This conclusion is in agreement with the conclusions reached in earlier studies,[23,24] but the present systems represent more dramatic examples.

It is probable that this difference in internal energies arises from differences in the mechanisms of excitation in low- and high-energy collisions. High-energy collisional activation of low mass ions is thought[32] to involve electronic excitation and it is highly probable that those ions which receive a large amount of electronic excitation energy will undergo electron detachment before the excitation energy can be redistributed as vibrational energy, thus leading to bond cleavage. In contrast to high-energy collisional activation, low-energy collisional activation is considered[33,34] to involve translational to vibrational energy transfer, and it appears that a greater amount of energy can be deposited in vibrational modes without leading to electron detachment; i.e., the rate of vibrational to electronic energy redistribution is relatively slow. This difference in mean internal energies attainable is likely to be a common phenomena for organic negative ions of low electron affinity. For such species low-energy collisional activation is likely to be more useful than high-energy collisional activation in the determination of the structures of negative ions by negative ion tandem mass spectrometry. It should be noted that the enolate ions studied in the present work have electron affinities of about 1.5 eV;[35] as the electron affinity of the anion under study increases, one would anticipate that the differences between high-energy and low-energy collisional activation should decrease. We also note that negative ions on high-energy collisions undergo charge inversion reactions[36,37] involving loss of two electrons, so clearly large amounts of energy can be transferred to the anion in a single collision; these charge inversion reactions can be used to identify ion structures.[38]

Finally, in contrast with the results of table I, Raftery and Bowie[27] have concluded that all fragmentation reactions of the enolate ions have a unimolecular component. This conclusion was reached as a result of applying a voltage to the collision cell under CID conditions and observing that all peaks split into two components, one displaced in electric sector voltage by an amount determined by the cell voltage and the other not displaced by the applied cell voltage. The latter component was assigned to "unimolecular" reactions occurring outside the cell while the displaced component was assigned to collision-induced reactions occurring within the cell. We are concerned that collision gas leaking from the cell may result in collision-induced reactions occurring outside the cell leading to an undetermined "collision-induced" component to the undisplaced and apparently "unimolecular" reaction. Accordingly, the results in Table I have been obtained without admitting collision gas to the cell (background pressure $\sim 2 \times 10^{-9}$ torr), and we believe that they represent more truly the unimolecular fragmentation reactions of the enolate ions.

Acknowledgment. The authors are indebted to the Natural Sciences and Engineering Research Council of Canada for financial support. A.G.H. gratefully acknowledges the award of a Killam Research Fellowship (1985-87) by the Canada Council.

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Electronic Spectroscopy of Small Tryptophan Peptides in Supersonic Molecular Beams

J. R. Cable, Michael J. Tubergen, and Donald H. Levy*

Contribution from the The James Franck Institute and the Department of Chemistry, The University of Chicago, Chicago, Illinois 60637. *Received April 6, 1988*

Abstract: Laser desorption provides an attractive approach to vaporizing thermally sensitive nonvolatile molecules. By coupling this vaporization technique with supersonic molecular beam methods, the high resolution electronic spectra of several tryptophan containing di- and tripeptides have been observed. The detailed spectra of these systems reveal the existence of a large number of stable conformations in the ground electronic state. In the majority of the peptides, strong vibronic activity is found in the spectrum of the conformer possessing the lowest energy electronic transition, suggesting that the excited-state potential energy surface is substantially displaced from the ground-state surface in this particular conformer. Since the electronic excitation in these peptides is localized on the indole ring system of tryptophan, differences in the electronic spectra reflect different perturbations to this chromophore arising from the various peptide environments. Comparison of the electronic spectra in a series of peptides having either an N-terminal or a C-terminal tryptophan residue suggests that the dominant perturbation to the indole chromophore is more closely associated with the location of the tryptophan residue in the chain than with the identity of the other amino acids of which the peptide is composed.

The use of seeded supersonic molecular beams as a means of simplifying complex electronic spectra is well documented.[1] By cooling the internal vibrational and rotational degrees of freedom and providing an isolated environment free from inhomogeneous site broadening, electronic spectra which show extensive congestion at room temperature may often be interpreted in great detail. One requirement of molecular beam studies is that the molecule of interest have sufficient vapor pressure to seed a supersonic expansion. For many nonvolatile, biologically relevant molecules this requirement presents a sufficient obstacle to limit spectroscopic studies to the condensed phase. The use of direct heating as a means of increasing vapor pressure is often not possible due to the instability of these compounds at elevated temperatures.

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A great deal of activity has been reported in the mass spectrometry literature concerning the development of alternative mechanisms for volatilizing large, thermally labile molecules.[2] Of the various techniques, laser desorption[3] seems most readily adaptable to conventional molecular beam technology. In fact, several studies have already demonstrated the utility of laser vaporization as a means of seeding a supersonic expansion with thermally sensitive molecules for which the usual oven techniques would not be appropriate.[4] Although these investigations have primarily emphasized the mass spectrometric analysis of the seeded expansion, such conditions are ideal for performing relatively high resolution electronic spectroscopy. In this report we demonstrate how resonantly enhanced two-photon ionization spectroscopy may be applied to examine at high resolution the electronic structure of the lowest excited states of some simple peptides which have been laser vaporized and seeded into a supersonic molecular beam. A preliminary report of part of this work has already appeared.[5]

These initial spectroscopic investigations are focused on several tryptophan containing di- and tripeptides. The tryptophyl residue represents a very important chromophore in protein spectroscopy since it is one of the few amino acids which is optically accessible in the near-ultraviolet wavelength region. Recently, high resolution optical studies have been carried out on isolated and jet-cooled tryptophan itself.[6] The sharp, well-resolved features seen in the electronic spectrum give direct evidence for the existence of several stable conformers in the ground electronic state as well for an appreciable excited-state distortion along a low-frequency vibrational coordinate in one specific conformer. The conditions found in a supersonic beam allow for the observation of subtle changes in the electronic spectrum that occur as the tryptophan chromophore is incorporated into a peptide environment. In many of the resonantly enhanced two-photon ionization spectra of the small peptides studied in this paper, sharp spectral features are also observed. Even though many of these features can be assigned to transitions terminating on vibrationally excited levels in the excited electronic state, the complexity of the spectra also suggests that a large conformational distribution exists in the ground electronic state.

