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SPIN-LABELED METACHROMATIC DYES. II. INTERACTIONS OF SPIN-LABELED PROFLAVINE WITH POLYPHOSPHATE AND SOME DYE-POLYPHOSPHATE COMPLEXES

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Metachromasy was studied by means of ESR and light absorption using a spin-labeled Proflavine (s1PF) which interacts with polyphosphate. The exchange interaction between bound s1PF's was observed. Qualitative correlations were found between the magnetic and optical data. s1PF could be utilized as a probe for dye-polyphosphate complexes.

The phenomenon metachromasy arises from the complicated optical behavior of a metachromatic dye bound to polyelectrolyte in aqueous solution and involves both the chemical structure of dye and polyelectrolyte and the binding equilibrium between the two components. Metachromasy has been studied by the optical method with sodium polyphosphate (NaPP) which is a simple polyelectrolyte and yet bound by a number of metachromatic dyes such as Crystal Violet (CV)[1] and Trypaflavine (TF).[2] No metachromatic change was observed for the dye-NaPP system at a very high mixing ratio of polymer residues to dye (P/D) where the dye was considered as totally bound. However, metachromasy fully developed in the P/D range 3-10 for CV and 2-10 for TF. These findings have led to the conclusion that the binding mode of dye to polyphosphate is general but the spectral behavior of the bound dye is specific.

As already emphasized,[3] the availability of a variety of physical methods not based on the optical principle is crucial for the further understanding of metachromasy. For example, a dye chemically coupled with a stable radical should be a useful probe with which the ESR study of metachromasy becomes possible. Since the ESR and optical properties of a spin-labeled Proflavine (s1PF) has been reported in the preceding paper,[3] it appears timely to pursue both the study of metachromasy with s1PF and polyphosphate and the possibility of utilizing s1PF as a structural probe for the unlabeled CV- and TF-NaPP complexes by the method of ESR. The present results showed that the ESR spectra of s1PF change drastically in the presence of NaPP in the P/D range 2-1000. At a low P/D range, the spin exchange interaction between the bound s1PF was clearly observed. In the s1PF-unlabeled dye-NaPP complex, the magnitude of the exchange interaction varied with the ratio of s1PF to either CV or TF. The possibility of dye-stacking on NaPP was discussed with the present ESR data.

Materials. NaPP samples are the same as used previously.[1] The number-average degrees of polymerization ($\bar{n}$) are 158 and 148. The dyes CV,[1] TF,[2] and s1PF [3-N-(3'-carbonyl-1'-oxyl-2',2',5',5'-tetramethylpyrrolidinyl)-3,6-diaminoacridinium chloride][3] are all the same samples as already described.

Procedures. The s1PF-NaPP solutions were prepared by adding the stock dye

solution (0.4 mM) dropwise to the aqueous NaPP solution. The final concentration of s1PF was kept constant at ca. 0.1 mM. s1PF was added to the CV- or TF-NaPP system at a given P/CV or P/TF to prepare the ternary s1PF-unlabeled dye-NaPP system at a desired P/D_t, where D_t is the total concentration of s1PF and either CV or TF. The pH of all the solutions was maintained in the range 6-7.

Measurements. Both ESR and absorption spectra were measured as before.[3]

P/D Dependence of Interactions between s1PF and NaPP. The interaction between s1PF and NaPP ($\bar{n}$=158) is shown in Fig. 1. Metachromasy develops effectively in the absorption spectra at a relatively high P/D (already at 200), as previously reported for TF.[2] The ESR spectrum of the s1PF-NaPP system at a P/D of 200 is composite of sharp and broad signals. Since almost all of s1PF in the solution appear to be bound,[2] the sharp triplet lines may be due to the rapid isotropic rotation of the loosely and isolatedly bound s1PF as a whole or the radical moiety thereof only. The broad spectrum, whose intensity increases with a decrease in P/D, reflects that some of the bound s1PF approach one another close enough to show the spin exchange interaction. In the ESR spectrum at a P/D of 50, which signifies only three s1PF per NaPP polymer on the average, the broad signal further enhances. The ESR spectrum at a P/D of 5 shows a very broad single line on which the sharp hyperfine structure is barely discernible. This broadening is mainly due to the exchange interaction which probably results from the stacking of s1PF on NaPP, whereas the sharp component lines result from both unbound and isolatedly bound s1PF. The fact that the broad signal does remain at a high P/D (~1000) suggests that some radical moieties at least can remain close together even at such a P/D giving rise to the spin exchange. Based on the result of the s1PF-NaPP system, the usefulness of s1PF as a structural probe was examined below in the CV- and TF-NaPP complexes.

