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Spectroscopic Evidence for Gas Phase Formation of Successive β-turns in a Three-Residue Peptide Chain

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Gas phase studies of small biomolecules[1-3] and peptides[4-9] have gained a considerable interest in the recent years as they provide experimental counterparts to the numerous high level calculations on short peptide chains,[10-15] documenting the competition between secondary structures such as β-strands, β-turns, 2_7 ribbons, α- and 3_{10} helices. In the present communication, we report an optical spectroscopic study in the gas phase on the three-residue peptide chain, Ac-Phe-Gly-Gly-NH_2, (Ac=acetyl). The conformational analysis carried out from the IR spectroscopy is backed by high-level theoretical calculations, which enables a structural assignment in terms of intramolecular H-bonding. In particular, we provide evidence for the spontaneous formation of successive entangled β-turns in the gas phase.

In the experiment, molecules are laser-desorbed and cooled in a supersonic expansion.[16,17] UV spectra (Fig. S1) are recorded in the absorption region of the phenylalanine UV chromophore using mass-selective resonant two-photon ionization (R2PI). At least five conformers, distinguishable in the UV spectrum, are populated in the jet (labeled A1-A4 and B in Fig. S1).

The IR spectrum of each conformer in the amide A region (3 μm) has been obtained in Saclay by the IR/UV double resonance technique[1,2] using an OPO (1 cm^{-1} resolution) as an IR source.[8] The IR spectra (Fig. 1a) of the five conformers A1-A4 and B of Ac-Phe-Gly-Gly-NH_2 show resolved absorption bands assigned to NH stretching modes of the molecule. Some bands are intense, broad and significantly red-shifted compared to the range of free or nearly free NH's (3420 - 3550 cm^{-1} [8]). Those characteristics are unambiguous signatures of intramolecular H-bonding. The presence of IR bands in an intermediate region (3420-3450 cm^{-1}) indicates weaker interactions of the corresponding NH groups, as for example in C_5 close contacts in β strand-like (β_L) conformations or in NH-aromatic interactions.[5,6,8] These basic considerations indicate that conformers A1-A4 in Fig. 1 contain strong intramolecular H-bonds that involve at least two NH moieties. The spectrum of conformer B is qualitatively different. It exhibits two narrow and intense bands that are moderately red-shifted (at 3380 and 3392 cm^{-1}), indicating that two medium-strength H-bonds are present. The amide I-II (5-8 μm) absorption spectrum of this conformer (Fig. 2) was recorded using the same IR/UV double resonance technique, employing the free electron laser FELIX[17,18] (15 cm^{-1} resolution) as the IR source. In contrast to the amide A region, the amide I region exhibits only one partially resolved feature.

The here proposed H-bonding assignment of these five conformers are consistent with recently reported IR spectra of shorter peptides.[19] The main conformer of Ac-Gly-Phe-NH_2, assigned to a double γ-fold structure, shows two broad C_7 bands in the 3300-3370 cm^{-1} region, bearing a striking resemblance to the red part of the present A1-A4 spectra (Fig. 1a). On the other hand, the minor conformers of Ac-Gly-Phe-NH_2 and Ac-Phe-Gly-NH_2, assigned to β-turns,[18,19] exhibit a C_{10} H-bond signature at 3388 and 3387 cm^{-1} respectively. This is very close to the slightly red shifted bands observed for conformer B.

This first analysis provides evidence for a large propensity of the Ac-Phe-Gly-Gly-NH_2 tripeptide chain to form an extended intramolecular H-bonding network in the gas phase, presumably containing at least two strong C_7 H-bonds (conformers A1-A4). Interestingly, however, the most intense feature observed in the UV spectrum (conformer B; Fig. S1) exhibits a radically different IR pattern, which might be due to β-turns.

