Resonance-Enhanced Multiphoton Ionization Spectroscopy of Dipeptides

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We report resonance-enhanced multiphoton ionization (REMPI) spectroscopy of laser-desorbed, jet-cooled dipeptides, containing either tyrosine or phenylalanine as an aromatic chromophore (C). The single amino acids have multiple origins that we interpret as arising from two types of conformations, with the carboxyl group either anti or gauche with respect to the ring. Spectra for dipeptides of the form X−C, for example, Ala-Tyr, are similar to those of the corresponding single amino acid. On the other hand, spectra for dipeptides of the form C−X, for example, Tyr-Ala, show significant changes in the peaks, which we associate with gauche conformations. This observation can be understood in terms of an interaction between the carboxyl terminus and the ring, associated with the molecule assuming a folded conformation.

Introduction

Dipeptides are the smallest unit containing a peptide bond, and as such, they afford an opportunity to study basic peptide properties. The attraction of gas-phase spectroscopy of dipeptides lies in the opportunity to study their intrinsic properties free of the solvent environment. The challenge lies in the difficulty of bringing dipeptides into the gas phase as intact neutral molecules. Because of this difficulty, most related work in the gas phase has been limited so far to the vibronic spectroscopy of single amino acids. A number of groups have studied the three aromatic amino acids, phenylalanine (Phe),[1−3] tyrosine (Tyr),[2,4,5] and tryptophan (Trp).[6−8] These data have been obtained by laser-induced fluorescence (LIF) or by resonance-enhanced multiphoton ionization (REMPI). We note that in the work of Teh and Sulkes[9] tyrosine decarboxylated to tyramine because they volatilized by heating and not by laser desorption. In addition to these studies, work has been reported on mass spectrometry of small peptides in the gas phase employing nonresonant ionization without vibronic spectroscopy.[10−17]

In the case of tryptophan, Rizzo et al. have shown the formation of an intramolecular exciplex, possibly involving excited-state proton transfer to form a zwitterion.[6] Evidence for such an exciplex follows from the appearance of a vibrational sequence in one of the conformers and broad, red-shifted fluorescence. It is conceivable that the carboxylic acid acts as a proton donor, while the nitrogen on the indole ring or the amine group can serve as a proton acceptor. In that case, zwitterion formation is similar to that in solution, with the ring system stabilizing the zwitterionic structure in lieu of solvent molecules. This model suggests a folded conformation for the exciplexes. As the observed vibrational sequences indicate, the same phenomenon occurs for Trp-Gly and for Trp-Gly-Gly, for which the −COOH group is further removed from the ring. This also suggests a folded conformation for the di- and the tripeptide. On the other hand, there is no indication of any type of exciplex formation in Tyr and in Phe. In these two cases there is no nitrogen in the ring, but an interaction that would stabilize an intramolecular complex could still occur with the π system of the phenyl ring, or with the −OH group in the case of Tyr. The question arises whether dipeptides containing Tyr or Phe might show evidence for interactions between either the carboxyl or the amino end group of the second peptide residue and the ring. Such interaction could be allowed if the molecule were to adopt a folded conformation. If this were to happen, we would expect a significant difference in the spectra depending on the sequence, since, for example, X-Tyr exposes the amino end group, while Tyr-X exposes the carboxyl end group. We have used a combination of laser desorption, jet cooling, and REMPI to study a series of such dipeptide pairs with tyrosine.

Experimental Section

The experimental setup has been described in detail elsewhere.[18] In brief, material is laser desorbed from a sample probe in front of a pulsed nozzle. All chemicals were obtained from Sigma-Aldrich Co. and used without further purificaton. The desorption laser is a Nd:YAG laser operated at its fundamental wavelength of 1064 nm. At this wavelength one does not expect photochemical interaction with the compounds that we desorb while the graphite substrate absorbs effectively. Typical laser fluences are of the order of 1 mJ/cm^2 or less, which is significantly less than the fluences normally used for ablation. The laser is focused to a spot of the order of 0.5 mm diameter within 2 mm in front of the nozzle. The nozzle consists of a pulsed valve with a nozzle diameter of 1 mm. We usually operate with Ar as a drive gas at a backing pressure of about 5 atm. In earlier work we optimized the geometry for effective entrainment by mapping entrained perylene with laser-induced fluorescence.[18,19]

