Chirality-controlled formation of β-turn secondary structures in short peptide chains: gas phase experiment vs. quantum chemistry

Valérie Brenner, François Piuzzi, Iliana Dimicoli, Benjamin Tardivel, and Michel Mons

Laboratoire Francis Perrin, URA CEA-CNRS 2453, Service des Photons, Atomes et Molécules, CEA Saclay, bât 522, Gif-sur-Yvette Cedex, France

michel.mons@cea.fr

Abstract

Supersonic expansion coupled to laser desorption of a solid sample enables one to isolate and cool down very efficiently flexible molecules in the gas phase. State-of-the-art optical laser techniques, in particular double resonance IR/UV spectroscopy, are then used to provide structural information about the conformers populated. The present work demonstrates the spontaneous formation of secondary structures, namely β-turns, in capped dipeptides mimicking a short protein fragment and provides insight on the role of backbone chirality on the type of turns formed. In some respects, expansion performs an experimental equivalent of the theoretical exploration of the conformational landscape of these flexible molecules. Comparison between experimentally observed conformers and lowest energy structures theoretically calculated therefore enables a precise assessment of the quantum chemistry methods used.

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Conformational preferences of short linear peptide chains, in particular the type of β-turn they can adopt, have been predicted very early 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 or used in peptidomimetics, for drug design purposes in particular, resulting 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] As a matter of fact, the conformational preferences of small peptides based on simple residues like alanine (Ala), are essentially known through quantum chemistry calculations, without much experimental counterpart.[7-10] The aim of the gas phase approach, which couples a supersonic expansion together with state-of-the-art 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 with the emphasis put on the role of the chirality of backbone residues on these preferences.

A supersonic expansion provides an original approach to investigate flexible molecules, which exhibit complex conformational landscapes.[11-18] Coupled with a desorption set-up[19] in order to vaporise the molecules without damage, this technique enables to start from hot disordered molecules and to cool them down thanks to the numerous collisions in the expansion. A highly efficient conformational relaxation enables to quench the high temperature populations into the potential wells of the most stable forms.[11-15, 18] The several conformers observed *in fine* are therefore low energy structures separated by high barriers which are not easily crossed during the cooling process. In some respects the expansion performs an 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 the quantum chemistry calculations, enabling a precise assessment of the theoretical methods used. [20]

The use of laser spectroscopy, including IR/UV double-resonance spectroscopy,[11] provides a cross-checking of IR and UV information and enables the spectroscopists to sort out the conformational families observed according to their intramolecular interactions, in particular H-bonding. Several types of biomolecules have been recently investigated using this technique, including DNA bases, sugars, amino-acids or short peptides, etc... [11-18] The issue of the formation of secondary structures in gas phase peptide chains has been addressed recently,[16-18] and evidence for the observation of β-turns in short capped peptides was reported.[18, 21-24] The effect of backbone chirality was also investigated on natural peptides,[25] but the uncapped character of these species did not allow documenting the formation of turns.

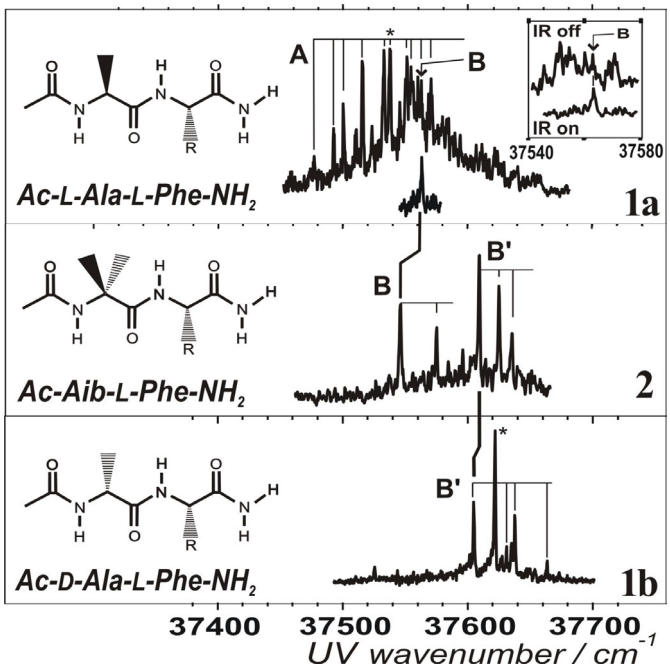

Figure 1. Near UV spectrum of jet-cooled Ala-Phe (**1a** and **b**) and Aib-Phe (**2**) capped dipeptides obtained by mass-selected resonant two-photon ionization. Conformers are labelled according to their structure : extended forms for A, β-turns for B and B' (see text). Progressions have been identified 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 carrying out 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 the excitation of red most C_7 H bond of the A conformer (Fig. 2).

