Characterization of 1La and 1Lb emission from indole-polar solvent complexes by supersonic jet spectroscopy
by Kurt William Short

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry
Montana State University
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Abstract:
Two-photon polarized fluorescence • excitation and vibronically resolved one-photon dispersed fluorescence from experiments in the supersonic jet have been used to characterize the lowest excited state of van der Waals complexes of indole and 3-methylindole with various polar solvents. For indole, in complexes with water, methanol, and formamide, the two-photon spectra for all of the complexes have circular/linear polarization ratios of absorptivity (Ω-values) that show the excitation is to the 1Lb state. The Franck-Condon patterns of the dispersed fluorescence for these same indole-polar solvent complexes show that emission is also from the 1Lb state. The complexes of 3-methylindoie with water, methanol, ethanol, butanol, diethyl ether, diethylamine, and triethylamine show that the nature of the emitting state is closely related to the proton affinity of the complexing solvent. As the. proton affinity increases there appears to be a shift from a 1Lb type excited state to one which has more 1La type characteristics. The point at which the excited state becomes more 1La-like than 1Lb-like appears to be around a proton affinity of 200 kcal/mol. Two-photon Ω-values decrease from 0.99 for water which has a proton affinity of 166.5 kcal/mol to 0.55 for triethylamine which has a proton affinity of 231.2 kcal/mol. The dispersed fluorescence spectra of the 3-methylindole-polar solvant complexes all show characteristics which can be associated with both 1La and 1Lb types of emission. However, for solvents with lower proton affinities the 1Lb type characteristics dominate, while solvents with higher proton affinities have emission more like that expected for the 1La state. Also reported here are results of supersonic jet experiments using 2,3-dimethylindole, 7-azaindole, bare indole, and bare 3-methylindole.

CHARACTERIZATION OF ^1L_a AND ^1L_b EMISSION FROM INDOLE-POLAR

SOLVENT COMPLEXES BY SUPERSONIC JET SPECTROSCOPY

by

Kurt William Short

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Kurt William Short

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Patrik R. Callis

_____ 12 Apr 99
Chairperson, Graduate Committee   Date

Approved for the Department of Chemistry

David M. Dooley

_____ 4/12/99
Head, Major Department   Date

Approved for the College of Graduate Studies

Bruce McLeod

_____ 4-16-99
Graduate Dean   Date

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Signature _Kurt W. Short_

Date _April 12, 1999_

The light shines on in darkness,
a darkness that did not overcome it.

John 1:5

To all those who are lights in my life.

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ABSTRACT

Two-photon polarized fluorescence excitation and vibronically resolved one-photon dispersed fluorescence from experiments in the supersonic jet have been used to characterize the lowest excited state of van der Waals complexes of indole and 3-methylindole with various polar solvents. For indole, in complexes with water, methanol, and formamide, the two-photon spectra for all of the complexes have circular/linear polarization ratios of absorptivity (Ω-values) that show the excitation is to the ^1L_b state. The Franck-Condon patterns of the dispersed fluorescence for these same indole-polar solvent complexes show that emission is also from the ^1L_b state. The complexes of 3-methylindole with water, methanol, ethanol, butanol, diethyl ether, diethylamine, and triethylamine show that the nature of the emitting state is closely related to the proton affinity of the complexing solvent. As the proton affinity increases there appears to be a shift from a ^1L_b type excited state to one which has more ^1L_a type characteristics. The point at which the excited state becomes more ^1L_a-like than ^1L_b-like appears to be around a proton affinity of 200 kcal/mol. Two-photon Ω-values decrease from 0.99 for water which has a proton affinity of 166.5 kcal/mol to 0.55 for triethylamine which has a proton affinity of 231.2 kcal/mol. The dispersed fluorescence spectra of the 3-methylindole-polar solvant complexes all show characteristics which can be associated with both ^1L_a and ^1L_b types of emission. However, for solvents with lower proton affinities the ^1L_b type characteristics dominate, while solvents with higher proton affinities have emission more like that expected for the ^1L_a state. Also reported here are results of supersonic jet experiments using 2,3-dimethylindole, 7-azaindole, bare indole, and bare 3-methylindole.

