

# Evidence for Dual $L_a$ and $L_b$ Emission in 5-Methylindole

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The steady-state fluorescence anisotropy of 5-methylindole is shown to depend on both excitation and emission wavelengths, at room temperature in glycerol. A simulation is presented that shows that this emission wavelength dependence of the anisotropy can be explained in terms of dual emission from both the  $L_a$  and the  $L_b$  transition moments of the indole ring. For such dual emission to occur, the lowest excited-state energy level of both of these oscillators must be very similar.

**KEY WORDS:** 5-Methylindole; electronic transitions; fluorescence anisotropy.

## INTRODUCTION

The luminescence of indole continues to be an important subject for investigation, since the side chain of tryptophan is the dominant fluorescing amino acid in proteins. The photophysics of indole is consistent with the existence of two overlapping  $\pi \rightarrow \pi^*$  electronic transitions, referred to by the Platt labels  $L_a$  and  $L_b$ . These transition moments lie at approximately  $90^\circ$  with respect to one another in indole [1,3,8,9]. In the vapor phase, the  $L_b$  transition is the  $S_1$  state (the lowest-energy excited-singlet state) for indole and its simple analogues. In polar solutions, there can be a reversal of the  $S_1$  state. The  $L_a$  transition is more sensitive to solvent polarity, and consequently,  $L_a$  can be the lowest-energy singlet state in polar solvents. The explanation for the solvent sensitivity of  $L_a$  is that its moment lies through the imino NH of the pyrrole ring of indole and that there is a large change in dipole moment upon excitation of the  $L_a$  transition. The  $L_b$  transition shows little dependence on solvent polarity [2,5-7].

In addition to the different solvent dependencies, there is thought to be a significant difference in the vibronic structure of the  $L_a$  and  $L_b$  transitions. Whereas  $L_a$

shows a broad and featureless absorption envelope,  $L_b$  shows a strong 0-0 transition and a series of higher-energy vibronic peaks [8,9].

In a recent publication we studied the photophysics of indole and its methyl and methoxyindole analogues in an attempt to determine the mutual orientation of the  $L_a$  and  $L_b$  moments and to correlate experimental spectral features with semiempirical molecular orbital calculations [3]. In the course of this investigation we noticed that a couple of methylindoles, particularly 5-methylindole, shows evidence of dual emission of the  $L_a$  and  $L_b$  transitions. This evidence was that the emission anisotropy of 5-methylindole shows a dependence on both the emission and the excitation wavelength, resulting in a crossing pattern (see Fig. 4 in Ref. 3). These data were obtained in a propylene glycol glass at  $-50^\circ\text{C}$ . We reinvestigated this phenomenon to see if it occurs at room temperature and we present simulations here of the photophysics of 5-methylindole to show the probable basis for the crossing pattern.

## MATERIALS AND METHODS

*Materials.* 5-Methylindole, indole, and spectral-grade glycerol were obtained from Sigma Chemical Co. The solids were sublimed before use. Solutions of the indoles

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in glycerol were prepared to have an absorbance of  $\sim 0.2$  at 280 nm.

**Methods.** Absorption spectra were obtained with a Shimadzu 160A spectrophotometer. Fluorescence spectra and anisotropy measurements were made with a SLM 4800 fluorometer, which has been modified to include an ISA spectrograph and a Princeton diode array detector and ST-120 controller. Excitation slits of 4 nm, Glan-Thompson polarizers, and 1-cm cuvettes were used. The emission anisotropy,  $r$ , values were calculated as

$$r = \frac{F_{V,V} - gF_{H,V}}{F_{V,V} + 2gF_{H,V}} \quad (1)$$

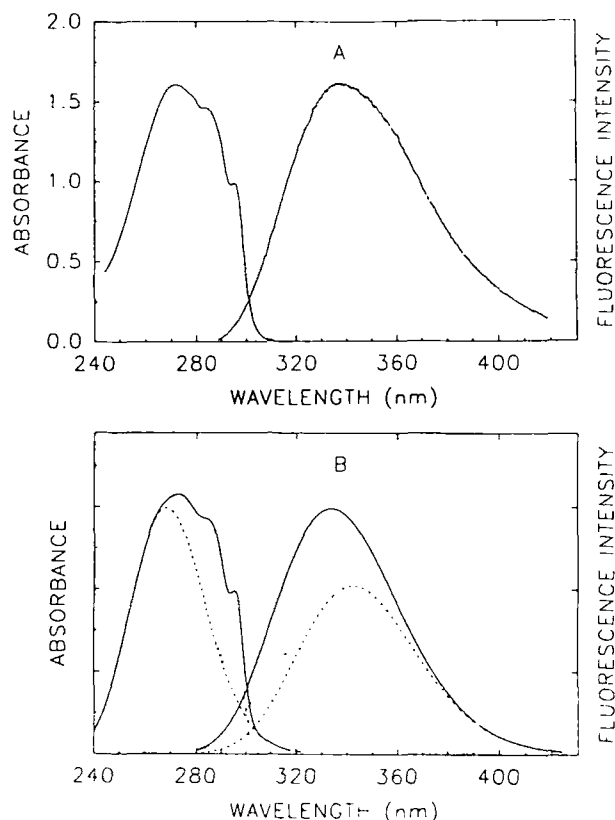
where  $F_{V,V}$  is vertically polarized emission with vertically polarized excitation,  $F_{H,V}$  is horizontally polarized emission with vertically polarized excitation, and  $g$  is an instrument correction factor, which is  $g = F_{V,H}/F_{H,H}$ , where  $F_{V,H}$  is vertical emission with horizontal excitation and  $F_{H,H}$  is horizontal emission with horizontal excitation.

