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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 biomolecules1−3 and peptides4−9 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, α-helices, and 310 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 (3300−3500 cm−1) has been obtained in Saclay by the IR/UV double resonance technique1,2 using an OPO (1 cm−1 resolution) as an IR source.5 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). 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 C3 close contacts in β strand-like (βL) conformations or in NH-aromatic interactions.3,6,8 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 the minor 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 (Figure 2) was recorded using the same IR/UV double resonance technique, employing the free electron laser Felix17,18 (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 α-β-turns.

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 C3 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 C3 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 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.
a set of selected conformations that exhibit only two H-bonds (Figure 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_13–C_10 order), (3) one central γ-turn accompanied by either a parallel C_13 or an antiparallel C_3 bond, and (4) having a β strand-like (β_L; C_9) conformation on Phe (very stable in the protected amino acid_8) followed by two successive γ-folds (C_1–C_7). For comparison, two remarkable secondary structures have also been considered (5) a triple C_7 structure (β_2 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-NH₂.19 For the 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. For the 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.

Finally, one can remark that nascent 3_10 helices (type I–type I conformations, Figure S2) are less stable than type II–type II 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-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.

References