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Vibrational spectroscopy of a non-aromatic amino acid-based model peptide: identification of the γ-turn motif of the peptide backbone

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The first infrared hole burning spectrum of a gas-phase neutral peptide not containing an aromatic amino acid is presented. In the model peptide Z–Pro–NHMe, the amide I and II bands in the 1500–1800 cm^{-1} region appear to be a clear diagnostic for the secondary structure of the backbone, while the analysis of a series of coupled CH bending modes in the 1000–1500 cm^{-1} region allows to distinguish between different possible orientations of the chromophore. The geometry of the peptide is strongly constrained by the proline and only one conformation of the backbone is observed, which is identified as a hydrogen bonded γ-turn.

Reverse turns are small motifs in the secondary structure of proteins, consisting of short sequences of amino acids that allow the peptide chain to reverse its overall direction. β-turns are the most common type of turns observed in native proteins. They are formed by four amino acids, the CO of the first peptide bond being hydrogen bonded to the NH of the last one. γ-turns and α-turns have similar H-bonded arrangements, but are composed of three and five amino acids, respectively. Short capped peptides have been widely used as models to study the stability of these structures in solution by infrared absorption and circular dichroism as well as by X-ray diffraction in crystals.[1] From these studies it appears that the amino acid proline strongly constrains the folding of the peptide backbone, leading to γ-turn or β-turn motifs.

Infrared/ultraviolet (IR/UV) double resonance techniques[2–4] have been applied to amino acids[5,6] and small peptides [7–10] in the gas phase to obtain IR spectroscopic information in the hydrogen stretching region and more recently also in the amide I (C=O stretch) region[11] as well as deeper in the IR.[12] The great advantage of these techniques is that they are species (*i.e.* mass) *and* conformer selective, but they were until now limited to peptides containing a UV chromophore in the form of an aromatic amino acid, namely tryptophane, phenylalanine or tyrosine. Infrared spectra of gas-phase ionic non-aromatic peptides have also been measured recently,[13,14] however, these spectra are not conformer selective.

In order to overcome this severe restriction, a model peptide including a chromophore in its cap is designed (see Fig. 1A). A similar approach has been used recently for sugars.[15–17] The model peptide used here is composed of the single amino acid proline; its C-terminus is protected with a methylamide (NHMe) function and its N-terminus is protected with a benzyloxycarbonyl (commonly referred to as Z cap). In this peptide, the only remarkable structure with an internal H-bond is a γ-turn. In addition, the conformational space is restricted by the proline ring: L-proline exclusively forms inverse γ-turns, while D-proline forms classic γ-turns, as shown in Fig. 1B. Similar γ-turn structures have been identified recently in the gas phase for phenylalanine-based peptides.[9,10]

Z–Pro–NHMe is synthesized from L-proline, and then purified and crystallized[18] (all compounds are purchased from Sigma-Aldrich). The sample is mixed with graphite powder, rubbed onto a graphite surface and laser-desorbed directly under the orifice of a pulsed valve. The IR absorption spectrum is measured using a pulsed molecular beam machine coupled to a UV/IR ion dip setup.[12] Briefly, the Z–Pro–NHMe molecules are internally cooled in the supersonic expansion of the carrier gas (argon at a backing pressure of 3.5 bar), after which the