Downstream, ionization lasers intersect the beam inside the source region of a reflectron time-of-flight (TOF) mass spectrometer (R. M. Jordan Co.). Two Nd:YAG-pumped dye lasers, used for two-photon ionization, intersect the beam at right angles. By monitoring specific mass peaks while varying the two-photon ionization wavelength, we obtain mass selected excitation spectra. We achieve spectral hole burning by implementing a delay of about 30 ns between the two dye laser pulses. This results in two peaks in the time-of-flight spectrum, which we can monitor individually. The first laser pulse then serves as a "burn" laser, while the second serves as a "probe" laser. When both lasers are tuned to a resonance of the same

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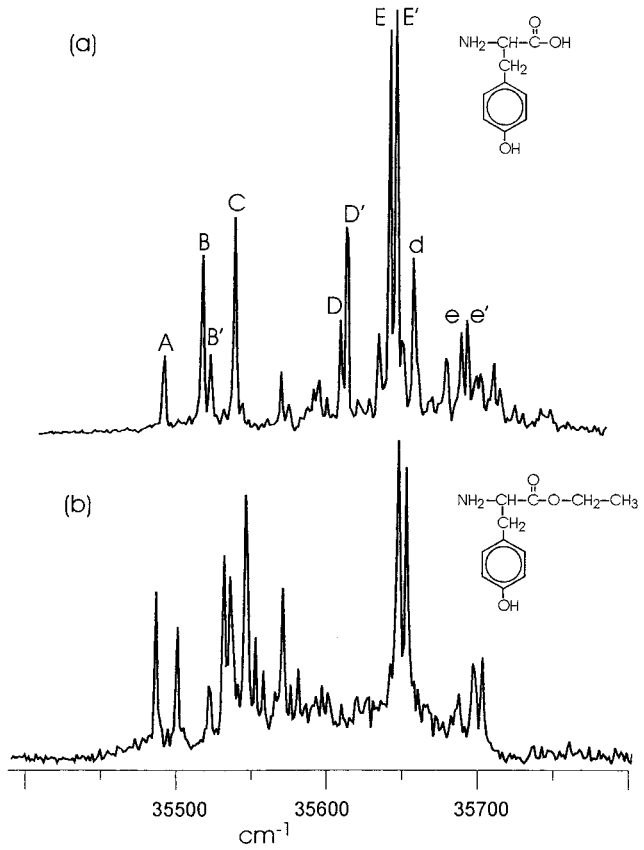

Figure 1. REMPI spectra of laser-desorbed, jet-cooled (a) tyrosine and (b) tyrosine ethylester, offset by +45 cm^{-1}.

conformer population the burn laser causes a decrease in the signal of the probe laser.

Results and Discussion

(A) Tyrosine. We first wish to discuss tyrosine, which serves as one of the chromophores in the dipeptides to be discussed in the following sections. Figure 1a shows the REMPI spectrum of tyrosine in the origin region only. We will report a detailed study of the vibrational excitation spectrum elsewhere. This spectrum is very similar to that reported by Martinez et al., obtained with laser-induced fluorescence.[2] On the basis of power dependence measurements, those authors interpreted 10 peaks as 10 different conformers, which they labeled A−J. They found no broadened fluorescence in any of these, suggesting no exciplex formation. In He droplets Lindinger et al. found only peaks A, D, E, and I.[4] This may be due to a different conformer distribution in the droplets, as compared to a jet, or to conformer selective interactions with the He matrix. Li and Lubman found only E, I, and J, which may have been due to different source conditions.[5] Given the spacings, E, H, I, and J could be a repeat of A, B, C, and D with an energy difference of 123 cm^{-1}. Martinez et al. interpret this pattern as arising from an −OH splitting. For reasons outlined below, we propose another interpretation and adopt a different labeling scheme. The peak positions relative to the redmost peak (at 35 485 cm^{-1}) are given in Table 1. For comparison we also give the labels as used by Martinez et al.

Different peaks within the origin region of a spectrum need not be due to different conformers. They may also arise from low-energy vibrations. To resolve this issue, we performed hole-burning experiments. As a result, we find pairs of peaks, related to the same conformers. These pairs are indicated in the figure

TABLE 1: Peaks in the Origin Region of Tyrosine

label	peak positions, cm^{-1} + 35 485 cm^{-1}	labels by Martinez et al.[2]
A	0	A
B	25	B
B′	31	C
C	48	D
D	118	
D′	121	E
	132	F
	142	G
E	150	H
E′	154	I
d	166	J
	187	
e	197	
e′	202	

by corresponding labels in upper- and lowercase. The assignment is based on the following observation: For each of the labeled pairs a probe laser is tuned to one of the peaks. When a burn dye laser pulse, preceding the probe by about 30 ns, is scanned over the corresponding peak, this causes the probe signal to decrease. This implies that both peaks are due to absorption by the same population in the beam, and thus that they are both associated with the same conformer, the one with higher energy representing a vibration. Since the spacings in these pairs are all about 45 cm^{-1}, this is likely to be a torsional mode.