INTRODUCTION

Motivation for this Research

The spectroscopic characterization of proteins and their components has long been a subject of great interest to physical and biological scientists. This interest is the result of the belief that it should be possible to determine much about the structure and local environment in a protein simply from the intensity, shape, and wavelength of the spectral signal. All of the amino acids in proteins show spectroscopic activity. However, of the twenty amino acids that commonly make up proteins, tryptophan has been of greatest importance in probing local protein structure and environment. This is because tryptophan has by far the largest spectroscopic activity (absorption) in the near UV(250-300nm)(1), and because it has the most sensitivity to changes in the local environment. So, even if a large protein contains only one tryptophan residue, it will dominate the protein spectrum in this wavelength range. The intensity and wavelength position of the maximum fluorescence signal will depend strongly on the local environment and conformation of the tryptophan residue. Thus, tryptophan (or one of its analogues) can be used as a sensitive local probe in proteins. In many instances, this is accomplished by using site specific mutagenesis in which specific insertions or deletions of tryptophan are made. For this reason, it is important to determine what changes in tryptophan fluorescence mean in terms of specific changes in its local environment and conformation.

In the absorption spectrum of aqueous tryptophan three of the important (π,π^*) transitions occur at about $220nm(45455cm^{-1})$, $280nm(35714cm^{-1})$, and $288nm(34722cm^{-1})$ {439}. These correspond

respectively to the electronic transitions $^1B_a \leftarrow {}^1A$, $^1L_a \leftarrow {}^1A$, and ^1L_b $\leftarrow {}^1A(2,3)$. The same three bands are also present in solution spectra of indole, which is the chromophore of tryptophan (see Figure 1).

Structures of Indole Derivatives
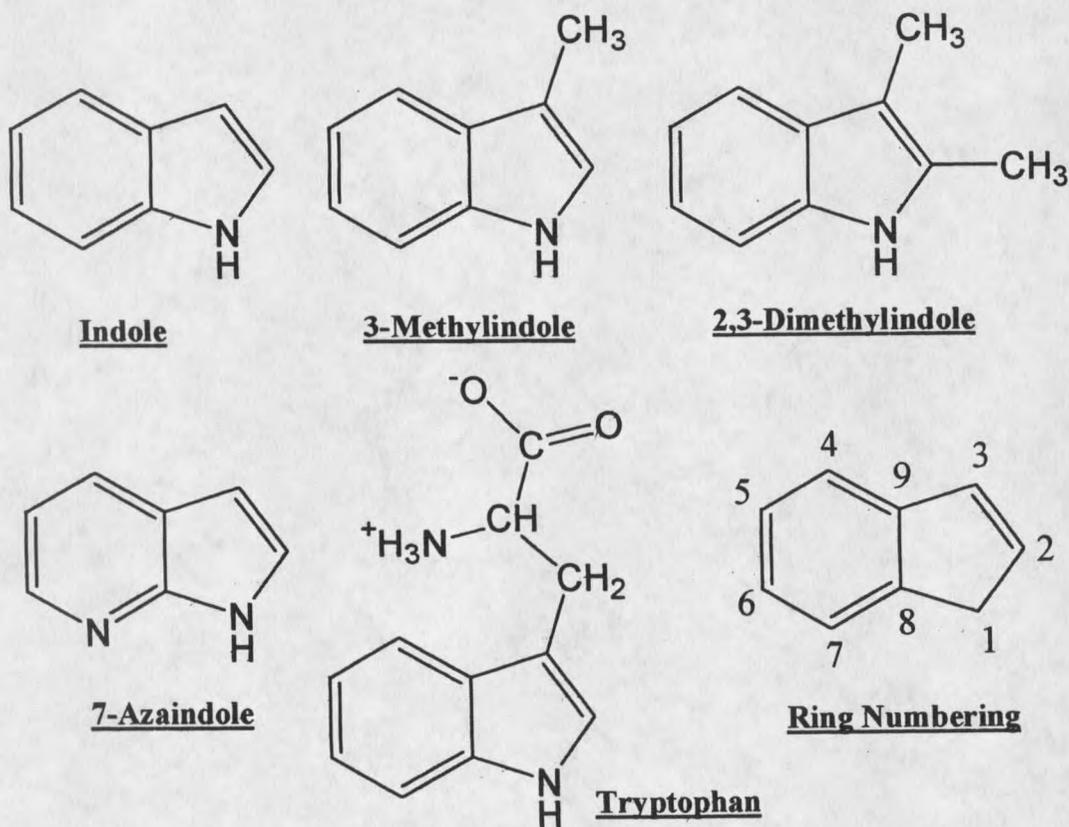
Figure 1. The structures of various indole derivatives and the numbering of the indole ring are shown.