Detailed analyses of the observed spectra have not been completed, and will almost certainly require additional studies of the dispersed emission arising from excitation of single excited-state levels. The present report is focused on obtaining the high resolution spectra of several different peptides to determine where further investigations are needed to accurately characterize the electronic structure of these biologically important systems. As an initial aid in understanding these complex spectra, optical saturation techniques have been applied. Such techniques allow one to determine whether different transitions originate from a common ground-state level, but the data are not as easily interpreted as dispersed emission spectra.

We first discuss several experimental conditions under which laser desorption of peptide samples was attempted and then the final configuration which evolved from this work. Next, the optical spectra of several jet-cooled tryptophan peptides, as monitored by resonance enhanced two-photon ionization are presented. Initial assignments for these complex spectra are suggested, and common characteristic features found among the different peptide systems are pointed out. Finally, the spectra are compared to what has recently been seen in the amino acid tryptophan.

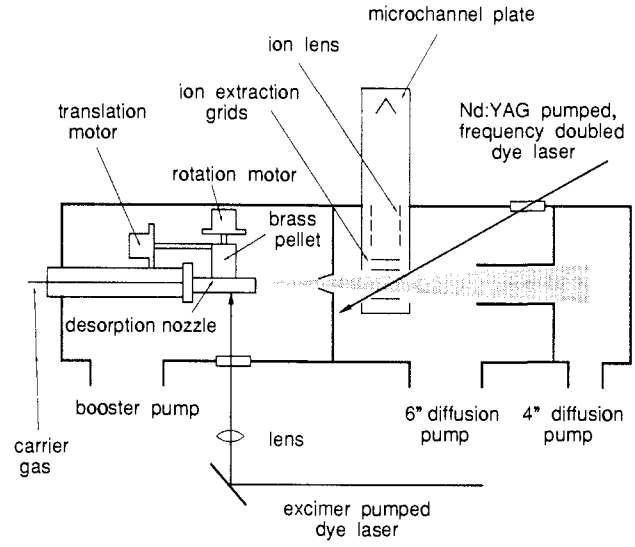

Figure 1. Schematic view of the molecular beam vacuum chamber showing the laser desorption source with rotating and translating brass sample target and the time-of-flight mass spectrometer.

Experimental Section

In laser desorption, the energy contained in a high-intensity pulsed laser is used to rapidly heat a nonvolatile sample. Depending on the conditions, both neutral and ionic parent and fragment species may be desorbed. In many mass spectrometric investigations a high intensity desorption source is used to both volatilize and ionize the compound of interest.[3,7] By using a gentler vaporization step, the production of neutral parent species should be enhanced.[4,8] To seed the supersonic expansion, a pulse of high-pressure carrier gas is passed over the sample as the desorption laser is fired and is then allowed to expand into a vacuum. Once cooled by the expansion, the seeded pulse is available for the usual spectroscopic interrogations, such as resonant multiphoton ionization or fluorescence excitation.

The initial phase of this study entailed the development of a reliable laser desorption source. Preliminary attempts at laser desorption of the amino acid tryptophan were made using a home-built Q-switched CO_2 laser. Tryptophan was pressed into a solid pellet and then irradiated with a focused 3-mJ laser pulse of 10.6-μ radiation. Under these conditions no evidence of tryptophan vaporization was seen. The pulsewidth of the rotating mirror Q-switched CO_2 laser is estimated to be on the order of a microsecond, leading to an intensity of approximately 3×10^5 W/cm^2 at the sample surface. Many studies have successfully used CO_2 lasers for the desorption of neutrals, although higher intensities have generally,[4] but not exclusively,[8] been employed. To investigate the effects of laser intensity on desorption efficiency, the second harmonic of a Q-switched Nd:YAG laser at 532 nm was then used in place of the CO_2 laser. With a similar pulse energy, but now at a focused intensity of greater than 10^8 W/cm^2, tryptophan desorption was readily observed.

Desorption of several tryptophan-containing dipeptides was also attempted with the Nd:YAG laser but without success. However, the addition of a small amount of the laser dye Rhodamine 6G to the dipeptide, prior to pressing the sample pellet, did allow desorption to occur. The strong absorption by the laser dye at this wavelength apparently allowed for better coupling of the laser energy into the sample pellet. The primary disadvantage for this type of sample handling was the large amount of substance necessary to produce a reasonably sized pellet ($^1/_4$ in. diameter by $^1/_{16}$ in. thick).

The final modification to the desorption source involved the use of a thin sample film instead of bulk sample pellets. Sample films approximately 10 μ thick were deposited by evaporation from methanol solutions onto a brass target. With these films, desorption was found to occur using pulse energies as low as 0.1 mJ focused to give intensities of 5×10^7 W/cm^2. Each desorption pulse completely penetrated the thin sample and desorbed all of the deposited film from the focal area on the brass target. For this reason the target was simultaneously translated and

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rotated to ensure that a fresh surface was seen by each desorption pulse.[9] The laser dye was also incorporated into the films, although it was not essential to the desorption process. Its presence, however, greatly aided in evaluating the uniformity of the sample film.

