CHARACTERIZATION OF PROTEIN TRIPLET STATES BY OPTICAL DETECTION OF MAGNETIC RESONANCE

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Received January 18, 1972

Summary The technique of optical detection of magnetic resonance (ODMR) is applied for the first time to the study of molecules of biological interest in frozen glassy solutions. We present results describing the triplet state properties of tryptophan, tyrosine, and the tryptophan and tyrosine residues of bovine serum albumin.

The development of the technique of optical detection of magnetic resonance (ODMR) [1-3] has resulted in a number of studies yielding much more detailed information about aromatic triplet states [4-6] than has been obtainable generally from conventional electron spin resonance experiments. Previous reports on ODMR have dealt with pure or doped single crystals of simple aromatic molecules, usually containing some elements of symmetry. We report here the first applications of ODMR to studies of frozen glassy solutions and to studies of molecules of biological interest. In particular, we have obtained preliminary ODMR signals from frozen ethylene glycol-water solutions of tryptophan, tyrosine and bovine serum albumin (BSA).

ODMR is possible because the phosphorescence emission

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from the excited triplet state is composed of three individual components, one from each of the three spin sublevels of the triplet. The radiative rate constants of the sublevels will differ, in general, due to selective spin-orbit mixing with the singlet mainfold. Thus, it becomes possible to affect the intensity of the phosphorescence by saturating the populations of a pair of spin sublevels using a microwave radio-frequency field at the appropriate magnetic resonance frequency. An ODMR effect will be observed a) if there is a difference in the populations of the sublevels, and b) if the spin sublevels have different radiative rate constants. At high temperatures, spin lattice relaxation (SLR) processes are fast enough to maintain approximately equal sublevel populations and ODMR effects are expected to be weak. At sufficiently low temperatures ($<2^{o}K$) SLR times will become longer than the decay times of the $T_1 \rightarrow S_o$ processes, and large polarizations of the spin-level populations may be found in the steady state [7]. If a pair of sublevel populations is saturated suddenly by a strong microwave pulse, or a rapid passage during continuous optical pumping, a transient fluctuation in the phosphorescence emission may be observed which will eventually decay back to the steady state emission level. It has been demonstrated in a previous communication [6] that analysis of the transient response under conditions that the SLR can be ignored yields the individual lifetimes of the spin sublevels, their relative radiative rate constants, their steady state populations, and the relative intersystem crossing rate constants.

The experiments described here are carried out in the absence of any external magnetic field. This eliminates broadening of the energy levels due to the Zeeman interaction, as well

as nearly all of the broadening due to hyperfine interactions. The frequencies at which ODMR effects are seen in zero field are a direct measure of the zero-field splittings (ZFS) of the triplet state. The samples are placed in a liquid helium dewar and subjected to constant u.v. irradiation from a PEK 100 watt mercury lamp filtered by a 1/4-meter Bausch and Lomb Monochromator. The phosphorescence is monitored at right angles to the excitation path using a McPherson Model 2051 1-meter grating Monochromator. Temperatures as low as 1.25°K are achieved by pumping on the liquid helium. Microwave power is conducted to the sample through a coaxial transmission line terminated with a helix containing the sample. A rotating sector is used to eliminate the fluorescence as well as the scattered light.

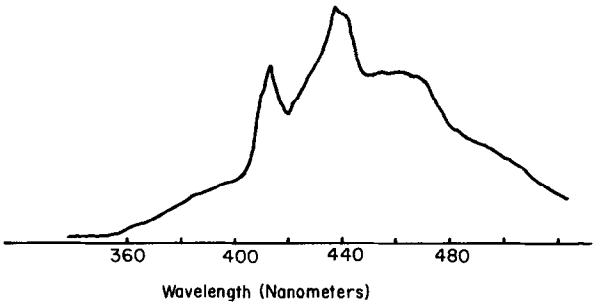

Figure 1 Phosphorescence spectrum at 1.3°K of 10^{-3}M bovine serum albumin in ethylene glycol-water (1:1). Excitation is at 290 nm.

The phosphorescence emission spectrum of a frozen solution of BSA (Pentex Biochemicals) in ethylene glycol-water (1:1) is shown in Figure 1. An ODMR signal obtained by monitoring the 414 nm peak of the emission is shown in Figure 2. The sudden increase in intensity occurs as the frequency-swept microwaves traverse a measured frequency of 1.657 GHz. A subsequent exponential decay of the transient occurs with a time constant of

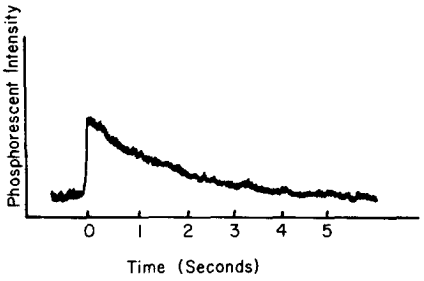

Figure 2 ODMR signal from bovine serum albumin solution at 1.3°K. Signal is obtained by monitoring the phosphorescence emission at 414 nm, and occurs at a microwave frequency of 1.657 GHz.

1.5 sec. A second signal occurs at a frequency of 2.605 GHz, again with a decay constant of 1.5 sec. Similar ODMR signals are found by monitoring the 442 nm peak of the phosphorescence spectrum. We identify these two signals as originating from the tryptophan residues of BSA. Indeed, when a solution of free tryptophan in the same solvent is measured, similar signals are found at only slightly shifted frequencies.

If the BSA phosphorescence is monitored at 390 nm where the emission is expected to be due to tyrosine residues, the two signals described above are not found. Instead, a small signal corresponding to a decrease in light is found at a frequency of 2.2 GHz. A solution of free tyrosine in the same solvent gives a similar response at this frequency, and we conclude that this BSA signal is due to the tyrosine residues of the protein.

Some interesting conclusions can be drawn at this preliminary stage of these investigations which suggest a direction for future work. First, there is a small but easily measurable (by zero-field ODMR) change in the ZFS between free tryptophan, and tryptophan residues of the protein. The ZFS parameters measured for free tryptophan are $|D|/hc = .0988\ cm^{-1}$ and $|E|/hc = .0408\ cm^{-1}$,

in very close agreement with the results reported by conventional electron spin resonance (ESR) [8]. The parameters from the tryptophan residues of BSA are found to be $|D|/hc = .0987\ cm^{-1}$ and $|E|/hc = .0434\ cm^{-1}$. This change in E, which is on the limit of resolution of the conventional ESR method [8], is substantial when considering the increased resolution of the zero-field ODMR method. Thus, it may be hoped that different protein sites could be resolved and identified by ODMR, and that conformational changes including those occurring on binding of enzymes with substrates could be detected by small changes in the ZFS. Finally, double resonance experiments to detect triplet-triplet energy transfer from tyrosine to tryptophan in protein molecules are suggested by our observation of signals from both residues in BSA. Such studies are currently in progress.

ACKNOWLEDGMENT. This work was supported by a Grant, GM 12327, from the National Institutes of Health, U. S. Public Health Service, and PHS Postdoctoral Fellowship, GM 37208, to JZ.

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