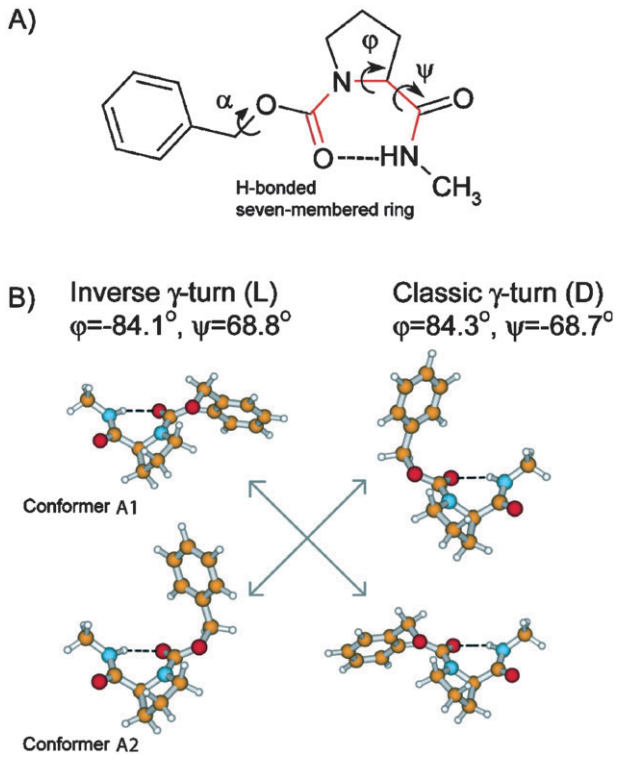

Fig. 1 (A) The only internally H-bonded structure expected in Z–Pro–NHMe is a seven-membered ring (also called γ-turn), in which the CO of the first peptide bond is bridged to the NH of the second one. (B) The geometric structures of the two lowest energy conformations calculated for a L-proline based peptide (left) are inverse γ-turns. The corresponding classic γ-turns (containing D-proline) are presented for completeness on the right. The arrows indicate the pairs of enantiomers.

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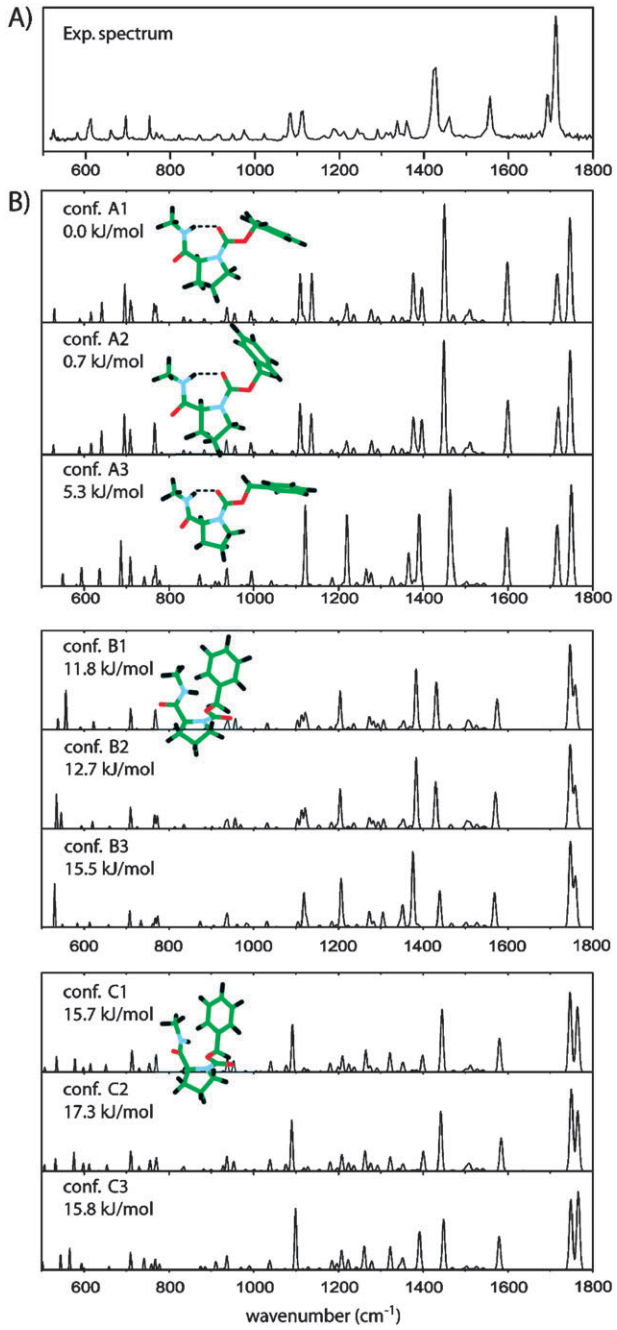

Fig. 2 (A) Experimental IR absorption spectrum of Z–Pro–NHMe. (B) Calculated IR absorption spectra, convoluted with the spectral profile of FELIX, for the nine lowest energy conformers found. The relative energies are given for each conformer. Note: A, B and C indicate the conformation of the backbone, while 1, 2 and 3 indicate the different orientations of the Z-cap for each conformation.

beam is skimmed and enters the extraction region of a linear time-of-flight mass spectrometer. A UV beam (frequency doubled output of a dye laser pumped by the third harmonic of a Nd:YAG laser) and a tunable IR laser beam produced by the free electron laser for infrared experiments (FELIX)[19] cross the molecular beam perpendicularly and are mutually counter propagating. The molecules are two-photon resonantly ionized (R2PI) *via* the S_1 state, thereby selecting one specific conformation. IR absorption prior to UV excitation induces a depopulation of the ground state, which is detected as a dip in the ion current on the mass/charge channel corresponding to singly charged Z–Pro–NHMe.