The detailed mechanism for laser desorption is not entirely understood although several theoretical treatments have been made. Levine and Zare suggest that for molecules physisorbed to a surface, the low-frequency physisorption bond can serve as a bottleneck for the flow of energy from the laser heated surface into the internal molecular vibrations.[10] Thus desorption can occur to produce gas-phase molecules having only moderately warm internal temperatures. The samples used in our studies are extremely thick on a monolayer scale, and thus the applicability of the above description is somewhat uncertain. However, the fact that thin film samples desorb equally well with or without the addition a laser dye dopant does point toward an effect involving energy transfer from the initially heated metal surface into the bulk sample. Since the initial deposition of laser energy into the brass surface should be fairly wavelength independent, the failure encountered with the CO_2 laser based desorption is blamed on its fairly low pulse energy and intensity rather than on any effect involving its wavelength.

A simplified schematic representing the final experimental configuration used for recording both the mass spectra and the resonantly enhanced two-photon ionization spectra of the tryptophan peptides is shown in Figure 1. In these experiments, an excimer pumped dye laser using the dye Coumarin 500 at 530 nm was used in place of the Nd:YAG laser as the desorption source. The output pulses of the dye laser were directed and focused onto the sample film by steering prisms and a 25-cm focal length lens external to the source chamber. Under typical conditions, each laser pulse completely removed the sample film from a circular spot having a diameter slightly larger than 150 μ.

After passing over the sample film, the pulse of helium carrier gas, now seeded with the vaporized peptide, expanded from the desorption nozzle into the first vacuum chamber. The free expansion was skimmed before entering a second differentially pumped chamber containing a linear time-of-flight mass spectrometer having unit mass resolution at mass 245. In the center of the extraction region of the mass spectrometer, the molecular beam was crossed by the frequency doubled output of Nd:YAG pumped tunable dye laser. Optical spectra were recorded by monitoring the resonantly enhanced two-photon ionization signal corresponding to the parent mass of the peptide, as the wavelength of the ionization laser was scanned. The details of the mass spectrometer and data collection system have been described in a previous publication.[11]

In these experiments the Nd:YAG laser was triggered internally at 10 Hz, and various synchronization outputs of the laser were used with digital delay generators to provide the necessary timing signals to the pulsed valve and the excimer laser. First the delay to the pulsed valve was adjusted to ensure coincidence of the seeded helium pulse with the pulsed ionization laser in the mass spectrometer. Then the delay to the excimer laser was set so that desorption occurred as the helium pulse passed over the sample and seeded that portion of the gas pulse that was being probed by the ionization laser. Gas pulses used in these experiments typically had 800-μs durations. Although the desorption energy was deposited into the film in roughly 10 ns, the distribution of the desorbed sample in the carrier gas pulse was found to spread over a range of approximately 20 μs. This range may reflect a distribution of times when the molecules actually become entrained in the main gas flow after desorbing from the film. Any turbulent mixing in the nozzle channel would also tend to broaden the time distribution.

A detailed view of the desorption nozzle is shown in Figure 2. This assembly was bolted to the downstream side of a pulsed valve having a 1-mm orifice, and consisted of a 2-in. long, 2-mm diameter gas channel crossed at right angles in a 1-mm diameter light channel which terminated on the brass sample target disk. Helium at 14 atm was discharged by the pulsed valve into the gas channel where it passed directly over the sample film. The desorbed sample was entrained in the helium and eventually expanded supersonically into the source chamber. The sample target was sealed against the body of the nozzle using a Teflon O-ring to prevent any flow of carrier gas out around the brass disk. To ensure that each desorption event occurred from a fresh film surface, the cylindrical brass disk on which the film was evaporated was simultaneously rotated about its axis and translated linearly across the light channel of the nozzle. The usable film surface was a circle of 1-in. diameter. Rotation occurred at a rate of 2 rpm, and translation was accomplished by a 0.25-rpm motor coupled to a 20 thread per inch screw, giving a linear translation speed of 0.032 cm/min or 0.016 cm per target rotation. Under these conditions each sample film could be used for approximately 30 min.

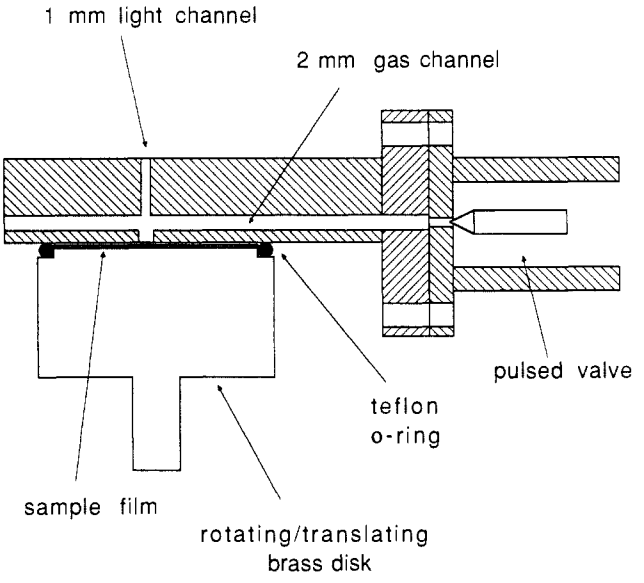

Figure 2. A detailed view of the laser desorption source. The pulsed beam valve is typically backed by 14 atm of helium. When triggered, an 800-μs gas pulse is discharged into the 2-mm gas channel, entraining the desorbed sample molecules. Peptide sample films are prepared by evaporating methanol solutions on the cylindrical brass target. During laser desorption the target is simultaneously rotated and translated so that each desorption event occurs from a fresh area of the film. A Teflon O-ring seals the target to the body of the desorption nozzle.

