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Intrinsic Folding of Small Peptide Chains: Spectroscopic Evidence for the Formation of β-Turns in the Gas Phase

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Abstract: Laser desorption of model peptides coupled to laser spectroscopic techniques enables the gas-phase observation of genuine secondary structures of biology. Spectroscopic evidence for the formation of β-turns in gas-phase peptide chains containing glycine and phenylalanine residues establishes the intrinsic stability of these forms and their ability to compete with other stable structures. The precise characterization of local minima on the potential energy surface from IR spectroscopy constitutes an acute assessment for the state-of-the-art quantum mechanical calculations also presented. The observation of different types of β-turns depending upon the residue order within the sequence is found to be consistent with the residue propensities in β-turns of proteins, which suggests that the prevalence of glycine in type II and II′ turns stems essentially from an energetic origin, already at play under isolated conditions.

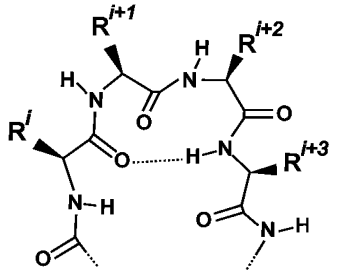
Introduction

β-turns, the smallest secondary structures of proteins, allow the peptide chain to fold back upon itself and therefore play a key role in the initiation of the folding process. X-ray structures of crystallized proteins have enabled biochemists to classify these chain reversals in terms of structure.[1−6] Spanning over four amino acid residues (labeled i, ..., $i + 3$), these folds are characterized by a H-bond bridging the CO(i) and NH($i + 3$) moieties, the so-called C_{10} interaction, named after the ten-membered ring formed (Schemes 1 and 2). Four major types of turns (labeled I, II, I′, and II′) have been distinguished, based on the Ramachandran φ and ψ dihedral angles controlling the relative orientations of the amide groups of the $i + 1$ and $i + 2$ central residues.[3−6] Types I and II mainly differ by a 180° flip of the central amide unit, and primed species have a backbone mirror image compared to unprimed species, the side-chain linkage to the backbone being unchanged.

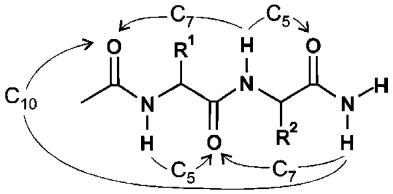
In proteins, these secondary structures are in competition with other structures, corresponding either to local conformational preferences of the backbone about each residue, such as β-strands or γ-turns (based on C_5 and C_7 interactions, respectively; see Scheme 2), or to helices (based on intramolecular H-bonding schemes between more remote sites such as C_{13} interactions).

To investigate the role of local interactions in the formation of β-turns, biochemists and chemists have studied the folding

Scheme 1. β-Turn within a Peptide Sequence



Scheme 2. Intramolecular Interactions in a Protected Peptide



properties of model peptide chains,[2,7−10] namely, short synthesized peptide sequences containing a complete set of amide bonds, i.e., protected peptides. Such systems were investigated in solution two decades ago, using traditional techniques, such as circular dichroism (CD), infrared (IR) absorption, or nuclear magnetic resonance (NMR).[2,7−10] The difficulty of these experiments, however, stems from the fact that, in solution, linear peptides present multiple forms, sometimes β-folded, but also

