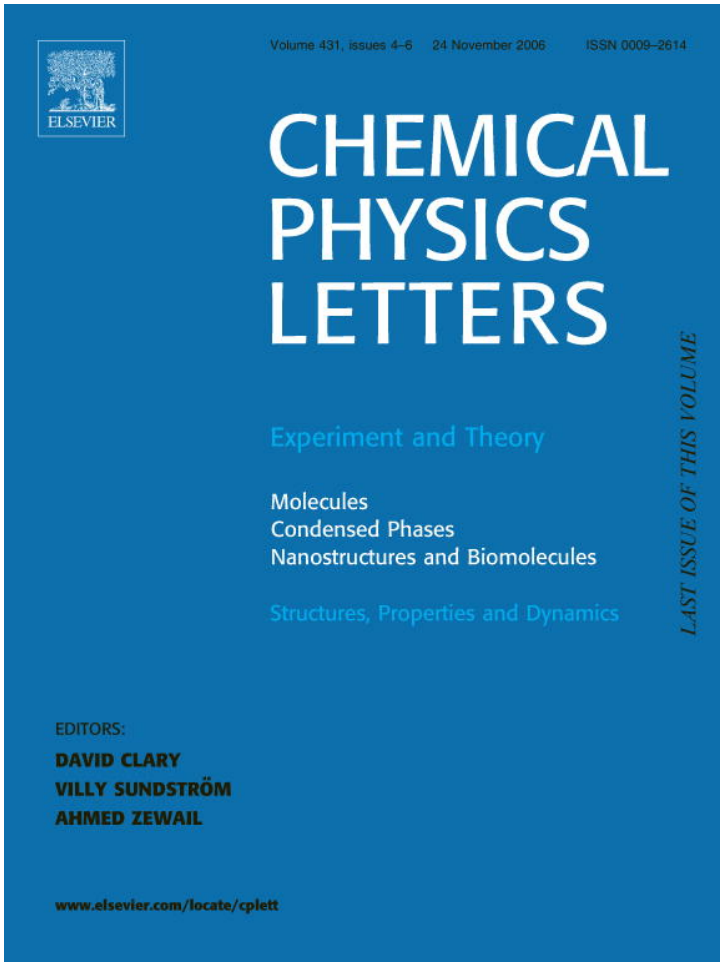

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Gas phase spectroscopy of the pentapeptide FDASV

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Abstract

IR–UV double resonance spectroscopy of the laser-desorbed, jet-cooled pentapeptide FDASV shows only one type of conformer in the gas phase. Comparison with computations at the B3LYP/6-31G** level suggests formation of an α-turn in the absence of solvent stabilization.

1. Introduction

Tight turns form an important folding motif in the secondary structure of proteins. They usually change the direction of the chain, causing it to fold back on itself. As shown in Fig. 1, γ-turns involve three amino acid residues, with an intramolecular hydrogen bond between the backbone CO(i) and the backbone NH($i+2$). β-Turns, involve four amino acid residues with an intramolecular hydrogen bond between the backbone CO(i) and the backbone NH($i+3$) [1]. α-Turns arise with five amino acid residues in which CO(i) forms an intramolecular hydrogen bond with NH($i+4$) [2]. Recent gas phase spectroscopy reports evidence for γ- and β-turns in small peptides with up to three amino acid residues [3–13]. Here we report the resonant two-photon ionization (R2PI) and the IR–UV double resonance spectra of the pentapeptide phe-asp-ala-ser-val (FDASV). We performed theoretical calculations using density functional theory (DFT) to compare the experimental infrared spectrum with the calculated frequencies of the lowest energy structures. The comparison between the experimental IR–UV spectrum and the calculated frequencies shows for the first time evidence of a possible α-turn formation in the gas phase.

2. Methods

We use laser desorption with jet cooling to vaporize the molecules, as described in detail elsewhere [14]. We perform mass selected spectroscopy by resonant two-photon ionization (R2PI), detecting the ions in a time-of-flight mass spectrometer. Fig. 2 shows the R2PI spectrum of the FDASV peptide with two main peaks at 37497 and 37555 cm^{-1}. We obtain the IR spectrum by IR–UV hole burning. A tunable IR 'burn' pulse precedes the UV R2PI 'probe' pulse by about 100 ns. IR resonances give rise to transitions that deplete the ground state, which we detect by a decrease in the ion signal [15,16].

