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Conformational Analysis

Chirality-Controlled Formation of β-Turn Secondary Structures in Short Peptide Chains: Gas-Phase Experiment versus Quantum Chemistry

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Conformational preferences of short linear peptide chains, in particular the type of β-turn they can adopt, were predicted early on by biochemists to be sensitive to the chirality of the backbone residues.[1–4] Achiral or D-conformation residues are indeed found in natural molecules and used in peptidomimetics, for drug-design purposes in particular, and they result in robust local-chain motifs. However, documenting the competition between the several conformers in solution turns out to be a difficult task, because data are often blurred by spectral overlap and solvent or temperature effects.[4–6] In fact, the conformational preferences of small peptides based on simple residues such as alanine (Ala) are essentially known through quantum chemical calculations, without much experimental counterpart.[7–10] The aim of the gas-phase approach, which couples supersonic expansion with optical laser techniques,[11,12] is to provide such experimental data. The intrinsic conformational preferences of capped dipeptides, the minimal peptide structure capable of forming β-turns, are reported here with emphasis on the role of the chirality of backbone residues in these preferences.

A supersonic expansion provides an original approach to investigate flexible molecules which exhibit complex conformational landscapes.[11–18] Coupled with a desorption setup[19] that vaporizes the molecules without damage, this technique starts from hot disordered molecules and cools them down through the numerous collisions in the expansion. Highly efficient conformational relaxation quenches the high-temperature populations into the potential wells of the most stable forms.[11–15,18] The several conformers finally observed are therefore low-energy structures separated by high barriers which are not easily crossed during the cooling process. In some respects the expansion performs the experimental equivalent of the theoretical search for minima on the potential-energy surface (PES). In addition, the cold isolated conformers observed this way are directly comparable to quantum chemistry calculations, and this enables precise assessment of the theoretical methods.[20]

The use of laser spectroscopy, including IR/UV double-resonance spectroscopy,[11] provides cross-checking of IR and UV information and enables the observed conformational families to be sorted according to their intramolecular interactions, in particular H-bonding. Biomolecules that have been recently investigated by using this technique include DNA bases, sugars, amino acids, and short peptides.[11–18] The formation of secondary structures in gas-phase peptide chains was addressed recently,[16–18] and evidence for β-turns in short capped peptides was reported.[18,21–24] The effect of backbone chirality was also investigated on natural peptides[25] but, owing to the uncapped nature of these species, the formation of turns could not be documented.

The present work is focused on capped dipeptides capable of mimicking a fragment of a peptide chain. The molecules chosen contain an aromatic residue (phenylalanine, L-Phe), which provides the UV chromophore necessary for IR/UV double resonance. The chiral L-Phe was associated with the simplest residues capable of testing backbone-chirality effects, namely, alanine, in either of its enantiomeric forms L- and D-Ala (to form homo- and heterochiral backbones) and its achiral α-methylated derivative, that is, α-aminoisobutyric acid (Aib), in order to form *N*-Ac-Xxx-L-Phe-NH_2 (**1a** and **1b**: Xxx = L- and D-Ala, respectively; **2**: Xxx = Aib).

The near-UV spectra of the three molecules, obtained by using the resonant two-photon ionization technique, are shown in Figure 1. The rovibrational cooling achieved in the expansion narrows the spectral features, and this enables us to resolve the individual contributions of the observed conformers, including the vibrational structure of their electronic transition, governed by the Franck–Condon (FC) principle. The IR/UV double-resonance spectra, recorded by probing IR absorption with the bands of the UV spectrum, then enable the conformers to be distinguished from their IR spectra (Figure 2). The UV spectrum of **1a**, already discussed in a former study,[22] was obtained here with better quality. A single conformer (A) is responsible for the majority of the spectral features observed.[22] The intense excitation achieved by the IR light allowed us to record "IR-purified" UV spectra, in which the contribution of the main conformer is selectively removed to reveal spectral features of minor forms (inset in the upper panel of Figure 1). This procedure provides clear evidence for the existence of a second, less populated conformer B, characterized by a single UV band. The IR spectra in the NH stretching region of these two conformers, obtained by double-resonance spectroscopy (Figure 2, top left) exhibit respectively two and one band(s) at frequencies lower than 3400 cm^{-1}, that is, red-shifted compared to the region usually assigned to free or weakly interacting N–H bonds.[18] Such a red shift clearly bears the signature of H-