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γ-folded (corresponding to a γ-turn) or extended. β-turns are usually minor conformers that are often difficult to detect among the condensed-phase broad IR or CD spectra of the major conformers.[2,7−10] The results of NMR studies are limited by the long time-scale characteristic of NMR relative to the time for interconversion between the conformers. Apart from those containing the proline residue, the small linear peptides are often considered as not structured in solution, and chemists interested in turn spectroscopy commonly used cyclic peptides to ensure the spontaneous formation of turns (generally two for each cyclic peptide, either two β-turns or a mixed β-turn−γ-turn structure).[7,10] As a result, the experimental data from condensed-phase studies of small peptides are not particularly useful tests of the intermolecular force fields used to study proteins. In addition, these condensed-phase data are not necessarily the most relevant for describing folding processes in a hydrophobic medium, such as the protein interior or lipidic membranes. In this respect, gas-phase investigations of model peptides (Scheme 2) offer a unique approach to the structural and dynamical properties of a protein chain in an apolar environment. The precise data collected by the most recent techniques developed by experimental groups working on small biomolecules in the gas phase[11−24] are moreover directly comparable to the most sophisticated calculations currently available.[16,17,23−35]

From a theoretical point of view, the potential energy surface (PES) of such small model systems is already complex. Its accurate exploration, description, and characterization, essential for a complete understanding of folding processes, remains a difficult task. Recent progress in fast computers makes possible quantum mechanical studies on modified amino acid and dipeptide model systems, launching new approaches[25,28,32,34] to study the formation of secondary structures and their competition with other forms in vacuo.[30,35] So far, however, no relevant gas-phase experimental counterpart data have been available on peptides long enough to exhibit β-folding. The numerous works devoted to gas-phase experimental studies of small peptides or model peptide chains[11−13,15−17,20−23] indeed essentially focused on the local conformational preferences about a unique residue.

Aiming to bridge the gap between experiment and high-level theoretical calculations on secondary structures of peptides, we report here the first spectroscopic observation of β-turns in gas-phase peptide chains and their characterization in terms of H-bonding.

The sequences studied (Scheme 2) exhibit two residues (side chains labeled R^1 and R^2 in Schemes 1 and 2): glycine (Gly), chosen for its known ability to induce β-turns,[5] and phenylalanine (Phe), which provides the UV chromophore needed in the experiment. The peptides are chemically protected on both ends, giving rise to an *N*-acetyl group (Ac−) on the N terminus and an amino group ($-NH_2$) on the C terminus, so that the species can be considered as model segments of a protein chain. Results on a simple protected amino acid, Ac-Phe-NH_2, are also presented to illustrate the intrinsic local conformational preferences of the peptide chain about an isolated Phe residue. The laser desorption technique coupled to a supersonic expansion[14] allows the peptide molecules to be vaporized and cooled rotationally and vibrationally. This very efficient cooling combined with the spectral selectivity achieved with lasers enables us to perform a conformer-selective spectroscopy using double-resonance IR/UV techniques,[36] in which the individual spectral contributions of each conformer, including minor ones, can be distinguished.[16−18,20−24] Spectroscopic evidence for the formation of a β-turn is first deduced from a qualitative analysis of the well-resolved IR features measured, and then confirmed by comparison with the results of a detailed theoretical characterization of the potential energy surface for these species.

Methods

Experimental Methods. The experimental setup has been described in detail previously.[14,18,21] A powder of the protected peptides (Epytop, Nîmes, France) is mixed and compressed with spectroscopic grade powder graphite. The pellet obtained is fixed below the nozzle of a pulsed valve (General Valve Co. operating at 10 Hz), and the sample molecules are desorbed from the pellet surface by the second harmonic output of a synchronized Nd:YAG laser (Continuum) guided to the sample through a multimode optical fiber. Molecules from the desorption plume are picked up and cooled by the pulsed supersonic expansion of argon (4 bar backing pressure) and introduced through a 1 mm diameter skimmer in the interaction region of a time-of-flight mass spectrometer. Molecules are photoionized in a resonant two-photon ionization process using the frequency-doubled output (typical pulse energy 1 mJ) of an excimer-pumped dye laser (Lambda Physik FL 2002). The corresponding ion signals are sorted by the mass spectrometer, collected, and averaged in a PC-controlled LeCroy oscilloscope. Near-UV spectra are obtained by scanning the dye laser in the origin region of the $S_1 \leftarrow S_0$ transition of the Phe UV chromophore. The IR spectra of UV-selected conformers of the peptide studied are obtained by the resonant ion-dip IR spectroscopy technique.[36] The IR source is a Pérot−Fabry etalon-narrowed $LiNbO_3$ optical parametric oscillator (OPO; Euroscan, 1 cm^{-1} line width, 3 mJ pulse energy) pumped by the fundamental beam of a Nd:YAG laser (Brilliant B, Quantel). The idler output, tunable in the 3 μm region, is used to induce IR absorption

