$$b_i{}^2 = (g_2/g_b)^2 a_{i2}{}^2 \cos^2 (\phi' + \alpha_i)$$
$$+ (g_3/g_b)^2 a_{i3}{}^2 \sin^2 (\phi' + \alpha_i) \quad \text{(A8)}$$

$$\cos \phi' = (g_2/g_b) \cos \phi \quad \text{(A9)}$$

$$\sin \phi' = (g_3/g_b) \sin \phi \quad \text{(A10)}$$

A Fortran program was written which computes the resonant field for each of 4096 orientations of the molecule using eq A2 and then constructs a histogram of the number of resonant conditions found in each of 1575 equal field increments spanning the range of the experimental spectrum. The component powder patterns are then added and artificially broadened by computing the convolution integral with the appropriate Gaussian or Lorentzian shape function. The result is then differentiated and plotted. The program is based on a general approach to magnetic resonance powder patterns developed by Taylor and Bray[26] but with provisions added for more than one nucleus and noncoincident tensor axes. Since for two spin $^7/_2$ nuclei there are 64 components, the program computes over 262 000 resonant field values for each spectrum and is therefore expensive to run, each spectrum requiring approximately 40 min on an IBM 370/138 computer.

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The Methylmercury Heavy Atom Effect. Phosphorescence Polarization and Triplet Spin Sublevel Radiative Activities of the CH₃Hg^{II}–Benzimidazole and CH₃Hg^{II}–Tryptophan Complexes

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Abstract: The triplet-state kinetics of the complexes of benzimidazole (HBlm) and tryptophan (HTrp) with CH₃Hg^{II} have been investigated. The sublevel relative radiative rate constants and total decay rate constants are reported for both complexes. The heavy-atom effect causes the largest radiative enhancements of in-plane spin sublevels. Results of the sublevel total decay rate constant measurements confirm the previously reported reduction factor of 40 in the triplet state lifetime of the CH₃HgHBlm⁺ complex. The triplet lifetime of CH₃HgTrp is reduced by a factor of 800 relative to that of HTrp. Polarized phosphorescence excitation measurements were made on both complexes. The CH₃HgTrp complex has a polarization of $P = -0.18$, constant over the ¹Lₐ and ¹L_b absorption bands indicating out-of-plane polarization, while CH₃HgHBlm⁺ has $P = 0.06$ over the ¹Lₐ band and $P = -0.06$ over the ¹L_b band indicating in-plane polarization. A new transient microwave-phosphorescence measurement is described which permits the measurement of average triplet lifetimes in the presence of optically competing chromophores.

I. Introduction

The uptake of CH₃Hg^{II} in biological systems[1-3] has been an area of great interest in recent years. Since the neurotoxicity of CH₃Hg^{II} to humans has been convincingly demonstrated,[4,5] a major concern has been to identify the critical sites of action. Potential candidates for CH₃Hg^{II} binding sites have been suggested and include the bases of DNA and RNA, primary amines, and sulfhydryl groups.[6-15]

It has been demonstrated previously[16,17] that the attachment of heavy closed-shell metal ions to aromatic chromophores of biopolymers results in an external heavy-atom effect.[18] The triplet quantum yield is enhanced with a corresponding quenching of fluorescence and a shortening of the triplet radiative lifetime. Frequently, the phosphorescence quantum yield is enhanced.[19] Optically detected magnetic resonance (ODMR) slow passage[20,21] and transient[22-24] methods may
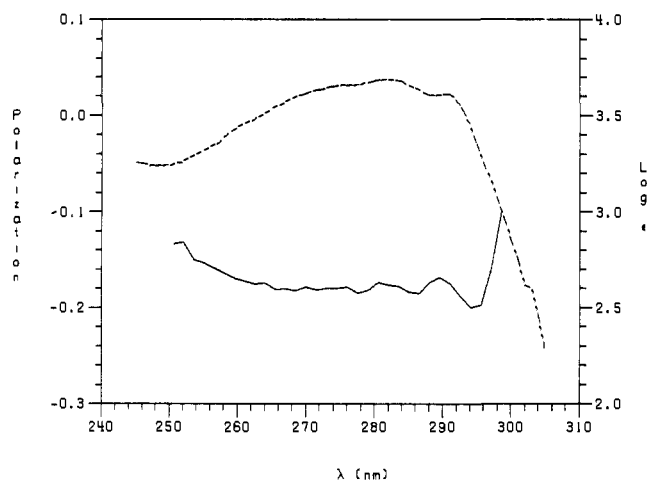
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Figure 1. Phosphorescence polarization excitation (PPE) spectrum (—) and absorption spectrum (- - -) of CH₃HgTrp: PPE λ_{obsd} 435 nm, T = 77 K.

