OPTICALLY DETECTED ZERO FIELD MAGNETIC RESONANCE STUDIES OF PROFLAVINE-DNA AND 3,4-BENZPYRENE-DNA COMPLEXES

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Summary

Optical detection of magnetic resonance has been used to observe the photoexcited triplet state zero-field transitions of proflavine in DNA and 3,4-benzpyrene in DNA at 2°K. The results suggest that optically detected magnetic resonance may be utilized for determining the local site distribution of small molecules bound to DNA.

Introduction

The interaction of small molecules with macromolecules is of central importance in many biochemical processes. To achieve an understanding of such interactions requires specific information on their nature and local site requirements involved. Much work on the interaction of planar aromatic molecules, particularly the acridine dyes, with nucleic acids has focused on the physical binding by intercalation in these systems (1). Since these complexes serve as models for the binding of a variety of similar systems, it is important to arrive at a detailed description of the interactions controlling the binding process.

Optical detection of magnetic resonance (ODMR) in the photoexcited triplet state of organic molecules has been shown to be a powerful spectroscopic technique, combining the sensitivity of optical spectroscopy with the resolution of magnetic resonance (2). Through the use of ODMR techniques, the triplet state of planar aromatic molecules bound to DNA might be util-

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ized as a nondestructive probe into the details of the surrounding environment and provide information on binding site selectivity or site distribution. In the present note we provide data which suggests the possibility of utilizing ODMR, specifically phosphorescence-microwave double resonance (PMDR) (2) and triplet absorption detection of magnetic resonance (TADMR) (3), to obtain information about the binding of polycyclic hydrocarbons and acridine dye molecules to DNA.

Methods and Materials

Solutions of DNA (Type I sodium salt DNA obtained from Sigma Chemical Co.) were made up to a concentration of approximately 1.0 mg/ml using 0.01 M phosphate buffer. The buffer consisted of 0.0025 M Na_2HPO_4 and 0.0050 M NaH_2PO_4, yielding a pH of 6.8. An appropriate volume of proflavine (Aldrich Chemical Co.) in the 0.01 M Phosphate buffer solution was added to the DNA solution to produce a concentration of 10^{-5} M proflavine in the sample. The proflavine-DNA samples were passed through a Sephadex G-25-80 (Sigma Chemical Co.) column and subsequently diluted 1:1 with ethylene glycol. Upon slow freezing the samples formed a good optical quality cracked glass for the low temperature ODMR experiments. Preparation of the 3,4-benzpyrene (Eastman-Kodak Co.) in DNA samples followed the procedures of T'so et al. (4). The samples were also diluted 1:1 with ethylene glycol and slowly cooled to liquid helium temperatures for the ODMR experiments. Details of the experimental arrangement for PMDR and TADMR have been previously described (3) (5).

Results and Discussion

Proflavine in various media (including DNA) is known to produce a long-lived phosphorescence at low temperatures in the spectral region of 520-600 nm (6). The proflavine absorption bands lie to longer wavelength than the DNA absorption, and the proflavine phosphorescence can be directly excited in DNA complexes with visible light. When the phosphorescence intensity of the proflavine-DNA complex is monitored at 2°K in the presence of a swept microwave field, three structured zero-field EPR transitions are observed at frequencies corresponding to the known proflavine triplet state D and E values ($|D|$ = 0.0734 cm^{-1}, $|E|$ = 0.0177 cm^{-1}) obtained in glycerine at 77°K (6). The middle energy ($|D| - |E|$) transition is shown in Figure 1. The $2|E|$ and $|D| - |E|$ transitions are of comparable intensity and are observed as increases in the phosphorescence intensity. The $|D| + |E|$ transition is considerably weaker.

For the 3,4-benzpyrene-DNA complex when one excites in the region of the

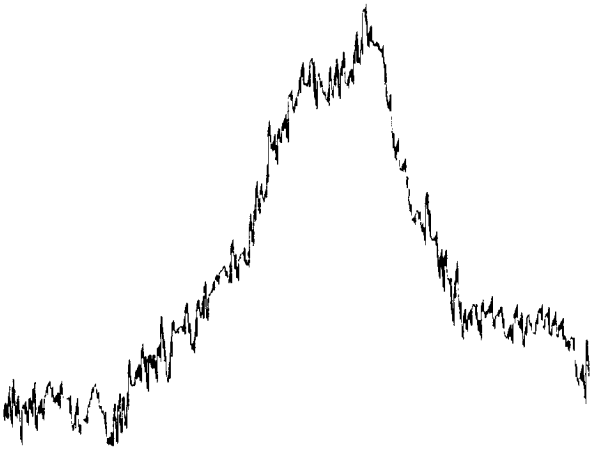

Figure 1. Phosphorescence-microwave double resonance spectrum in the region of the $|D|-|E|$ transition of the triplet state of proflavine in DNA at 2°K. The phosphorescence intensity was monitored at 560 nm. The frequency range across the figure is from 1400 MHz (left) to 1800 MHz (right).

benzpyrene singlet state (to lower energy than the DNA absorption), no phosphorescence is observed at 2°K, but by monitoring the light transmitted through the sample in the region of the benzpyrene triplet-triplet absorption maximum (469 nm) as microwaves are applied, a weak, but reproducible, peak is observed at 1100 MHz. The frequency of the transition corresponds to the $2|E|$ transition of the benzpyrene triplet observed by TADMR in n-octane solutions at 2°K (7) and appears sharper than those observed for the proflavine-DNA complex.

The most interesting feature of the ODMR spectra obtained is the structure of the zero-field bands, as shown in Figure 1. The ODMR of proflavine in buffer solution displays no structure comparable to that observed in DNA. Further, if argon laser excitation at 457.9 nm is used to produce proflavine emission in DNA, the ODMR spectrum in Figure 1 sharpens to a single band located at 1600 MHz with a linewidth of $\sim$100 MHz. Using broadband light excitation (1000 W Hg-Xe lamp) filtered to match the proflavine absorption, the more complex ODMR spectrum of Figure 1 is obtained with its strongest features at 1600 and 1690 MHz. These observations suggest that the structure being observed in the ODMR spectra is due to proflavine molecules in different local environments within the DNA and

that the intensity distribution with the ODMR peaks reflects the site distribution of proflavine molecules in DNA.

These results suggest the interesting possibility of using the triplet state as a probe for the selectivity and distribution among binding sites of small molecules in nucleic acids. Work on complexes of synthetic polynucleotides is underway in an attempt to correlate the ODMR structure observed with selectivity of specific base pair sites by planar aromatic molecueles.

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