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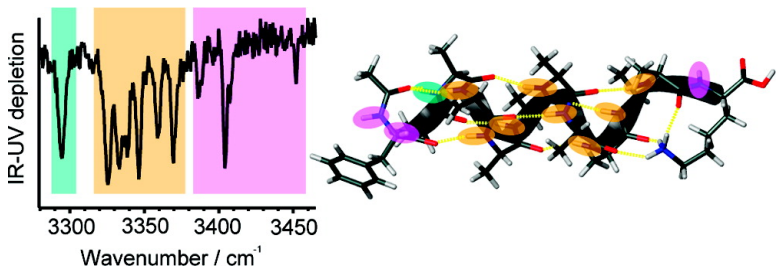

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Spectroscopic Signatures of Gas-Phase Helices: Ac-Phe-(Ala)$_5$-Lys-H$^+$ and Ac-Phe-(Ala)$_{10}$-Lys-H$^+$

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The α-helix is a common secondary structural element in proteins and represents the first level of complexity in the folding process. One approach to studying the folding preferences of such structures is to remove them from solution and investigate them in vacuo. Jarrold[1−4] pioneered the study of gas-phase helices using ion mobility techniques, discovering that one key to forming stable gas-phase protonated polyalanine helices is to include a lysine cap at the C-terminus. The Lys serves two functions: first, it provides hydrogen-bonding sites for three carbonyls that are not otherwise engaged; second, the ammonium charge stabilizes the macrodipole, which is created by alignment of the amide groups. On the basis of their mobility, protonated polyalanines without Lys or with Lys in other positions seem to be globular, whereas those with the sequence Ac-(Ala)$_n$-Lys-H$^+$ seem to be helical. Several groups have used spectroscopic techniques in supersonic jet expansions to study secondary structural elements of smaller neutral peptides,[5−8] with a particular emphasis on infrared (IR) spectroscopy to provide structural information by comparison to calculated vibrational frequencies. Evidence of a 3_{10}-helical hydrogen-bonding structure in neutral capped tripeptides was recently identified.[7,9] Others have studied larger protonated peptides at room temperature using resonant IRMPD spectroscopy to extract structural information.[10−13]

Here we study the IR spectroscopy in the N−H stretch (amide A) region of gas-phase Ac-Phe-(Ala)$_5$-Lys-H$^+$ and Ac-Phe-(Ala)$_{10}$-Lys-H$^+$ to determine the spectroscopic features associated with helical structures. On the basis of the work of Jarrold and co-workers,[2−4] we anticipate these molecules to be helical in the gas phase. It is noteworthy that these ions are of a length that is biologically relevant, given that the average span of a helix in a protein is about 10 residues.[14]

Our experimental apparatus has been described elsewhere.[15] Briefly, we transfer the protonated peptides to the gas phase using nanoelectrospray from an acidic solution in methanol or methanol/water and collect them in a hexapole ion trap. The ions of interest are released from this trap, selected in a quadrupole mass filter and steered into a cold 22-pole ion trap, where they are collisionally cooled to ∼10 K and interrogated with an ultraviolet (UV) laser. The trap is then emptied, and any UV-induced fragment ions are selected using a second quadrupole and detected. We record the photofragmentation signal as a function of UV-laser frequency to obtain a UV-excitation spectrum. The peptides were synthesized using solid-phase Fmoc chemistry on an Applied Biosystems 433A synthesizer.

Figure 1 shows the first 120 cm^{-1} of the UV photofragmentation spectra of Ac-Phe-(Ala)$_5$-Lys-H$^+$ and Ac-Phe-(Ala)$_{10}$-Lys-H$^+$. Despite the large size of the ions, the UV spectra still show sharp features without significant congestion from Franck−Condon activity, a large number conformers, or thermal congestion. Each spectrum is dominated by two intense transitions with several minor transitions, which may represent minor conformers or low-frequency vibronic activity.