We performed calculations on the neutral form of FDASV using a two-step approach, calculating candidate structures using a simple molecular mechanics force field followed by geometry optimization using density functional theory. Fig. 3 shows the optimized structures calculated with B3LYP/6-31G**, with the relative energies listed above each structure in kcal/mol. We used the Amber force field as implemented in the AMBER 7 program suite to perform simulated annealing. Low energy candidate structures from Amber then served as starting structures for subsequent optimization with the B3LYP hybrid density functional and the 6-31G** basis set using the GAUSSIAN03 program [17]. We also chose several starting structures (Fig. 3e and j–l) based on the fact that the experimental IR–UV spectrum, as described below, suggests that the

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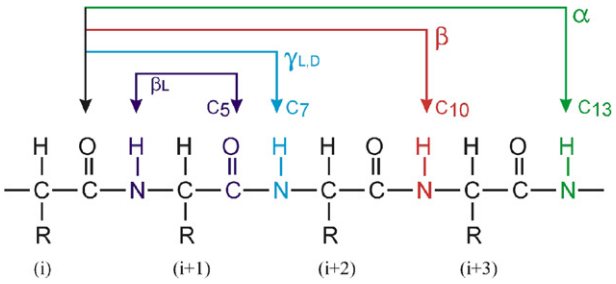

Fig. 1. Schematic peptide structure indicating the hydrogen bonds involved in different type turns and the nomenclature for the respective structural motifs as α-, β-, or γ-turns or C_n rings.

hydroxyl of the Serine residue is H-bonded and at least one and probably both of the carboxylic hydroxyls are free. Finally, we calculated the frequencies for all optimized

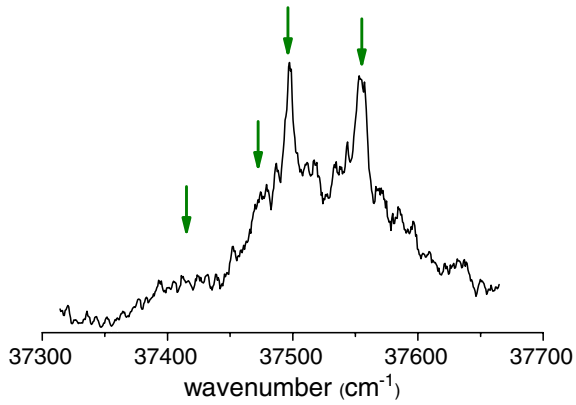

Fig. 2. R2PI spectrum of FDASV. The green arrows indicate the wavelengths at which we recorded IR–UV double resonant spectra.