## RESULTS AND DISCUSSION

Figure 1A shows the absorption and emission spectra of 5-methylindole in glycerol at 25°C. Figure 2A shows the emission wavelength dependence of the anisotropy of the fluorescence of 5-methylindole, with excitation at 270, 280, 290, and 295 nm. Note the crossing pattern. Figure 2B shows similar data for indole in glycerol; no emission wavelength dependence is seen for indole. Thus there is a distinctive pattern for 5-methylindole. This pattern had been reported previously to exist at  $-50^\circ\text{C}$  in a propylene glycol glass [3]. The present data show the pattern also to exist at room temperature and in a fluid, polar solvent. Note that glycerol was used in this study because it has a high viscosity, which reduces the rotational correlation time of the fluorophore and results in there being a high enough anisotropy for the pattern in Fig. 2A to be seen.

Previously we suggested that this crossing pattern is due to the existence of dual emission from the  $L_a$  and  $L_b$  states in 5-methylindole. For dual emission to occur, the  $L_a$  and  $L_b$   $S_1$  energy levels must be almost-identical, whereas for most indoles either  $L_a$  or  $L_b$  will have the lower origin (and hence will be  $S_1$ ) in a given solvent environment. The existence of dual emission has also been suggested in a couple of other publications [2,4].

To support the argument that dual emission occurs in 5-methylindole, we present the following simulation of spectra. In Fig. 1B we show a simulated absorption



**Fig. 1.** (A) Absorption and emission spectra of 5-methylindole in glycerol at 25°C. (B) Simulated spectrum for a hypothetical indole with contributions from  $L_a$  (---) and  $L_b$  (····) components. The  $L_a$  component is simulated by a Gaussian shape [Eq. (2)] with  $\bar{\nu} = 37.3$  kK and  $\Delta = 2.85$  kK. The  $L_b$  component is simulated with three Gaussian shapes with  $\bar{\nu} = 33.7$ ,  $\bar{\nu} = 34.66$ , and  $\bar{\nu} = 35.8$  kK, respectively, and  $\Delta = 0.4$ ,  $\Delta = 0.77$ , and  $\Delta = 1.09$  kK, respectively. The simulated absorption spectrum was generated by performing a nonlinear least-squares fit of the Gaussian peak model to the raw absorption spectrum. Also shown is a simulated emission spectrum for a hypothetical indole with contributions from  $L_a$  (---) and  $L_b$  (····) components. The  $L_a$  component is a Gaussian shape [Eq. (1)] with  $\bar{\nu} = 29.2$  kK and  $\Delta = 2.8$  kK. The  $L_b$  component is simulated with  $\bar{\nu} = 31$  kK and  $\Delta = 2.5$  kK. It is assumed that the fluorescence quantum yield of the  $L_a$  and  $L_b$  components is 0.2 and 0.1, respectively. These simulated absorption and emission spectra was used to simulate the anisotropy pattern in Fig. 3.

spectrum, which is intended to represent roughly the absorption of 5-methylindole. This was simulated by representing the absorption bands by a Gaussian shape in wavenumber by the following equation:

$$C\nu = \frac{\exp\left(\frac{-(\nu - \bar{\nu})^2}{\Delta^2}\right)}{\Delta\sqrt{2\pi}} \quad (2)$$