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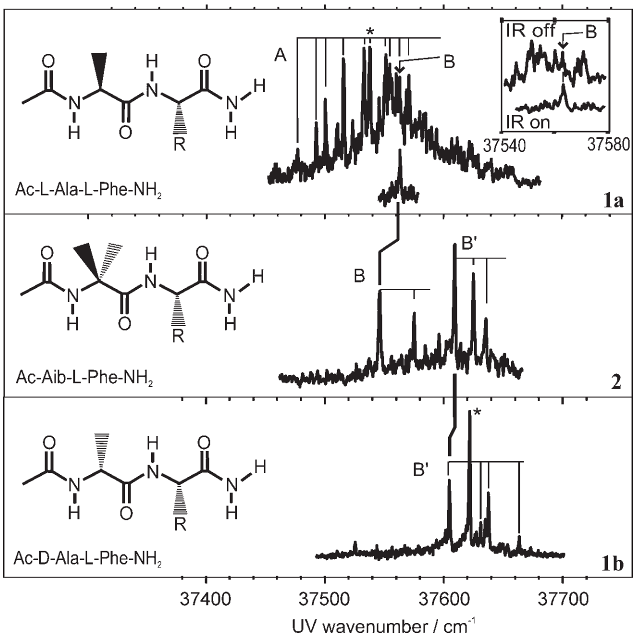

Figure 1. Near-UV spectrum of jet-cooled Ala-Phe (**1a** and **1b**) and Aib-Phe (**2**) capped dipeptides obtained by mass-selected resonant two-photon ionization. Conformers are labeled according to their structure: extended forms for A, and β-turns for B and B′ (see text). Progressions were identified by checking the IR absorptions by IR/UV double resonance. For **1a**, the band of the minor conformer B (inset in upper panel) was isolated by recording an "IR-purified" UV spectrum (see text), in which the population of the cold A species was removed by selective IR excitation prior to UV absorption. The intense IR transition used (at 3309 cm^{-1}) corresponds to excitation of the lowest frequency C$_7$ H-bond of the A conformer (Figure 2).

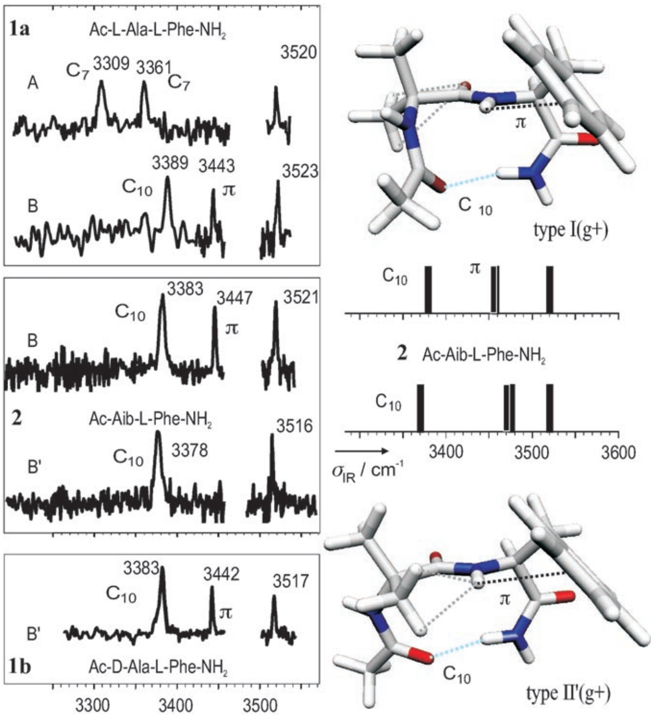

Figure 2. Left: Double-resonance IR/UV spectra (amide NH stretching region) of conformers A, B, and B′ of **1a**, **1b**, and **2** obtained by pumping either the origin band or the most intense bands, labeled by asterisks in Figure 1. Right: DFT-optimized [B3LYP/6-31+G(d)] conformations of the most stable type I and type II′ β-turn forms of **2**, together with the corresponding calculated harmonic frequencies, scaled by a factor of 0.960 to account for anharmonicity.[18] The label g+ refers to the *gauche*+ orientation of the Phe side chain. The interactions playing a role in the conformational stability are indicated by colored dots (blue: H-bond, black: NH–π interaction, gray: close contacts. The 3460–3500-cm^{-1} region, not covered owing to absorption in the LiNbO$_3$ crystal of the OPO, corresponds to free N–H bonds and does not carry significant information about H-bonding.