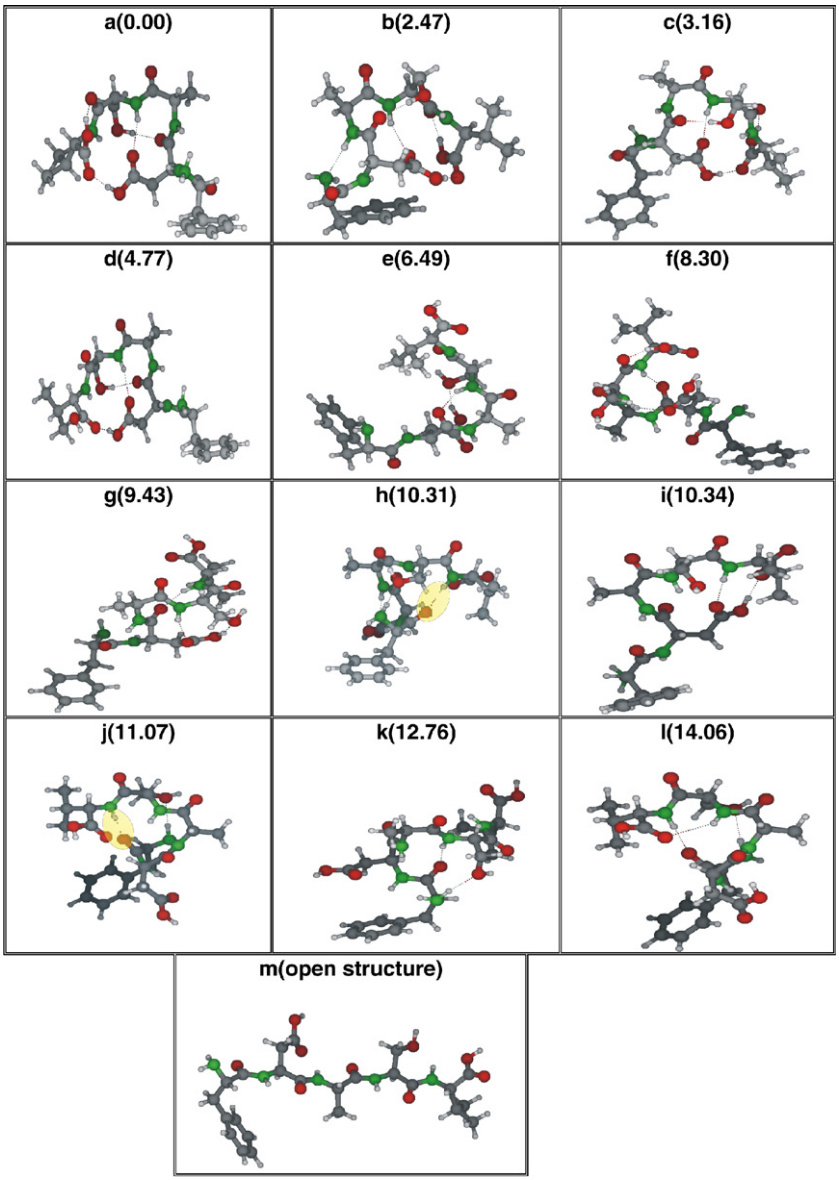

Fig. 3. Optimized structures calculated by B3LYP/6-31G**. The relative energy in kcal/mol is listed above each structure. In structures (h) and (j) shading indicates the CO(i)...HN($i+4$) hydrogen bond that forms an α-turn.

structures (with a scaling factor of 0.956) and compared them with the IR–UV double resonance spectrum. As a reference, we also calculated frequencies for the completely open structure (structure (m) in Fig. 3).

3. Results and discussion

Fig. 4 shows the IR–UV double resonance spectrum of FDASV (upper trace) and the calculated frequencies of the different structures presented as stick spectra (lower traces a–m). For this experiment, the UV laser was tuned to 37490 cm^{-1}, which is one of the transitions marked by a green[1] arrow on the R2PI spectrum in Fig. 2, while we scanned the IR laser in the region of 3100–3800 cm^{-1}. For all the different UV transitions we obtained identical IR–UV spectra. This indicates that we observe only one conformer or family of closely related conformers. The colored sticks in Fig. 4 represent the different local modes indicated in the scheme at the top of the figure by matching colored circles around each mode. Other possible modes, such as the C=O stretch have frequencies outside the 3100–3800 cm^{-1} range covered in this experiment.

We observe from the experimental IR–UV spectrum that the hydroxyl of the serine residue is H-bonded. This conclusion follows from our previous study of the dipeptide phe-ser (F–S) [18]. In this peptide, the free hydroxyl of the serine residue appears at 3620 cm^{-1}, while in the IR–UV spectrum of the FDASV this peak is absent. We also notice a strong peak around 3590 cm^{-1} which is typical for a free carboxylic acid hydroxyl group. In FDASV, there are two carboxylic hydroxyls (one on the aspartic acid and one on the C terminus). The intensity of the peak in the IR–UV spectrum suggests that this peak is a result of contributions from both carboxylic hydroxyls.

By comparing the experimental IR–UV spectrum and the calculated frequencies for the different structures (a–m), we can exclude structures (a–c) and (f) because in those structures both carboxylic hydroxyls are hydrogen bonded, leading to the absence of a peak at 3590 cm^{-1}. In structures (e), (g), (i), (k) and the open structure (m), the hydroxyl of the serine residue is free or only weakly bound. This is indicated by the unshifted or only slightly shifted black peak in the stick spectrum of each structure. Of the remaining three structures (d) is the least likely because the carboxylic hydroxyl of the aspartic acid is strongly hydrogen bonded. This implies that the peak at 3590 cm^{-1} would result from only one COOH group, in which case we would expect it to be much less intense.

The remaining two structures, (h) and (j), exhibit an α-turn as shown in Fig. 3 by the shading of the dashed line that connects the C=O of the phenylalanine residue with the NH of the valine residue (CO(i)–NH($i+4$)). Structure (h) is folded back to the right side whereas structure (j) is folded back to the left side. In both structures, the hydroxyls of the carboxylic groups are free and the hydroxyl of the serine residue is strongly hydrogen bonded to C=O($i+2$).