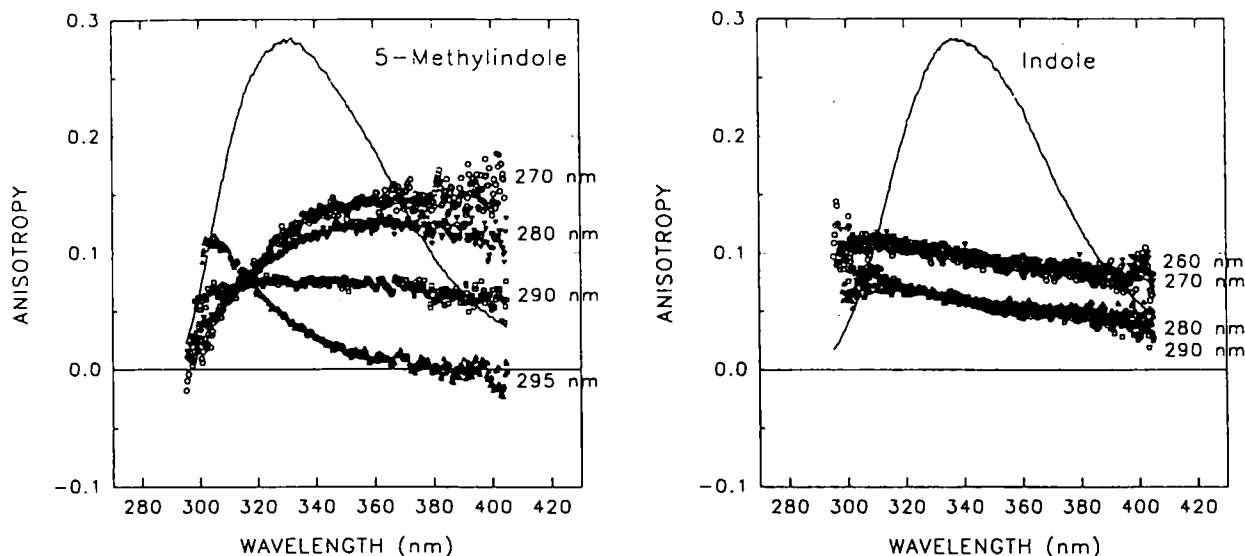


Fig. 2. (A) Emission wavelength dependence of the fluorescence anisotropy of 5-methylindole in glycerol at 25°C, with excitation wavelengths of 270 (○), 280 (▽), 290 (□), and 295 (△) nm. (B) Same type of data for indole in glycerol, with excitation wavelengths of 260 (○), 270 (▽), 280 (△), and 290 (□) nm.

where  $C_\nu$  is the normalized spectral shape function,  $\nu$  is the wavenumber in  $\text{kK}$  ( $=1000 \text{ cm}^{-1}$ ),  $\bar{\nu}$  is the wavenumber for the center of the shape, and  $\Delta$  is the width of the shape. We simulated the  $L_a$  band as a single peak with  $\bar{\nu} = 37.3 \text{ kK}$  (268 nm) and  $\Delta = 2.85 \text{ kK}$ . For the  $L_b$  component, we added three narrow Gaussian shapes, to represent the vibronic pattern of this transition. These included the 0–0 transition, with  $\bar{\nu} = 33.7 \text{ kK}$  (297 nm), plus two others, at  $\bar{\nu} = 34.7 \text{ kK}$  (288 nm) and  $35.9 \text{ kK}$  (278 nm). For these peaks we used  $\Delta = 0.40, 0.77,$  and  $1.09 \text{ kK}$ , respectively, and we adjusted the height of the three peaks to approximate the expected  $L_b$  pattern. Actually, a nonlinear least-squares program was used to fit the above Gaussian shapes (one for  $L_a$  plus three for  $L_b$ ) to the absorption spectrum. The dashed and dotted lines in Fig. 1B represent the simulated  $L_a$  and  $L_b$  components. Further, we simulated the emission spectra for the pure  $L_a$  and  $L_b$  states as illustrated in Fig. 1B. These emission spectra were also Gaussian in shape, with  $\bar{\nu} = 29.2 \text{ kK}$  (343 nm) and  $\Delta = 2.8 \text{ kK}$  for  $L_a$  and  $\bar{\nu} = 31 \text{ kK}$  (322.6 nm) and  $\Delta = 2.5 \text{ kK}$  for  $L_b$ . Thus  $L_b$  is simulated to be bluer emitting than  $L_a$ , as expected if  $L_a$  has the larger change in transition dipole (upon excitation) and shows the larger Stokes shift in a polar solvent.

If there is only a single transition moment in a fluorophore, then the steady-state emission anisotropy is equal to the maximum anisotropy,  $r_o = 0.40$ , times depolar-

izing factors,  $d_i$ . There is an electronic depolarizing factor,  $d_{ac}$ , for the noncoincidence of the orientations of the absorption and emission transition moments (where  $\Theta_{ac}$  is the angle between the absorption and the emission oscillators), and a rotational depolarizing factor,  $d_{rot}$ , for rotation (assumed to be isotropic for simplicity) of the molecule (and hence the emission dipole) in the excited state.

