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SPIN-LABELED METACHROMATIC DYES. III. INTERACTION OF SPIN-LABELED PROFLAVINE WITH NATIVE AND HEAT-DENATURED DNA

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The binding of spin-labeled Proflavine to native and denatured DNA was evidenced by the line broadening and anisotropic pattern in ESR and by the absorption changes and extrinsic Cotton effects in optical spectra. The tilt angle and rotational diffusion constants of the bound spin-label were obtained by the simulation of ESR spectra.

Acridine dyes are known for a variety of biological activities such as photodynamism, mutation, and chemotherapy. While these activities have been studied mostly by means of optical techniques, it is always desirable to pursue the same studies by other independent methods to verify the conclusions or hypotheses advanced solely on the basis of the optical data. Structural information can be readily obtained by magnetic resonances in which NMR is more detailed but less sensitive than ESR and difficult to apply to the macromolecular system in dilute aqueous solutions. Only a few ESR studies have been reported on the dye-biopolymer interactions, e. g., the chlorpromazine[1)] and 5-methylphenazinium[2)] radicals with DNA. In these cases, however, the ESR signals were too complicated to obtain detailed information by simulation, or else the optical data were unavailable because of the weak absorption.

Since optical and ESR properties of spin-labeled Proflavine (slPF) and the application of this dye to metachromasy have been reported in the previous papers,[3a,b)] the ESR and optical study of the interaction of slPF with DNA, a typical biopolymer, is now appropriate. In this paper, slPF is shown to bind to both native and heat-denatured DNA. The interaction between them was evidenced in the ESR spectra by the change of widths and positions of the resonance lines of slPF and was reflected in the absorption spectra by the bathochromic and hypochromic changes of band intensity relative to the free slPF. The extrinsic Cotton effects were also observed in the slPF-DNA complex. Since the ESR signal of slPF was simple, the useful parameters of the slPF bound to DNA such as rotational diffusion constants could be obtained by simulation. The data obtained by the optical and ESR methods under the same experimental conditions yielded specific and detailed information on the dye-DNA complex.

Materials. slPF, 3-N-(3'-carbonyl-1'-oxyl-2',2',5',5'-tetramethylpyrrolidinyl)-3,6-diaminoacridinium chloride, was described in Part I.[3a)] Calf thymus DNA (Worthington Biochemical Corp.) has a molecular weight of ca. 4.5×10^6 daltons.

Procedures. The stock native and heat-denatured DNA solutions were prepared as described elsewhere.[4)] The mixing ratio of polymer residues to dye, P/D, was adjusted by addition of an appropriate amount of the stock slPF to DNA solution. The final concentration of slPF in the dye-DNA solution was kept constant at ca. 0.1 mM. The solution contained 1.5 mM NaCl at pH ca. 6.5, unless otherwise specified.

Measurements. ESR and absorption spectra were measured as before.3a) The circular dichroism was measured on a JASCO J-20 CD/ORD spectropolarimeter at 20°C.

Absorption and Circular Dichroism of slPF in the Presence of DNA. The interaction of the slPF chromophore with DNA is manifested in the light absorption and circular dichroic (CD) spectra as shown in Fig. 1. Although the spectrum of slPF differs from that of Proflavine (PF), the spectral behavior and P/D dependence of the slPF-DNA system are closely related with those of the PF-DNA system. For example, each of the maxima of slPF is shifted toward the long wavelength with the concomitant loss of intensity in the presence of DNA in the P/D range 18-2. The appearance of the extrinsic Cotton effect, which is sensitive to P/D and the denaturation of DNA, supports the notion that slPF binds to DNA in an ordered array. However, the molar ellipticity [θ] in terms of the slPF concentration is at least one order smaller than that of other acridine dye-DNA complexes,4) despite that most of the slPF are bound in the P/D range 18-2. At P/D=2, the amount of the unbound slPF was estimated to be only ca. 8% from ultrafiltration5) and by the double integration of simulated ESR lines (see below). The complicated and feeble CD spectra of the slPF-DNA complex may be partly due to the fact that slPF is a racemic mixture3a) and partly due to the substitution of a nitroxyl radical in one of the amino groups of PF. It is thus clear that slPF interacts with DNA in a like manner as other acridine dyes.4)

ESR Spectra of slPF in the Presence of Native and Denatured DNA. As shown in Fig. 2, the interaction of slPF with the native and denatured DNA is also evidenced by the ESR spectra, the change of which depends on the P/D value. The ESR spectra of the native and denatured slPF-DNA at high P/D values of 18-17 show almost no sharp