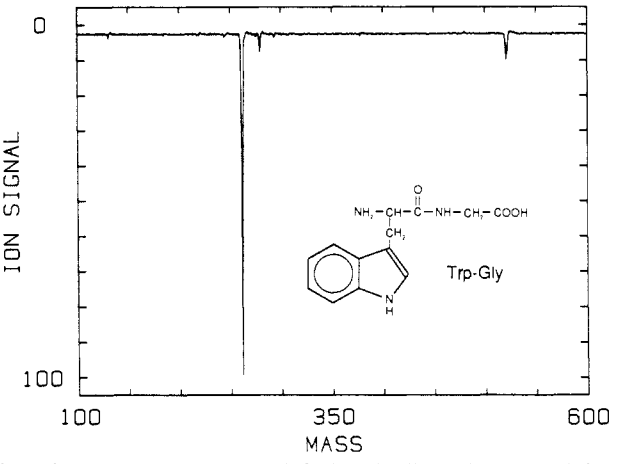

Figure 3. A mass spectrum recorded when the dipeptide Trp-Gly is laser desorbed and seeded into the supersonic molecular beam. The ionization laser is at a wavelength of 2882 Å. The largest peak in the spectrum occurs at a mass of 261 and corresponds to the parent dipeptide ion. A second peak is seen at a mass of 522 which reflects the formation of the peptide dimer during the supersonic expansion. Of particular interest is the lack of a peak at mass 243, corresponding to the loss of water from the parent dipeptide, which is a major decomposition product when thermal vaporization methods are used. The small peak at the parent mass + 32 may be a cluster formed with any residual methanol that was incorporated into the sample film.

The peptides used in these studies were all commercially available and used without further purification. Trp-Gly, Gly-Trp, Trp-Gly-Gly, Trp-Trp, and Trp-Phe were obtained from Sigma, while Phe-Trp and Gly-Gly-Trp were purchased from Chemical Dynamics. All films were prepared by evaporation from 1 mg/mL methanol solutions except for Gly-Gly-Trp which required an aqueous solution to obtain adequate solubility. Some of the Phe-Trp peptide was apparently received as an acetate salt as was revealed by a characteristic odor and the appearance of a peak in the mass spectrum corresponding to an ion having a mass equal to the sum of the masses of the parent dipeptide and acetic acid. It appeared that the presence of this species could contaminate the optical spectrum of the parent dipeptide via resonant ionization and subsequent dissociation to the peptide ion.

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Results

Figure 3 displays the mass spectrum of the dipeptide Trp-Gly seeded in a supersonic expansion. The peptide was vaporized by the focused desorption laser (approximately 5×10^7 W/cm^2) into a helium gas pulse which was expanded from a stagnation pressure of 14 atm. The pulse energy of the unfocused ionization laser was 0.3 mJ (10-ns pulsewidth) and ions were collected from 600 laser shots and averaged. These expansion conditions and laser powers are typical of those used throughout the study. The mass spectrum clearly shows a strong parent ion peak at a mass of 261 amu with very little evidence for fragmentation by either the desorption or ionization process. For all of the peptides studied, the mass spectra were dominated by the parent ion peak although some show a greater degree of fragmentation than Trp-Gly. When fragmentation was seen, no strong dependence on the ionizing wavelength was apparent over the limited wavelength regions of the excitation spectra. In no cases, though, was a large peak seen at a mass 18 less than the parent peptide. Such a fragment is seen very prominently if thermal vaporization is attempted and presumably is formed by dehydration. A second peak in the Trp-Gly spectrum is found at mass 522 which corresponds to a cluster of two peptides. The intensity of this higher mass peak, relative to the parent, showed some dependence on the energy in the desorption laser and could be minimized at lower pulse energies.

In all cases, resonantly enhanced two-photon ionization spectra were recorded by monitoring the ion signal corresponding to the parent peptide as a function of excitation wavelength. Interpretation of these two-photon ionization spectra as being representative of the normal absorption spectrum requires that the ionization efficiency from the excited electronic state be independent of wavelength. Since the wavelength regions covered by the excitation spectra are fairly small and the two-photon energies at these wavelengths exceed the ionization potential of the indole chromophore (7.76 eV[12]) common to all the tryptophan peptides by approximately 1 eV, it is doubtful that any structure in the observed spectra can be attributed to the second photon in the sequential ionization process.

The electronic excitation in all of the systems studied corresponds to a transition to the lowest excited state of the indole chromophore in the amino acid tryptophan, perturbed by interactions with the peptide backbone and the other amino acid residues. Only the lower energy vibronic transitions in this electronic manifold have been studied. The spectra displayed typically cover a range of only 600 cm^{-1}. Few ring vibrations associated with the rigid indole chromophore have vibrational frequencies low enough to be observed in this small wavelength region. Thus the complexity seen in these spectra must be associated with low-frequency vibrations and torsions associated with the peptide chain as well as the existence of a number of stable conformations in the ground electronic state, each responsible for a slightly different perturbation of the indole chromophore.

One of the goals of this initial study is to begin to understand the origin of the complications seen in the electronic spectra. In a peptide, the perturbations on the tryptophan chromophore may arise from the various substituents which distinguish the different amino acids or they may originate from the peptide chain itself. In the former case, peptides composed from different amino acids would be expected to each have a characteristic spectrum while in the latter case the electronic spectrum would more closely reflect the location of the tryptophan chromophore along the peptide chain. To distinguish between the two cases, a series of peptides have been studied where both the location of the tryptophyl residue and the composition of the remaining peptide have been systematically varied. Before any conclusions can be made, though, an understanding of these complex spectra must be developed. In this study the initial assignments for these electronic spectra are made in terms of contributions from different ground-state conformers and low-frequency vibronic progressions in the excited electronic state. More detailed assignments based, for example, on dispersed emission experiments would certainly aid in the assignment process.

