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Solvent Complexes of Jet-Cooled β-Phenylethylamine

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Laser-induced fluorescence spectroscopy was used to investigate β-phenylethylamine molecules that were cooled via supersonic gas expansions. The bare molecule spectra reveal 0–0 transitions that can be attributed to four separate conformers. Upon the addition of water or alcohol solvents, a series of new peaks is induced that appear to be built off separate bare molecule transitions. This is a pattern that is markedly different from the one seen for solvent addition to tyramine, which includes an –OH in the para position. As a result, definite hypotheses can be made to explain the influence of the para –OH in affecting solvent induced conformations.

KEY WORDS: Supersonic gas expansions; conformers; laser-induced fluorescence.

INTRODUCTION

The fluorescence properties of tryptophan residues have proved to be useful as probes of microenvironment in proteins and peptides [1]. As a result, there has been considerable interest in explaining the fluorescence decay of individual tryptophan zwitterions, which has been seen to be multiexponential [2]. A plausible and widely accepted explanation is the conformer model, which assumes that emission occurs from populations of different rotamers in solution, each with a different lifetime [2–4]. It is difficult, however, to obtain a detailed understanding between individual conformations and lifetime.

Supersonic gas expansion techniques can meaningfully address the relationship between fluorescence lifetime and conformation for tryptophan and related molecules. In a supersonic expansion, the seeded molecules of interest (volatilized tryptophan molecules, for example) have virtually all of their internal energy removed by collisions with the carrier gas in the early portion of the expansion process. One or more laser beams queries the gas jet farther downstream, by which time the sample molecules are in free molecular flow (collisions per molecule/ns, $< < 1$). At this point it is typically the case that the vibrational "temperature" is <50 K for a vibronic mode and <5 K for rotations; virtually all the molecules are therefore in the ground vibronic state. There are now populations of molecules "frozen" in the conformations that existed in Boltzmann equilibrium prior to expansion, probably in nearly the same proportions.

It is the case that different conformations of chemical derivatives of tryptophan can generally be distinguished by carrying out laser-induced fluorescence scans (LIF) in the region of the S_0–S_1 origin–origin transition. As was first seen with jet-cooled alkylbenzenes [5], the side chain may interact with the chromophore to varying degrees, depending on whether it is extended away from it or folded back to interact with it. The result will be different degrees of stabilization for S_0 and S_1 for different geometries of the side chain, leading to a set of separate 0–0 transitions, each at a slightly different frequency. For example, scanning to the blue, LIF spectra of tryptamine begin with six major peaks from 34834 to 34918 cm^{-1}, which can be shown to be vibrationally independent of one another [6]. Each must correspond to a 0–0 transition for a different conformer geometry. (Rotationally resolved spectra show that one vibronic

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band actually consists of two separate sets of rotational transitions, due to different geometries, that happen to overlap [7,8].)

For jet-cooled bare molecules this brings up the possibility of selectively exciting the S_1 origins of different conformer populations and measuring the ensuing fluorescence decay curves. Precise data can be obtained using time-correlated photon counting. This has now been done for chemical derivatives of tryptophan[9–13] as well as bare molecule tryptophan itself [13,14], and it is evident that each conformation shows a clean single exponential decay with generally different lifetime values. The largest lifetime differences occur for derivatives containing a carbonyl, in accord with the hypothesis of the conformer theory that intramolecular charge transfer from the chromophore to the carbonyl is an important radiationless process [2–4]. For further interpretation there is the need to be able to associate a geometry with each distinguishable 0–0 transition. Rotationally resolved LIF can potentially provide information of this sort. Experimental spectra must be matched with simulated spectra generated as a function of side-chain geometry driven through all angular variables. This extremely laborious process was actually carried out for the bare molecule conformers of jet-cooled tryptamine [7,8], but it is not practical in general. As a far less demanding alternative, the application of semiempirical methods such as molecular mechanics needs to be evaluated further.