Figure 2. Phosphorescence polarization excitation (PPE) spectrum (—) and absorption spectrum (- - -) of CH₃HgHBlm⁺: PPE λ_{obsd} 415 nm, T = 77 K.

be used to detect heavy atom perturbed chromophores with radiatively enhanced triplet states, since ODMR sensitivity usually increases with increasing triplet radiative quantum yield. We have found previously[25-27] that selective detection of heavy-atom perturbed chromophores can be readily carried out in the presence of uncomplexed molecules.

The mechanism of the heavy-atom effect induced by heavy metal binding to an aromatic chromophore is not well understood. In particular, it would be desirable to obtain information regarding the heavy-atom effect on individual triplet sublevels, the polarization of the phosphorescence, and the effect of metal binding geometry on these properties. To this end we report measurements on two model complexes, the cation methylmercury complex of benzimidazole ($CH_3HgHBlm^+$) and the methylmercury complex of tryptophan (CH_3HgTrp). Our measurements include the individual triplet sublevel total decay rate constants, the relative radiative rate constants, and the polarized phosphorescence excitation (PPE) spectra.

The two model complexes represent differing structural types. The complex $CH_3HgHBlm^+$ involves σ binding of CH_3Hg^{II} to an aromatic ring nitrogen, and is analogous to complexes which occur with the nucleic bases.[26] The complex CH_3HgTrp, on the other hand, involves σ binding with the side-chain amine and secondary interactions of the Hg atom with the π-electron system of the heterocyclic ring.[27]

II. Experimental Section

L-Tryptophan (HTrp, Calbiochem, Inc., A grade, chromatographically homogeneous) was used as obtained. Benzimidazole (HBlm, Eastman, Inc.) was recrystallized from deionized, doubly distilled water. Methylmercury hydroxide (Alfa Products, Inc., 97%) was dissolved in deionized, doubly distilled water and the stock solution was standardized by the method of Waugh et al.[28] Ethylene glycol (EG) was Matheson Coleman and Bell, Inc., chromatoquality.

For transient ODMR measurements the solutions were composed as follows: 1 mM CH₃HgOH with 1 mM HBlm in 0.01 M pH 5.5 PO₄ buffer mixed 1:1 with EG; 5 mM CH₃HgOH with 1 mM HTrp in 0.01 M pH 7.0 PO₄ buffer mixed 1:1 with EG.