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transitions in the NH stretch (amide A) spectral region. The corresponding excitation leads to a transient depletion of the vibrational ground level population in the jet, which is detected by a depletion (ion dip) in the ion signal due to the UV "probe" laser, shot typically 50 ns later. IR spectra are obtained by recording ion signals, both with and without the IR laser, using a PC-controlled shutter (Vincent Associates), and by normalizing the difference with the ion signal in the absence of IR light, therefore providing zero-baseline depletion spectra. Because of absorption by impurities in the OPO crystal, a large drop in IR intensity, due to the nonlinearity of light generation, is observed in the 3470−3500 cm^{-1} region, forbidding any spectral recording. This is indicated by the absence of an experimental trace in the ion-dip IR spectra.

Theoretical Methods. To provide a refined picture of the conformational landscape of the peptides studied, a set of low-energy conformations are first generated using several strategies: by exploring the PES using the AMBER force field,[37] by combining local conformational preferences known for each residue, and by simply considering the four canonical types of β-turns as determined by biochemists.[5] All these structures are then refined using the density functional theory (DFT) method (B3LYP/6-31+G(d) level of theory), and their harmonic vibrational frequencies are calculated using the Gaussian 98 program.[38] A post-Hartree-Fock method (single-point MP2/6-31+G(d) on the B3LYP optimized geometry) is finally used to account for electronic correlation.

Experimental Results

The UV spectra of the peptides studied (Figure 1) measured in the absorption region of Phe (first ππ* transition) present resolved narrow features, illustrating the efficiency of the cooling process during the expansion, ending up with a very small number of finally populated structures. The vibrational progressions observed correspond to a significant vibrational excitation simultaneous to electronic excitation. This Franck-Condon vibrational activity, controlled by geometry changes between the ground and excited states, reveals information on the Phe chromophore environment. In this respect spectra of the most populated conformers of Ac-Phe-NH_2 and Ac-Phe-Gly-NH_2 (labeled A) are similar, suggesting comparable Phe environments in these two species. In contrast, the qualitatively different spectrum of Ac-Gly-Phe-NH_2 suggests a different geometrical arrangement. Besides the most populated species of these dipeptides, one minor conformer (labeled B) was also evidenced from its weak UV signature and identified from its IR spectroscopy.

The IR spectra of the protected peptides studied (Figure 2) exhibit well-resolved absorption bands, all corresponding to the stretching of the NH bonds in the molecule. Compared to the broad IR spectra in solution,[2,7−9] the present results emphasize the efficiency of the laser-selective gas-phase data to provide resolved features and to distinguish each IR chromophore of each conformer detected in the jet.