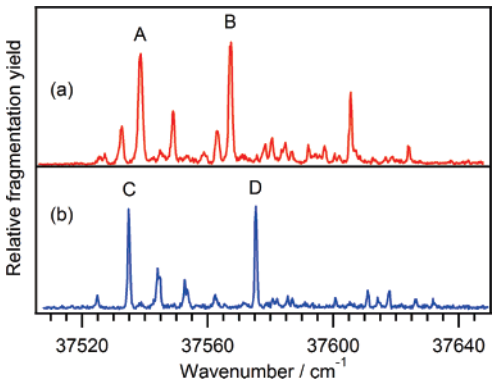

Figure 1. Ultraviolet photofragmentation spectra of (a) Ac-Phe-(Ala)$_5$-Lys-H$^+$ and (b) Ac-Phe-(Ala)$_{10}$-Lys-H$^+$. The four labeled transitions give rise to the IR spectra shown in Figure 2.

We record the IR spectrum of the conformation giving rise to a particular transition in the UV spectrum by fixing the UV laser on that transition while introducing an IR laser 100 ns earlier.[16] When the IR is resonant with a vibrational transition of the ion, a fraction of the population will be removed to vibrationally excited states. UV excitation out of these states appears to be less efficient than that out of the ground state, resulting in a depletion in the UV photofragmentation signal only when the IR and UV lasers are tuned to transitions of the same conformer. By recording the photofragmentation signal as a function of IR wavenumber, we obtain a conformer-specific IR spectrum.

Figures 2a and 2b show the IR spectra in the N−H stretch region of Ac-Phe-(Ala)$_5$-Lys-H$^+$ recorded with the UV laser fixed on transitions A and B in Figure 1a. Vibrations of the ammonium N−H bonds are red-shifted below 3200 cm^{-1} and broadened,[17] but the seven amide N−H bands all appear in Figure 2a and 2b. They can be classified into two general groups: those above ∼3380 cm^{-1} that are narrow, weaker, and characteristic of free or weakly interacting N−H bonds, and those further to the red that are more intense, broad, and typical of strongly hydrogen-bonded N−H groups. The similarity of the two spectra in the lower-frequency region suggests similar hydrogen-bonding patterns, with differences only in the more weakly interacting parts of the molecule.

We assigned the vibrations by comparing the experimental frequencies to harmonic frequency calculations[18] at the B3LYP/6-31G** level, which were scaled by 0.952. The conformers whose frequencies provide the best match to the experiment are 3_{10} helices, with three amide N−H bonds involved in 10-membered hydrogen-bonding rings (C_{10}, top of Figure 2). Figure 2a and 2b show the calculated frequencies as stick spectra. The vibrations are all calculated to be local modes, with the exception of the N−H vibrations of the fourth and fifth alanines, which are somewhat

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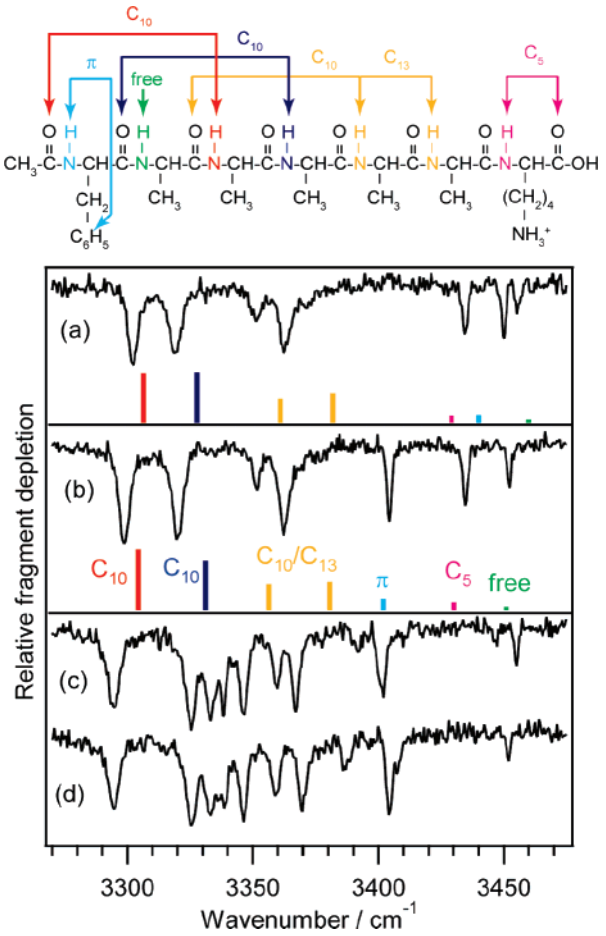