bonds: the A form is assigned to a conformation with two successive C$_7$ bonds along the backbone, reminiscent of the 2$_7$ ribbon secondary structure conjectured by biochemists.[22] Conformer B, however, exhibits a unique H-bonding band. The similarity with those observed along a series of capped dipeptides previously studied[22] provides evidence for the formation of a folded β-turn structure stabilized by a C$_{10}$ H-bond linking the two ends of the molecule. In addition, the presence of a slightly red shifted band (3443 cm^{-1}) indicates stabilizing π interaction between the central amide group and the phenyl ring of Phe.

The most interesting result of the present study stems from the difference between the UV spectra of **1b** and **2**, as compared to **1a**. The IR/UV double-resonance spectra recorded at the UV bands show that only two conformers, B and B′, are present in **2**, whereas **1b** exhibits a unique form (B′). The IR spectra of **1b** and **2** (Figure 2) show a striking resemblance to that of conformer B of **1a**; in particular, they feature the same H-bonding band, characteristic of a C$_{10}$ bond (the absence of the π-interaction feature in the spectrum of **2**(B′) is discussed below).

This unambiguous spectral signature enables us to assign all these conformers to β-turns. At this stage, the UV spectral shifts provide an additional clue to the structure. Figure 1 shows that the B and B′ UV bands of **1a** and **1b**, respectively, are spectrally very close to the corresponding bands in **2**. Also, the **2**(B′) and **1b**(B′) forms exhibit similarities in their FC patterns, in particular with two low-frequency modes of about 16 and about 26 cm^{-1}. These spectral shifts and FC activities suggest the same type of environment for the Phe UV chromophore within each family (B or B′), but different types from one family to another. One can therefore draw the following conclusions:

- The chain **2**, with its achiral first residue (Aib), exhibits two β-turn conformers of similar stability.
- Only one of these forms remains when the first residue is chiral: B in **1a**, as a secondary conformer, less stable than a prominent C$_7$–C$_7$ form (A), and B′ in **1b** as the major observed conformer.

These qualitative assignments compare nicely to the results of quantum chemical calculations. For **2**, type I and II′ β-turns with a *gauche*+ orientation of the Phe side chain are found to be the most stable forms among all possible I, I′, II, and II′ types of turns (Supporting Information). They exhibit very comparable stabilities (Figure 3) and are much more stable than extended C$_7$–C$_7$ structures (found to be 2.6 kcal mol^{-1} higher). In contrast, the corresponding type I

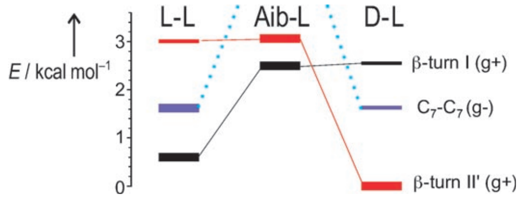

Figure 3. Energy diagram of type I and II′ (g+) turns and C$_7$–C$_7$ forms of **1a**, **1b**, and **2** at the LMP2/6-31+G(d)//B3LYP/6-31+G(d) level of theory (corrected for zero-point energy at the DFT level; see Supporting Information). The scale for **2** (Aib–L) has been arbitrarily taken so that the turn forms coincide with the sterically hindered turns of **1a**,**b**. Structures observed experimentally are indicated by thick bars.

and II′ β-turns of **1a** and **1b** exhibit dramatically different stabilities: in the L–L backbone (**1a**), type I becomes the most stable β-turn form but is challenged by a C$_7$–C$_7$ conformation (only 1 kcal mol^{-1} higher), whereas the D–L backbone (**1b**) predominantly accommodates a type II′ turn (Figure 3). The striking similarity of this energetic pattern with the gas-phase experimental abundances allows us to assign unambiguously the B form observed with the L–L backbone to a type I turn, whereas the B′ feature, favored with the D–L backbone, is a type II′ form.