The R2PI spectrum of the molecule recorded in the 37 500–37 660 cm^{-1} range shows several different absorption features. IR absorption spectra are taken with the UV laser on these different bands. These IR spectra are identical, indicating that only one unique conformer is detected in the beam. The IR spectrum presented in Fig. 2A) is obtained with the UV laser tuned to the main resonance of the R2PI spectrum around 37 550 cm^{-1}. The IR spectrum consists of over 30 distinct resonances, with the most intense ones in the 1400–1800 cm^{-1} range. Two amide I (C═O stretching) bands due to the two C═O groups appear at 1712 cm^{-1} and 1693 cm^{-1} and one amide II (NH bending) band due to the NH group appears at 1554 cm^{-1}. These amide bands are sensitive to the hydrogen bonding and can serve as a probe of the secondary structure.[20]

The potential energy surface of the molecule is explored using the Amber 99 force field by randomly scanning all the dihedral angles. The large set of structures obtained is then reduced by re-optimizing the geometries using the AM1 semi-empirical method. The highest energy structures are rejected since they lead to the very unlikely *cis* configuration of the second peptide bond. For the first peptide bond, both *trans* and *cis* isomers were considered due to the presence of the proline.[21] These structures are re-optimized and the vibrational frequencies are calculated by density functional theory (DFT) at the B3LYP/6-31+G(d) level using Gaussian03.[22] Of the nine conformers so obtained, the three most stable (labeled A1, A2 and A3) are H-bonded γ-turns, while the six least stable (labeled B1, B2, B3 and C1, C2, C3) are non H-bonded with the first peptide bond being in the *cis* configuration.

Unscaled calculated vibrational spectra, convoluted with a Gaussian line profile (full width at half maximum = 0.3% of the bandcenter, corresponding to the experimental bandwidth of FELIX), are presented for each conformer in Fig. 2B, and can be compared to the experimental spectrum. Conformations B and C can be rejected since the positions and the relative intensities of the amide I and II bands do not match the experiment. On the other hand, a very good agreement is observed in this region for the conformers A1, A2 and A3. These three structures exhibit the same γ-turn motif but differ by the orientation of the benzene ring. For conformer A3, the dihedral angle α (see Fig. 1A) is 0°, whereas it is −93° and +90° for conformers A1 and A2, respectively.

The bands appearing between 1000 cm^{-1} and 1500 cm^{-1} are mainly due to CH bending modes, most of which are strongly delocalized over the proline ring and the Z-cap. Comparison with the experimental spectrum in this range allows us to reject conformer A3. The experimental spectrum also shows three intense bands in the far IR region at 610, 695 and 749 cm^{-1}, which are less well reproduced in the calculated spectra. A possible reason is that in this region the NH out-of-plane bending modes are present, which can be strongly anharmonic and which will therefore be poorly reproduced by the harmonic calculations.[12] Nonetheless, based on the overall agreement the observed experimental spectrum can clearly be assigned to a γ-turn motif: either one of the two lowest energy conformers A1 and A2, or a mixture of these two.

In order to evaluate the influence of the aromatic chromophore on the structure of the backbone as well as on the IR spectra, the model molecule Ac–Pro–NHMe (*N*-acetyl capping group) is investigated theoretically, at the same level of theory as Z–Pro–NHMe. The same three backbone configurations calculated for Z–Pro–NHMe are found to be stable structures for Ac–Pro–NHMe as well. The ordering in energy is the same and the relative energies are quite similar (see Fig. 3). The H-bonded γ-turn, corresponding to the conformation A of Z–Pro–NHMe, is found to be the most stable structure, while the two non H-bonded structures corresponding to the conformations B and C are higher in energy. In the case of structures A and B, the backbone structure is almost the same for Z–Pro–NHMe and Ac–Pro–NHMe. Small differences exist for the highest energy conformation C: the orientation of the

