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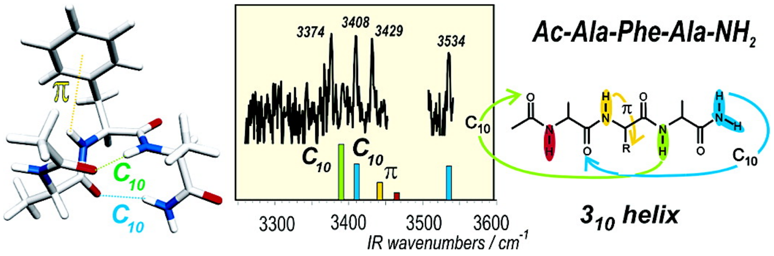

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Gas Phase Formation of a 3_{10}-Helix in a Three-Residue Peptide Chain: Role of Side Chain-Backbone Interactions as Evidenced by IR−UV Double Resonance Experiments

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Hydrogen bonding enables amino acid residues, the elementary bricks of proteins, to assemble with one another and to build up a large diversity of secondary structures. Its theoretical treatment is therefore a crucial feature for protein simulation. State-of-the-art quantum chemistry methods have recently been used to address the issue of competition between several secondary structures (γ- and β-turns, 3_{10}- and α- helices) of peptide chains, i.e., the competition between the C_7, C_{10}, and C_{13} H-bonds, which are responsible for their stability (Figure 1).[1−6]

Thus far, however, no experimental gas-phase data directly comparable to these precise computations are available. The recent progress in optical spectroscopy of large molecules in the gas phase has made it possible to record vibrationally resolved spectra (especially in the amide A, I and II regions) of selected conformations of biomolecules.[7,8] The combination of laser-desorption to vaporize fragile molecules and a supersonic expansion, in which an efficient conformational relaxation takes place, populates only very stable conformers so that the information collected is directly comparable to quantum chemistry optimized geometries and vibrational frequencies.

Concerning *peptide chains*, i.e., capped peptides containing at least three *complete* amide units, gas-phase optical spectroscopy has mainly been carried out on dipeptide chains to date.[9−13] The spontaneous formation of β-turns was reported very recently in capped dipeptides, thus providing information on the competition between a C_{10} bond and a sequence of two C_7 H-bonds.[11,12] Capped tripeptides (exhibiting four amide bonds; Figure 1) are the shortest species in which helical structures can be observed. In this case a competition is anticipated[3,4,6,13] which involves several types of H-bonding: (i) sequential C_7 bonds having a two-by-two common amide bond ($C_7-C_7-C_7$, a series of sequential γ-turns also referred to as a 2_7-ribbon), (ii) intertwined β-turns with C_{10} bonds ($C_{10}-C_{10}$) and (iii) mixed structures with sequential C_{10} and C_7 bonds. The second type of structures may eventually lead to a pseudoperiodic 3_{10}-helical structure, depending upon the type of β-turns involved. A fourth type involving a unique C_{13} bond (corresponding to the α-helix) is discarded essentially for energetic reasons.[6]

The aim of the present communication is to document the spontaneous formation of helical structures in the gas phase, i.e., in the absence of a solvent, by selectively characterizing the H-bonding of each conformer by infrared (IR) spectroscopy. The three chemically protected peptides studied here are all based on the same Ac-(Ala)$_3$-NH$_2$, (Ac = acetyl, Ala = alanine) tripeptide, in which one Ala residue is substituted by the aromatic phenylalanine (Phe) residue. Such an ultraviolet (UV) chromophore makes it possible to perform IR−UV double resonance experiments.[7,8] The present study on a series of tripeptides also provides insight into interactions between the peptide backbone and the aromatic side chain (SC).