The present work is focussed 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 the IR/UV double resonance. The chiral L-Phe was associated with the simplest type of residues capable of testing backbone chirality effects, namely alanine, in either of its enantiomeric forms, L- or D-Ala (to form homo- and hetero-chiral backbones) and its achiral α-methylated derivative, i.e., the α-aminoisobutyric acid (Aib), in order to form: *N*-Acetyl-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 using the resonant two-photon ionization techniques are shown in Figure 1. The rovibrational cooling achieved in the expansion narrows the

spectral features, which 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. IR/UV double resonance spectra, performed by probing IR absorption using the bands of the UV spectrum, then enable to distinguish the conformers from their IR spectrum (Figure 2). The UV spectrum of **1a**, already discussed in a former study,[22] is obtained here with a better quality. A single conformer (labelled A) is responsible for the majority of the spectral features observed.[22]In the present work, the intense excitation achieved by the IR light allows us to carry out "IR-purified" UV spectra, in

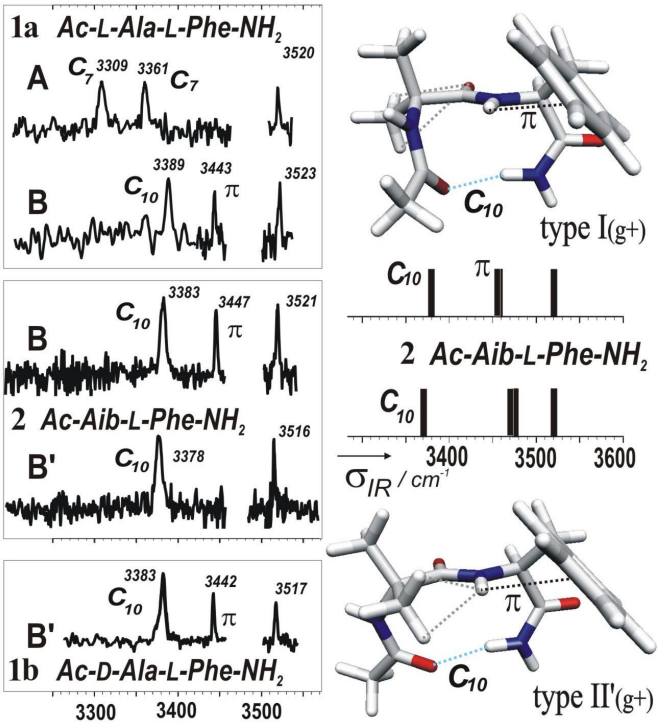

Figure 2. *Left panel :* Double resonance IR/UV spectrum (amide NH stretch region) of conformers A, B and B' of **1a**, **1b** and **2** obtained by pumping either the origin band or the most intense bands labelled by asterisks in Fig. 1. *Right panel:* DFT-optimized (B3LYP/6-31+G(d)) conformations of 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 conformation stability are indicated by color dots : *blue*: H-bond, *black* : NH - π interaction, *grey*: close contacts. The 3460-3500 cm^{-1} region, not covered due to absorption in the $LiNbO_3$ crystal of the OPO, corresponds to free NH bonds and does not carry significant information about H-bonding.

which the contribution of the main conformer can be selectively removed, revealing spectral features of minor forms (insert in upper panel of Fig. 1). This procedure provides clear evidence for the existence of a second, less populated, conformer B, characterised by a single UV band. The NH stretch IR spectra of these two conformers obtained by double resonance spectroscopy (Fig. 2, left panel, top traces) exhibit respectively two and one band(s) appearing at frequencies shorter than 3400 cm^{-1}, i.e., red-shifted compared to the region usually assigned to free or weakly interacting NH bonds.[18] Such a red-shift clearly bears the signature of H-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 stabilised 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 a 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**. IR/UV double resonance spectra carried out on the UV bands show that only two conformers, labelled B and B', are present in species **2**, whereas **1b** exhibits a unique form populated (labelled B'). The IR spectra of **1b** and **2** (Fig. 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')** will be 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. Fig. 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 present similarities in their FC patterns, in particular with two low frequency modes of ~16 and ~26 cm^{-1}. These two points, spectral shifts and FC activities, suggest a same type of environment for the Phe UV chromophore within each family (B or B'), but differing from one family to the other. One can therefore draw the following conclusions:

- The chain **2**, with its achiral first residue (Aib), exhibits two β-turn conformers of similar stabilities.
- Only one of these forms remains present when the first residue is chiral : B in **1a,** as a secondary conformer, less stable than a prominent C_7-C_7 form, and B' in **1b** as the major conformer observed.

These qualitative assignments compare nicely to the results of quantum chemistry 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' type of turns (Supporting Information). They exhibit very comparable stabilities (Fig. 3) and are much more stable than extended C_7-C_7 structures (found to be 2.6

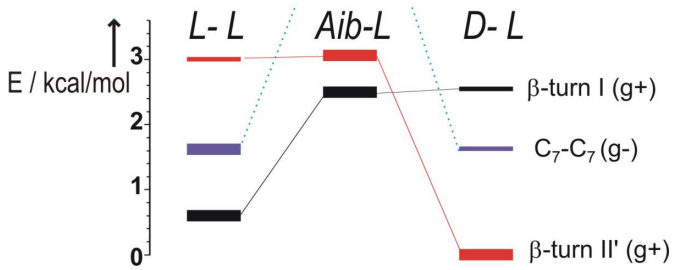

Figure 3. Energy diagram of type I and II' (g+) turns and C_7-C_7 forms of **1a**, **1b** and **2**, at the LMP2/6-31+G(d)//B3LYP/6-31+G(d) level of theory (corrected for zero-point energy at the DFT level; see Supp. Info.). The scale for **2** (Aib-L) has been arbitrarily taken so that the turn forms coincide with the sterically hindered turns of **1a, b**. Structures observed experimentally are indicated by thick bars.

kcal/mol higher). In contrast, the corresponding type I 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 higher), whereas the D-L backbone (**1b**) predominantly accommodates a type II' turn (Fig. 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, favoured with the D-L backbone, is a type II' form.

A 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, eventually resulting in a chirality-selective distortion of the backbone. With Aib (**2**), both turn types I and II' are destabilized by steric hindrances generated by its two Me groups on the α-C. The alternative extended forms, based on C_7-C_7 bonds, being also highly destabilised because of similar steric effects occurring from the C_7 conformation of Aib, the turn structures are then the most stable forms. When removing one of the Me groups, leading to either L- or D-Ala, one of these strains is lifted so that only one type of β-turn becomes prominent in the corresponding backbone, eventually competing 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 on energies is of the order of 1 kcal/mol 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 above that of the β-turn form (Fig. 3).

The calculated harmonic vibrations of these species (Fig. 2) are in fair agreement with the IR spectra. In particular, the absence of a red shift for the NH of the central amide bond in **2** B' (type II') in spite of a geometry favourable to a π interaction, is well reproduced by calculation. Comparison with other backbones (Supplementary 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 close-by Me group in type II' turns (Fig. 2, right panel).

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 favours 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, reflecting the structural flexibility required by functional biological systems.

The present case study illustrates the interest of a laser desorption gas phase spectroscopic approach. Working with small samples (a few mg), it enables to isolate and characterize experimentally with precision the most stable structures within a complex conformational landscape, providing 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 an experimental precise measurement of the energy barrier between two turn conformations, using a recently developed pump-probe technique,[28] which will be a qualitative step forward to a further characterisation of the PES of these systems.

Methodology Section

The experimental set-up for the gas phase preparation of the peptides and their spectral analysis was described previously.[19, 22] The powder of the Fmoc-synthesised peptides (Altergen Co.) is mixed with a graphite powder, compressed in a pellet, which is then placed downstream a pulsed valve. Peptides are desorbed from the pellet by the 2nd 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 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 $LiNbO_3$ OPO system (Euroscan Co.).

Quantum chemistry calculations were performed using the pseudospectral 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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