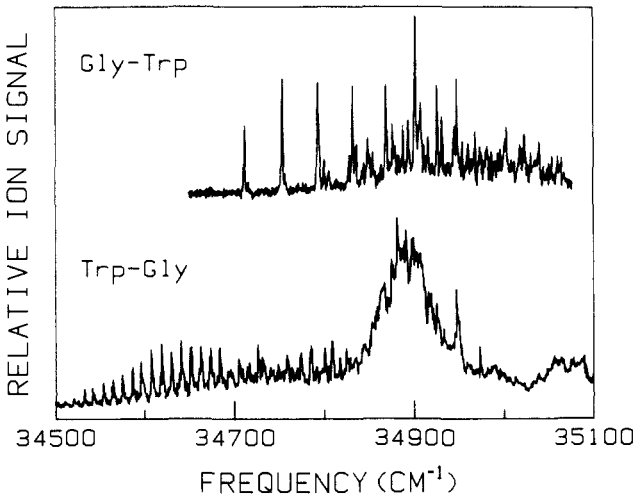

Figure 4. Resonantly enhanced two-photon ionization spectrum of Trp-Gly (lower trace) and Gly-Trp (upper trace) obtained by monitoring the ion signal corresponding to the parent dipeptide at mass 261. Helium is used as the carrier gas at a stagnation pressure of 14 atm.

Trp-Gly and Gly-Trp. The simplest tryptophan containing peptides are Trp-Gly and Gly-Trp. These dipeptides differ only in the ordering of the two amino acids along the peptide chain. In Figure 4 the resonantly enhanced ionization spectra of these two isomers are compared. It should be emphasized that in both cases the optical chromophore is the heterocyclic indole ring system, and thus differences between the two spectra arise solely from the different perturbations associated with the peptide chain.

The spectrum of the N-terminal tryptophan dipeptide Trp-Gly (lower trace in Figure 4) can roughly be divided into two distinct regions. A low-energy transition originates near 34 500 cm^{-1} and consists of a series of at least 18 evenly spaced, sharp peaks. These appear to be members of a very harmonic vibrational progression in a single normal mode having an excited-state vibrational frequency of 11 cm^{-1}. Such progressions typically develop their Franck-Codon activity from a shift in the excited-state potential energy surface, relative to the ground-state surface, along that particular mode. If the shift is quite large, the transition to the vibrationless excited-state level can be very weak. For this reason it is difficult to assign an origin to this low-energy progression. Four hundred wavenumbers to the blue, a broad unresolved band is found. The apparent structure in this band does not seem to be reproducible in different scans and therefore probably results from fluctuations in the vaporization process. Two additional sharp features are also seen to the blue of the broad band at 34 947 and 34 973 cm^{-1}. The fact that sharp vibronic structure is seen elsewhere in the spectrum means that the broad band cannot be accounted for by poor internal cooling in the supersonic expansion. Variations in the expansion conditions are found have no effect on the appearance of this band. More likely, the occurrence of the broad absorption arises from multiple sharp transitions associated with different ground-state conformers that are significantly overlapped.

Although occurring at roughly the same frequency, the ionization spectrum of Gly-Trp is quite different from that of Trp-Gly. As shown in the upper trace of Figure 4, the lowest energy transition occurs at 34 712 cm^{-1} and associated with it are at least four addtional members of a vibrational progression at 34 754, 34 793, 34 832, and 34 869 cm^{-1}. The vibrational frequency of the active normal mode is seen to drop from 42 cm^{-1} at the fundamental to 37 cm^{-1} at the third overtone. Near 34 900 cm^{-1} the spectrum becomes increasingly complex. Although sharp spectral features remain, it is difficult to relate them to the lower energy vibrational progression. The strongest feature in the spectrum occurs at 34 902 cm^{-1} and appears to represent the fourth

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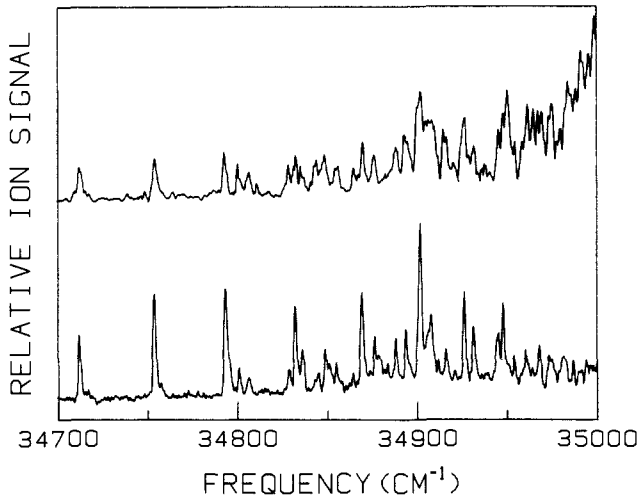

Figure 5. Effect of ionization laser intensity on the resonantly enhanced two-photon ionization spectrum of Gly-Trp. The lower spectrum is identical with that shown in Figure 4. The upper trace shows the spectrum seen when the laser intensity is increased by roughly a factor of 100.

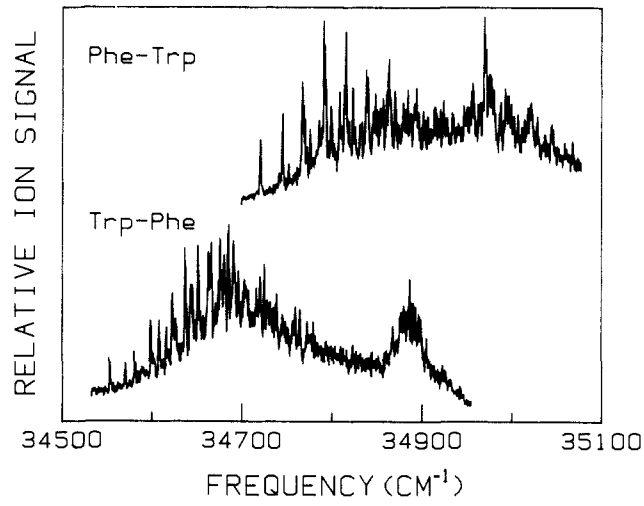

Figure 6. Resonantly enhanced two-photon ionization spectrum of Trp-Phe (lower trace) and Phe-Trp (upper trace) obtained by monitoring the ion signal corresponding to the parent dipeptide at mass 351. Expansion conditions are the same as in Figure 4.

overtone of the above-mentioned progression. On closer inspection, such an assignment would require a drop in the excited-state vibrational frequency to 33 cm^{-1}. This fact and the lack of any obvious higher members of the progression point against such an assignment.