Cold complexes with solvent molecules can also be produced via supersonic expansion methods by introducing a vapor pressure of the desired solvent into the expansion mixture. In the early, high-collision portion of the gas expansion, solvent complexes form with the parent molecule. They can subsequently be studied in the "cold" low-collision regime of the gas expansion, farther downstream. The first solvent-induced features to appear should correspond to $n=1$ solvent complexes, with $n>1$ features growing in more strongly with increased solvent vapor pressure. For some tryptophan derivatives as well as tryptophan itself [10–12,14,15], the $n=1$ solvent complexes have been characterized by LIF as well as mass sensitive excitation spectroscopies. Generally a number of origin-region $n=1$ features grow in, indicative of different solvent complex geometries. However, there is a striking departure from this pattern in the case of tryptamine. Here the addition of water or a variety of alcohols causes *only one* solvent-induced feature to grow in, always at the nearly the same frequency. The implication is that interactions take place that "pull" each initially formed complex into the same final geometry. We recently repeated these experiments with several derivatives of tyrosine, 3-(4-hydroxyphenyl) propionic acid (HPA), the analogue of 3-indole propionic acid, and tyramine, the analogue of tryptamine [16]. For addition of water or alcohols HPA showed a pattern of multiple solvent-induced features in the origin region similar to the one seen with 3-indole propionic acid [10,15]. Tyramine, however, showed only one solvent-induced feature, in a pattern very analogous to the one seen with tryptamine [10,15].

We now report similar solvent addition experiments to the phenylanaline analogue to tryptamine, β-phenylethylamine (hereafter PEA). (See Fig. 1 for relevant structures.) There is now a striking departure from the pattern seen for tyramine and tryptamine. These experimental results and their implications are the subject of this paper.

EXPERIMENTAL

He at 2–3 atm was bubbled through liquid PEA at room temperature and subsequently expanded through 200-μm pinhole orifices. LIF were excited by a frequency-doubled Hansch-type dye laser (linewidth, <1 cm^{-1}) pumped by a 50-mJ XeCl excimer (Lambda Physik EMG 52 MSC). Total fluorescence was collected through a bandpass filter to the red of the exciting line (typically a Schott W295 filter). LIF spectra were calibrated against hollow cathode optagalvanic sources, with wavenumber assignments over the full range of scans consistent to <2 cm^{-1}. Dispersed emission spectra were obtained using a 0.64-m Instruments, SA, monochromator.

RESULTS

Since PEA is a liquid at room temperature, there was no need for heating to obtain sufficient sample vapor

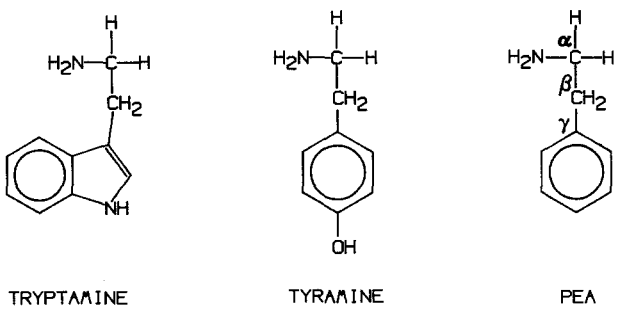

Fig. 1. Structures of tryptamine, tyramine, and β-phenylethylamine (PEA). The side-chain carbon designations are shown for PEA.

pressure. This fact and the consistent reproducibility of the LIF scans indicate that decomposition peaks in the spectra are unlikely. Scanning from red to blue, the first four prominent peaks occur at 37,547, 37,559, 37,609, and 37, 637 cm^{-1}, respectively. (See lower scan in Fig. 2.) These transitions nearly coincide in frequency with some 0–0 peaks that we observed for phenylalanine [17]. Based on the dispersed emission spectra (Fig. 3), each of the four major peaks is the 0–0 transition for a separate conformer. If, instead, one or more had been a low-frequency vibration built on an S_1 origin to the red, the dispersed emission spectra would be expected to show analogous low-frequency bands, but they do not.

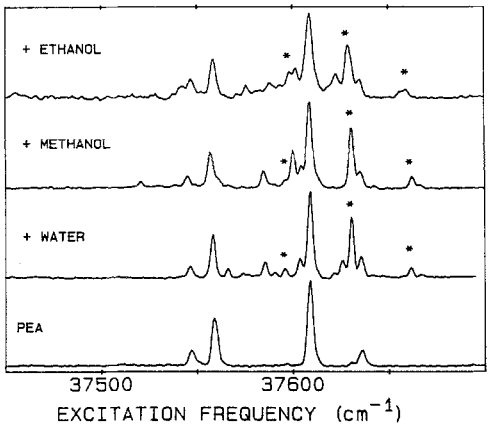

Fig. 2. LIF spectra of PEA without (lower scan) and with solvent addition. Solvent-induced peaks assigned to $n=1$ complexes are starred.

