the visible absorption band 340-500 nm in aqueous solution.

Linear Dichroic Spectra of slPF Oriented in the Stretched PVA Film. The slPF, which remains protonated and unassociated in the PVA film, can be oriented unidirectionally along the stretching direction of the film as shown in Fig. 2. The linearly-polarized absorption spectra of slPF parallel $A_{\parallel}$ and perpendicular $A_{\perp}$ to the direction are shown together with an isotropic spectrum A at a stretch ratio of 4.3. Throughout the visible wavelength region, $A_{\parallel}$ is always intense as compared with $A_{\perp}$, while the reduced dichroic spectrum $(A_{\parallel} - A_{\perp})/A$ is essentially flat. By analyzing the stretch ratio dependence of $(A_{\parallel} - A_{\perp})/A$,4) the transition moments responsible for the maxima at 460 and 380 nm were both found to make angles of ±(23.0 ± 0.4°) with respect to an orientation axis. If the orientation axis of slPF as a whole is assumed to be in the direction in the plane of the dye chromophore as given by an arrow in Fig. 2, the directions of the two transition moments can possibly be each along the double-arrows A and B. The corresponding ESR spectra of slPF in the stretched PVA film are lacking in the angular dependence with respect to the magnetic field (Fig. 2). The radical moiety of slPF should be immobilized in the film and oriented randomly relative to the dye chromophore. Together with the ESR data in solutions, the dichroic study leads to the conclusion that the rotation of the bond between the dye and radical halves is practically unrestricted in the absence of external perturbations.

Anisotropic ESR Spectra of slPF Oriented in the Liquid Crystal. slPF can be oriented almost perfectly in the caprylate-decanol-water system as shown in Fig. 3, where the ESR spectra parallel and perpendicular to the direction of the magnetic field are given. The presence of a small amount of unoriented slPF is indicated by arrows. The orientation order S_{33} of the $2p_{\pi}$ orbital (taken as the z-axis) of the nitroxyl radical with respect to the director (*i.e.*, the long axis of the oriented molecules constituting the smectic mesophase liquid crystal) was evaluated to be 0.44.6b) With the value of S_{33}, the average angle between the z-axis and the director was estimated to be ca. 40°. Because of a large effect of light scattering, no information was obtained on the orientation of the dye chromophore of slPF or on the relative disposition between the dye and radical halves in the liquid crystal.

slPF Solubilized in the Micelle of SDS. An application of slPF to nonpolymeric system is demonstrated in Fig. 4. The charged or uncharged slPF was equally solubilized in SDS (11.0 mM) above CMC (8.3 mM), although the precipitates appeared at the concentration of SDS below the CMC. The τ_c values for the SDS-slPF system were estimated to be 0.79 and 0.66 nsec at pH 6.2 and 11.0, respectively.6a) The absorption spectra of the charged and uncharged slPF in SDS each are bathochromic and hyperchromic relative to slPF (Fig. 4). These optical features indicate that both charged and uncharged dye chromophores of slPF may be located within an apolar environment of the micelle.2) The polar exonuclear amino groups of slPF are considered to extrude toward the polar region of the water layer surrounding the

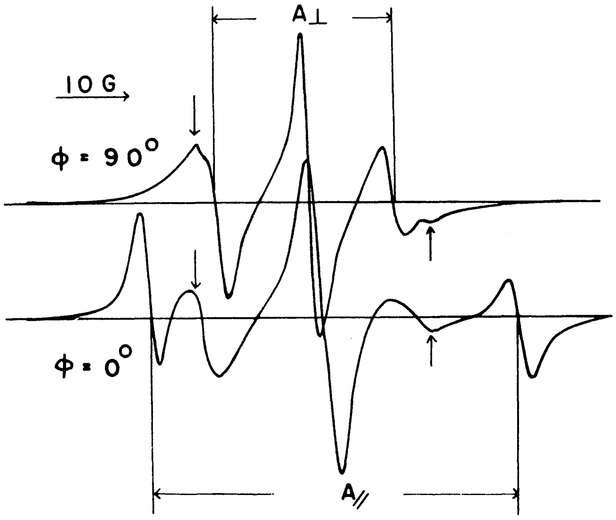

Fig. 3. The ESR spectra of slPF oriented in the smectic liquid crystal. φ is defined as the angle between the normal to the bilayers of the mesophase and the magnetic field.

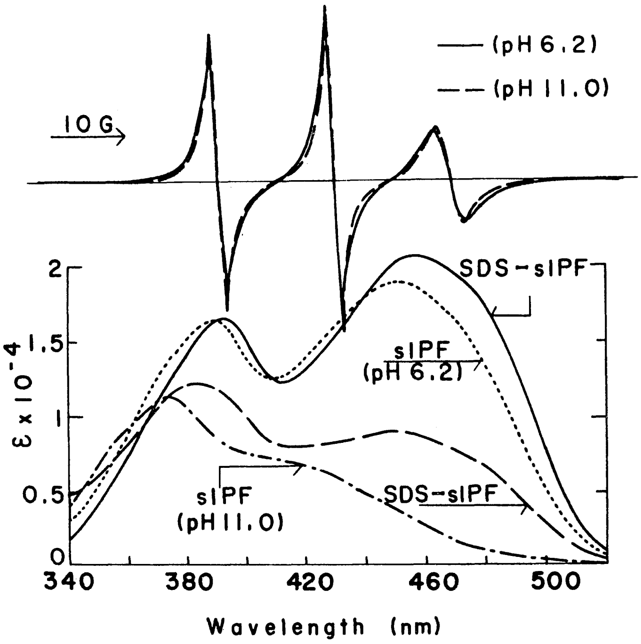

Fig. 4. The ESR and absorption spectra of charged (pH 6.2) and uncharged (pH 11.0) slPF in aqueous SDS solutions above CMC.

micelle on the ground that the rotational motion of the radical moiety is not strongly restricted.

The use of slPF appears to be promising for the study of metachromasy and interaction between dye and polyelectrolyte and to give information which may not be obtained by the conventional optical spectroscopic method. When the optical and magnetic properties of a well-designed metachromatic spin-labeled dye are fully understood, the dye may be utilized as an optical-magnetic double probe for the conformation of macromolecules and their complexes with small compounds.

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