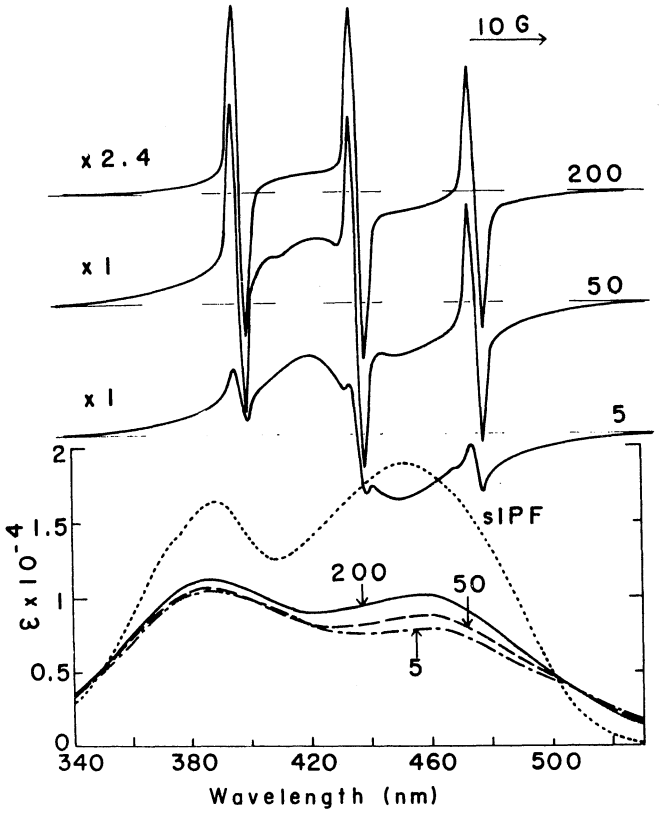

Fig. 1. The ESR and absorption spectra of some s1PF-NaPP systems in aqueous solution. Numerals indicate the P/D value.

ESR of s1PF Incorporated into the TF-NaPP Complex. Since metachromasy of TF bound to NaPP is most pronounced in the P/TF range 2-10,[2] s1PF was incorporated into the TF-NaPP system by keeping the P/D_t at ca. 3 and varying the TF/s1PF value from 0 to 14. As shown in Fig. 2, the absorption spectrum of the s1PF-TF-NaPP system is hypo- and hypsochromic relative to TF indicating that the ternary system

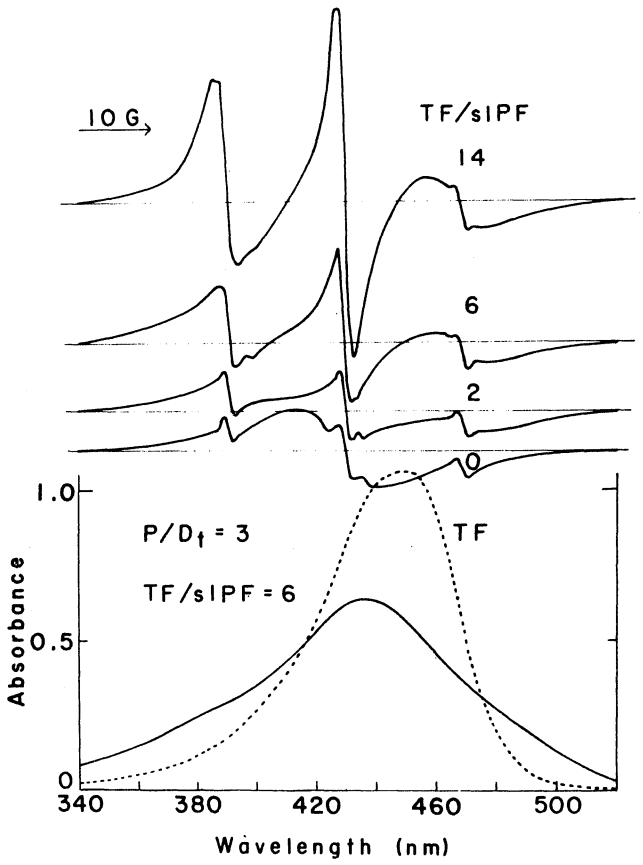

Fig. 2. The ESR spectra of s1PF incorporated into TF-NaPP complexes at various TF/s1PF, the values of which are indicated by numerals, and the absorption spectra of a s1PF-TF-NaPP system at TF/s1PF 6 and TF (------). P/D_t was kept constant at 3.

behaves like the TF-NaPP system. The ESR spectrum of the s1PF-TF-NaPP complex at a TF/s1PF of 14 is composite of two signals: the broad and sharp triplets. In comparison with the ESR spectra previously reported,[4a,b] the broad signal, which is strong but almost void of the exchange broadening, indicates that the radical moiety of s1PF rotates with a rotational correlation time of 4∿7 nsec. Consequently, s1PF is separately distributed and embraced by some of TF in the complex. The sharp signal, which is always weak, may be attributed to both unbound and monomerically- and isolatedly-bound s1PF. As the TF/s1PF value decreases, the broad triplet steadily broadens and eventually converges to the broad singlet which is overlapped by the sharp triplet (TF/s1PF=0 in Fig. 2 or P/D=5 in Fig. 1).