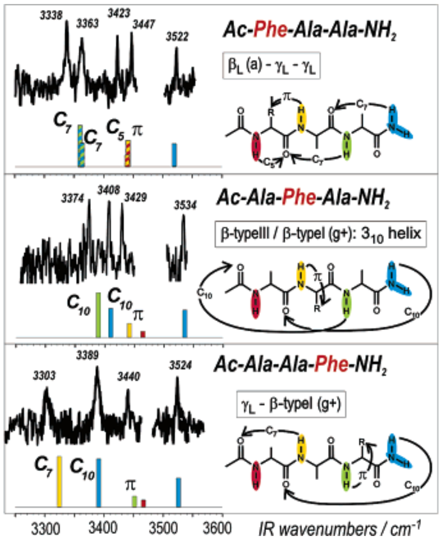

Figure 1. (Left) IR spectrum of the main conformers observed for the three tripeptides studied in the NH stretch region (amide A), as obtained by IR−UV double resonance experiments. Spectra are obtained by tuning the UV laser to the most intense band of the respective tripeptide. For comparison, DFT calculated stick spectra of selected conformations are also given. Harmonic frequencies are scaled by a factor of 0.96 to allow for anharmonicity.[10] (Right) Scheme of the H-bonding network in the corresponding conformations. Colors indicate the local NH stretch mode involved (two accidental near-degeneracies of local modes cause mode mixings for Ac-Phe-Ala-Ala-NH$_2$).

The UV absorption spectrum of the three peptides in the supersonic expansion, as obtained from one-color resonant two-photon ionization (Figure S1), is composed of a limited number of narrow features (either an isolated band or a short progression), which indicates that, despite the large flexibility of these species, only one main conformer is observed following conformational relaxation in the jet. In the double resonance experiment, the most intense of these features have been used to selectively probe the corresponding conformer, yielding conformer-selective IR spectra (Figure 1) in the amide A region, at a spectral resolution much better than that of usual condensed phase data. The setup and procedure are similar to those reported previously.[11,12] Four or five IR bands are observed in our spectra, all corresponding to NH stretch oscillators of each probed conformer.

Tentative assignments can be proposed on the basis of classic principles of IR spectroscopy, in conjunction with recent IR gas-phase data on shorter peptides.[10−13] Free amide NH's are usually found to be in the 3460−3490 cm^{-1} region. Bearing in mind that the IR source did not operate in this range, one can conclude that conformers that exhibit only four vibrational bands in their IR spectrum actually possess one free NH. Significantly red-shifted bands (typically at wavenumbers < 3410 cm^{-1}) correspond to

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H-bonded NH's. Weaker interactions, such as a C_5-close contact between CO and NH of the same residue (in a β_L, β-strand-like conformation) or a NH-aromatic interaction, are in the 3420−3450 cm^{-1} range. In addition, because the vibrational coupling which leads to symmetric and antisymmetric normal modes of a *free* -$CONH_2$ C-terminus amide is still active when one of the NH's is engaged in an H-bond, a frequency correlation still exists between the normal modes in the presence of an H-bond: both frequencies decrease and their splitting increases as the H-bond strength increases (see Appendix S1). The highest frequency band of the spectrum, which correlates to the NH_2 antisymmetric stretch for weak H-bonds and to the free NH stretch local mode for the limiting case of strong H-bonds, provides therefore a clear signature of the eventual involvement of the NH_2 group in the H-bonding. Moreover the doublet splitting enables us to evaluate the H-bond strength. Such an assignment strategy has already been successfully applied to provide evidence for the formation of β-turns.[10−13]

Using these basic rules, we conclude that the H-bonding patterns of the three conformers observed differ by the strength and the type of the H-bonds: two medium strength H-bonds for Ac-Phe-Ala-Ala-NH_2 , one strong and one weak for Ac-Ala-Ala-Phe-NH_2 and one medium and one very weak for Ac-Ala-Phe-Ala-NH_2 . In all cases, the C-terminus NH_2 is involved in the H-bonding. A more precise assignment can be proposed, based on a comparison with the spectroscopy of shorter peptides and DFT (B3LYP/6-31+G(d) level) geometry optimization of selected conformations and harmonic frequencies computations (Figure S2, Tables S1 and S2).