Their exact positions depend on the particular indole derivative and the polarity of the solvent environment. We are interested here in the nearly degenerate ^1L_a and ^1L_b transitions. For many indole derivatives in polar solutions(4) or low temperature solvent glasses(5-7), absorption spectra show that the ^1L_a maximum occurs at higher energy than the ^1L_b maximum. However, the ^1L_a band often has a low energy tail

that extends beyond the red edge of the ^1L_b transition (Figure 2). The work described in this thesis has helped to firmly establish the view that, for most proteins, the ^1L_a 0-0 transition occurs at a lower energy than the ^1L_b 0-0 transition(8).

Resolution of ^1L_a and ^1L_b Bands
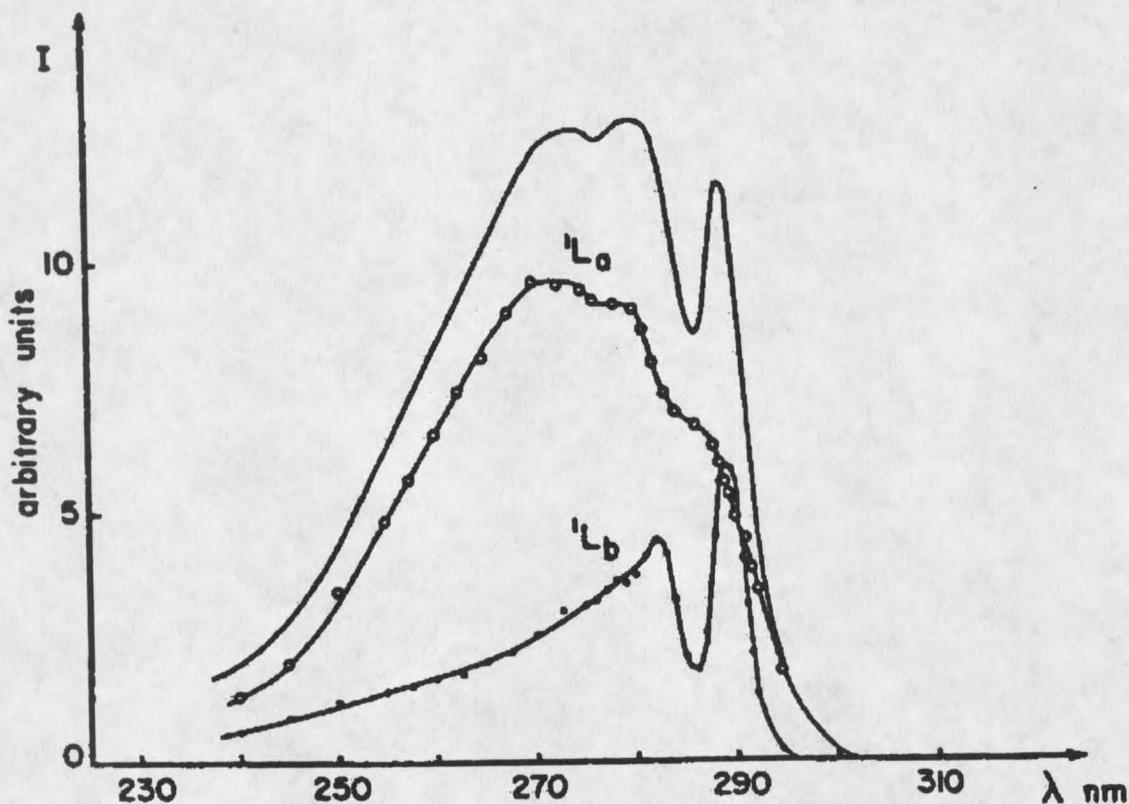
Figure 2. The absorption spectrum of indole is shown resolved into its ^1L_a and ^1L_b components. This figure is taken from reference(6).

