THEORETICAL AND MATRIX-ISOLATION STUDY ON SMALL ALPHA AND BETA PEPTIDES

Doctoral (Ph. D.) thesis statements

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Abstract

In order to study the functions of enzymes or the possibilities of the artificial synthesis of certain proteins (e.g. silk) it is important to study their structural properties. Glicine and alanine are the smallest achiral and chiral building blocks of proteins, while the unique structure of proline makes it a helix or β-sheet breaker structural element.

Two major conformer, γ_L and β_L, were identified in the matrixisolated IR spectra of Ac-Gly-NHMe and Ac-L-Ala-NHMe. In the case of the glicine derivative, a third minor conformer, δ_{DL} was also found. Experimental and computed spectra were in good correlation.

In the experimental and computed MI-VCD spectra of Ac-L-Ala-NHMe one of the peaks of the β_L conformer had opposite sign. Theoretical calculations have shown, that the rotatory strengths of the symmetric and antisymmetric vibrational modes of the β_L conformer are very sensitive to small variations in backbone geometry. This phenomenon has to be taken into the account during the analysis of VCD spectra of α-aminoacids.

One major, tγ_L+ and one minor, cα_L+ conformer was identified in the MI-IR and MI-VCD spectra of Ac-L-Pro-NH_2. The predicted tγ_L- conformer is converted to tγ_L+ during the decomposition to the cold window. The presence of only one major conformer further confirms the rigid structure and α-helix or β-sheet breaker properties of proline.

Recently, β-peptides were subjects of an increasing number of studies as their unique characteristics make them promising candidates in drug design.

Four possible sheet forming strand structure was identified with computational analysis of the For-(β-Ala)$_4$-NH_2 model peptide: H8$_M$ (A), H8$_P$ (B), S$_P$ (C), S$_M$ (D). In the case of C and D strands polar sheets are formed, with carbonyl groups facing same spatial orientation. Polar sheets were found to adopt a twisted nanotube structure. A and B strands however form novel apolar sheet structures with alternating carbonyl groups, not described in previous publications. These apolar sheets belong to a new conformational group of β-peptides, and their planar structure makes them ideal sheet forming structural elements.

In accordance with previous results, computations on For-β-Ala-β^{2,3}-hAla-β-Ala-NH_2 model peptides have shown that heterochirally disubstituted derivatives have the greatest tendency of sheet forming. However, in contrast to previous hypotheses, it has been shown that homochirally disubstituted strands can also form sheet structures, not only in vacuum but also in polar and apolar solvents. Based on these results, an easy-to-use guide is offered for the rational design of hairpin or sheet containing β-peptides.

Thesis statements

1. Based on the analysis of the matrix isolation spectra, both the β_{DL} and the $\gamma_{D=L}$ conformers of Ac-Gly-NHMe and the $\beta_{L(D)}$ and the γ_{l} conformers Ac-L-Ala-NHMe have been unambiguously identified. Although this is consistent with the former results of Grenie et al., some of the spectral features, mainly in the amide A region, have been reassigned. Furthermore, the better resolution of our spectra, the considerably lower ratio of aggregates, the auxiliary Kr matrix experiments, as well as the reliable quantum chemical predictions allowed us to search for less abundant conformers. Although in the case of of Ac-Gly-NHMe the presence of this third conformer, $\delta_{D=L}$, is not perfectly definite, there are several spectral features, which can most probably be assigned to this conformer. In the case of Ac-L-Ala-NHMe, the presence of δ_{L} conformer in the matrix could not be proved with certainty.

2. In DCM solution the two main conformers of both the Ac-Gly-NHMe and Ac-L-Ala-NHMe could be identified. In contrast to this, in solutions in DMSO, which is a stronger coordination agent, a single dominant conformer was observed. This conformer most probably has a strained structure, like the $\beta_{DL}/\beta_{L(D)}$ conformer, since in this structure the amide groups can strongly be coordinated by the solvent. The amide I region of the VCD spectra observed in DCM and DMSO solutions are also found to be consistent with conformational assignment above. In contrast to this, in the amide II region the discrepancy between the calculations and the observed spectra could most probably be interpreted by the effect of the coordination on the rotatory strengths.

3. In order to understand better this discrepancy, the VCD spectra have also been recorded in Ar and Kr matrices. The only discrepancy between the calculations and the MI-VCD spectra was observed in the high-frequency part of the amide I region. It was demonstrated that this discrepancy is caused by the $\beta_{DL}/\beta_{L(D)}$ conformer, since the rotatory strengths of this conformer's amide I modes are extremely sensitive to the backbone torsional coordinates. In addition to this the PES along these coordinates is very flat. That means that even a small perturbation, like the interaction with the matrix or the conformational variation of the side chain can result in an unexpectedly large change in the VCD spectrum. From the computational point of view this shows that in a case like this all the perturbations have to be taken into account. Furthermore, the rotatory strengths should be calculated using very accurate geometric parameters, and the vibrationally averaged (r_0) structure instead of the equilibrium (r_e) structure should be considered.