Careful analysis of the backbone dihedral angles (see Supporting Information) confirm the origin of the relative stabilities of the several forms: steric hindrances lead to close contacts between one of the Me groups of the first residue and either the NH or the CO moiety of the central amide bond, and this eventually results in chirality-selective distortion of the backbone. With Aib (**2**), turn types I and II′ are both destabilized by steric hindrance generated by its two Me groups on the α-C atom. Since the alternative extended forms, based on C$_7$–C$_7$ bonds, are also highly destabilized because of similar steric effects occurring in the C$_7$ conformation of Aib, the turn structures are then the most stable forms. When one of the Me groups is removed to give either L- or D-Ala, one of the steric strains is removed, so that only one type of β-turn becomes prominent in the corresponding backbone and eventually competes with C$_7$-C$_7$ extended forms (case of **1a**).

The good agreement between the experimental and theoretical stability patterns provides an assessment of the theoretical data and suggests that the precision in energies is on the order of 1 kcal mol^{-1} at this level of theory; the largest discrepancy is indeed observed for the experimentally prominent C$_7$–C$_7$ form of **1a**, whose energy is found to be 1 kcal mol^{-1} above that of the β-turn form (Figure 3).

The calculated harmonic vibrations of these species (Figure 2) are in fair agreement with the IR spectra. In particular, the absence of a red shift for the NH group of the central amide bond in **2**(B′) (type II′) in spite of a geometry favorable to a π interaction, is well reproduced by calculation. Comparison with other backbones (Supporting Information) shows that this effect, also present with L–L but absent with D–L, should be ascribed to the presence of close contacts between this NH bond and the nearby Me group in type II′ turns (Figure 2, right).

The present experiment provides a clear illustration for the origin of the chirality-controlled conformational preference of short peptide chains. Heterochiral dipeptides are confirmed to be the most selective promoters of turns (of type II′ in the present D–L backbone and therefore of type II in the L–D mirror image). The Aib residue also favors turn structures but does not induce much selectivity in terms of type. Finally in the homochiral dipeptide the competition between turns and extended open forms is much more important, and it reflects the structural flexibility required by functional biological systems.

The present case study illustrates the capabilities of the laser-desorption gas-phase spectroscopic approach. Working with small samples (a few milligrams), it enables the most stable structures within a complex conformational landscape to be isolated and characterized experimentally with precision, provided that the molecule bears a UV chromophore. Apart from the spectroscopic congestion, no severe limitation in terms of maximum size is expected, as testified by experiments on larger species.[15,16,24,26,27] In the present case, the observation of a system with two major conformers (**2**) opens up a route towards precise experimental measurement of the energy barrier between two turn conformations, by using a recently developed pump–probe technique,[28] which will be a qualitative step forward to further characterization of the PES of these systems.

Experimental Section

The experimental setup for gas-phase preparation of the peptides and their spectral analysis was described previously.[19,22] An Fmoc-synthesized peptide (Altergen Co.) powder is mixed with graphite powder and compressed into a pellet, which is then placed downstream of a pulsed valve. Peptides are desorbed from the pellet by the second-harmonic light of a Nd$^+$:YAG laser and entrained by the pulsed supersonic expansion. The UV spectra of the expansion-cooled molecules are recorded by scanning a frequency-doubled pulsed dye laser. The corresponding R2PI signal is then mass-selected by using a time-of-flight mass spectrometer. IR/UV double-resonance spectroscopy is carried out by scanning the IR idler output of a Nd$^+$:YAG-pumped LiNbO$_3$ optical parametric oscillator (OPO) system (Euroscan Co.).

Quantum chemical calculations were performed by the pseudo-spectral method with the Jaguar program package.[29] First, typical β-turn[1] and C$_7$–C$_7$ conformations[18] were fully optimized at the B3LYP/6-31+G(d) level. Second, for each configuration, harmonic vibrational frequencies were calculated at the same level of theory. Finally, single-point refined energy calculations were performed at the LMP2/6-31+G(d) level.

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