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ESR AND OPTICAL STUDY OF SPIN-LABELED ACRIDINE DYE-DNA COMPLEXES: LOCAL ENVIRONMENT OF THE DYE-DNA BINDING SITE[1)]

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Interactions of five spin-labeled acridine dyes with DNA were studied by the ESR and optical methods. The binding of the dye chromophore to DNA was evident from the change of absorption and the appearance of extrinsic circular dichroism. The radical moiety was found to rotate anisotropically regardless of the labeled positions.

Aminoacridines and other related dyes are known to be antibacterial and mutagenic. Interactions of these dyes with biopolymers such as nucleic acids have been studied extensively by means of optical spectroscopy.[2,3)] However, the results obtained from optical techniques alone are often insufficient to discuss the structural features of acridine dye-DNA complexes. The interaction of a spin-labeled dye with DNA can be detected by two independent methods of light absorption and ESR. A spin-labeled Proflavine (slPF) has been shown to give detailed structural information on the dye-DNA interaction not obtained by ESR or optical method alone: The radical of slPF bound to DNA revealed local motions around the labeled amino group.[1)]

Since the radical moiety of a spin-labeled dye acts as a reporter group giving accounts of the rotational freedom of the local environment in which it resides, the spin-labeled position of the dye is very important in investigating the geometry of the site of the dye bound to DNA. Until now no systematic investigation has been reported on the relation between the rotation of a radical and the labeled position of a dye bound to DNA. We wish to present some results of structural features around the binding site of the dye-DNA complex obtained with a series of spin-labeled dyes whose synthesis will be described elsewhere.

Materials and Procedure. Newly synthesized spin-labeled acridine dyes were 3,6-bis(dimethylamino)-10-(N'-(1'-oxyl-2',2',6',6'-tetramethyl-4'-piperidyl) carbamoylmethyl)acridinium chloride (I), 3,6-bis(dimethylamino)-10-(4'-(1'-oxyl-2', 2',6',6'-tetramethylpiperidyl)ethyl)acridinium chloride (II), 3-(1'-oxyl-2',2',5', 5'-tetramethylpyrrolinyl-3'-carboxamide)-6-aminoacridinium chloride (IV), and 3,6-bis(1'-oxyl-2',2',5',5'-tetramethylpyrrolidinyl-3'-carboxamide)acridinium chloride (V). A spin-labeled 9-aminoacridine, 9-N-(1'-oxyl-2',2',6',6'-tetramethylpiperidyl) aminoacridinium chloride (III), was synthesized and purified according to Sinha *et al.*[4)] The molecular weight of calf thymus DNA (Worthington Biochemical Corp.) was *ca.* 4.5×10^6 daltons. The preparation of the stock native DNA solution and the definition of the mixing ratio of the DNA phosphate to dye (P/D) were given elsewhere.[5)] In each sample solution containing 0.75 mM(=mmol·dm^{-3}) NaCl (pH 6.4-6.8), the final