Ac-Phe-Ala-Ala-NH_2. The two medium-strength H-bonds, reminiscent of the C_7 bonds of successive γ-turns ($\gamma-\gamma$ form, C_7-C_7) in Ac-Ala-Phe-NH_2[12] together with the occurrence of a vibrational progression in the UV spectrum, similar to that of the β_L conformation of Ac-Phe-NH_2,[10] suggest that this conformer should be assigned to a $\beta_L-\gamma-\gamma$ form ($C_5-C_7-C_7$ pattern). A fair agreement is observed between IR data and computation (Figure 1, top, Table S2), in particular two doublets are predicted, even if the splitting within each doublet is not reproduced: the near degeneracy found theoretically for these modes (which leads to a significant mixing of the corresponding local NH stretch modes) is not observed experimentally. One should note that neither the IR spectral features of $\gamma-\gamma$ forms nor those of NH−π interactions have been satisfactorily reproduced by calculation thus far in shorter peptides.[11,12] The observation of this conformer, which is topologically very close to one of the patterns anticipated ($C_7-C_7-C_7$, Figure S2), is actually due to the occurrence of a C_5 conformation of the Phe residue, induced by a stabilizing NH−π interaction when the Phe side chain adopts an *anti* (a) orientation (Table S1).

Ac-Ala-Ala-Phe-NH_2. The position of the highest-frequency band (3524 cm^{-1}) together with a rather small NH_2 doublet splitting (Figure 1, bottom) is characteristic of a β-turn band.[11,12] This, combined with the large red-shift of the lowest-frequency band suggests a C_7-C_{10} pattern, which is nicely confirmed by computation (Figure 1, Table S2). Interestingly, the significant red-shift of the C_7 bond close to the N-terminus is a trend already observed for the C_7-C_7 moieties in Ac-Phe-Ala-Ala-NH_2 and Ac-Ala-Phe-NH_2[12] and is assigned to cooperative polarization effects when the same amide moiety acts both as a proton donor and as an acceptor. Schemes of Figures 1 and S1 show that this form turns out to be close to a helical $C_{10}-C_{10}$ arrangement. However the NH−π interaction, which is found again, hinders the establishment of a second C_{10} bond and furthermore energetically stabilizes the resulting C_7-C_{10} conformation.

Ac-Ala-Phe-Ala-NH_2. The highest-frequency band (3534 cm^{-1}, Figure 1, center), significantly blue-shifted relative to those assigned thus far to β-turn structures,[11,12] indicates a fairly weak H-bond. Therefore, the presence of two rather weakly red-shifted bands in the conformer, exhibiting large intensities but narrower bandwidths compared to those of the other species, suggests a $C_{10}-C_{10}$ pattern. Experimental data have been compared to calculated of intertwined β-turns of different types, starting from a helical 3_{10} form (type III−type III), as well as a variant of it (type II′−type I), differing by the orientation of the N-terminus amide group (see Figure S2). Calculations (Tables S1 and S2) show that only a helical form can account for the apparent blue-shift of the NH_2 antisymmetric stretch band, which seems to be a specific signature of it. The corresponding C_{10} bond is quite weak, significantly weaker than the other C_{10} bond of the molecule. This can be ascribed to a distortion of the helical 3_{10} form into a type III−type I form (Figure S2, Table S1), which seems to be a robust characteristic of these helices as suggested by other theoretical studies.[6] Present calculations (Table S1) show that the other C_{10} bond is in general also rather weak in the helix, with a frequency in the 3395 cm^{-1} range. The slight red-shift observed here for the lowest frequency (3374 cm^{-1}) is ascribed to an effect of the NH−π interaction (also responsible for the 3429 cm^{-1} band), made possible by the *gauche*+ Phe orientation, which significantly distorts the C_{10} bond and energetically stabilizes the conformation. Such an effect is reproduced by calculation, which shows a slight increase of the red-shift of the lowest-frequency band for this orientation of the Phe SC (Table S2).

The present work illustrates the intrinsic ability of short peptide chains to form helical structures in the gas phase. It also emphasizes that 3_{10}-helix formation does compete with other archetypal H-bonding patterns, such as 2_7-ribbon or mixed structures, whose energetics can be greatly influenced by a modest interaction such as an NH-aromatic π H-bond. This work finally demonstrates that gas-phase IR−UV double resonance experiments on lased-desorbed, jet-cooled species provides very precise information on the H-bonding pattern, which turns out to be a stringent test for accurate quantum chemistry approaches. It also opens up the route for a better understanding of the cooperative effects driving the $3_{10}/\alpha$-helix transition in larger peptides.

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Supporting Information Available: Figures S1 and S2, Tables S1 and S2, Appendix S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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