The 1.1 K ODMR measurements performed consisted of fast passage transients,[23,24] saturation recovery transients,[22] and pulse transients done under conditions of continuous optical pumping. The output of a Hewlett-Packard 8690 sweeper and plug-in combination was amplitude modulated by use of a PIN diode driven by a Hewlett-Packard Model 8403A PIN modulator driver in the saturation recovery transient and pulsed transient measurements. The microwave power output from the PIN was amplified by use of a TWT microwave amplifier (Alfred Electronics, 2.0–4.0 GHz, or Alto Scientific, 4.0–8.0 GHz) to ensure saturation of the sublevel populations. To enhance the signals from the inhomogeneously broadened resonance, the mi-
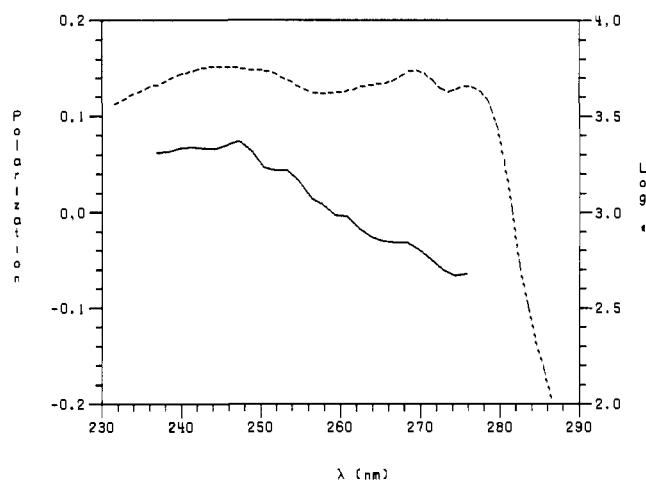
crowave generator was frequency modulated over a selected bandwidth either by internal or by external FM sources. For measurements on CH₃HgTrp, FM of 3 kHz was used with a deviation of ±35 MHz. The microwave pulse width was ca. 500 μs for the pulsed transient measurements.

The sample was optically pumped using a high-pressure mercury arc (Osram, Inc., HBO-100) filtered through a 5-cm water filter, a 0.25-m monochromator (Bausch and Lomb, Inc. 33-86-75) set for ca. 10-nm band-pass, and a CS 7-54 glass filter. Emission was observed at right angles using a 1-m monochromator (McPherson Model 2051) set for ca. 3.2-nm band-pass and detected using a cooled EMI 6256S photomultiplier. The photocurrent was measured using a Pacific Photometrics Model 124 photometer the output of which was fed into a Nicolet, Inc., Model 1072, 1024 channel computer for average transients. The transients were signal averaged and the resulting curves analyzed on an Interdata 7/16 minicomputer data analysis system using previously described techniques.[29]

Phosphorescence polarization excitation (PPE) measurements[30-32] were made at 77 K on solutions of H₂Blm⁺, CH₃HgHBlm⁺, and CH₃HgTrp. The solutions were made as described above except that the concentration of the aromatic was 0.1 mM. The excitation source was a high-pressure xenon arc (Osram XBO-450) filtered through a Schoeffel, Inc., GM-250 0.25-m monochromator set for a band-pass of ca. 1.5 nm and a CS 7-54 glass filter. The excitation light was polarized using a Glan-Taylor UV prism polarizer (Karl Lambrecht Corp. MGTYA12). The sample was contained in a 5-mm square fluorescence cuvette (Precision Cells, Inc., Type 4H) immersed in liquid N₂. A relatively strain-free portion of the cracked glass sample was selected and the emission at right angles to the excitation was collected. The components of light with electric vectors normal to and within the plane formed by the excitation and emission paths were monitored with an analyzing polarizer (Polacoat) with the direction of its polarization suitably oriented. The polarized emission passed through a glass filter (Schott WG-360, 3 mm) and the observation wavelength was selected with the 1-m monochromator having ca. 3.2 nm band-pass. Detection was with a cooled EMI, Inc., 6256S photomultiplier. The selective response of the detection system to light of parallel and perpendicular polarization was corrected using the method of Azumi and McGlynn.[33] Absorbance spectra were obtained on a Beckman, Inc., Model 25 spectrophotometer.

III. Results

The results of the PPE measurements follow. A calibration experiment on benzimidazole cation was performed to ensure the accuracy of the modified instrumentation for PPE spectra. H₂Blm⁺ has been shown previously[34] to exist in the cationic form under our experimental conditions. The PPE spectrum obtained (λ_{obsd} 415 nm) reveals that the polarization is negative ($P = -0.08$) over both the ^1L_a and ^1L_b[35] absorption bands. This result is in good agreement with the report of Schütt and Zimmermann,[36] who also found $P = -0.08$.