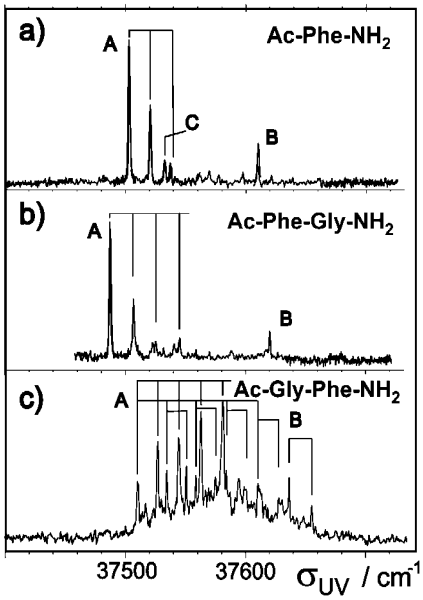

Figure 1. UV spectra of Ac-Phe-NH_2 (a), Ac-Phe-Gly-NH_2 (b), and Ac-Gly-Phe-NH_2 (c) in the origin region of the first electronic transition (ππ*), as obtained in supersonic expansion by mass-selected resonant two-photon ionization. Double-resonance IR/UV experiments demonstrate that only a few conformers are responsible for the spectra observed. The origin transition of each conformer is identified by its label (A, B, or C).

A qualitative assignment can be derived from the sensitivity of the NH stretch vibration to the environment of the NH bond, in particular its involvement in a H-bond. Whereas unperturbed NH stretches absorb in the 3450−3500 cm^{-1} range, the occurrence of intense, broadened, and significantly red-shifted bands (frequency <3420 cm^{-1}) is the unambiguous signature of intramolecular H-bonding.[21] The presence of IR bands in the intermediate (3420−3450 cm^{-1}) region indicates weaker interactions upon the NH group considered, such as C_5 or NH−π interactions. Crucial structural assignment information is also derived from the IR signature of the C-terminal amino group and its dependence upon H-bonding.[21] When free from H-bonding, the vibrationally coupled NH oscillators give rise to a doublet split by 117 cm^{-1}.[21,39,40] Any increase of this splitting, together with the slight red shift of the blue antisymmetric component in the 3500−3530 cm^{-1} range, provides information on the involvement of one of the NH oscillators within the intramolecular H-bonding network and enables us to estimate the H-bond strength.[21,40]

This is exemplified by the IR spectrum of Ac-Phe-NH_2 (Figure 2a), which reflects the local conformational preferences for a Phe residue[21,22,41] and will be used as a guide for the assignment of the dipeptide spectra. The most populated form A corresponds to an extended β-strand-like peptide backbone (labeled β_L according to Ramachandran terminology), stabilized by two rather weak interactions, one C_5 close contact, taking place between the close-lying NH and CO moieties of the Phe residue, and an NH_2−π interaction made possible in the *anti* orientation of the Phe side chain. Both interactions are observable in the spectra as causing only a slight red shift of the NH stretch bands. On the other hand, minor B and C conformers

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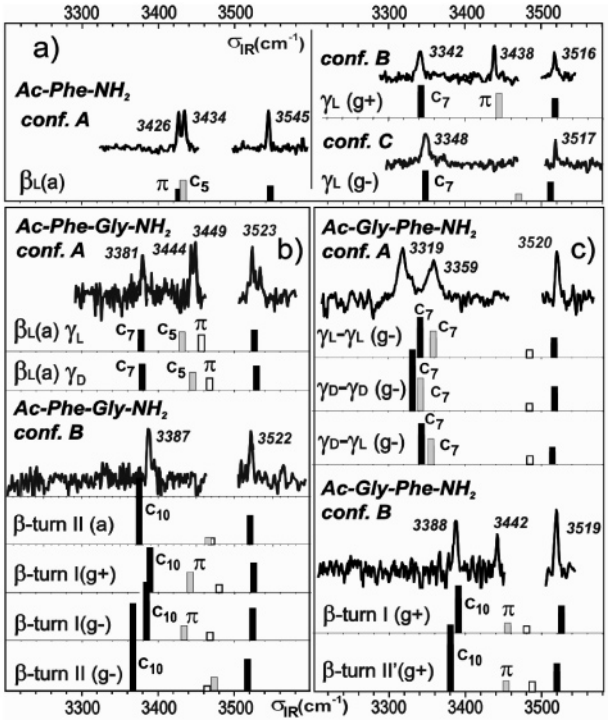

