Aromatic amino acids confer folding propensity to a nine-residue peptide

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Introduction

We investigated how individual amino acids affect the structural propensities of short peptides. We interpreted the results of molecular dynamics simulations (MD) through comparison with NMR measurements of peptides of sequence EGAAXAASS [1], where 15 different amino acids were tested at position X. Here we focus on the two peptides with X = Trp and Gly, especially on their residual dipolar couplings (RDC). The pattern of the peptide with X = Gly was rather flat, suggesting an extended or unfolded peptide, while the pattern of the peptide with X = Trp was particularly contrasted, characterized by a changing sign value in the middle of the chain, suggesting for us the formation of a helical turn. The molecular dynamics (MD) simulations confirmed theses hypotheses. In the simulations, the peptide with X = Gly was extended most of the time and calculated RDCs were in good agreement with the experimental one. In contrast, the peptide with X = Trp showed many different conformations, mostly folded but with a non negligible number of extended conformations. After clustering the conformations according to the dihedral angles of the main chain, we found that the clusters with theoretical RDCs that better fit the experimental data were those forming a helical turn (Fig. 2). We also show that the driving force leading to such folded conformation could arise from the lack of hydration of the peptide chain on either side of the bulky aromatic residue (Fig. 4).

Methods

The peptide, built with the Molmol package [2], was simulated in a box of about 8700 water molecules using the Gromacs simulation package [3] and the Amber03 force field [4]. RDCs were calculated from coordinates (at 20 ps intervals) using an in-house C program [5].

The simulations show a good fit to the experimental data when X= Gly, but large fluctuations among simulations when X = Trp.

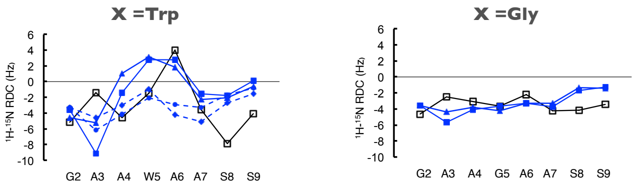

Fig.1. Experimental (open symbols) and MD predicted (filled blue symbols) RDC patterns of peptides EGAAXAASS. Left: The patterns from four different simulations with X = Trp are shown. Notice that two reproduce partly the contrasted experimentally observed pattern (continuous lines). Right: Two illustrative patterns with X = Gly are shown (all 7 performed simulations with X = Gly were similar).

Inferring the structure of the peptide with X = Trp through comparison with the experimental values

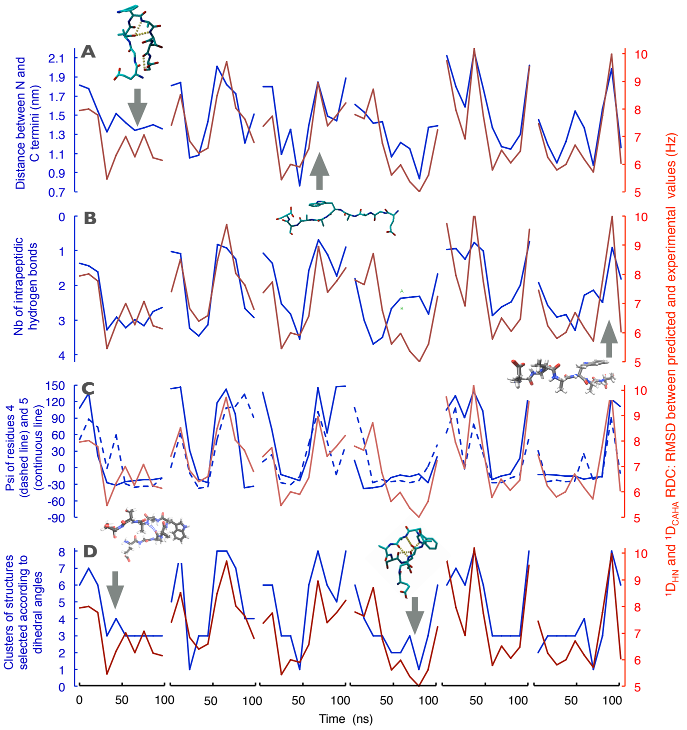

Fig. 2. The fluctuations of various structural parameters (blue) over 6 simulations (100 ns each) of the peptide with X = TRP correlate strongly with the root mean square deviation (RMSD) from experimentally measured RDCs (red). This relation suggests that the experimentally investigated peptide was characterized by:
A: a distance between the N and C termini below approximately 1.3 nm
B: more intrapeptidic hydrogen bonds
C: Psi angle values of residues 4 and 5 of about -30°
D: We clustered the structures according to the backbone dihedrals of residues 3 to 7. This figure shows that it is possible to sort these clusters according to the RMSD from experimental RDCs. Three out of eight clusters contain mainly structures with low RMSD from experimental RDCs.