Considering all basic conformations, we would expect 3 orientations for the $C_\alpha-C_\beta$ bond: 2 −OH orientations, 2 −COOH orientations, and 2 NH_2 orientations for a total of 24 possible conformations. Of these we observe only what apparently are the 8 lowest energy ones. We have performed computations of the lowest energy conformers in the ground state, using GAUSSIAN. We found pairs of conformers with a very small energy difference, which correspond to two possible orientations of the −OH group on the ring. All the spectra show doublets for most peaks, which we assign to this conformational −OH splitting, and which we have labeled with the same letter, distinguished by a prime. The 10 lowest energy conformations are shown as Newman projection diagrams in Figure 2, with their respective energies. Each pair in the figure differs in the −OH orientation on the ring. Configurations that appear alike in the figure differ in the orientation of either the −COOH or the $-NH_2$. We may assume that the lowest energy conformations are frozen in the supersonic beam and are thus the ones that appear in our spectra. Since the order of the energies can change in the excited state, we cannot necessarily use ground-state computations to assign the peaks to specific conformers. The torsional vibration that we observe also appears in the ground-state calculation at 45 cm^{-1}, as a torsion of the amino acid chain with respect to the ring.

Figure 1b shows the spectrum of the tyrosine ethyl ester. In comparison with tyrosine the carboxylic acid group is blocked. The spectrum is red shifted with respect to that of tyrosine and is displayed shifted +45 cm^{-1} in Figure 1. Peaks E and E′ appear unchanged, with the associated vibrational peaks e and e′. The pair D, D′ is missing. Peaks A−C are present, with B and C shifted by a few cm^{-1}. An additional peak appears to the red of peak A. We assume that the conformers least affected by the substitution will be those for which the carboxyl group has the least interaction with the rest of the molecule. Therefore, we tentatively assign peaks E as belonging to the conformation with the −COOH group in the anti position with respect to the ring. This assumption will guide us in the interpretation of the dipeptide spectra in the following two sections.

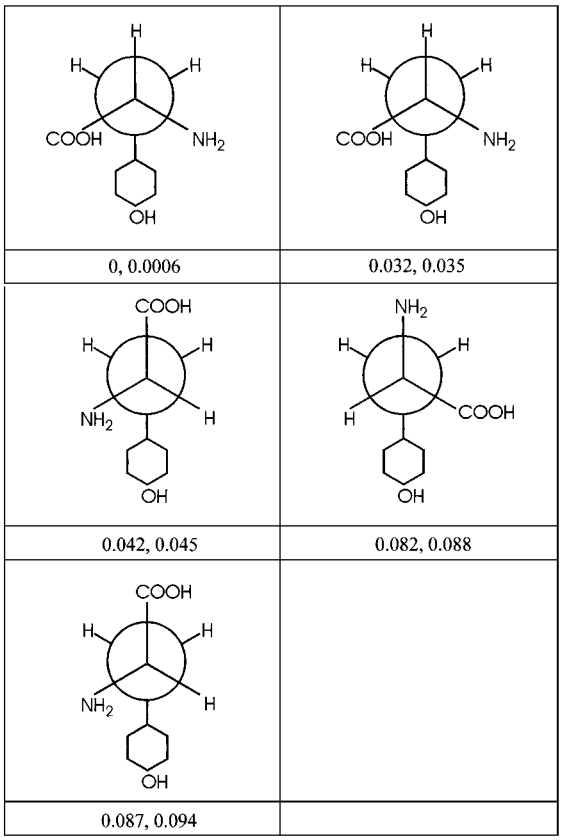

Figure 2. Lowest-energy conformations of tyrosine as calculated with the GAUSSIAN program. Each diagram corresponds to a pair of conformations with two different orientations of the phenol −OH group. Relative energies are given in eV.