Figure 2. Resonant ion-dip IR spectroscopy (amide NH stretch region) of the conformers of Ac-Phe-NH_2 (a), Ac-Phe-Gly-NH_2 (b), and Ac-Gly-Phe-NH_2 (c) selected from the origin bands identified in the UV spectra of Figure 1. For comparison are also given DFT-calculated stick spectra (Supporting Information Tables S3 and S4) of the most stable conformations (Figures 3 and 4) matching the experimental number of H-bonds (C_7 or C_{10}). Conformations are labeled according to the successive residue conformations (γ_L, γ_D, β_L) along the backbone, or to the corresponding type for β-turns, and using the classical terminology to describe the Phe side-chain orientation (a, g+ or g−, standing for *anti*, *gauche*+, or *gauche*−, respectively). Harmonic frequencies are scaled by a factor of 0.960, adjusted on Ac-Phe-NH_2 data,[21] to account for anharmonicity. The weak coupling between the several NH stretch oscillators enables a local assignment of the vibrational motions indicated by a color code (black, C-terminus NH_2; gray, NH(Phe); white, NH(Gly)). The assignment of the red-shifted bands in terms of type of interaction is given on the stick spectra.

exhibit significantly red-shifted IR bands, which are assigned to intramolecular C_7 H-bonds. These two γ-folded species (γ_L conformations) differ by the orientation of the Phe side chain. The local backbone conformational preferences observed for a Phe residue, β-strand-like β_L and γ-folded, are satisfactorily found as the lowest energy conformers (Figure 2a) in MP2 calculations, which illustrates the ability of this method to account for dispersive and weakly polar interactions, like NH−π contacts.[21,42] Interestingly, gas-phase microwave experiments and ab initio calculations on the protected amino acids glycine[27] and alanine[27,43] show that the conformational preference of both Gly and Ala residues is the γ_L-folded C_7 form alone, which demonstrates, in the case of Phe, the active role of the side-chain/backbone interactions in stabilizing β_L conformations.[42]

The knowledge of the spectral signatures of C_7 H-bonds and of the weaker C_5 and π interactions enables us to propose a qualitative assignment for the protected dipeptides.

(i) For the main conformers A, red shifts in the IR spectra of Ac-Phe-Gly-NH_2 and Ac-Gly-Phe-NH_2 (Figure 2b,c) indicate

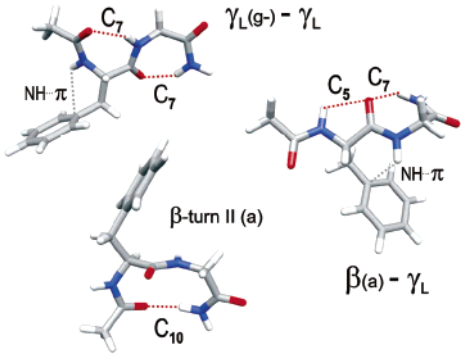

Figure 3. Selected DFT-optimized (B3LYP/6-31+G(d)) conformations of the model peptide Ac-Phe-Gly-NH_2, showing the C_7 and C_{10} H-bonds and the weak (NH−π and C_5) intramolecular interactions.

the presence of one and two H-bonds, respectively, as well as in both cases the involvement of the terminal NH_2 group in these H-bonds. Because C_7 and C_{10} H-bonds cannot occur simultaneously on the same CO acceptor for evident geometrical reasons,[3] only C_7−C_7 structures can account for the doubly bonded Ac-Gly-Phe-NH_2 main form. In the case of Ac-Phe-Gly-NH_2, however, either a C_7 or a C_{10} H-bond involving the NH_2 group can be responsible a priori for the spectrum observed. The striking similarities of the UV spectra of Ac-Phe-NH_2 (A) and Ac-Phe-Gly-NH_2 (A) (Figure 1a,b) nevertheless suggest a very similar backbone environment for the Phe ring in both species, namely, the same β_L(a) conformation. In such a case, because of the extended nature of the β_L conformation, a C_{10} H-bond can be ruled out, suggesting that a C_7 bond is located on the Gly residue. This assignment is corroborated by the IR spectrum of Ac-Phe-Gly-NH_2 (A) (Figure 2b): the presence of red-shifted bands at positions close to those observed in the spectra of the γ_L and β_L conformations of Ac-Phe-NH_2 (B/C and A) are consistent with a weak C_7 bond as well as C_5 and NH−π interactions taking place simultaneously in the molecule.