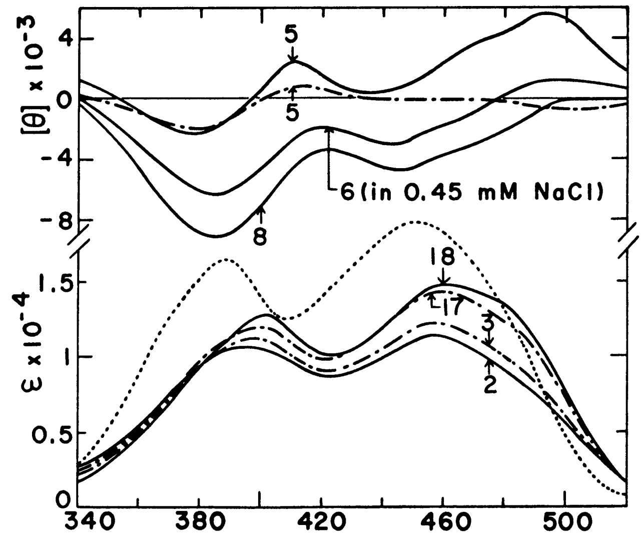

Fig. 1. The absorption and CD spectra of slPF in the presence of native (———) and denatured (— · —) DNA. Numerals indicate P/D values. The absorption of slPF (-----) is also shown. The CD of slPF itself was undetected.

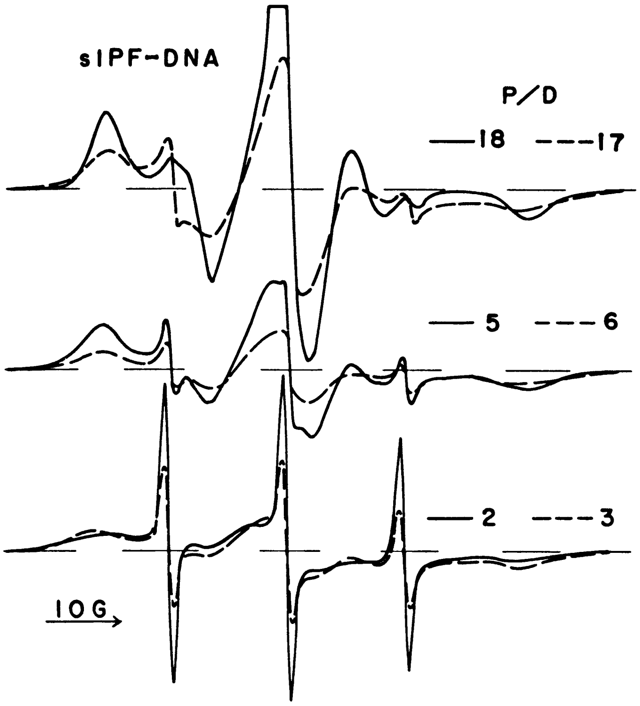

Fig. 2. The observed ESR spectra of slPF in the presence of native (———) and denatured (— —) DNA.

triplet lines but a strong anisotropy manifesting that slPF is bound to and possibly immobilized on DNA. The sharp lines are enhanced at lower P/D values, while the anisotropic lines become broader progressively. Thus, the ESR spectra of slPF-DNA are probably composite of at least two component spectra. The broadening of the anisotropic spectra with decreasing P/D values suggests that either of the exchange and dipole-dipole interactions or both of them become effective as the distance between bound dyes shortens. It is also worth noting that the ESR spectrum of slPF-denatured DNA at a P/D resembles that of slPF-native DNA at a P/D lower than that.

Computer Simulation of ESR Spectra of slPF-DNA. In order to obtain detailed structural information on the bound slPF, simulations of the observed ESR spectra of slPF-DNA were carried out with use of the computer program[6a)] in which the rotational reorientation of slPF was assumed to be axially symmetric Brownian diffusion. To this program the contributions of exchange interaction[6b)] and the sharp triplet lines were newly added. Computations were performed on a HITAC-8700 in OS7 FORTRAN language with the following parameters: the hyperfine coupling constants, $A_x (=A_y)$ and A_z; the g-values, g_x, g_y, and g_z; the rotational diffusion constants, $D_\perp$ and $D_{/\!/}$; the tilt angle between the z-axis of the magnetic tensors and the z'-axis of the rotational diffusion tensor,[6a)] θ; the peak-to-peak residual derivative width, δ; the exchange frequency, W_{ex}. To estimate the relative amount of the sharp lines, the parameters of free slPF[3a)] were used. Best-fitted simulated spectra are shown in Fig. 3, together with the values of parameters used for the simulations.