(B) X-Tyr. Figure 3 shows the origin region of two dipeptides of tyrosine, with glycine (Gly) and with alanine (Ala), both with the peptide bond at the N-terminus of the tyrosine. Figure 3a shows Gly-Tyr, while Figure 3b shows Ala-Tyr. Figure 3c shows a portion of the Ala-Tyr spectrum in the 800 cm^{-1} region, where the tyrosine ring "breathing" vibrational mode appears. The origins of both Ala-Tyr and Gly-Tyr appear red shifted with respect to that of tyrosine by about 50 cm^{-1}. The spectrum in 3c is shown shifted by −800 cm^{-1} for comparison. The spectra are very similar to that of tyrosine itself. The major difference is in the frequency of the torsion, built on the D and E conformers. The vibrational sequences are indicated in the figure, and they exhibit a spacing of 17 cm^{-1}. Once again, this is consistent with our calculation of the chain torsion frequency in the ground state of these dipeptides. The frequency is reduced relative to the 45 cm^{-1} of tyrosine by the increased mass.

(C) Tyr-X. Figure 4 compares the spectra of Tyr, Ala-Tyr, and Tyr-Ala. The origin of the latter appears red shifted with respect to that of tyrosine by about 140 cm^{-1}. In the figure the spectrum of Tyr-Ala is shown shifted by +100 cm^{-1}, to facilitate comparison. The spectral region of the E conformers of Tyr-Ala is very similar to that of Ala-Tyr, showing the same 17 cm^{-1} sequence. There is only one such sequence, consistent with the absence of the equivalent of a D conformer, as seen in the tyrosine ester. The region to the red of the E conformer, which in Ala-Tyr is similar to that in tyrosine, is very different in Tyr-Ala. It is much weaker and shifted further to the red relative to the E conformers. While the Ala-Tyr peaks in this region resemble those for the A, B, and C conformers of Tyr, no such parallel appears to exist for Tyr-Ala. It is possible that not all of the peaks for Tyr-Ala are separate conformers, but the signal was too weak to perform reliable hole-burning experiments, so far.

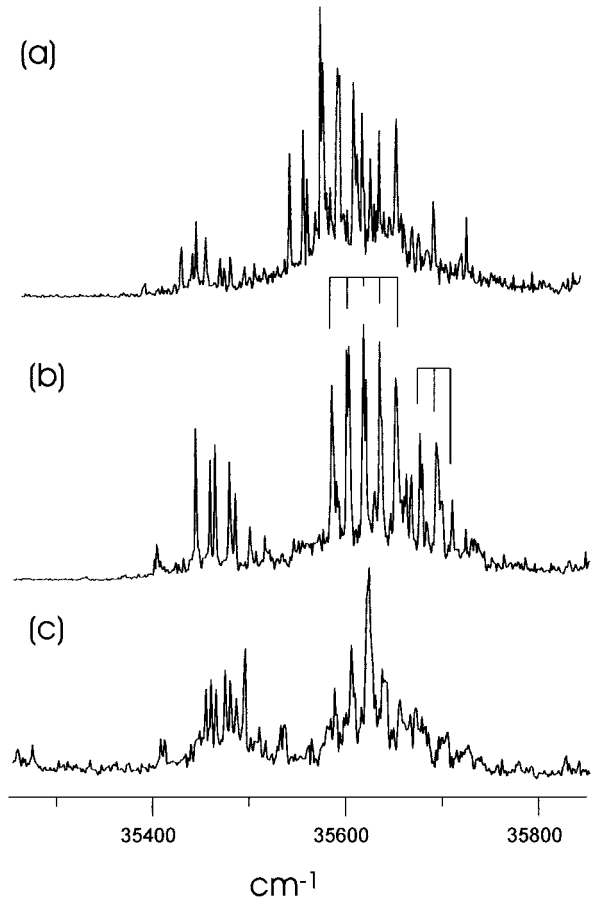

Figure 3. REMPI spectra of laser-desorbed, jet-cooled (a) Gly-Tyr, (b) Ala-Tyr, and (c) Ala-Tyr in the region of its ring "breathing" vibration. The latter trace is offset by −800 cm^{-1}.