(ii) Minor conformers B are both characterized by a single H-bond, originating from the terminal NH_2 group as testified by the red shift of the antisymmetric NH_2 stretch band. Moreover, the significantly smaller splitting of the NH_2 doublet, compared to that in γ_L conformers (B and C) of Ac-Phe-NH_2 (Figure 2), suggests a rather weak type of H-bonding for this group, in particular differing from that observed in the main conformers. Owing to the qualitatively different character of the H-bond established by the NH_2 group compared to the case of main conformers, a C_{10}-type assignment, i.e., β-turn-like structures, can be proposed for these forms.

Theoretical Results

The present assignment has been assessed by comparison to theoretical investigations. The energetic landscape of a protected dipeptide is complex, owing to the large number of conformations a priori expected ($3^5 = 243$), on the basis of three minima per C−C or C−N bond and three possible Phe side-chain orientations. The PES explorations, however, allowed us to distinguish two groups of conformations, or basins (Figures 3 and 4; Supporting Information, Tables S1 and S2), able to play a role under jet conditions (providing that all the peptide units are in the *trans* form, as usually observed in peptides and proteins not containing proline residues[44]). The first basin is

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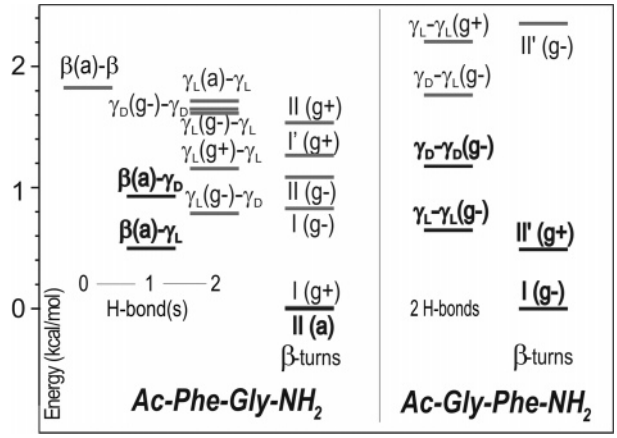

Figure 4. Energetic landscape (MP2/6-31+G(d)//B3LYP/6-31+G(d) level corrected for zero-point vibrational energy) of the protected dipeptides Ac-Phe-Gly-NH_2 (left) and Ac-Gly-Phe-NH_2 (right), showing the relative energies of the two main basins (open forms vs β-turns). Conformations are identified by their local conformation about each residue according to Ramachandran classification; Phe side-chain orientations are given in parentheses. In the first basin, competition between local backbone preferences (γ, β_L) gives rise to both energetically and topologically close structures ($\gamma-\gamma$ vs $\beta_L-\gamma$ or $\gamma-\beta_L$). For both peptides, the β-turn basin is very close in energy to the open form basin. One will notice however that the most stable type of turn changes with the sequence. The lowest calculated forms in agreement with experimental data are indicated in bold.

composed of conformations in which the local preferences (γ_L or β_L) are retained on both residues. These structures exhibit two, one, or no H-bond(s), depending upon the number of γ-folds that are present, and lead to "open" backbone structures, in which both termini do not interact. The second class (β-folds) contains the four types of β-turns, which all exhibit C_{10} H-bonds and are characterized by average H···O distances (at the B3LYP/6-31+G(d) level) of 2.04 and 2.11 Å for Ac-Phe-Gly-NH_2 and Ac-Gly-Phe-NH_2, respectively.