Table I. Relative Radiative and Total Sublevel Decay Rate Constants of Methylmercury Complexes of Benzimidazole and Tryptophan[a]

compd[b]	λ_{exc}, nm	λ_{obsd}, nm	k_x, s^{-1} [c]	k_y, s^{-1} [c]	k_z, s^{-1} [c]	$\bar{k}_{exp}$, s^{-1}	$\bar{k}_{calcd}$, s^{-1} [d]	$k^r_{x,rel}$	$k^r_{y,rel}$	$k^r_{z,rel}$	ref
HBIm	280.	373.4	0.250	0.189	0.043	0.159	0.161	1.0	≤0.1	≤0.1	34
CH$_3$HgH-BIm$^+$	270.	372.3	9.82	12.5	2.39	6.65[e]	8.24	0.56	1.0	0.098	
HTrp	295.	406.0	0.240	0.119	0.038	0.136	0.132	1.0	≤0.1	≤0.1	51
CH$_3$HgTrp	290.	409.2	303.	28.5	26.4	107.[e]	119.	1.0	0.078	0.084	

[a] T = 1.1–1.2 K. [b] 1 mM in 1:1 (v/v) EG–H$_2$O. [c] x and y are in-plane principal axes, with T$_x$ intermediate in energy. z is the out-of-plane principal axis except that for CH$_3$HgTrp y may be the out-of-plane principal axis. [d] $\bar{k}_{calcd} = (k_x + k_y + k_z)/3$. [e] Obtained from modified saturation recovery transient.

The PPE spectrum for CH$_3$HgTrp is shown in Figure 1. We find $P = -0.18$ across both the ^1L_a and ^1L_b absorption bands. This value is close to the value $P = -0.20$ across ^1L_a and ^1L_b previously published[36] for indole.

According to the theory,[37,38] a large negative polarization with respect to two mutually perpendicular in-plane absorption oscillators such as the ^1L_a and ^1L_b absorption oscillators implies that the emission oscillator is oriented perpendicular to the molecular plane.[39] Hence we assign the orientation of the emission oscillator in the CH$_3$HgTrp complex to be perpendicular to the molecular plane.

The PPE spectrum of CH$_3$HgHBIm$^+$ is given in Figure 2. The polarization is negative with respect to the ^1L_b and positive with respect to the ^1L_a oscillator. Since it was shown that H$_2$BIm$^+$ had *only* negative polarization with respect to both absorption oscillators (and was therefore perpendicular to both), then in the complex the emission oscillator is oriented significantly more parallel to the ^1L_a oscillator while remaining relatively perpendicular to the ^1L_b oscillator. We conclude that the emission oscillator has a significant component along the axis of the ^1L_a (short axis[35,40]) oscillator.

The results of the ODMR pulse and saturation recovery transient measurements have been combined and are summarized for both complexes in Table I. For CH$_3$HgHBIm$^+$ previously reported[27] results for the sublevel decay rate constants (k_i), measured by microwave-induced delayed phosphorescence (MIDP),[41] have been combined with the results from the present continuous optical pumping measurements. The MIDP, pulse, and saturation recovery transient methods give sublevel lifetimes which are in good agreement with each other. The measured relative radiative rate constants (k_i^r) of CH$_3$HgHBIm$^+$ are also presented in Table I. The microwave pulse and saturation recovery transient measurements give results which are in reasonable agreement.

In the case of CH$_3$HgTrp, the microwave pulse and the saturation recovery transient measurements yield values of the k_i and relative k_i^r which are in good agreement. An independent measurement of $\bar{k} \equiv \frac{1}{3}(k_i + k_j + k_k)$ was carried out by a method described in Appendix I, and is in good agreement with that calculated from the individually determined k_i. In a previous communication,[27] an extremely short and intense component of the pulsed-laser excited phosphorescence decay of CH$_3$HgTrp was interpreted as arising from a sublevel with a decay constant several times larger than those observed in the present work. We have repeated the pulsed-laser excited decay measurements and invariably observe this anomalously short decay component. The short decay component is not the result of stimulated emission, since its relative amplitude is not affected by large variations in solute concentration or in the intensity of the laser pulse. We will return to this point in the Discussion.