The fluorescence spectrum of indole is extremely sensitive to the polarity of its environment. The spectral position of ^1L_a emission is particularly sensitive to local environment. This is due to the large permanent dipole moment of this state. The larger dipole will have more interaction with the local electric field caused by the neighboring atoms, stabilizing or destabilizing the excited state that affects the properties of the fluorescence(9). The magnitude and directions of the

ground, ^1L_a, ^1L_b, and ^3L_a permanent dipoles are shown in figure 3. The ground state dipole has a value of 2.1D, the ^1L_a 5.1D, and the ^1L_b 2.2D(2). More recent measurements have put the ground state to excited state permanent dipole changes at $^1L_a|\Delta\mu|=5.9\pm0.3D$ and $^1L_b|\Delta\mu|=1.3\pm0.3D(10)$. So, it is not surprising that the spectral position of ^1L_a emission is very sensitive to its environment.

Permanent Dipoles of Indole
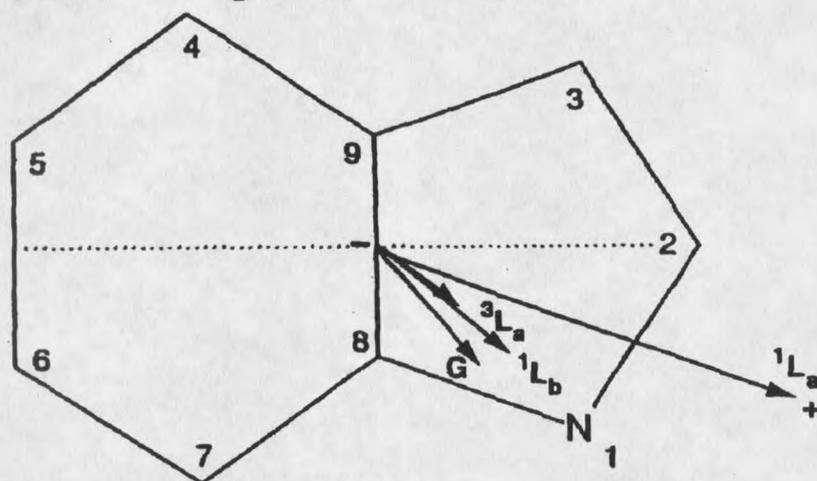
Figure 3. The ^1L_a, ^1L_b, ^3L_a, and ground state(G) permanent dipoles of indole are shown. This figure is taken from reference(9).

This sensitivity to environment makes indole amenable to studies in which it is formed into van der Waals complexes with various solvents in the environment of a supersonic jet. Jet spectroscopy allows high resolution vibronically (and rotationally) resolved spectra to be collected in the gas phase. Its characteristics will be reviewed in the Experimental section. Since complexes can be formed that contain only one or two solvent molecules, it is possible to determine how specific solvent interactions affect ^1L_a and ^1L_b emission. Information of this type will be very important in establishing how specific interactions in proteins affect tryptophan fluorescence.