Discussion

The UV and IR data of Ac-Phe-Gly-NH_2 (Figures 1b and 2b) and Ac-Gly-Phe-NH_2 (Figures 1c and 2c) can be accounted for by one of the lowest energy structures found for these species.

Comparison with calculated spectra of the lowest structures of the open basin (Figures 3 and 4) shows that the successive local preferences of the backbone (β_L or γ_L for Phe, $\gamma_{L/D}$ for Gly) are retained in the dipeptide, leading to the competing $\gamma-\gamma$ (C_7-C_7) and $\beta_L-\gamma$ (C_5-C_7) forms. Depending upon the NH$-\pi$ interactions occurring, either one or the other form is finally populated in the jet: $\gamma-\gamma$ for Ac-Gly-Phe-NH_2 and $\beta_L-\gamma$ for Ac-Phe-Gly-NH_2. The latter structure explains the resemblance of the UV spectrum of Ac-Phe-Gly-NH_2 (A) to that of the β_L (C_5) conformer of Ac-Phe-NH_2, due to their similar Phe environments. The repeated γ-turn pattern of Ac-Gly-Phe-NH_2 corresponds to a short segment of the so-called 2_7 ribbon, conjectured by biochemists a long time ago and observed only rarely in proteins as transition structures at the ends of longitudinally compressed β-strands.[45] One will notice that the most stable structure of the open type experimentally observed for both peptides does match the minimum of the corresponding basin at the MP2 level (Figure 4), in contrast to DFT predictions (Tables S1 and S2), which seem to overestimate the β_L stability and underestimate NH$-\pi$ interactions. This finding illustrates the interest of the gas-phase experiments as benchmark data in assessing the quality of high-accuracy energetic calculations on peptide chains.

For the minor conformers B, comparison of IR data with calculations (Figure 2b,c) leads to a fair agreement with the C_{10} H-bond of the most stable β-turns found at the MP2 level. In Ac-Gly-Phe-NH_2, the two lowest calculated β-turns (of types I and II′) do possess the same NH$-\pi$ signature so that no further experimental assignment is possible. For Ac-Phe-Gly-NH_2, however, the two most stable types of β-turns (type I and II), found to be nearly isoenergetic at the MP2 level, exhibit different NH$-\pi$ interactions. This enables us to assign the actual most stable β-turn to type II, and indicates the limitations of the present level of theory for energetic relevance.

The small number of conformers observed in the experiment is consistent with the probably small barrier existing between conformations belonging to the same basin, as illustrated by the competition between the close-lying $\gamma-\gamma$ and $\beta_L-\gamma$ forms, which seems to be controlled by a rather weak NH$-\pi$ interaction. On the other hand, the simultaneous observation of conformers belonging to distinct basins suggests that these basins are probably separated by high-lying barriers that may not be easily crossed over during the desorption/expansion process. As a result, the final basin populations, as estimated by the A/B population ratio, do more probably reflect a free energy ordering pertaining to the early times of the expansion rather than relative stabilities under cold conditions.

Concluding Remarks

The present work on gas-phase peptide models reveals several properties of the peptide chain, from well-resolved details of the conformer-selective IR spectroscopy. Both β- and γ-foldings appear as intrinsic properties of the peptide chain, stemming from the flexible nature of the backbone. This turns out to be a general feature, still observed when Gly is replaced by a more bulky side chain (Ala or Val).[46] The particular role of the side-chain/backbone interactions (namely, NH$-\pi$ interactions) is also emphasized since they are shown to alter backbone preferences by controlling the competition between several nearly isoenergetic minima, such as the topologically close $\gamma-\gamma$ and $\beta_L-\gamma$ structures, or the orientations of the Phe side chain with respect to the backbone. These findings are not accessible in liquid-phase data, owing to the poor resolution achieved in this medium. In addition, the present data illustrate the resemblance between the spectral IR features of β-turns and $\beta_L-\gamma$ structures, which makes problematic their distinction in the liquid phase. In this respect, one should notice that the use of an $-NH_2$ protection on the C-terminus, instead of the more canonical $-N(H)CH_3$ ending, makes unambiguous the assignment of the β-turn C_{10} band thanks to the additional red shift due to the NH_2 vibrational coupling.[21] In the absence of such an effect, the small red shift of the weak C_{10} band might be confused, in solution and the gas phase as well, with weaker interactions, such as the C_5 bond of a β_L conformation for instance.