Optical saturation techniques provide some additional insight into the interpretation of the Gly-Trp spectrum. Such an approach was shown to be extremely useful in understanding the contributions from multiple ground-state conformers to the optical spectrum of jet-cooled tryptophan.[6] Under conditions of intense excitation, the intensities of different vibronic transitions having a common initial state saturate to a common value. Any differences in intensity observed among the various peaks in a saturated spectrum must then reflect differences in the populations of the levels on which the transitions originate. Thus transitions associated with a common conformer will saturate to a common intensity while those originating from different conformations having different populations will saturate to different intensities.

Figure 5 compares the two-photon resonance enhanced ionization spectra of Gly-Trp under different excitation conditions. The lower trace is the same as in Figure 4 and was obtained using unfocused excitation. The upper trace is seen when the excitation laser is focused by a 50-cm lens, resulting in a power density approximately 100 times greater than in the unfocused case. Under focused excitation, all of the peaks of the proposed vibrational progression appear to reach a common intensity. Several peaks with energies less than 34 900 cm^{-1} that appear only weakly in the lower trace approach this same intensity, suggesting their assignment to weakly Franck-Condon active modes associated with the same conformer. Two of these transitions seem readily assignable to excitations of 89- and 94-cm^{-1} vibrations upon which additional combinations involving the main 40-cm^{-1} vibration can be found. In contrast, the 34 902 cm^{-1} peak and several others at higher frequencies seem to be saturating to a common but higher intensity, preventing their assignment to additional members of the strong 40 cm^{-1} vibrational progression. Instead, these features must be associated with transitions in different ground-state conformers. At frequencies near 35 000 cm^{-1}, the upper trace shows a dramatically rising base line where little intensity is seen in the unfocused spectrum, making the comparison of different peak heights difficult.

Trp-Phe and Phe-Trp. Figure 6 displays the resonantly enhanced ionization spectra of the two dipeptide isomers which may be formed from the amino acids tryptophan and phenylalanine. Although the phenylalanyl residue represents a second ultraviolet chromophore, in this particular wavelength region the electronic excitation must be localized on the indole ring system of tryptophan. As with Trp-Gly and Gly-Trp, the optical spectra of the two dipeptide isomers display considerable differences. Although both exhibit a large number of sharp spectral features, there also appears to be a substantial amount of broad absorption underlying this structure. The broad background hinders attempts to interpret the sharper features by applying saturation techniques.

The spectrum of Phe-Trp (upper trace of Figure 6) has its lowest energy sharp feature at 34 720 cm^{-1}. Associated with this peak is a series of six transitions, each member separated by approximately 24 cm^{-1}. Between the members of this series numerous other features are found. Many of these features can be assigned to vibrational excitation of a 32-cm^{-1} mode in combination with the previously mentioned 24-cm^{-1} vibration. By 34 900 cm^{-1}, individual sharp transitions are no longer distinguishable and the broad background has become quite intense. Despite this background a second structured region is seen beginning at 34 950 cm^{-1}. The peaks at this energy are substantially broader than those in the lower energy series and seem to be associated with a characteristic 25-cm^{-1} spacing.

The lower trace in Figure 6 represents the resonance enhanced ionization spectrum of Trp-Phe. In the lower energy region, beginning at 34 550 cm^{-1}, a large number of sharp spectral features are found. Underlying these is a very broad peak centered near 34 700 cm^{-1}. A second very distinct, unresolved band is also found near 34 900 cm^{-1}. Unlike all of the previous spectra, the lower energy sharp spectral features are not easily characterized by a single vibronic progression. The lowest energy transitions occur at 34 553 and 34 570 cm^{-1}. Assignment of the second transition to a 17-cm^{-1} fundamental vibration is not appropriate since there is no evidence for any higher vibrational overtones, and yet the similar intensities of the two peaks would suggest that strong vibronic activity should be seen. An alternative assignment suggests that the two transitions originate from distinct ground-state conformers. Using the two lowest features as origin transitions of distinct conformers, strong 27-cm^{-1} vibrational progressions can be found based on each. In Trp-Phe there thus appear to be two readily identifiable conformers having slightly different electronic transition frequencies but very similar equilibrium distortions in their excited electronic state.

Trp-Gly-Gly and Gly-Gly-Trp. The spectrum of the tripeptide Trp-Gly-Gly is shown by the lower trace in Figure 7. At 34 800 cm^{-1} an intense, unresolved peak is seen having a width (fwhm) of approximately 10 cm^{-1}. To the red, a series of sharp resolved features is found. The lowest energy feature occurs at 34 542 cm^{-1}, and at least seven other peaks in a vibrational progression, spaced by 27 cm^{-1}, can be associated with this transition. Since such a progression must indicate a substantial shift in the potential energy surface, it is not certan that the lowest feature can be associated with a transition terminating on the vibrationless level of the electronically excited state. Three other peaks also appear at 34 640, 34 645, and 34 689 cm^{-1} upon which additional 27-cm^{-1}