Various derivatives of indole can be formed by attaching substituents at different positions around the five- or six-membered rings. Addition at each position affects the fluorescence emission in different ways(5). It is important to understand these changes in fluorescence for the following reasons. First, for tryptophan in proteins the connection of the indole group to the peptide backbone is via the 3-position, thus 3-methylindole is the indole analogue most like tryptophan. Second, it has recently become possible to incorporate tryptophan analogues into proteins in place of unmodified tryptophan. These analogues include 7-azaindole(11) and 4-fluoroindole(12). Since these modified tryptophans can be used as reporter groups in proteins it is important to understand their spectroscopic properties. Finally, it is interesting from a fundamental point to understand how an identical perturbation at different ring positions affects indole fluorescence. The mono-methylated indoles have been useful in this regard(5).

Jet spectroscopy measurements of indole have characterized the vibronic structure of both the ground(13) and excited states(14). Using only jet data, it initially proved impossible to determine the location of the ^1L_a origin(15-18). However, the combination of polarized two-photon jet spectra with matrix isolation studies of indole in an argon matrix(19), has found the location of the ^1L_a origin of indole to be displaced about 1400 cm^{-1} above that of the ^1L_b origin. This is in agreement with the seminal work done by Strickland et.al.(20) more than twenty-five years ago on indole dissolved in polar and non-polar solvents.

The maximum wavelength of tryptophan fluorescence in proteins, in solution, varies from 308-355nm, depending primarily on the exposure of the indole chromophore to water(8,21-25). Therefore, of the jet studies that have been done on indole and its various derivatives in van der

Waals complexes with one or more polar or non-polar solvent molecules(16,17,26-36), the ones involving water complexes(16,26,28-34,36) have been of most interest. Hager et al. first reported that indole formed two van der Waals complexes with water(30) and alcohols(31): one whose origin was shifted 132-214 cm^{-1} below the bare molecule origin(the σ-complex), and another whose origin was shifted 450-518 cm^{-1} below the bare origin and showed greater Franck-Condon(FC) activity. The σ-complex was assigned to a complex in which the solvent acted as a proton acceptor from the indole NH group. The second complex was at first attributed to an indole associated with two solvent molecules because of its concentration dependence(31), but the subsequent observation that this signal was confined to the indole + H_2O mass channel during mass selected experiments(30) led to its assignment as a second 1:1 complex. Because no redshifted complexes were found for N-methylindole, Hager et al. suggested that the second 1:1 complex also involved a hydrogen bond with the water or methanol acting as a proton acceptor(30). Levy and co-workers(35,37) then discovered that a special conformer of tryptophan and related molecules exhibited a long FC progression in a 20-30 cm^{-1} vibration in the excitation spectrum, and that excitation of the special conformer resulted in a unique redshifted and diffuse dispersed fluorescence in comparison to that from other conformers. In the course of their extensive investigation, the Levy group came to the interpretation that this behavior was associated with an intramolecular π-complex between a polar side chain and the indole π system. They also concluded (despite no real proof) that the interaction was sufficient to invert the order of the nearly degenerate ^1L_a and ^1L_b $\pi\pi^*$ excited states whose mixing was responsible for the large FC activity and the diffuse redshifted fluorescence. This interpretation was then extended to those complexes of indole with water, methanol,

formamide, and acetamide that exhibited long, low-frequency progressions and broad, redshifted fluorescence(29). Independently, Arnold and Sulkes(28,36) applied the same interpretation to a larger set of methylated indoles complexed with various polar molecules, concluding that solvent-induced ^1L_a emission in jet-cooled indole complexes is more the rule than the exception. Finally, the Levy group(29) tried to correlate the two types of complexes observed in cold jets with the two exciplexes postulated by Lumry and co-workers(38,39) to explain the pronounced redshifts of fluorescence for solutions of indole in hydrocarbons at room temperature when mM quantities of alcohols were added.

