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Amphiphilic spectroscopic probes utilizing metal chelates

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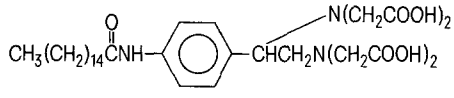
Summary. The synthesis and initial applications are reported for 1-[p-(palmitamido)-phenyl]ethylenedinitrilotetraacetic acid. The results demonstrate the versatility of this spectroscopic probe molecule, which allows choice of a particular technique for a particular system as well as use of multiple spectroscopic techniques for complementary information about hydrophobic regions in biological systems.

The class of molecules exemplified by 1-[p-(palmitamido)-phenyl]-ethylenedinitrilotetraacetic acid (**1**) incorporate a fatty acyl chain and an aromatic ring adjacent to a powerful metal-chelating group.



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The ethylenedinitrilotetraacetic acid (EDTA) moiety forms stable chelates with many different metal ions, permitting the same basic molecule - using different chelated metal ions - to be used to study a given system with different spectroscopic techniques. For example, paramagnetic metal ions such as VO^{+2} and Cu^{+2} provide useful electron paramagnetic resonance (EPR) spectra[2]; lanthanide metal ions such as Tb^{+3} and Eu^{+3} allow use of luminescence techniques[3]; paramagnetic metal ions such as Mn^{+2} and Gd^{+3} cause characteristic perturbations of nuclear magnetic resonance spectra[4]; and certain radionuclides (which decay by sequential emission of gamma rays) such as $^{111}In^{+3}$, $^{62}Zn^{+2}$, and $^{204}Pb^{+2}$ give information from perturbed angular correlations (PAC)[5].

Using luminescence, EPR, and PAC, we have studied **1** chelates in aqueous media as micelles, as monomer, and as a complex with the protein human serum albumin.

Compound **1** was prepared by acylation with palmitoyl chloride in acetonitrile of the tetraethylammonium salt of 1-(p-aminophenyl)-ethylenedinitrilotetraacetate[6]. Chelates were prepared by adding metal ions to **1** in 0.1 M ammonium citrate, pH 6.1 except where noted. All spectra were taken at room temperature. Under these conditions, the critical micelle concentration of **1** is approximately 2×10^{-4} M, as determined by the dye rhodamine 6G[7]. The behavior of **1** micelles at various degrees of saturation with oxovanadium (IV) ions was observed by EPR. A typical spectrum, given in figure 1a, indicates slow, anisotropic tumbling of the chelate on the EPR time scale[8]. No change other than a linear increase in amplitude was observed as the added

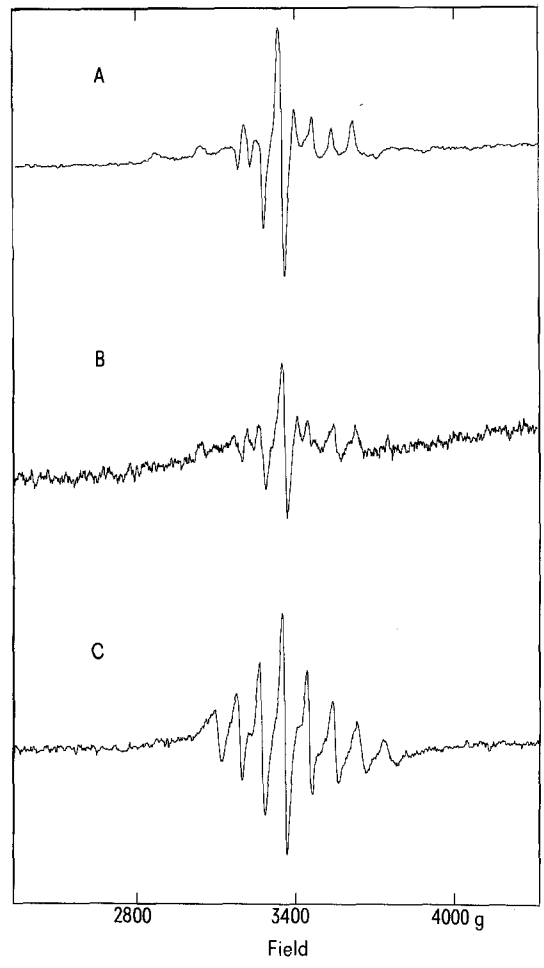

Fig. 1. EPR spectra of **1**-VO^{+2}. *a* 0.5 mM **1**-VO^{+2} in 47 mM **1** (micelles). *b* 0.6 mM **1**-VO^{+2} in 0.2 mM human serum albumin. *c* 0.7 mM **1**-VO^{+2} in 0.06 mM albumin.