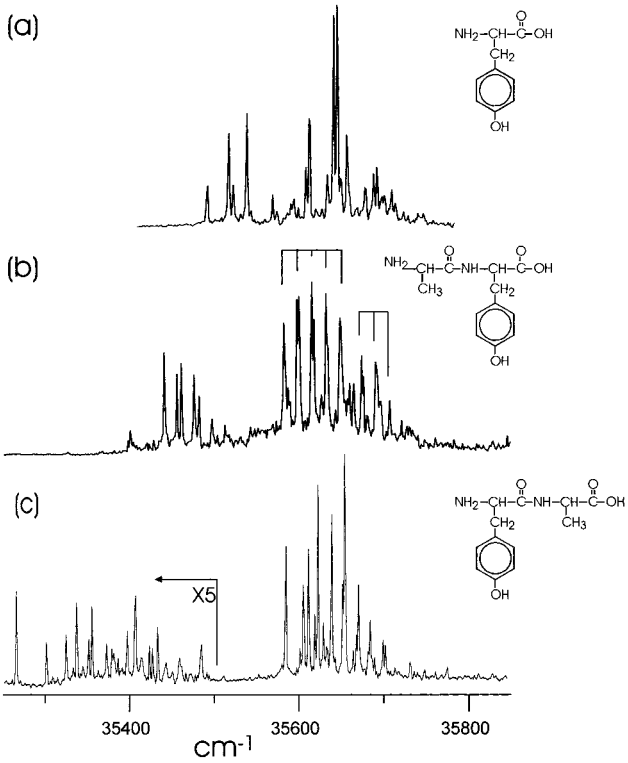

Figure 4. REMPI spectra of laser-desorbed, jet-cooled (a) Tyr, (b) Ala-Tyr, and (c) Tyr-Ala, offset by +100 cm^{-1}.

These findings suggest a model, based on two assumptions. (i) The carboxyl terminus can interact with the aromatic chromophore, while the amino terminus does not. (ii) There are two types of conformers, those with the chain stretched away from the ring (anti) and those with the chain folded back toward the ring (gauche). In this picture the E conformations in tyrosine are associated with the carboxyl group being in the anti position. Therefore, they are not affected by any substitution; neither the ethyl ester, nor the Tyr-X peptides affect these peaks, except for the torsional frequencies, which depend on the mass. For these conformations the torsion is unhindered, because the carboxyl terminus is as far removed from the ring as possible and does not interact with it. We associate the D conformer with a strong interaction between the −COOH group and the ring. It is strongly affected by esterification as well as by formation of a peptide bond at the carboxyl terminus. The A, B, and C conformations are also likely to be anti for the tyrosine and lead to folded conformations for the dipeptide.

It is worth noting that we find the ring breathing vibration of tyrosine at 809 cm^{-1} for conformations A, B, and C, while we find it at 803 cm^{-1} for conformations D, D, and E. This difference may be the result of the fact that one group of conformations has some interaction with the ring, while the other group does not. We also note that Ala-Tyr and Tyr-Ala are isomers and thus cannot be distinguished in conventional forms of mass spectrometry. The additional dimension of wavelength dispersion in the laser desorption REMPI technique allows for clear isomeric identification.

(D) Phe-X. Phenylalanine (Phe) is comparable to tyrosine without the −OH at the ring, which should reduce the number of possible conformations by a factor of 2. Figure 5 compares the spectra of Phe, Phe-Gly, and Phe-Gly-Gly. The origins of the latter two are red shifted 45 cm^{-1} with respect to that of Phe, which is displayed at −45 cm^{-1} for easy comparison. Phe absorbs about 2000 cm^{-1} to the red of Tyr, consistent with the difference in absorption between phenol and benzene, as well as between toluene and *p*-cresol.[1,20−24] We may expect the conformer distribution to be similar to that of tyrosine without the −OH splitting. Indeed, the peak splitting, which is characteristic for the Tyr spectrum is absent in the spectrum of Phe. We find the same peaks as do Martinez et al.[2] at 37 535 cm^{-1} plus 0, 21, 33, 63, 70, and 76 cm^{-1}. Our relative intensities are somewhat different, which may be the result of different source conditions, and we find a few extra peaks at slightly higher energy and at relatively low intensity. By analogy with Tyr we can tentatively identify two groups of peaks. The three red-most peaks, labeled A, B, and C, may once again be associated with molecules that have the carboxyl group in the gauche conformation, while the other peaks would correspond to the anti conformation for the carboxyl group. This tentative assignment is consistent with the spectra of Phe-Gly and of Phe-Gly-Gly. The presence of glycene at the carboxyl terminus strongly affects peaks A, B, and C. In their place a new set of peaks appears at lower energy in the spectrum of Phe-Gly; These same peaks can also be seen with much more intensity in the spectrum of Phe-Gly-Gly. Tyr and Phe differ only in the ring hydroxyl, which is present in Tyr and absent in Phe. Both the Tyr-X and the Phe-X spectra show additional peaks at lower energy, compared with the spectra of Tyr and Phe, respectively. This suggests an interaction of the glycene carboxyl terminus with the π electrons of the ring in both cases. In other words, the change of conformation, which follows from the change in the REMPI spectra, indicates folding of the peptide. The fact that these peaks are much more intense for the tripeptide than for the dipeptide suggests that the longer chain allows a more facile approach of the acid terminus on one end of the chain to the aromatic chromophore at the other end of the chain. In future experiments we aim to extend the chain to further study the effect of chain length on folding.