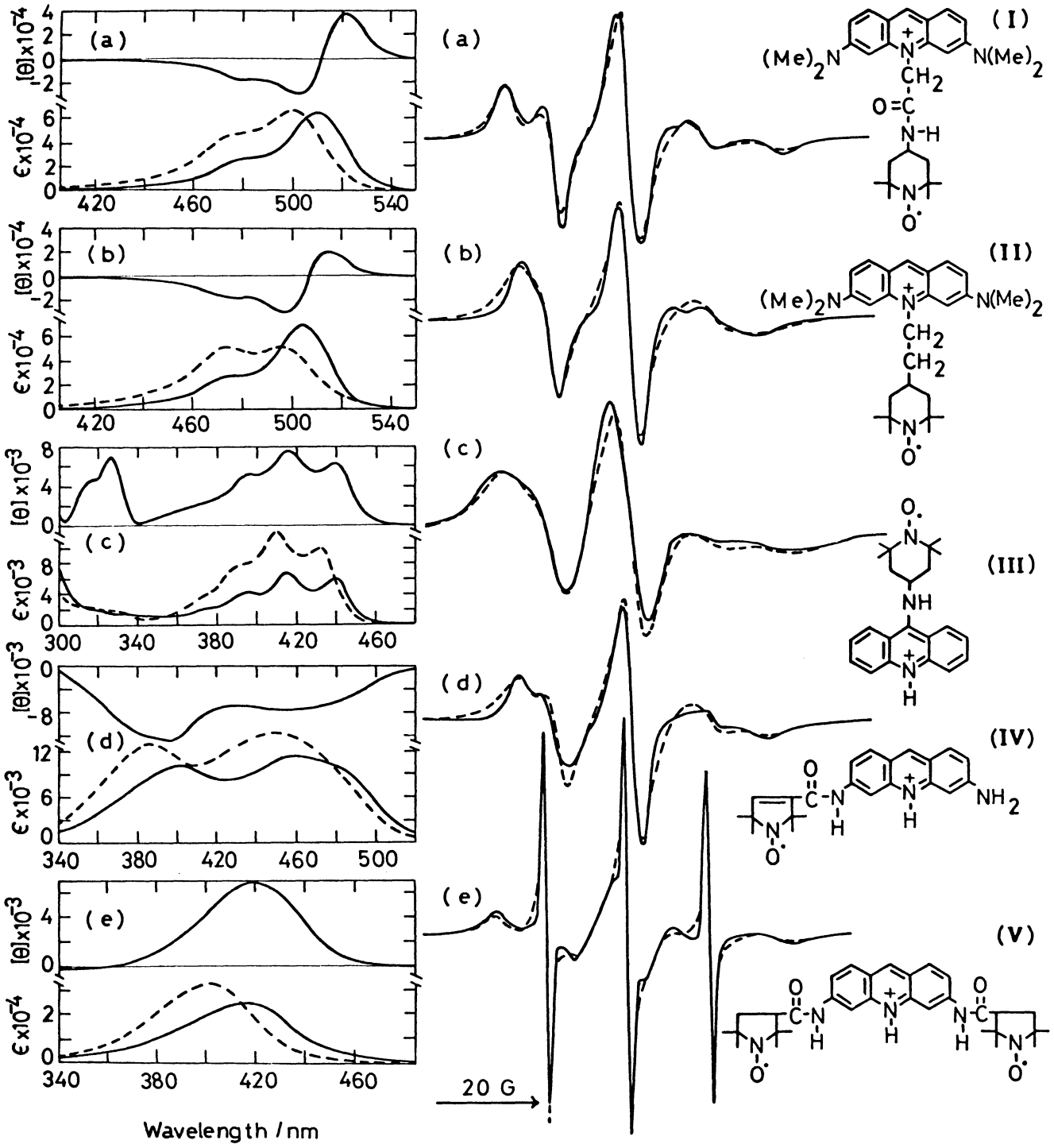

Fig. 1. The absorption and CD spectra of spin-labeled dyes in the presence of DNA (——): (a), I; (b), II; (c), III; (d), IV; and (e), V. P/D values are listed in Table 1. The absorption spectra of dyes alone are also shown (----).

Fig. 2. Observed (——) and simulated (----) ESR spectra of spin-labeled dye-DNA. P/D values and the ESR parameters are listed in Table 1. The modulation widths were 1.0 G for (a), (b), and (d), 3.2 G for (c), and 0.5 G for (e).

concentration of DNA phosphate was 2.1 mM and that of dye was ca. 0.1 mM.

Measurements and ESR Simulation. ESR and absorption spectra were measured at 20°C and ESR simulation was carried out in the same manner as before.[1] Circular dichroism (CD) was measured on a JASCO J-40S spectropolarimeter at 20°C.

Absorption and CD of Spin-labeled Dyes in the Presence of DNA. The absorption and CD spectra of spin-labeled dyes (I-V) in the presence of DNA are shown in Fig. 1, where ε is the molar absorption coefficient in $cm^{-1}mol^{-1}dm^3$ and $[\theta]$ is the molar ellipticity in $deg \cdot cm^2 decimol^{-1}$ in terms of the total dye concentration in solution. Binding with DNA is evident from the bathochromic shift of absorption maxima and the appearance of extrinsic Cotton effects.[1,2,3] The binding property of the ring-N- and amino-substituted acridine dyes to DNA has been shown to be nearly independent of the kind and bulkiness of the substituents.[6] Our spin-labeled acridine dyes are probably bound to DNA in the same manner as nonspin-labeled, substituted derivatives. Since the monocationic form of a dye is an essential factor for the extrinsic Cotton effect to appear,[2] the positively charged ring nitrogen and, possibly, the amino group seem to be directed towards the negatively charged phosphate group of DNA.