IV. Discussion

The effects of complexing CH$_3$Hg^{II} with HBIm and HTrp are a dramatic decrease in the overall triplet lifetime and a marked enhancement in the radiative triplet yield.[27] We have

found that the heavy-atom effect in these complexes leads to a shortening of the individual sublevel lifetimes which is strongly sublevel selective. Additionally, we observe that the reduction in lifetime is due primarily to an increase in the radiative rate constant. The increase in the radiative rate constant is reflected in the observation that the relative sublevel radiative quantum yields, $Q^r_{i,rel}$, defined by $Q^r_{i,rel} = k^r_{i,rel}/k_i$ are about the same for all sublevels in both complexes examined. In contrast, for HTrp, and H$_2$BIm$^+$, two of the sublevels have $Q^r_{i,rel} \sim 0$, i.e., $k_i^r \sim 0$ within our sensitivity limits.[34,42] The observed overall radiative enhancement of triplet states due to heavy-atom perturbation has been noted in a number of other studies.[43,44]

Cheng et al.,[45] from MIDP measurements on quinoxaline triplet in a heavy-atom host crystal (p-dibromobenzene), have concluded that a significant degree of radiationless deactivation of the triplet state occurs as a result of the external heavy-atom effect. Furthermore, they conclude that radiative enhancement of the out-of-plane sublevel is dominant. These conclusions are at variance with our present findings regarding the heavy-atom effect of CH$_3$Hg^{II} complexing. Radiative enhancement of triplet-state decay is dominant, and the in-plane sublevels are the most affected in both complexes studied. Azumi has found, based upon theoretical work,[46] that the radiative enhancement of an aromatic triplet state should be largest for the out-of-plane sublevel. The theory, however, presumes noncoplanarity of the aromatic molecule and perturber, and that the perturber is itself an aromatic molecule. It is clear that Azumi's results cannot be generalized to all types of perturbers and molecule-perturber geometries. For instance, recently published measurements[47] on the heavy-atom effect of Ag$^+$ on the inorganic ion NO$_2^-$ show that the greatest radiative enhancement occurs for an in-plane sublevel.

It has been observed previously[19] that the degree of polarization of the phosphorescence of aromatic molecules decreases upon introduction of heavy atom containing solvents. These observations are in contrast with our results from the PPE spectra, where the same degree of phosphorescence polarization is retained upon formation of CH$_3$Hg^{II} complexes. These different observations can be explained by assuming random molecule-perturber geometries in the heavy-atom solvent in contrast with specific complexes with CH$_3$Hg^{II}, as well as a dependence of the phosphorescence polarization axis on the molecule-perturber geometry. Significant polarization of the heavy atom perturbed phosphorescence of NaNO$_2$ doped with Ag$^+$ also has been observed by Clark and Tinti.[47] Here, a specific molecule-perturber geometry also presumably exists.

A convenient classification of heavy atom perturbed complexes which include charge-transfer character has been suggested by Gronkiewicz et al.[44] Class I consists of complexes which have essentially a locally excited triplet of one of the complex components, while class II consists of complexes in which the triplet state is described as a charge-transfer (CT) triplet composed of both donor and acceptor components. Class II complex phosphorescence has been shown[48] to be polarized
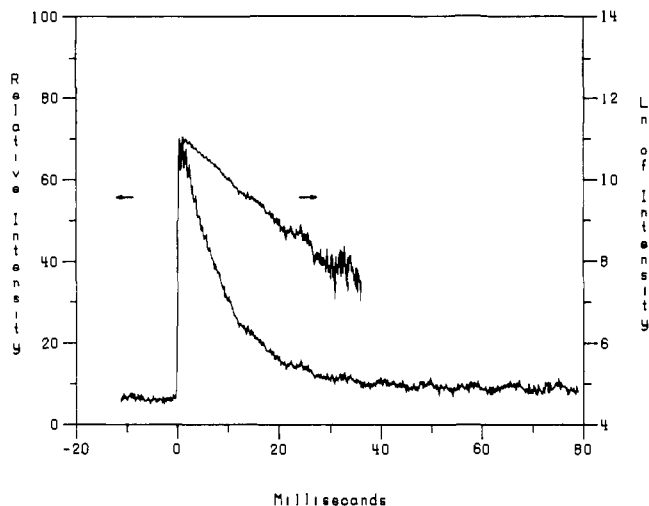
Figure 3. Lower curve is a modified microwave saturation recovery transient. Upper curve is semilogarithmic plot of lower curve. Sample is CH₃HgTrp observed monitoring at 409.2 nm. Temperature ~ 1.13 K. Signal is result of continuously saturating ν_3 transition and gating ν_2 transition on at $t = 0$. Signal is result of 25 000 accumulations.