An achievement of this thesis has been to show unequivocally that the above picture is incorrect. We have used polarized two-photon excitation and dispersed fluorescence, to show that jet-cooled complexes of indole with the polar solvents water, methanol, and formamide are both excited to and emit from the ^1L_b state. We have also shown that exciplexes are not involved in the redshifted indole polar-solvent complexes.

Despite no real proof of its structure, it has become well accepted to refer to the indole-water complex with the redshifted spectrum as the π-complex(28,29,36). Recently, however Carney, Hagemeister, and Zwier, have found from resonant ion-dip infrared spectra, infrared-ultraviolet holeburning, and supporting *ab initio* calculations(40) that this complex has the formula indole-$(H_2O)_2$, with one water accepting a hydrogen bond from the indole N-H and the other accepting a hydrogen bond from the first water and bridging to the π-system. Because of this, the complexes showing redshifted spectra will be referred to as "ρ-complexes," with the "ρ" standing for redshifted. This red shift is what the indole-polar

solvent complexes have in common with the intramolecular complexes reported by the Levy group.

Although fluorescence from some complexes of different indole derivatives with polar solvents is believed to originate in the ^1L_a state, one question has remained. What is the vibronic structure of ^1L_a fluorescence in a jet environment? It was originally suggested, based on the unexpectedly short fluorescence lifetimes for the higher vibronic modes of 2,3-dimethylindole(13,28,33), that the ^1L_a transitions could not be observed in the jet because this state is dissociative through breaking of the N-H bond. Further studies with 3-methylindole(34) and negative results in a mass-resolved search for the possible fragment of N-H bond dissociation in 2,3-dimethylindole(36), led to the suggestion that the ^1L_a state is not dissociative, but is coupled to a third state that is probably a highly excited ground state vibronic level. So the short lifetimes in 2,3-dimethylindole were explained by non-radiative decay to this state. Independent of this idea, as mentioned above, the idea that emission from the ^1L_a state is broad and redshifted was being proposed(28,29,36). During this same time, experimental and theoretical results from the Callis group had started to suggest a slightly different picture of ^1L_a emission. Muiño and Callis(32) showed that dispersed fluorescence from an indole-H_2O complex, that was believed to originate from the ^1L_a state, could be well represented simply by adding the appropriate progression onto each line of the emission spectrum from bare indole(which has ^1L_b emission) and broadening each line by 50 cm^{-1} to simulate typical monochrometer resolution. This suggested that the emission could not be ^1L_a because it was constructed from ^1L_b emission. Also, *ab initio* calculations of the vibronic spectra for indole(41) had suggested that ^1L_a emission while different from ^1L_b emission should have a well defined vibronic structure in which the 0-0 transition and should

have the largest FC activity. Initial experimental evidence for these ideas has come from well resolved phosphorescence spectra of indole in an argon matrix(42). Indole phosphorescence is known to originate from the ^3L_a state and so should have a FC pattern very similar to that expected for ^1L_a fluorescence. This is exactly what was found for the ^3L_a phosphorescence spectrum of indole. It is against this background that the research to be presented here was started.

Subject of this Research

The research described here was started at a time when the nature of the emission from the ρ-complex was undefined. A major goal was to clearly define the state to which this complex was excited and from which it emitted, using polarized two-photon excitation and high resolution dispersed fluorescence. It should be noted that the sensitivity of the measurements had to be extended well beyond what had been previously obtained in our lab(two-photon measurements) or in other labs(dispersed fluorescence).

Another goal in this research was to more clearly define the vibronic structure of the $^1L_a \leftarrow$ ground transition and to prove that it can be observed in the supersonic jet. Also we wanted to form a better picture of the way in which polar solvents influence the energies of the ^1L_a and ^1L_b states relative to one another. This has been done by studying 3-methylindole in the supersonic jet, either as a bare molecule or as a van der Waals complex with polar solvent molecules.