Figure 2. Infrared-ultraviolet double resonance depletion spectra in the N−H stretch region: (a and b) Ac-Phe-$(Ala)_5$-Lys-H^+; (c and d) Ac-Phe-$(Ala)_{10}$-Lys-H^+. The spectra were recorded with the UV laser fixed on the transitions labeled correspondingly in Figure 1. The stick spectra in panels a and b were calculated using B3LYP/6-31G** with a scale factor of 0.952. The calculated peaks are labeled by the type of interaction and correspond to the color-coded oscillators and hydrogen-bonding patterns shown schematically above.

mixed by virtue of being hydrogen-bonded to the same carbonyl. The conformers associated with the two observed spectra differ mainly in the orientation of the Phe side chain owing to rotation about the Phe $C_\alpha-C_\beta$ bond. These conformers lie 3 and 7 kJ/mol above the global minimum, which is a helix with two C_{10} and two C_{13} rings. However, the minimum-energy structure lacks the intense IR transition near 3300 cm^{-1} and thus cannot be responsible for the experimental spectra. Given the modest level of theory that we have used, it is not surprising that we do not recover the proper relative energies of the lowest energy conformers to better than several kJ/mol.

The power of our IR−UV double resonance technique for even larger molecules is demonstrated in Figure 2c and 2d, which show the IR spectra associated with the UV transitions C and D (Figure 1b) of Ac-Phe-$(Ala)_{10}$-Lys-H^+. At least 10 of the 12 amide N−H vibrations are sufficiently resolved to be identified. Comparison between these spectra and those in Figure 2a and 2b allows us to assign many of the features. The vibrations below ∼3380 cm^{-1}, which account for most of the activity in these spectra, are associated with vibrations of N−H groups in strong hydrogen bonds. A cluster of intense transitions between 3320 and 3350 cm^{-1} appears in Ac-Phe-$(Ala)_{10}$-Lys-H^+ but not in Ac-Phe-$(Ala)_5$-Lys-H^+, which strongly suggests that we assign these transitions to the internal N−H groups of an α-helix, all of which are in very similar environments with C_{13} hydrogen-bonded rings. As with Ac-Phe-$(Ala)_5$-Lys-H^+, there is an intense N−H stretch near 3290 cm^{-1}, which we again attribute to the second alanine in a C_{10} arrangement. Finally, the amide N−H bonds at the C- and N-termini are weakly bound or free, giving rise to the vibrational transitions above ∼3380 cm^{-1}.

We have thus identified several characteristics of the IR spectra of gas-phase helices, in the process demonstrating that high-resolution spectroscopic techniques are applicable to peptides of biologically relevant size and result in sharp electronic and vibrational spectra. Calculations enable us to assign the N−H stretches of Ac-Phe-$(Ala)_5$-Lys-H^+, providing a foundation to make inferential assignments for the Ac-Phe-$(Ala)_{10}$-Lys-H^+ IR spectra. The N−H stretches appear as primarily local-mode oscillators, meaning that even for molecules of this size, IR spectroscopy can provide structural information at the level of a single bond. With this powerful combination of theory and intuition to interpret spectra, we anticipate extending these techniques to even larger systems incorporating multiple elements such as the helix-turn-helix and other functionally important motifs.

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Supporting Information Available: Computational methods, calculated geometries, and complete ref 18. This material is available free of charge via the Internet at http://pubs.acs.org.

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