along the line of centers between donor and acceptor. Our CH₃Hg[II] complexes are of class I, since the spectra and triplet zero field splitting (ZFS) parameters are hardly perturbed. Avouris and Sheng[49] note that large changes in ZFS need not result from the formation of class I complexes. It has been pointed out recently[50] that for complexes of class I enhanced radiative triplet decay can be secured via the mixing of the locally excited triplet state with radiative singlet charge transfer states involving the heavy-atom perturber by a second-order mechansim involving one-center spin–orbit coupling terms.

Our observed phosphorescence polarization directions (perpendicular to the plane for CH₃HgTrp, and generally along the short in-plane direction for CH₃HgHBIm⁺) appear to fit in well with a mechanism such as this in which the radiative intensity is obtained from an aromatic → metal atom singlet charge transfer state. Chandra et al.[50] have studied the triplet lifetimes of a series of rigid bromoalkyl derivatives of naphthalene and benzene, and have observed a great sensitivity of the heavy-atom effect to the distance of the heavy atom and its orientation relative to the aromatic chromophore. This sensitivity also is reflected in the perturbed triplet state lifetimes of our complexes. The reduction of triplet lifetimes is a factor of 40 for CH₃HgHBIm⁺, while it is a factor of 800 for CH₃HgTrp.

In a previous communication[27] we reported the average triplet total decay rate constant of CH₃HgTrp to be 910 s⁻¹ determined by pulsed-excitation phosphorescence decay, while in the present work it is reported as 107 s⁻¹ measured under continuous optical pumping. We suggest that the presence of a small quantity of a second complex with a very short-lived triplet state is responsible for the apparent discrepancy in triplet decay rate constants. We estimate (cf. Appendix II) that the approximate steady-state ratio of long- to short-lived triplet populations is at least 20:1. This population ratio would make detection of a short-lived component in steady state difficult within the available signal-to-noise ratio. With pulsed laser excitation (ca. 2 μs), however, the populations of the two types of complex are comparable and the short-lived complex would be dominant in the decay.

We have attempted to observe wavelength shifts in the time-resolved phosphorescence spectrum at 77 K using a rotating sector which selects the phosphorescence emission occurring during the first 1–2 ms following the excitation pulse.

Within our experimental error, the spectra of the short-lived and long-lived components of the phosphorescence are the same. Thus the short-lived species cannot be resolved optically. ODMR measurements at 1.1 K made using a rotating sector which is selective for signals from shorter lived species, however, reveal two broad peaks at ca. 4.5 and 6.6 GHz, well resolved from the D + E signal previously reported[27] for CH₃HgTrp (4.23 GHz). In order to determine whether these signals are associated with shorter lived triplet states than that reported here, we have measured saturation recovery transients,[22] pulsing the microwaves at 4.5 and at 6.6 GHz. The 6.6-GHz transient, in particular, has components of ca. 500 μs and 4 ms, consistent with a short-lived species which would decay with $\tau_p \sim 1$ ms at high temperature. Details of these measurements will be published in due course.