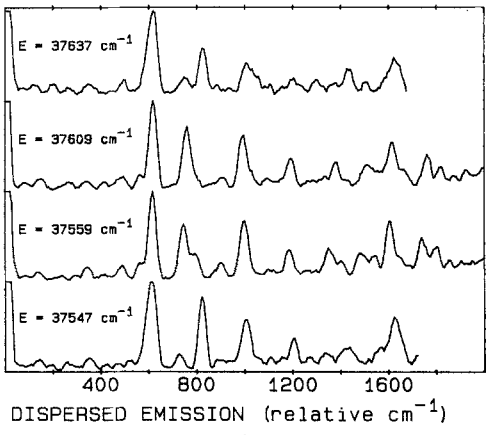

Fig. 3. Dispersed emission spectra for the four major origin region peaks of bare molecule PEA. Spectral resolution is ~20 cm^{-1}. A significant amount of laser light was present at the origins, and these peaks have been truncated.

We have also carried out molecular mechanics calculations on PEA using a modified version of MM2 (1987) [18]. The bond angles C_γ–C_β, C_β–C_α, and C_α–N (see Fig. 1 for C designations and Table 1, footnote *a*, for further angle specifications) were systematically driven to find global conformational minima which were then subsequently refined individually. The object is to attempt to correlate the molecular mechanics results with the LIF specra [18]. First, the numer of predicted conformers should be compared. To do this, we take all the molecular mechanics conformers that are "spectroscopically indistinguishable" and group them together. All conformations with the side chain fully extended away from the chromophore but with different values of C_α–N were assumed to have the same 0–0 frequency. (This would definitely be a highly dubious assumption if the side chain were not fully extended from the chromophore.) By this method we obtained the prediction of four major 0–0 transitions, the number actually seen. It would be desirable, though not necessarily possible, to go further and correlate the predicted conformer popu-

Table I. Predictions of Fractional Abundance (Room T) and Geometries of the Spectroscopically Distinguishable Conformers of PEA, Based on MM2 (1987) Calculations[a]

	Torsional angles (degrees)		
Conformer %	$C_\gamma - C_\beta$	$C_\beta - C_\alpha$	$C_\alpha - N$
41.2	±(83.595 to 85.803)	±(57.335 to 57.799)	±(68.518 to 68.668)
38.8	±(84.165 to 86.312)	±(55.668 to 56.110)	∓(177.815 to 177.549)
7.6	±(81.289 to 84.561)	±(59.974 to 60.355)	∓(66.895 to 67.300)
12.4	86.041 to 87.417	±(179.373 to 179.430)	±(62.201 to 62.335)
	87.121 to 87.344	179.960 to 180.00	179.937

[a] The ± signs refer to mirror images. Use the top or bottom sign for every angle of a conformer. When no sign precedes a range, that angle is always positive. Angles are defined as follows. C_γ–C_β: Orient the phenyl ring horizontally with the para C directly in front of the C_γ and the side chain below the plane of the phenyl ring. C_γ–C_β is defined as the angle formed between C_α and the plane of the ring. The angle is positive if C_α is clockwise from the ring and negative if it is counterclockwise from the ring. Angles range from 0 to ±90°. C_β–C_α: Orient C_β directly in front of C_α. C_β–C_α is defined as the angle formed by N and C_γ. The angle is positive if the N is clockwise from C_γ and negative if it is counterclockwise from C_γ. Angles range from 0 to ±180°. C_α–N: Orient C_α directly in front of N. C_α–N is defined as the angle formed by the nitrogen lone pair and C_β. The angle is positive if the lone pair is clockwise from C_β and negative if it is counterclockwise from C_β. Angles range from 0 to ±180°.

lations from molecular mechanics with observed LIF peak sizes. To try to do so, we make the following assumptions. First, we assume that for each 0–0 transition, population and absorbance scale equally (i.e., same transition dipoles and Franck–Condon factors). Second, we assume that "frozen" conformer population fractions are the same as those that obtained for the room T Boltzmann distribution in the preexpansion mixture. Finally, we assume that the LIF spectra closely follow absorbance spectra in the 0–0 region. Based on lifetime measurements of tyramine conformers [16], there are probably not large differences in PEA conformer lifetimes, which would likely mean an absorbance-like LIF spectrum. With these assumptions the molecular mechanics predictions of conformer fractions in Table I can be compared with the LIF spectrum. It is gratifying that there are in fact four LIF peaks with similar relative intensities. Whether one-to-one peak associations between the two data sets can be taken seriously is not yet clear. It is the case, however, that we have similarly found reasonable correlations between predicted and actual LIF 0–0 bands for other tryptophan and tyrosine derivatives [18].