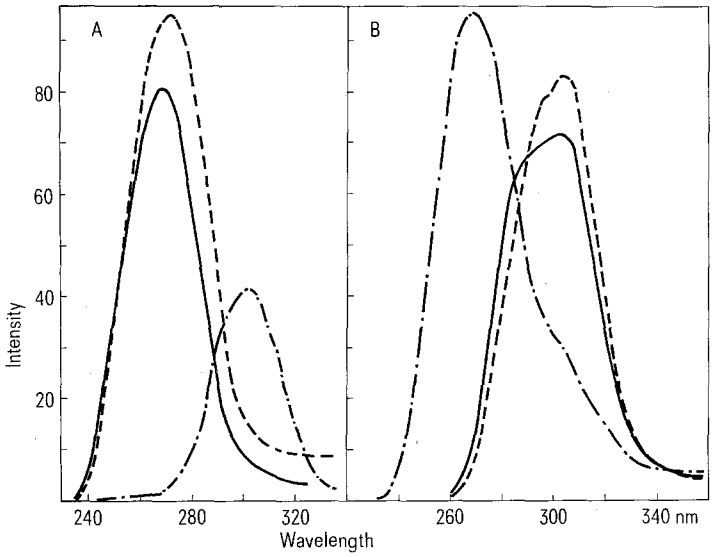

Fig. 2. Lanthanide luminescence excitation spectra. *a* **1**-Tb^{+3} (emission at 546 nm) as monomer (——), micelles (–·–·–), albumin complex (– – –). *b* **1**-Eu^{+3} (emission at 618 nm) as monomer (–·–·–), micelles (——), albumin complex (0.6 mM **1**-Eu^{+3} in 0.4 mM albumin, – – –).

VO^{+2} concentration was varied from 0.5×10^{-3} M to 9.0×10^{-3} M in a 0.04 M solution of **1**, ruling out spin-spin interactions in this range.

The binding of fatty acid anions such as stearate and palmitate by serum albumin has been described[9]. We observed the binding of the VO^{+2} chelate of **1** by defatted[10] human serum albumin. Different spectra were observed at different ratios of **1**-VO^{+2} to albumin, an effect which evidently is due to a sum of signals from **1**-VO^{+2} at several binding sites on the protein. Figure 1b and c give spectra of **1**-VO^{+2}: albumin with molar ratios of 3:1 and 12:1.

The gamma-ray perturbed angular correlation measurements were performed using $^{111}In^{+3}$, which decays by emission of 2 gamma rays in succession. Perturbation of the correlation between the directions of propagation of the 1st and 2nd gamma rays is caused by the ligands around the metal nucleus[11]. The integral perturbation factor $G_{22}(\infty)$ gives a measure of the rational correlation time of the molecule to which the $^{111}In^{+3}$ nucleus is attached. For comparison, the $G_{22}(\infty)$ values of the simple EDTA chelate of $^{111}In^{+3}$ in glycerol over a large range of viscosity are given by Leung et al.[12]; for EDTA (^{111}In), $G_{22}(\infty)=0.34$ when $\eta=0.06$ poise. Carrier-free $^{111}In^{+3}$ in a (micellar) 1.4×10^{-3} M solution of **1** gave a value of $G_{22}(\infty)=0.35$. A 4.5×10^{-4} M solution of albumin with 1–3 equivalents of **1**-In^{+3} chelate, shown to be over 98% bound by gel exclusion chromatography, gave $G_{22}(\infty)$ values of 0.32–0.37. These results are consistent with tumbling which is moderately rapid on the PAC time scale[12].

It has been shown that when human serum albumin is conjugated with **1**-(p-benzenediazonium)-ethylenedinitrilotetraacetic acid and subsequently titrated with Tb^{+3} or Eu^{+3}, lanthanide luminescence is easily observed[13]. A basic feature of the system is a transfer of energy from the adjacent aromatic ring to the chelated lanthanide. The excitation spectra of 4×10^{-6} M to 3×10^{-3} M solutions of **1** (50% saturated with Tb^{+3}) were examined. As illustrated in figure 2a, single excitation peaks whose maximum wavelengths ranged from 266 nm to 302 nm were observed. The wavelength of maximum excitation changed most rapidly near 2×10^{-4} M, the critical micelle concentration as determined using rhodamine 6G[7]. It is interesting to note that small amounts of **1**-Tb^{+3} in sodium dodecyl sulfate micelles exhibited an excitation maximum at 269 nm. A μmolar defatted albumin solution with 1 **1**-Tb^{+3} chelate bound per albumin molecule gave an excitation maximum at 271 nm with a shoulder at longer wavelengths, as shown in figure 2a. Similar results were found for experiments with **1**-Eu^{+3} (figure 2b).

These results provide compelling evidence that different metal chelates of **1**, while quite similar in their chemical properties, can provide complementary spectroscopic information about their environment. We have found that **1** and its metal chelates are readily incorporated into phospholipid vesicles and into the membranes of intact human red blood cells; thus this compound is well suited to studies of hydrophobic regions in biological systems by a variety of physical techniques. There are advantages to the application of a particular technique in a given situation: for example, PAC using $^{111}In^{+3}$ is sensitive to quadrupolar interactions with electric field gradients[11]; the VO^{+2} EPR spectrum to dipolar interactions with magnetic fields[8]; and the lanthanide luminescence properties to energy transfer and environmental effects[3].

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