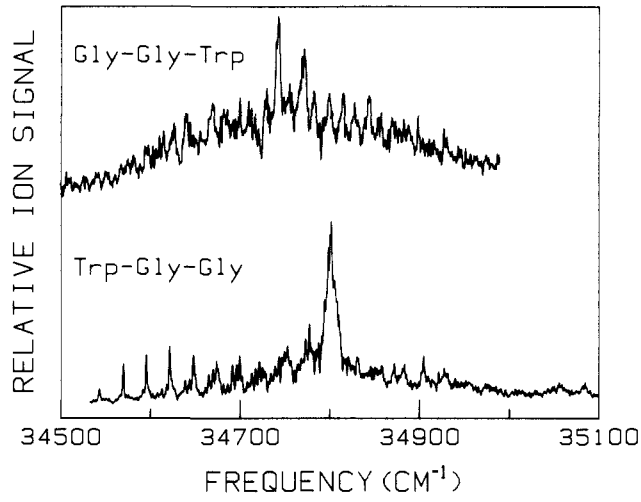

Figure 7. Resonantly enhanced two-photon ionization spectrum of Trp-Gly-Gly (lower trace) and Gly-Gly-Trp (upper trace) obtained by monitoring the ion signal corresponding to the parent tripeptide at mass 318. Expansion conditions are the same as in Figure 4.

progressions are built. If the transition at 34 542 cm^{-1} is actually the origin, then these three vibrations would have excited-state frequencies of 97, 102, and 147 cm^{-1}. It thus appears that all of the sharp structure to the red of broad peak at 34 800 cm^{-1} can be assigned to vibronic transitions associated with a single conformer.

The width of the most intense peak prevents its assignment to a single vibronic transition. Most likely it arises from the overlapped origin transitions of several different conformers. No low-frequency vibronic structure is seen to the blue of this peak, indicating little shift in the excited-state geometry. When the power density of the excitation laser is significantly increased the sharp, red structure and the main peak at 34 800 cm^{-1} remain at different intensities, in support of their assignment to different conformers. A broad, unstructured absorption also begins to appear, similar to what was seen in the saturated spectrum of Gly-Trp. This background makes saturation studies on these congested spectra more difficult to interpret than was the case when applied to the amino acid tryptophan.[6]

In the upper trace of Figure 7 the spectrum of the C-terminal tryptophan tripeptide Gly-Gly-Trp is displayed. In contrast to all of the previous peptides, this spectrum is dominated by a broad, structureless band upon which only two weak sharp features may be recognized. These two peaks are found at 34 743 and 34 772 cm^{-1} and show considerably larger widths than the sharp single vibronic transitions seen, for example, in the structured region of the Trp-Gly-Gly spectrum. Although additional sharp structure may actually be present, the poor signal-to-noise ratio in Figure 7 makes its identification difficult.

Trp-Trp. The ionization spectrum of the dipeptide Trp-Trp (Figure 8) is best characterized by its extremely diffuse absorption. This absorption is composed of three broad peaks centered at 34 550, 34 700, and 34 900 cm^{-1}. A region of weakly structured features appears to be superimposed on the central peak, but noise in the underlying signal hinders an accurate characterization of this region. In Trp-Trp both amino acid residues may act as chromophores, and complications in the optical spectrum are to be expected. In the simplest case, the observed spectrum might reflect a simple superposition of the spectrum of a typical dipeptide with an N-terminal tryptophan with that of a peptide containing a C-terminal tryptophan. If a strong interaction exists between the two chromophores, a more complex spectrum would be predicted.

Discussion

Despite their complexity, the electronic spectra of the different di- and tripeptides show some striking similarities. In all of the peptides where sharp resolved features are observed, the lowest energy transition appears to be associated with a particular

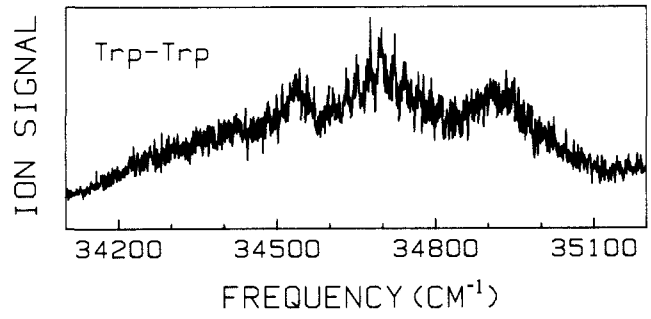

Figure 8. Resonantly enhanced two-photon ionization spectrum of Trp-Trp obtained by monitoring the ion signal corresponding to the parent dipeptide at mass 390. Expansion conditions are the same as in Figure 4.

Table I. Summary of Peptide Spectra

	lowest energy feature, cm^{-1}	prominent vibrations,[a] cm^{-1}	broad feature,[b] cm^{-1}
N-terminal Trp			
Trp-Gly	34 519	11	34 900
Trp-Phe	34 553	27	37 900
	34 570[c]	27	
Trp-Gly-Gly	34 542	29 (97, 102, 147)	34 800
C-terminal Trp			
Gly-Trp	34 712	40 (89, 94)	
	34 902[c]		
Phe-Trp	34 720	24 (32)	
Gly-Gly-Trp	34 743		
Tryptophan[d]	34 873	26	
	34 909[c]		
	34 936		
	34 965		
	34 971		
	34 980		

[a] Strong progression forming vibrations are listed. Weaker vibrations are given in parentheses. [b] A broad feature is seen most predominantly in the N-terminal tryptophan peptides. [c] In Gly-Trp and Trp-Phe lowest energy transitions for a second distinct conformer are also listed. Five additional conformers have been identified in the tryptophan spectrum. [d] From ref 6 and 13.

ground-state conformer in which the excited electronic state is appreciably distorted along a single low-frequency normal coordinate. The evidence for this distortion is derived from the long vibronic progressions found in the electronic spectra. Except for Trp-Trp, all of the peptides studied can be classified as having either an N-terminal or a C-terminal tryptophan residue. Even stronger correlations in the optical spectra are found within these two classifications. Details of the different peptide spectra are summarized in Table I.