The β-turn motifs of biology are observed as minor conformers in the gas phase but are common in a series of dipeptides experimentally investigated in our group.[46] Their most striking

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feature is the C_{10} H-bond, whose strength, as revealed by IR red shifts, is rather weak compared to that of the C_7 bond, but independent of the sequence in marked contrast with the γ-folding (Figure 2). The energetic competition between β-turns and doubly γ-folded forms is probably due to a different balance between H-bonding and backbone strains: the relative weakness of the C_{10} H-bond compared to C_7 bonds is compensated for by weaker backbone strains in β-turns compared to γ-folding. Calculations indeed show that, in $\gamma-\gamma$ and $\beta_L-\gamma$ structures, the C−N bond of the central amide is slightly but significantly shortened by typically 0.01 Å, compared to those of the terminal amide groups. No such differences are found in β-turns.

Comparison of gas-phase IR spectra with quantum chemistry calculations indicates that the intrinsically most stable type of β-turn is sequence-dependent. More generally, when averaged over the three possible orientations of the Phe side chain, calculations lead to the following type preferences: type II for Ac-Phe-Gly-NH_2 and type II′ immediately followed by type I for Ac-Gly-Phe-NH_2 (Tables S1 and S2). This result can be compared to the so-called "residue potentials" derived by biochemists from data mining on X-ray diffraction spectra of crystallized proteins.[1,5,47] Indeed, types II and II′, which represent, respectively, 20% and 4% of all the classified turns, have been recognized as nearly exclusively specific of the glycine residue in one of the central positions of the turn, namely, $i + 2$ and $i + 1$ positions, respectively (Scheme 1). Whereas biochemistry potentials illustrate the occurrence frequency of residues in turns in a biochemical environment, the conformational preferences deduced from the present gas-phase studies reflect the *intrinsic* β-turn-inducing power of the Gly residue, free of any environmental effect (neighboring residues, remote parts of the protein, etc.). The correlation observed between gas-phase and protein data suggests therefore that, for the small Gly residue, (i) the preference for types II and II′ is an intrinsic property, already at play in the gas phase, in the absence of an environment, and (ii) this preference is not affected in biological environments: the eventual environmental effects, usually acting through interactions of remote parts of the protein upon the side chain, remain negligible with Gly.

The present UV and IR optical studies of β-turns illustrate the relevance of gas-phase experiments to probe minima on the PES of a small biomolecule in detail. Combined with the most recently developed experimental approaches[17,23] focused on the dynamics on the electronic ground-state surface, such investigations also open up the way to a direct probe of the folding process of a small peptide chain. Finally, the spectral resolution achieved with lasers in gas-phase experiments enables us to envisage applying the same methodologies to biomolecules of larger size, large enough to allow the formation of more complex secondary structures, such as 3_{10}- or α-helices.

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Supporting Information Available: Energetic (B3LYP/6-31+G(d) and MP2/6-31+G(d)//B3LYP/6-31+G(d)) and geometrical parameters of the minimum-energy structures found for Ac-Phe-Gly-NH_2 and Ac-Gly-Phe-NH_2 (Tables S1 and S2) and the corresponding calculated harmonic frequencies and IR intensities for the most stable forms and comparison of the calculated frequencies to experimental frequencies (Tables S3 and S4) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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