Appendix I. A Transient ODMR Method for Determination of Average Triplet Lifetimes

The method described is a modification of the saturation recovery transient method described by Shain and Sharnoff.[22] The experiment is performed with continuous optical pumping by first applying continuous microwave power to saturate one pair of sublevels. The transient response of the phosphorescence intensity is allowed to decay to a new steady value in the presence of the single saturation. At $t = 0$ a second microwave frequency is applied to saturate all levels of the triplet state. From $t = 0$, the triplet state is maintained by the double microwave saturation as a pseudo-one-level system which initially is out of steady-state equilibrium, but which approaches the steady state exponentially with a decay constant given by $\bar{k}$. For a one- (or pseudo-one-) level system the population is regulated by

$$\dot{n}(t) = P - \bar{k}n(t) \tag{I.1}$$

where n is the total triplet population and P is the populating rate. Under steady-state conditions, i.e., saturation of all sublevel populations

$$n^0 = P/\bar{k}$$

The steady-state intensity is then given by

$$I^0 = s\bar{k}^r n^0$$

where s is a constant depending on the experimental arrangement and $\bar{k}^r$ is the mean radiative rate constant. If the population is not at the steady-state value, the intensity is given by

$$\Delta I(t) = s\bar{k}^r \Delta n(t) \tag{I.2}$$

where

$$\Delta n(t) = n(t) - n^0 \text{ and } \Delta I(t) = I(t) - I^0 \tag{I.3}$$

Substitution of (I.3) into (I.1) gives

$$\dot{n}(t) = P - \bar{k}[\Delta n(t) + n^0] = -\bar{k}\Delta n(t)$$

We assume that $\Delta n(t)$ is always sufficiently small that P is constant. Since $\dot{n}(t) = \Delta\dot{n}(t)$, we get

$$\Delta\dot{n}(t) = -\bar{k}\Delta n(t)$$

Integration gives

$$\Delta n(t) = \Delta n(0)e^{-\bar{k}t} \tag{I.4}$$

Substitution of (I.4) into (I.2) gives

$$\Delta I(t) = s\overline{k}^r \Delta n(0) e^{-\overline{k}t} \qquad (1.5)$$

As an example of this measurement, we include both the phosphorescence transient and a semilogarithmic plot of the transient in Figure 3.

The advantage of the modified saturation recovery transient method over conventional phosphorescence decay measurements for the determination of average triplet decay lifetimes lies in the double-resonance property of ODMR. Since one uses both microwave and optical selection of the molecule to be probed, it is possible under many conditions to selectively detect a target chromophore in the presence of competing emission sources. We intend to apply this method in further ODMR studies of the binding of CH_3HgOH to synthetic and naturally occurring polynucleotides, where the presence of competitive binding sites will be likely to yield complex luminescence emission.

Appendix II. Relative Triplet State Populations under Pulsed and Steady-State Conditions

The time rate of change of a triplet population is given by

$$\frac{d[T_1]}{dt} = K[S_0] - k[T_1] \qquad (II.1)$$

where K is the probability per unit time that a molecule is excited from $S_0 \rightarrow T_1$ and k is the total triplet decay rate constant. The solution to the differential equation is

$$[T_1] = \frac{K[S_0]}{k}[1 - e^{-kt}] \qquad (II.2)$$

For times which are short compared to k^{-1}, e.g., pulsed excitation, the exponential may be expanded and the resulting approximate form is

$$[T_1]^P = K[S_0]t_p \qquad (II.3)$$

Under steady-state conditions, i.e., in the limit $t \rightarrow \infty$

$$[T_1]^0 = K[S_0]/k \qquad (II.4)$$

For triplets whose radiative quantum yield is unity

$$[T_1]^P = \int_0^\infty A^P e^{-kt} dt = A^P/k \qquad (II.5)$$

where A^P is the magnitude of the pulsed decay component at $t = 0$. For a decay curve of two components, originating from a long-lived and short-lived triplet state, use of eq II.3, II.4, and II.5 readily leads to the following expression for the ratio of steady-state populations of the triplet states:

$$\frac{[T_1]_s^0}{[T_1]_l^0} = \frac{A_s^P}{A_l^P}\left(\frac{k_l}{k_s}\right)^2 \qquad (II.6)$$

where the assumption has been made that the ratio of extinction coefficients of the two species is the same for the pulsed and steady-state measurements.

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