Observed and Simulated ESR Spectra of Spin-labeled Dyes Bound to DNA. The observed ESR spectra of spin-labeled dyes in the presence of DNA are shown in Fig. 2 (——). Each of ESR spectra (I-IV) shows a strong anisotropy indicating that the spin-labeled dye is bound to and probably immobilized on DNA. However, the line shapes of the ESR spectra differ from one another. In particular, the ESR spectrum of doubly labeled dye V is a composite of two components consisting of the sharp and broad triplet lines. Despite that the unbound V in solution was estimated to be only 0.2% from ultrafiltration, the sharp lines amount to ca. 18% estimated by the double integration of the simulated ESR lines.[1] Thus, the sharp lines result from freely rotating labels of bound V. This suggests that some of V are bound to DNA but mobile on DNA or that the rotation of V as a whole is restricted but either of the two labels, or both, substituted on the 3,6-positions can rotate rapidly and isotropically, whereas the broad lines result from the restrictedly rotating labels.

In order to obtain information on the motion of the radical moiety of a bound dye, the observed ESR spectra were simulated with the computer program in which the rotational reorientation of a radical moiety was assumed to be the axially symmetric Brownian diffusion.[1] When the radical moiety undergoes a rapid rotation about a single bond but the labeled dye reorients slowly, the rotational diffusion constants

Table 1. Parameters used for simulation of observed ESR spectra

Dye	P/D	$D_\perp \times 10^{-8}$ / s^{-1}	$\tau_\perp$ / ns	$D_{\parallel} \times 10^{-8}$ / s^{-1}	$\tau_{\parallel}$ / ns	θ / deg	δ / G	A_o / G	$A_x(=A_y)$ / G	A_z / G
I	21	0.01	170	2.7	0.62	42	0.8	17.0	7.2	36.6
II	19	0.06	28	3.6	0.46	48	0.5	16.7	5.5	39.1
III	16	0.12	14	2.5	0.67	40	2.3	16.9	5.8	39.1
IV	20	0.03	56	3.3	0.51	46	0.7	15.9	5.1	37.6
V	21	0.03	56	2.5	0.67	38	1.0	16.0	5.0	38.0
s1PF[1]	18	0.01	170	3.0	0.56	35	1.0	16.0	6.6	35.5

$D_{\parallel}$ and $D_{\perp}$ refer to the bond rotation and to the overall rotation of the dye chromophore, respectively. The best-fitted simulated spectra are shown in Fig. 2 (-----). The parameters used are listed in Table 1, where $\tau_{\perp}=(6D_{\perp})^{-1}$ and $\tau_{\parallel}=(6D_{\parallel})^{-1}$, θ is the tilt angle between the z-axis of the magnetic tensors (the $2p_{\pi}$ orbital of a radical) and the z'-axis or symmetry axis of the rotational diffusion tensor, δ is the peak-to-peak residual width, and A_i is the hyperfine coupling constant along the i-axis.[7] (For g-values, see Note 8).

Although the values of parameters differ from one another, the radical moiety of each dye bound to DNA shows a very anisotropic rotation. The slower rotation ($\tau_{\perp}$ = 14 − 170 ns) can possibly be attributed to the overall motion of the dye chromophore. The $\tau_{\perp}$ values are in the same order of magnitudes with the rotational relaxation times of bound nonlabeled acridine dyes (estimated to be ca. 20-50 ns from the fluorescence anisotropy[9]). The overall motion of bound dye chromophore may, for instance, result from a local flexibility of DNA or rotation about the helical axis. The faster rotation ($\tau_{\parallel}$ = 0.46-0.67 ns) should be attributed to the internal rotation about the linkage between the moieties of dye chromophore and spin-label, since the rotational correlation time about a single bond has been estimated to be ca. 0.2 ns.[10] This rapid rotation of the radical moiety is nearly independent of the labeled positions. It is thus concluded that the environment of each of bound acridines (I-V) at the DNA site is open so that the radical (ca. 0.5 nm in diameter) of the bound dye can rotate at a relatively fast rate.

A number of structurally related spin-labeled dyes have been shown to provide information on the dye-binding site of DNA. We believe that those dyes are applicable to other macromolecular and aggregate systems as the optical-magnetic double probe to obtain the insight into local structure and dynamics. By utilizing electric dichroism and fluorescence techniques in addition to ESR, CD and absorption, more detailed structural knowledge will be accumulated. Some work is now in progress.

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