Solvent addition to PEA was studied with water, methanol, and ethanol. To interpret these spectra, several trends evident from solvent addition to tyrosine derivatives [16] are useful to keep in mind. First, solvent addition to polar groups on the alkyl side chain of the chromophore results in red or blue shifts of tens of cm^{-1}. Much larger shifts, generally to the red, take place for solvent binding in closer proximity to the chromophore. Polar solvent binding readily occurs at the phenolic –OH of tyrosine derivatives but no analogous binding possibility exists in phenylalanine derivatives. Indeed, no such solvent-induced peaks were observed with PEA when scans were carried out to the red. Finally, it is observed that proton donor solvent binding at the amine results in solvent blue shifts.

For solvent addition to both tryptophan [10–12,14–16] and tyrosine [16] derivatives, strong separate $n=1$ bands arising in the 0–0 region have always been interpretable as separate solvent complex conformers. Features at slightly higher frequencies due to intracomplex motions appear to be weak for $n=1$ complexes since they have not been readily evident in previous LIF spectra. Similarly, strong Franck–Condon progressions for a complex origin are not usual, and in a case like this the pattern itself would be quite evident (Fig. 2).

For each solvent added to PEA a number of LIF scans were made under different solvent flow conditions, in order to correlate the initial appearance and growth of new bands. With water, tandem growth of solvent-induced features is always seen to appear first at 37,597, 37,630, and 37,662 cm^{-1}. Similar initial growth of features is seen at nearly the same frequencies for methanol and ethanol. (For ethanol the last solvent-induced peak is at 37,659 cm^{-1}.) On this basis it is likely that each feature corresponds to a different $n=1$ solvent complex. The bands at 37,630 and 37,662 cm^{-1} are shifted to the blue by roughly equal amounts relative to the bare conformer peaks at 37,609 and 37,637 cm^{-1}, respectively, and their relative intensities also scale roughly the same as these bare conformer peaks. For these reasons each of these solvent bands appears interpretable as a satellite of one of the bare conformer peaks, with the geometry of each solvent complex resembling the bare molecule conformer to which it is a satellite. The solvent peak at 37,597 cm^{-1} likely arises from binding to one of the two bare conformers to the red, probably the larger one at 37,559 cm^{-1}. The solvent addition spectra shown in Fig. 2 correspond to conditions of strong solvent addition, to make solvent complex bands more evident. The three $n=1$ assignments are starred. Other relatively large solvent induced features that subsequently appear are not necessarily $n=1$.

DISCUSSION

When an –OH-bearing solvent molecule is added to PEA, at least three different bands are induced. The blue shifts of tens of cm^{-1} are characteristic of solvent proton donation to the amine. Each solvent peak appears to be correlated to a separate bare molecule conformer. This pattern of solvent induced peaks represents a striking departure from the one seen in tyramine [16]. Here water or alcohol addition induced *only one* prominent solvent feature, to the blue of all the bare conformer peaks and not obviously related to any one of them. The implication is that the tyramine–solvent complexes formed in the early portions of the supersonic expansion, where they still retained significant vibrational energy, always relaxed into a unique conformation. Evidently interaction possibilities are present that can "lock" tyramine complexes into one conformation, but removal of the para –OH, in PEA, removes this possibility.

While the phenolic –OH apparently plays some role in inducing a single solvent complex in tyramine, this group appears to be too distant for atom–atom interactions with the amine to be important. On the other hand, it does act as a charge donor to the benzene ring. A plausible possibility is that the partially protonated amine interacts with enhanced electron density at C-1. It was also the case that water or alcohol addition to tryptamine induced only one $n=1$ solvent peak, and a similar mech-

anism can be postulated. In this case the pyrrole nitrogen acts as an electron donor to that ring, in analogy to the phenolic –OH in tyramine.

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