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While Trp favors helical propensities, the peptide with X = Gly remains unfolded

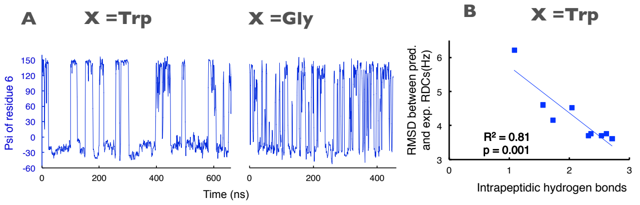


Fig. 3. A) X = Trp: There is a tendency to maintain the psi value of Ala6 at about -30 (and phi between -50 and -70, not shown), which is typical of turns or Helices.
This is not observed for X = Gly.
B) Each points represents the averaged value over a 100 ns simulation. For X = Trp, the RMSD to the experimental values is strongly related to the number of intrapeptidic hydrogen bonds. These were H-bonds between carbonyl and amide groups of residues n_i and n_{i+3} or n_{i+4}, typically residues A3, A4, W5 and A6, A7, S8. For X = Gly, there is no such relation.

Toward a mechanistic explanation?

As shown above, aromatic amino acids a position X tend to increase the folding propensity of the peptide through, among other observations, an increased intrapeptidic hydrogen bonding. To explain this, we postulated that the bulky side-chain of Trp could limit the access of water molecules to the carbonyl and amide groups of the neighboring residues. Consequently, these backbone atoms would interact more with each other, leading to an increased folding propensity. If this is true, one should be able to observe, when X = Trp, a reduced interaction between these particular atoms and water molecules even in conformations for which the peptide is extended. To verify this hypothesis we compared the number of water molecules in the first solvation shell of CO and NH groups for clusters of structures where X = Trp which were "folded" (in the figure as Trp fold), or extended (Trp ext), and of structures where X = Gly, which are mainly extended.

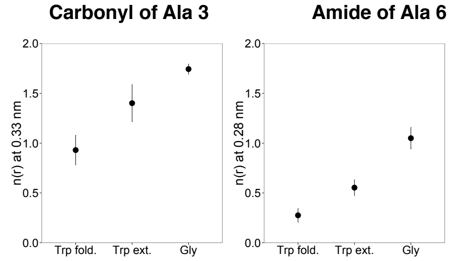


Fig. 4. The number of water molecules interacting with the CO of A3 as well as with the NH of A6 in the peptide where X = Trp is significantly lower than in the case where X = Gly. Particularly, this tendency remains true even when the Trp containing peptide is extended. (mean values ± sd over 7 to 9 100 ns simulations).

Conclusions

The aromatic amino acid Trp favors the folded state of the 9 residue peptide EGAAXAASS, whereas the peptide containing Gly at position X is essentially unfolded.
More precisely, when X = Trp, we observe trends toward a more compact structure, as evidenced by shorter N to C termini distances and radius of gyration, the presence of hydrogen bonds and dihedral angle values typical of turn or helices in the middle of the chain.
We investigated on the mechanism that may explain this folding propensity. In the case of small side chain residues,like X = Gly, surrounding backbone atoms are well hydrated. When X is a bulky amino acid, though, the interactions between water and the CO of A3 or the NH of A6 are strongly hindered. We observe a reduction of interaction with water even when the peptide is extended. Because these atoms are partially prevented from interacting with water molecules, they are more likely to form intrapeptidic hydrogen bonds.

Turn forming peptide

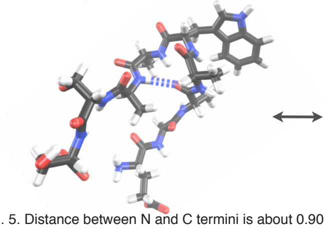
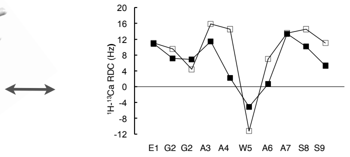


Fig. 5. Distance between N and C termini is about 0.90 nm
Hydrogen bond between CO of A3 and NH of A6
Both psi values of residues 4 and 5 around -30
This structure was taken from cluster 1 (bottom of Fig. 2)

Contrasted RDC pattern



Open symbols: experimental $^1D_{CAHA}$ RDCs
Filled symbols: MD predicted RDCs from dihedral cluster 1 (bottom of Fig. 2)

References:

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