The lowest energy transition in each electronic spectrum of the different N-terminal tryptophan peptides (Trp-Gly, Trp-Gly-Gly, and Trp-Phe) always occurs within 17 cm^{-1} of 34 536 cm^{-1}. In the dipeptide Trp-Phe, a second conformer is found which also shows a low-energy transition and considerable vibronic activity in a low-frequency excited-state mode. The geometries of the two Trp-Phe conformers must be quite similar since their electronic transition energies differ by only 17 cm^{-1}, and their excited-state potential energies show very similar distortions along a 27-cm^{-1} normal coordinate.

In addition to the lower energy structured transition, a broad unresolved band is always seen at higher frequencies in the optical spectra of the N-terminal tryptophan peptides. This feature occurs at 34 900 cm^{-1} in Trp-Gly and Trp-Phe, and at 34 800 cm^{-1} in Trp-Gly-Gly. The broad spectral feature is believed to arise from several overlapped transitions associated with different ground-state conformers and not from poor cooling conditions in the supersonic expansion. The location of this band is very similar to where vibrationless transitions for several distinct conformers in tryptophan itself have been observed.[6] No vibronic activity appears to be associated with the broad feature in the peptide spectra nor with any of the tryptophan conformer transitions

occurring at similar wavelengths.

The C-terminal tryptophan peptides (Gly-Trp, Phe-Trp, and Gly-Gly-Trp) also show similar energies for the lowest energy sharp spectral feature in their resonantly enhanced ionization spectra. These peaks all occur within 16 cm^{-1} of 34 728 cm^{-1}, and thus are roughly 200 cm^{-1} higher in energy than the corresponding peaks in the N-terminal tryptophan peptides. The Gly-Gly-Trp spectrum is unique in that it also contains a significant broad contribution and very few sharp features. With both Gly-Trp and Phe-Trp strong vibronic progressions can be identified in 40- and 24-cm^{-1} vibrational modes, respectively. At higher energies both spectra become quite congested and are thus difficult to interpret. In Gly-Trp, optical saturation studies clearly identify at least one additional conformer although the importance of vibronic activity in this conformer cannot be determined. The primary reason for the spectral congestion at higher transition frequencies in both Gly-Trp and Phe-Trp is thought to reflect the existence of a number of different conformations in the ground electronic state.

The similarities in electronic structure between the tryptophan peptides and the free amino acid tryptophan have been alluded to above. In tryptophan, six conformations were found to contribute to the electronic spectrum with origins ranging from 34 873 to 34 980 cm^{-1}. Associated with the lowest energy transition were three additional members of a 26-cm^{-1} vibrational progression. No significant vibronic activity was found to be associated with the other conformers. This pattern is repeated in many of the peptide spectra, where even stronger vibronic progressions are observed for the particular conformer possessing the lowest energy electronic transition. In the N-terminal tryptophan peptides, this transition occurs at an energy 350 cm^{-1} lower than where the similar transition in the vibronically active conformer of tryptophan is seen. The analogous, but somewhat smaller, shift to lower energy of the electronic transitions in the vibronically active conformers of the C-terminal tryptophan peptides is approximately 150 cm^{-1}. The magnitude of the perturbation seen by the indole chromophore in the vibronically active conformer of the various tryptophan peptides is apparently much greater than is pesent in the free amino acid, resulting in both a larger shift in the electronic transition frequency and a greater displacement in the excited-state potential energy surface along a characteristic low-frequency normal coordinate.

The photophysics of electronically excited tryptophan has been studied in some detail. Dispersed emission spectra contain a broad red-shifted component when the conformer showing strong vibronic activity in its excitation spectrum is excited. The emission from other excited conformers, in contrast, shows very little of this broad component. Studies of several tryptophan derivatives demonstrate that the appearance of a low-energy transition carrying a strong vibrational progression in the absorption spectrum and the existence of a broad, red-shifted component in the emission spectrum correlates with the ability of the molecule to exist as a zwitterion.[13] The initial electronic absorption in tryptophan almost certainly occurs to a neutral excited state since the transition energy is very similar to what is seen in methylindole itself. A subsequent proton transfer and crossing to a zwitterionic excited-state potential surface may then be relatively facile in one particular conformer, resulting in the unusual emission properties.

The frequencies of the electronic transitions in the tryptophan peptides also suggest that absorption is occuring to a neutral form. However, at least one particular conformer in the peptides studied here appears to correlate well with the tryptophan conformer showing the anomalous fluorescence properties. For this reason, current studies are focused on observing and characterizing the emission from the different electronically excited conformers in the various di- and tripeptides. It should be realized that either the amine or the carboxylic acid group of tryptophan is blocked when incorporated into a peptide, and if proton transfer is to occur it must now take place between different amino acid residues.

Conclusion

A primary conclusion from this study is that laser desorption may be successfully applied to the problem of seeding nonvolatile, thermally labile molecules into a supersonic expansion for interrogation by various spectroscopic techniques. In this manner, spectra which have only been studied at low resolution in condensed phases may now be recorded at high resolution. The internal cooling afforded by the expansion dramatically increases the intrinsic spectral resolution and therefore allows one to study small perturbations that would otherwise be very difficult to observe. Laser desorption has been applied to increasingly larger molecules in the field of mass spectroscopy and may prove equally well suited as a vaporization source for molecular beam studies.

The optical spectra of the tryptophan peptides emphasize the importance of molecular conformation and the effects that different geometries can have on electronic structure. The clear distinction between the spectra of the N-terminal and C-terminal tryptophan peptides is surprising and suggests that the primary perturbation to the indole chromophore is associated with location along the peptide backbone rather than with the detailed nature of the other amino acid residues. Whatever the nature of this perturbation on the indole ring system of tryptophan, its magnitude is significantly greater in the tryptophan peptides than in the free amino acid, as evidenced by the larger shifts in electronic transition frequencies and the more pronounced low-frequency vibronic activity. The greater flexibility resulting from the longer backbones of the peptides may be a significant factor in determining the details of the perturbation.

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