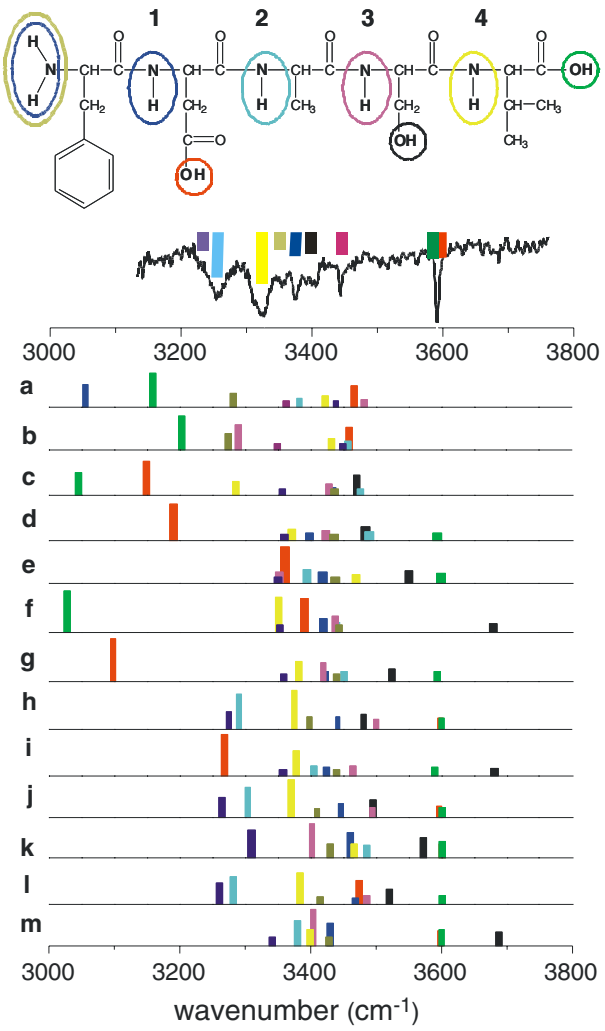

Fig. 4. IR–UV double resonance spectrum of FDASV (top trace). Bottom traces (a–m) represent the frequencies for the 13 different optimized structures.

To further facilitate comparison, we have overlaid a color coded stick spectrum on top of the IR–UV spectrum in Fig. 4. This pattern fits well with the frequencies of structure (h). The spectral modes resulting from this structure can be summarized as follows: according to this assignment, the free carboxylic hydroxyls appear at 3591 cm^{-1}, while the hydroxyl of the serine residue appears at 3403 cm^{-1}. This large shift to the red (about 210 cm^{-1}) is a result of strong hydrogen bonding of this hydroxyl with C=O($i+2$). The NH stretches of the backbone can be classified into two different types based on whether they undergo hydrogen bonding or not. NH($i+3$) does not hydrogen bond, corresponding to the mode at 3443 cm^{-1} (typical for the free NH of a peptide bond). NH(i), however, is weakly bonded to the carboxylic oxygen (C=O) of the aspartic acid residue, causing a small shift to the red. This corresponds to the mode at 3376 cm^{-1}. NH($i+4$) is strongly bonded to C=O(i), forming an

[1] For interpretation of color in Figs. 1–3, the reader is referred to the web version of this article.

α-turn. This strong hydrogen bond causes a large red shift corresponding to the broad peak at 3322 cm^{-1}. NH($i + 2$) is also involved in a very strong hydrogen bond, leading to a large shift to the red of about 3254 cm^{-1}. The size of the shift may be due in part to the fact that the NH–NH_2 and NH_2 symmetric stretch are coupled, and that the NH_2 is also a hydrogen donor to an OH group while the amide NH is hydrogen bonded to the lone pair of electrons of the amine group of the N terminus.

4. Summary

We observe that certain qualitative assignments, such as the presence or absence of specific free vs hydrogen bonded modes can be made with reasonable confidence for a peptide of this size. While this ability facilitates structural assignment, complete and accurate interpretation still poses a considerable computational challenge. In this work, we have reported the R2PI spectrum as well the IR–UV double resonance spectrum of the pentapeptide FDASV. The combination of these spectroscopic techniques with high level density functional theory calculation shows, for the first time, evidence of the formation of an α-turn in a peptide in the gas phase, free of solvent. This structure is stabilized by internal hydrogen bonding.

Acknowledgement

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