$$r = r_o d_{ac} d_{rot} \quad (3)$$

$$d_{ac} = \frac{3\cos^2\Theta_{ac} - 1}{2} \quad (4)$$

If there are two degenerate and rapidly interconverting  $S_1$  emitting states, of  $L_a$  and  $L_b$  origin, then the steady-state anisotropy is given by the following equation:

$$r = (f_a f_{a\lambda'} + f_b f_{b\lambda'}) r_o d_{ac} d_{rot} + \frac{(f_a f_{b\lambda'} + f_b f_{a\lambda'}) r_o d_{ac} d_{rot} d_{ba}}{2} \quad (5)$$

where  $d_{ba}$  is the depolarizing factor for the interconversion of the  $L_a$  and  $L_b$  excited-state oscillators and is given by

$$d_{ba} = \frac{3\cos^2\Theta_{ba} - 1}{2} \quad (6)$$

where  $\Theta_{ba}$  is the angle between the  $L_a$  and the  $L_b$  emission oscillators. Also in Eq. (5),  $f_{a\lambda}$  and  $f_{b\lambda}$  are the fractional contributions of  $L_a$  and  $L_b$  to the total absorbance

at wavelength  $\lambda$ , and  $f_{a\lambda}$  and  $f_{b\lambda}$  are the fractional contributions of  $L_a$  and  $L_b$  emission oscillators to the total fluorescence at wavelength  $\lambda$ . The first half of Eq. (5) represents direct excitation and emission from a given oscillator (with  $d_{ac}$  and  $d_{rot}$  depolarizing factors), and the second half of Eq. (5) represents the contribution from interconverting states (i.e., absorption into  $L_a$  with emission from  $L_b$ , and vice versa), with the additional depolarizing factor,  $d_{ba}$ . (If emission occurs from only one excited state, i.e.,  $L_a$ , then one of the fractional emission contributions, i.e.,  $f_{b\lambda}$  will be zero and the equation collapses to the relationship used in Ref. 8.)

Figure 3 shows a simulation of a pattern for dual emission using the above equation, the simulated absorbance and emission spectra in Fig. 1B, and values of  $d_{ac} = 0.886$  (corresponding to  $\Theta_{ac} = \pi/8$ ) for both  $L_a$  and  $L_b$ ,  $d_{rot} = 0.5$  for both  $L_a$  and  $L_b$ , and  $d_{ba} = -0.5$  (corresponding to  $\Theta_{ba} = \pi/2$ ). The latter value of  $\Theta_{ba}$  was selected based on molecular orbital calculations [3].

The simulated patterns in Fig. 3 are very similar to the experimentally observed patterns in Fig. 2A. The patterns in Fig. 3 are not nonlinear least-squares fits, due to the large number of adjustable parameters, and

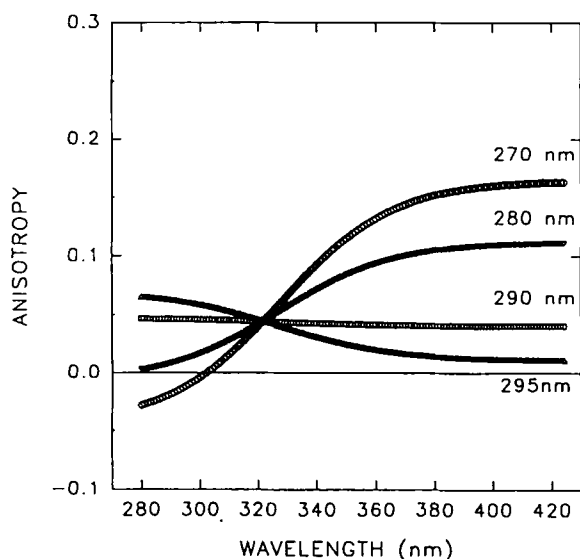


Fig. 3. Simulated emission and excitation wavelength dependence of the fluorescence anisotropy, using simulated spectra in Fig. 1B and the parameters given in the text.

we do not claim the parameters used in the simulation to be unique. We present the simulation to support the notion that there is dual emission from the  $L_a$  and  $L_b$  states of 5-methylindole. Such dual emission occurs both in low-temperature glasses and in a polar solvent at room temperature for this chromophore.

## ACKNOWLEDGMENT

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## NOMENCLATURE

$C_v$	Gaussian spectral shape function
$d_{ac}, d_{rot}, d_{ba}$	Depolarizing factors due to the angle between the absorption and the emission moments, rotational motion, and the angle between the $L_a$ and the $L_b$ moments
$\Delta$	Width of shape function
$L_a, L_b$	Electronic transitions, following Platt labels
$r$	Steady-state fluorescence anisotropy
$r_o$	Limiting anisotropy in the absence of depolarizing processes
$\Theta_{ac}, \Theta_{ba}$	Angle between absorption and emission moments and between $L_a$ and $L_b$ moments
$\nu, \bar{\nu}$	Frequency and central frequency for spectral shape function ( $\text{cm}^{-1}$ )

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