Focusing onto this conformer B, quantum mechanical geometry optimizations (DFT B3LYP/6-31+G(d)) have been carried out for a set of selected conformations that exhibit only two H-bonds (Fig. 1b; Table S1). Considered were: structures with (1) two C_{10} bonds (successive entangled β-turns), (2) one C_{10} and one C_7 bond (successive entangled β-turns and γ-turns in C_{10}-C_7 as well as C_7-C_{10} order), (3) one central C_7 γ-turn accompanied by either a parallel C_{13} or an antiparallel C_{11} bond, and (4) having a β strand-like (β_L; C_5) conformation on Phe (very stable in the protected amino acid[6,8]) followed by two successive γ-folds (C_7-C_7). For comparison, two remarkable secondary structures have also been considered: (5) a triple C_7 structure (2_7 ribbon) and (6) a triple C_5 structure (β-strand). The orientation (anti, gauche+ and gauche-) of the Phe side-chain relative to the backbone was chosen either arbitrarily or, when possible, to allow stabilizing NH-π interactions. In structures (1-2), among the four canonical types of β-turns, types I or II were chosen for the Phe-Gly part, as they have been shown to be the most stable forms in Ac-Phe-Gly-NH_2.[19] For the Gly-Gly part, all the four types were *a priori* considered, but steric considerations led to retain only I-I and II-I' combinations. 3_{10} helices (which also belong to family (1)) were found to converge to I-I forms. For γ-turns on Gly residue (families (2) and (5)), both inverse or direct, i.e., γ_L or γ_D respectively, were considered, in contrast to γ-turns on Phe for which γ_L are much more stable.[8] Figure 1c displays calculated absorption spectra in the amide A region for selected conformations characteristic of the six families described above. A large variety of red shifts and hence of H-bond strengths is found and the calculated spectra can be compared to the experimental absorption pattern of conformer B. This comparison (Fig. 1c and Table S2) suggests that the central γ-fold (3), 2_7-ribbon (5) and β-strand (6) secondary structures can be rejected, although one should remark that in the case of the A1-A4 conformers, the 2_7-ribbon family accounts for the observed red-shifts, in agreement with the qualitative analysis. For a further analysis of the spectrum of conformer B, only conformations of

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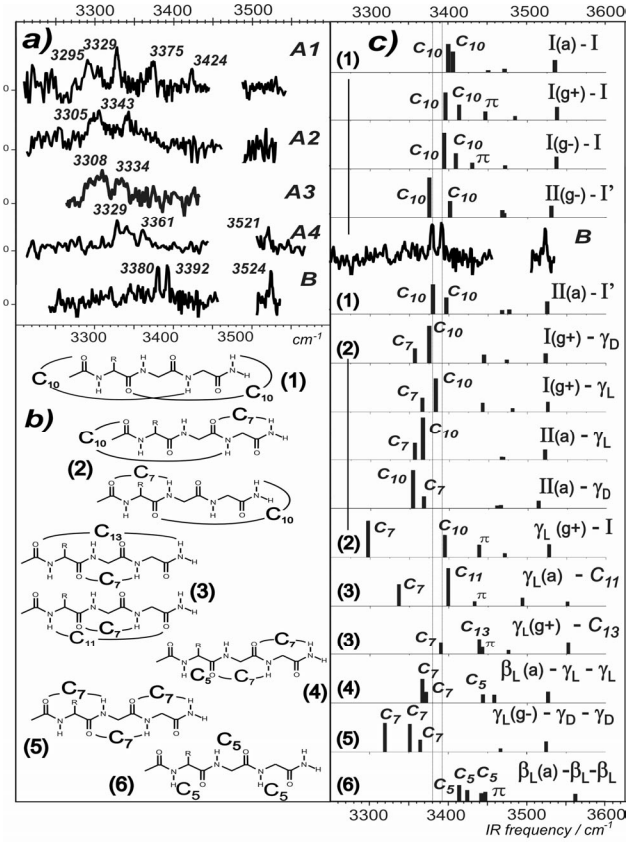

Figure 1. a) IR/UV double resonance spectrum in the amide A region of five conformers of Ac-Phe-Gly-Gly-NH_2 labeled A1-A4 and B. The spectral gap in the 3470-3500 cm^{-1} region is due to an undesired absorption in the crystal of the IR source. b) H-bonding schemes in a series of conformations exhibiting 2 H-bonds (labeled (1) to (4)), three H-bonds (5) or none (6); c) DFT B3LYP/6-31+G(d) calculated stick spectra (scaling factor 0.960) of selected conformations (see text and Table S1) for comparison with the spectrum of conformer B.