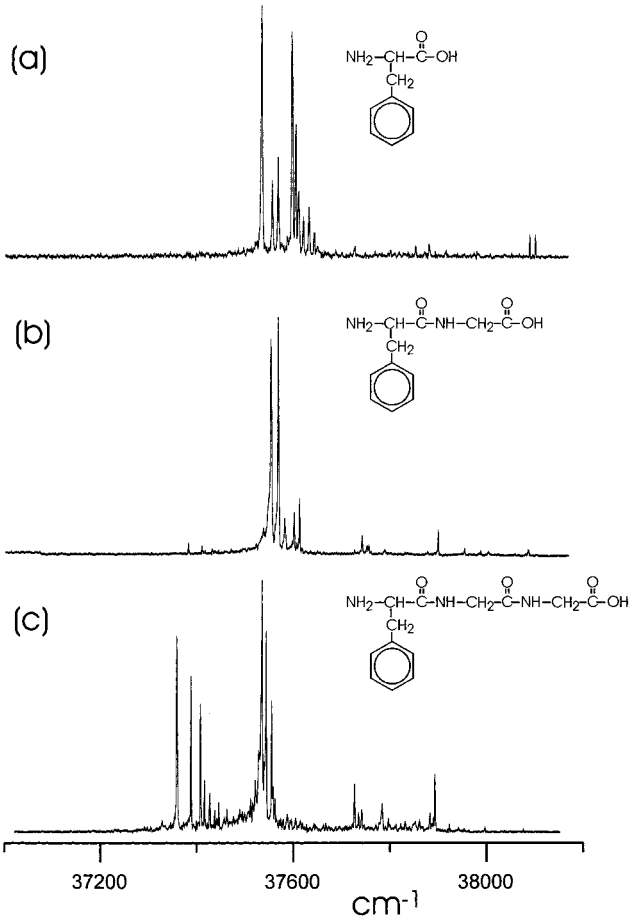

Figure 5. REMPI spectra of laser-desorbed, jet-cooled (a) Phe, (b) Phe-Gly offset by −45 cm^{-1}, and (c) Phe-Gly-Gly offset by −45 cm^{-1}.

Summary

We have used Phe and Tyr as aromatic chromophores (C) to probe a number of dipeptides and one tripeptide, containing one of those amino acids. By combining laser desorption, jet cooling, and REMPI, we have obtained vibronic spectroscopy of these compounds. The single amino acids have multiple origins that we interpret as arising from two types of conformations. We associate one set of peaks with conformations in which the amino acid chain is stretched away from the ring (anti) and the other set of peaks with the acid terminus approaching the ring (gauche). Spectra for dipeptides of the form X−C, for example, Ala-Tyr, are similar to those of the corresponding single amino acid. On the other hand, spectra for dipeptides of the form C−X, for example, Tyr-Ala, show a change in the peaks, which we associate with gauche conformations. This observation can be understood in terms of an interaction between the carboxyl terminus and the ring, which is associated with a folded conformation. This effect is more pronounced for the tripeptide Phe-Gly-Gly than for the dipeptide Phe-Gly.

It is clear that combining optical spectroscopy with mass spectrometry adds to the latter a very sensitive approach to isomeric distinction between peptides containing the same amino acids. These results also demonstrate that sequence reversal can lead to a fundamentally different molecular structure. A single

aromatic chromophore in an otherwise nonaromatic peptide sequence can serve as a sensitive conformational probe. For example, one may wonder whether in the gas phase the bond angles on either side of the peptide bonds are constrained in ways described by the Ramchandran maps, which hold for peptides in solution.[25,26] If so, one might expect peptides with more than about four amino acids to not be able to fold back on themselves as effectively as the short sequences that we studied here. Therefore, we will extend these studies to larger peptides as well as to their clusters with water.

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