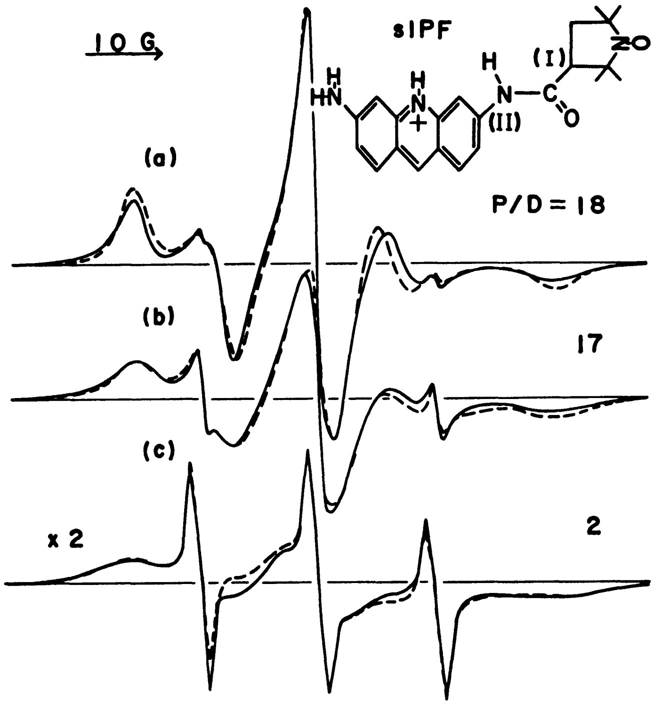

Fig. 3. Comparison between observed (— — —) and simulated (———) spectra of slPF-native DNA at P/D=18 and 2 and -denatured DNA at P/D=17. The fixed parameters are: $A_x=A_y=6.58$ G, $A_z=35.3$ G;[3a)] $g_x=2.0092$, $g_y=2.0061$, and $g_z=2.0032$ (slightly modified from the values in Ref. 6c). The adjusted parameters are: (a) $D_\perp=1\times10^6$ s^{-1}, $D_{/\!/}=3\times10^8$ s^{-1}, $\theta=35°$, and $\delta=1$ G; (b) $D_\perp=8\times10^6$ s^{-1}, $D_{/\!/}=3\times10^8$ s^{-1}, $\theta=32°$, and $\delta=2$ G; (c) $D_\perp=8\times10^6$ s^{-1}, $D_{/\!/}=3\times10^8$ s^{-1}, $\theta=32°$, and $\delta=7$ G (modulation width 3.2 G). The line intensity (c) should be enlarged proportionally by the multiplier given in the left. The same should also apply to Figs. 1 and 3 of Part II.[3b)]

The ratio of the area of the simulated sharp lines to that of the broad lines at P/D=2 was computed to be 0.08 by the respective double integrations. This result indicates that most of the dyes are bound (>92%) and contribute to the broad ESR lines at the P/D range 18-2. At P/D=18 (a), the radical part rotates very anisotropically with an average angle θ of ca. 35° between the nitrogen $2p_\pi$ orbital that holds the odd electron (the z-axis) and a symmetry axis around which the faster rotation occurs. The most acceptable axes for this faster rotation are the NHCO-C(3') bond (I) which makes an average angle θ of ca. 35°[7)] and the C(3)-N bond (II) in the trans conformation ($\theta \cong 36°$) as shown in Fig. 3. The cis conformation is excluded because the angle (ca. 65°) differs largely from the simulated value (ca. 35°). Thus, the faster rotation of the $2p_{\pi}$ orbital is likely to result from the internal rotations around (I) and/or (II), both of which are nearly in parallel. This interpretation is also supported by the result that the values of $D_{//}$ and θ are almost independent of P/D and the conformation of slPF-DNA. The faster rotation of the chromophore of the bound slPF is permissible only around the axes parallel to (I) and (II); otherwise an isotropic ESR spectrum should be observed.[7)] Consequently, the slower rotation of the chromophore should be attributed to the motion around the axes perpendicular to (I) and (II). Such a rotation of the chromophore would have a correlation time, τ, nearly equal to the value of $\tau_{\perp} = (6D_{\perp})^{-1}$ for the nitroxide. The reorientation of bound slPF with a τ value of 170 ns suggests that the binding site of the slPF on native DNA is either flexible due to the local motions or rotates around the double-stranded helical axis.

The parameters which were used to simulate the spectrum of slPF-denatured DNA at P/D=17 (b) are practically the same as the above except the larger values of $D_{\perp}$ and δ (the latter is the measure of dipole-dipole broadening). This result indicates that slPF may be bound to denatured DNA more loosely than to native DNA and that the distance between bound dyes is shorter in denatured DNA at a given P/D value. With use of a value of δ between 6 and 8 G and an increased amount of the sharp triplet lines, the simulated ESR spectrum of slPF-native DNA at P/D=2 (c) is fitted very closely to the observed one when the exchange interaction is not considered at all ($W_{ex}=0$). Thus, the observed broadening of ESR spectra of slPF-DNA with decreasing P/D values should result from the dipole-dipole interaction between the radical moieties of slPF but not from the exchange interaction as previously noted for slPF-polyphosphates.[3b)]

A spin-labeled dye has been shown to be able to give structural information on dye-biopolymer interactions not obtainable by either ESR or optical method alone. In consideration of the present results, the use of slPF and other spin-labeled dyes such as Ethidium bromide,[8)] appears to be hopeful for resolving the long-pending problem of the intercalation of some acridine dyes into the double-stranded DNA.

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