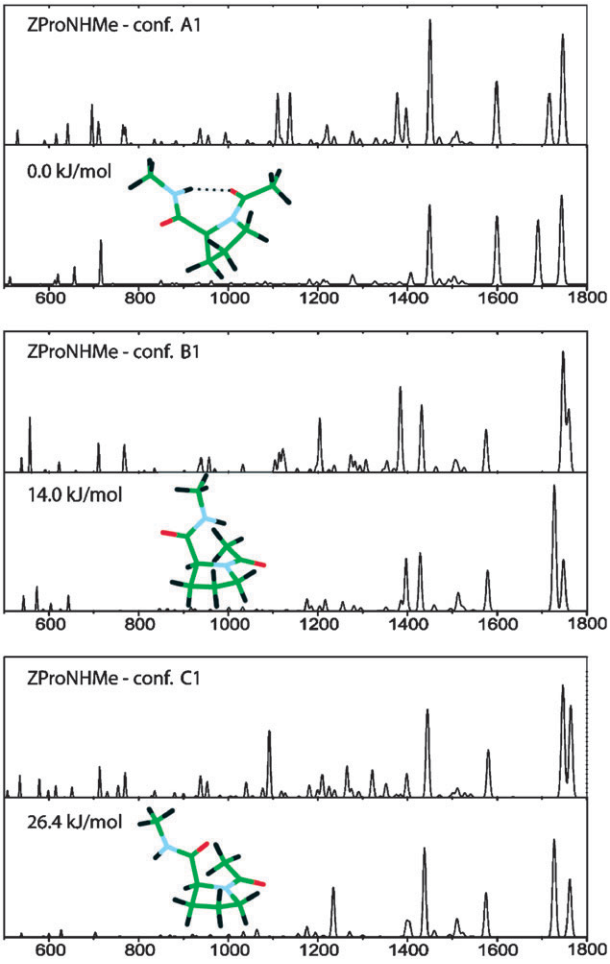

Fig. 3 The calculated spectrum of the model molecule Ac–Pro–NHMe (lower part of the panels) is compared to the spectrum of Z–Pro–NHMe (upper part of the panels) having the same backbone conformation.

NHMe group is influenced by an interaction between the NH group and the ester oxygen atom of the Z cap.

The calculated spectra of Ac–Pro–NHMe are displayed in Fig. 3 and compared to the corresponding spectra of Z–Pro–NHMe. For all three conformers, the spectra in the amide region look very similar for the two molecules. The NH bending mode is found near 1600 cm^{-1} and its position is not influenced by the choice of the capping group. For both capping groups, this peak is observed to be slightly blue shifted when the NH group is involved in an H-bond (conformation A). Similarly, in both molecules, the position of the C═O stretching mode is slightly red shifted when that group is engaged in an H-bond. The position of one of the two C═O stretching bands is observed to be slightly influenced by the cap. When the Z-cap is present, it can be seen that the position of the peak resulting from the first C═O group is systematically blue shifted by 10 to 20 cm^{-1}, compared to the Ac cap. This difference is due to the presence of the ester oxygen atom as a direct neighbour. To the red of the amide region, the spectra of Z and Ac terminated Pro–NHMe differ sometimes substantially and a direct comparison of the spectra is no longer useful. This leads to the conclusion that the Z-chromophore affects the backbone structure of the peptide and the relative energies of the different conformers only slightly. The spectra in the amide (C═O stretch and NH bend) region are also only weakly perturbed and the Z-cap can, for this study, be considered as a spectator.

The design of this model peptide extends the UV and IR spectroscopy of gas-phase peptides to any sequence of amino acids, since the chromophore is included in the protecting function. The data presented here constitute the first IR hole burning spectrum of a gas phase peptide without an aromatic amino acid. The high propensity of proline to form turns[1] is confirmed for proline in the gas phase, where interactions with the environment are absent. The amide I and II bands in the 1500–1800 cm^{-1} region appear to be a clear diagnostic for the conformation of the backbone, although on this basis one would not be able to distinguish a classic turn from an inverse turn if both were present in the beam. The spectrum in the 1000–1500 cm^{-1} region is rather sensitive to subtle differences in the structure, and allows to distinguish between possible orientations of the benzene ring. The bands observed between 500 and 800 cm^{-1} are less well reproduced by the calculations used thus far.

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