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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-NH₂, (Ac = *N*-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 (Figure 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 Figure S1).

The IR spectrum of each conformer in the amide A region (3 μ m) 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 (Figure 1a) of the five conformers A1–A4 and B of Ac-Phe-Gly-Gly-NH₂ 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 C₅ 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 Figure 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 μ m) absorption spectrum of this conformer (Figure 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 is consistent with recently reported IR spectra of shorter peptides.¹⁹ The main conformer of Ac-Gly-Phe-NH₂, assigned to a

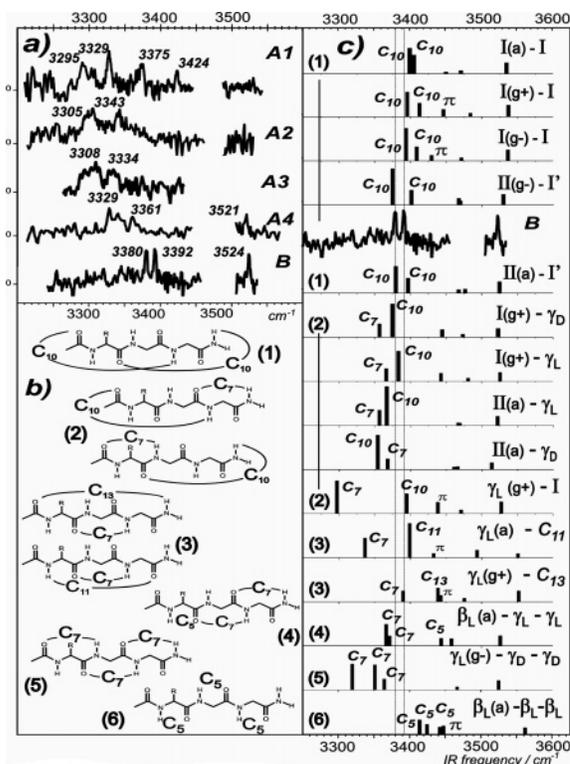


Figure 1. (a) IR/UV double resonance spectrum in the amide A region of five conformers of Ac-Phe-Gly-Gly-NH₂ labeled A1–A4 and B. The spectral gap in the 3470–3500 cm⁻¹ region is due to an undesired absorption in the crystal of the IR source. (b) H-bonding schemes in a series of conformations exhibiting two H-bonds (labeled 1–4), 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.

double γ -fold structure, shows two broad C₇ bands in the 3300–3370 cm⁻¹ region, bearing a striking resemblance to the red part of the present A1–A4 spectra (Figure 1a). On the other hand, the minor conformers of Ac-Gly-Phe-NH₂ and Ac-Phe-Gly-NH₂, assigned to β -turns,^{18,19} exhibit a C₁₀ 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-NH₂ tripeptide chain to form an extended intramolecular H-bonding network in the gas phase, presumably containing at least two strong C₇ H-bonds (conformers A1–A4). Interestingly, however, the most intense feature observed in the UV spectrum (conformer B; Figure 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

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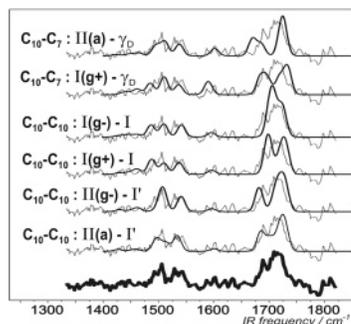


Figure 2. Amide I and II IR spectrum of conformer B of Ac-Phe-Gly-Gly-NH₂ (lower bold trace and dots) and DFT B3LYP/6-31+G(d) calculated spectra (narrow lines) of selected conformations (see text; Table S1) obtained by convoluting the stick spectra by a 15 cm⁻¹ width Gaussian-like function to account for the experimental line widths. Scaling factors of 0.980 and 0.970 have been used in the amide I and II regions, respectively.

a set of selected conformations that exhibit only two H-bonds (Figure 1b; Table S1). Considered were structures with (1) two C₁₀ bonds (successive entangled β -turns), (2) one C₁₀ and one C₇ bond (successive entangled β -turns and γ -turns in C₁₀-C₇ as well as C₇-C₁₀ order), (3) one central C₇ γ -turn accompanied by either a parallel C₁₃ or an antiparallel C₁₁ bond, and (4) having a β strand-like (β_L ; C₅) conformation on Phe (very stable in the protected amino acid^{6,8}) followed by two successive γ -folds (C₇-C₇). For comparison, two remarkable secondary structures have also been considered (5) a triple C₇ structure (2₇ ribbon) and (6) a triple C₅ 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-NH₂.¹⁹ 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. 3₁₀ 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 (Figure 1c and Table S2) suggests that the central γ -fold (3), 2₇ 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 2₇ 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 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 (Figure 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, such as II(a)-I' and II(g-)-I', or II(a)-I' and II(a)- γ_D (Figure S2). The best fit to the experimental amide I and II spectrum (Figure 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 3₁₀ helices (type I-type I conformations, Figure 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 3₁₀ 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.

Acknowledgment. This work was supported by the Stichting voor Fundamenteel Onderzoek der Materie (FOM) in providing the required beam time on FELIX and by the F.P.6 "Structuring the European Research Area" Program of the European Community. We also thank the FELIX staff for their skillful assistance.

Supporting Information Available: UV spectrum of Ac-Phe-Gly-Gly-NH₂ (Figure S1), structural and energetic parameters (Table S1), calculated IR data (Table S2), and relevant DFT optimized structures (Figure S2). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA042860B