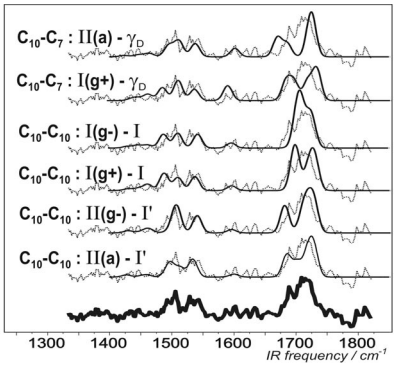

Figure 2. Amide I and II IR spectrum of conformer B of Ac-Phe-Gly-Gly-NH_2 (lower bold trace and dots) and DFT B3LYP/6-31+G(d) calculated spectra (narrow lines) of selected conformations (see text; Table S1) obtained by convoluting the stick spectra by a 15 cm^{-1} width gaussian-like function to account for the experimental line widths. Scaling factors of 0.980 and 0.970 have been used in the amide I and II regions respectively.

families (1), (2) and (4) are considered, as the agreement between their amide A spectra and the experiment is within the expected range (usually better than 15 cm^{-1}).[8,19] The best agreement is found for structures exhibiting *two successive β-turns, of type II and I' respectively*, and a Phe side chain in either (a) or (g-) orientation. This backbone structural assignment has been crossed-checked against spectroscopic data from the amide I/II regions as well as energetic considerations.

Calculations (Fig. 2, Table S2) show that the amide I absorption spectrum is sensitive to both H-bonding and Phe side-chain orientation. Comparison to the experiment allows us to reject conformations possessing one or two γ-folds (Families (2) and (4)), as their spectra have too large amide I splittings. We also can distinguish between very similar structures, like II(a)-I' and II(g-)-I', or II(a)-I' and II(a)-γ_D (Fig. S2). The best fit to the experimental amide I and II spectrum (Fig. 2) is provided by the double β-turn type II(a)-type I' conformation.

This assignment is further supported by refined energetic calculations (MP2/6-31+G(d)// B3LYP/6-31+G(d); Table S1): the type II(a)-type I' conformation is the most stable form among all the conformations here considered.

As a conclusion, for the first time for a peptide chain model of this size, a detailed conformational analysis has been carried out, revealing the intrinsic folding properties of the chain. The abundance of the double β-turn form observed indicates a significant evolution of the folding preferences by sequence lengthening. Indeed, a previous study revealed that a Phe-Gly chain preferentially adopts an extended β_L-γ_L conformation; the type II β-turn conformation had only minor abundance.[19] This propensity is reversed in the present longer peptide, presumably because of the rather high stability of the β-turns in the Gly-Gly part, as suggested by recent high level calculations.[15,20]

Finally, one can remark that nascent 3_{10} helices (type I-type I conformations, Fig. S2) are less stable than type II-type I' conformations, probably because the central Gly residue strongly favors the type II β-turn of the Phe-Gly part of the chain. The absence of the 3_{10} helix conformation should probably be viewed as a specific effect of the Gly residue. This result might be of importance since the Gly residue, which is less demanding in terms of calculation facility, is generally considered as a case study and a popular model in theoretical studies.

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Supporting Information Available: UV spectrum of Ac-Phe-Gly-Gly-NH_2 (Fig. S1); Structural and energetic parameters (Table S1), calculated IR data (Table S2) and relevant DFT optimized structures (Fig. S2).

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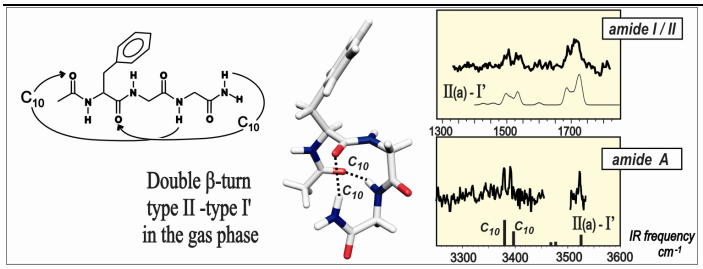

We report the first gas phase spectroscopic study of a three-residue model of a peptide chain, Ac-Phe-Gly-Gly-NH_2 (Ac= acetyl), using the IR/UV double resonance technique. The existence of at least five different conformers under supersonic expansion conditions is established, most of them exhibiting rather strong intramolecular H-bonds. One of the most populated conformers, however, exhibits a different H-bonding network characterized by two weak H-bonds. Comparison of the amide A and I/II experimental data with Density Functional Theory calculations carried out on a series of selected conformations enables us to assign this conformer to two successive β-turns along the peptide chain; the two H-bonds being of C_{10} type, i.e., each of them closing a 10-atom ring in the molecule. The corresponding form is found to be more stable than the 3_{10} helix secondary structure (not observed), presumably because of specific effects due to the glycine residues.