This fact indicates that the magnitude of the exchange interaction of the s1PF incorporated into the TF-NaPP complex varies with the value of TF/s1PF. In the ternary complex, s1PF is unlikely to form self-associative clusters which are likely to exist in the s1PF-NaPP complex. Hence, the average distance between the radical moieties (or the dye chromophores) of s1PF probably shortens in the ternary complex with a decrease in TF/s1PF values.

ESR of s1PF Incorporated into the CV-NaPP Complex. Figure 3 shows some results obtained by the use of another dye CV which structurally differs from TF and exhibits a distinct metachromasy band at 506 nm in the P/CV range 3-10 in the presence of NaPP.[1] The presence of s1PF in the CV-NaPP complex hardly affects the spectral features except that the band is located at 520 nm. The ESR spectra of s1PF added to the CV-NaPP complex differ from those in Fig. 2 showing a marked anisotropy in the CV/s1PF range 10-5. This anisotropy results from the restricted motion of the radical moiety probably due to the steric hindrance imposed on s1PF by the surrounding CV. With a decrease in CV/s1PF, the anisotropic features gradually disappear and a broad singlet emerges (CV/s1PF=1 in Fig. 3). This trend of broadening was likewise observed in the s1PF-TF-NaPP complex (Fig. 2). However, the rotational correlation time of the bound s1PF is longer in the CV-NaPP system than in the TF-NaPP system. This difference must be attributed to the chemical structure

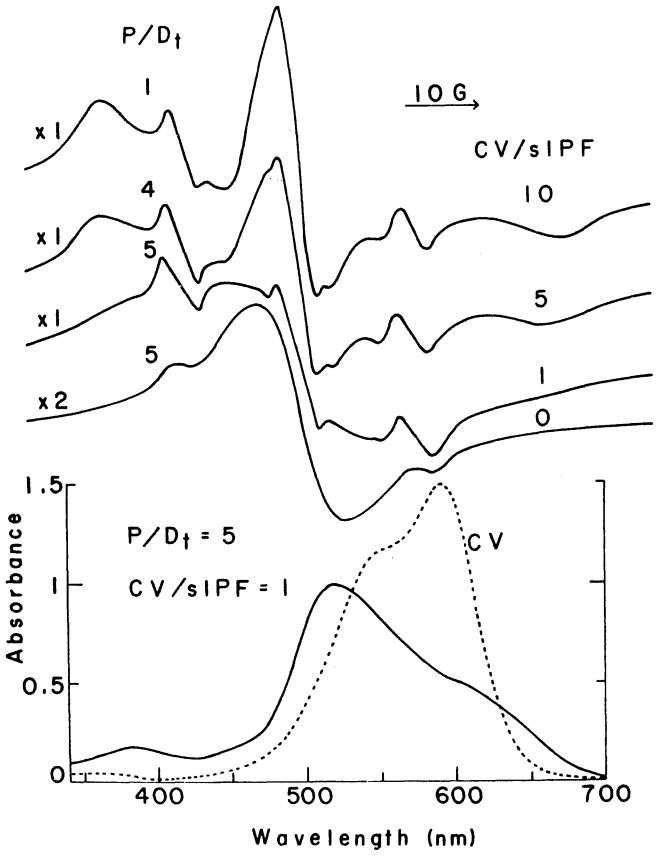

Fig. 3. The ESR spectra of sIPF incorporated into CV-NaPP complexes. Modulation width was 5 G. The absorption spectra of the sIPF-CV-NaPP system at CV/sIPF=1 and CV (------). Numerals are P/D_t and CV/sIPF.

and binding ability of CV and TF and influenced by both the rotational restriction of the radical moiety of sIPF and the closest distance between sIPF and either CV or TF in the complex.

If the broad ESR signal (the exchange interaction and/or slow motion) of sIPF represents the dye aggregates on polymer in the above three examples, sIPF should be clustered together or incorporated into the bound CV or TF in a low P/D_t range. Thus, the remaining problems are to clarify, on a quantitative basis, if there exists a relation between the line broadening of ESR signals and the formation of dye aggregates and if such broadening is correlated to the development of metachromasy. The structure of the aggregates and the metachromatic change of spectra exhibited by the bound dye should be finally elucidated on the molecular basis. In addition to sIPF, which has been proven a useful magnetic probe, spin-labeled dyes of different types should no doubt be necessary. Qualifications of a spin-labeled dye as the optical-magnetic double probe, which is our ultimate objective, are that it should be sensitive both optically and magnetically to respond to conformational changes of polymers, that the absorption coefficient should be high and that both absorption and ESR spectra are simple and isolated. Detailed work is now in progress and will be reported shortly.

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