4. The analysis of the MI-IR and MI-VCD spectra of Ac-L-Pro-NH_2 revealed that two backbone conformers, the major tγ_L+ and the minor cα_L+ forms exist in observable amounts(>1%). Theoretical calculations predict an additional low energy conformer, tγ_L-, but that conformer converts to the tγ_L+ structure when freezing onto the cold surface. The computed spectra based on this conformational model show a very good agreement with the measurements. Our results are in accord with former IR and NMR investigation of Ac-L-Pro-NH_2 solutions, which showed that in apolar solvents the trans forms are dominant. The observation that the proline diamide has hardly any alternative conformer than tγ_L+ not only supports the general view on the elevated rigidity of this amino acid residue, but also explains how it can be an effective α-helix breaker and a β-edge distorter preventing β-proteins to aggregate.

5. Local backbone folds of For-(β-Ala)$_3$-NH_2 and For-(β-Ala)$_4$-NH_2 with μ torsion angle set to *anti* orientation were probed at the M052X/6-311++G(d,p)//M052X/6-31G(d) and B3LYP/6-311++G(d,p)//B3LYP/6-31G(d) levels of theory. Four possible sheet forming monomers were used, namely $H8_M$, $H8_P$, S_P, S_M. $H8_M$ or $H8_P$ strands were shown to form novel foldamers of β-peptides: apolar sheets possessing alternating carbonyl groups. These apolar sheets belong to a new conformational group of β-peptides, and were not described in previous publications. Their planar structure makes them ideal sheet forming structural elements.

6. S_P and S_M structural monomers form polar sheets containing strands with carbonyl groups monotonically facing the same spatial direction. Despite of the predictions of former works, sheets built of polar strands possess a strongly twisted nanotube-like structure. Although previous works argue in favor of polar sheets, it should be noted that only shorter hairpin like structures were studied in which the two strands are connected by a turn motif. These model systems are too short to present the characteristic twisting clearly present in longer systems.

7. The effect of chirality on apolar sheet stability has been investigated on [For-β-Ala-$\beta^{2,3}$-hAla-β-Ala-NH_2]$_2$ peptide model. In accordance with previous predictions, heterochirally disubstituted sheets have the greatest preference for sheet formation. However, in contrast to previous predictions, homochiral substitution itself does not hinder sheet formation. There is no theoretical evidence for the non-existence of homochiral apolar sheets, it depends on the orientation of the *gauche* methyl groups.

8. The aforementioned characteristics of disubstituted apolar sheets were found to hold in water and heptane as well. Even though the effect of destabilization was higher the formation of heterochirally disubstituted apolar sheets were predicted to be thermodynamically favoured in both water and heptane.

Publications related to the dissertation

Pohl, G., Beke-Somfai, T., Csizmadia I. G., Perczel, A.: „*Exploiting Diverse Stereochemistry of β-Amino Acids: Toward a Rational Design of Sheet-Forming β-Peptide Systems*", Amino Acids (2011) submitted to editorial office

Pohl, G.; Beke, T.; Csizmadia I. G.; Perczel, A.: „*Extended apolar β-peptide foldamers; the role of axis chirality on β-peptide sheet stability*", J.Phys.Chem. B *(2010)*, 114, 9338

Pohl, G.; Perczel, A.; Vass, E.; Magyarfalvi, G.; Tarczay, G.: *"A matrix isolation study on Ac-L-Pro-NH_2, a frequent structural element of β- and γ-turns of peptides and proteins"*, Tetrahedron *(2008)*, 64: 2126

Pohl, G.; Perczel, A.; Vass, E.; Magyarfalvi, G.; Tarczay, G.: *"A matrix isolation study on Ac-Gly-NHMe and Ac-L-Ala-NHMe, the simplest chiral and achiral building blocks of peptides and proteins"*, Phys.Chem.Chem.Phys.: *(2007)*, 9: 4698

Other publications

Pohl, G., Jákli, I., Csizmadia I. G., Matías, G. F., Perczel, A.: „*A first principle study on oligopeptide dimerization: An Examination of the Role of Entropy In Initializing Plaque Formation in Alzheimer Diseases*", J. Comp. Chem. (2011) under consideration

Pohl, G.; Beke, T.; Borbély, J.; Perczel, A.:
"Prediction of folding preference of 10kDa silk-like proteins using a lego-approach and ab initio calculations", J.Am.Chem.Soc. *(2006)* 128:14548