Study of β -sheet helix interactions based on conformational properties of synthetic D,L-alternating decapeptides

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Abstract

De novo peptide design is based on the ability to construct peptide sequences with predictable folding patterns. Helices and β -sheet have been the focus of considerable synthetic attention in attempts to assemble mimics for helical bundles and all β -motifs. In contrast, strategies for construction of β -sheet helix have been less widely explored. The β -sheet helical architecture is constructed from polypeptides that are coiled into a large helix, formed by stacks of β -sheets separated by loops. β -Sheet helices are present in the fibrous form of transyretin, that play an important role in bovine spongiform encephalopathy (BSE) and form the crucial structural elements in insect antifreeze proteins. We report on synthetic analogues able to form β -sheet helices and on the structural comparison with β -helices formed by D,L-alternating peptides.

Introduction

The β -sheet helical architecture is constructed from polypeptides that are coiled into a large helix, formed by stacks of β -sheets separated by loops. β -Sheet helix is also known to occur in Nature, for example, form the crucial structural elements in insect antifreeze proteins. Antifreeze protein consists of an irregular β -sheet helix with a triangular cross-section and a long α -helix that runs parallel on one side of the β -sheet helix, both domains are stabilized by hydrophobic interactions. Several studies have indicated that the ion-conducting transmembrane channels formed by gramicidin A in lipid bilayer originated through a dimerization process, and Urry [1] has proposed that the channels are helical dimers consisting of two single stranded β -helices (6,3 residues per turn) connected head-to-head (formyl-end-to-formyl-end). Urry's proposal is consistent with a number of experimental observations, and the ability of single- and double-stranded β -helices to give head-to-head dimers has been demonstrated either with gramicidin A [1,2] as with synthetic N-formyl oligopeptides [3-4]. A previous work on elastin fragments has clearly revealed the presence of type I and type II β -turns. Based on these results, Urry proposed that elastin adopts a β -spiral structure that contains approximately three pentamers (VPGVG) per turn and the repeating type II Pro-Gly β -turns function as spacer between turns of the β -spiral, utilizing dominantly the Val $\longleftrightarrow$ Pro hydrophobic interactions. Proline is the most frequent residue that occurs at the i+1 position of type I and type II β -turn in proteins. Here we investigated the role played from proline in the conformational behavior of β -turn-containing peptides.

Method

Hydrogen-bonding vs Hydrophobic interactions: there are many occasions in the literature where conformational preference (propensity) is varied through hydrophobic interactions, even at the expense of hydrogen-bonds. The balance between hydrogen bonds and hydrophobic interactions is addressed to differing extents by existing molecular modeling packages. The relative importance of hydrophobic and polar interactions is especially important when attempting to predict the orientation of conformational behavior in the absence of experimental observations. The conformational behavior of D,L-alternating peptides clearly show the conformational changes due to feeble shift of the interactions equilibrium. Oligonorleucines, that on theoretical ground would prefer to form double stranded β -helix, form instead a extended chain structure that is slightly arched (or has a shape of large incomplete ring). This contrast is due to the prevalence of hydrophobic interaction of the linear side chain (interdigitation) that stabilize the extended structure and is prevalent respect to the effect of hydrogen bonds in stabilize the helical structure. Boc-(D-Nle-L-Nle)-5-OMe (X) form aggregates which are unsoluble in common organic solvents even at moderate chain lengths Fig.1 [3]. An N-methylated residue at the n-3 position reduce the maximum number of H-bonds realizable by Boc-(D-Nle-L-Nle)-3-D-NMeNle-L-Nle-D-Nle-L-Nle-OMe that forms a single β -helix in solution Fig.2 [4].

Insertion of a Leu residue in central position of decapeptide backbone reduce hydrophobic interaction between norleucine side chains Boc-(D-Nle-L-Nle)3-D-Leu-L-Nle-(D-Nle-L-Nle)-OMe forms double stranded β -helix in solution [5].

Figure 1: Backbone structure of single β -helix[4].

Results. Here we report about conformational study of synthetic analogue of β -casomorphin-8; Boc-Tyr1(tBu)-Pro2-Phe3-Pro4-Gly5-Pro6-Ile7-Pro8-OMe(I). NMR spectra of peptide I show an equilibrium of five different conformations. Internuclear distances between not vicinal nuclei have been obtained from spectral data, by measuring correlation peak's volumes that are proportional to the inverse of the internuclear distance's sixth potency (Fig.4). The results show the presence of a conformation having all the amide bonds Xxx-Pro trans (64% events). After deprotection of the phenolic group, NMR spectra show the presence of 3 H-bond (Fig.): (Y1)C=O—H-N(F3), 1.820 Å; (F3)C=O—H-N(G5) 1.821 Å (with formation of a γ -turn in both cases); (Y1)O-H—O=C(G5) 1.454. The contribution of steric/hydrophobic interactions to the stability of pseudocyclic structure of β CM-8 (Fig.6) can be well described with homochiral sequences Boc-(Xxx-Pro)2-OMe with Xxx=bulky, hydrophobic residue. The results suggest that, like occur in β -spiral, the balance between hydrogen bonding and hydrophobic interaction is particularly important for the stability of pseudocyclic structure. All peptide bonds Xxx-Pro are trans.

Figure 2: ROESY (CDCl₃) spectrum of Boc-Tyr(tBu)-Pro-Phe-Pro-Gly-Pro-Ile-Pro-OMe.

Dihedral angles H-N-C-H(Karplus eq.)

F3 139.5° (calc.) vs 151.7° (exp.)

G5 120.9° (calc.) vs 117.4° (exp.)

I7 146.7° (calc.) vs 153.7° (exp.)

Figure 3: Molecular Dynamic $(Y1)C=O \cdots H-N(F3)$; $(F3)C=O \cdots H-N(G5)$

Figure 4: Molecular Dynamic $(G5) CO \cdots HO(Y1)$.

NMR spectral data of Boc-Gly-Tyr-Tyr-Pro-OMe and Boc-Gly-Tyr-Tyr-Pro-Thr-OMe reported in this work show a predominant equilibrium between two β -turn structures and a very low presence of the cis/trans isomers. The gluten exorphins A4 and A5 are δ -selective ligands; these two peptides had not affinity for κ -receptors. The gluten exorphin A4 is found 21 times in the repetitive domains of glutenin and structural prediction studies suggest the presence of β -turns (fig. 5).

Figure 5: Boc-Gly1-Tyr2-Tyr3-Pro4-OMe equilibrium of structures

Conclusions

The results presented above provided a possible method for relating the propensity of amino acid residues with the structural features of single stranded β -helix (formed by D,L-alternating peptides) and of β -sheet helix (formed by all-L-peptides). The data of conformational analysis of peptides here reported suggest that important determinants of β -sheet helix formation are amino acid residues with high β propensity, well-defined turns, and inter- and intra-strand side chain interactions between hydrophobic residues. Our interest in this peptide is from the standpoint of deducing the structure and dynamics of homopeptides that assume preferentially a β -sheet helical structure. These oligopeptides can be considered as synthetic analogs of naturally occurring β -sheet helices. The cooperative behavior of the hydrogen-bonded network in these oligopeptides allows their architecture and thus their properties to be finely tuned. The availability of a large number of natural and unnatural amino acids opens the possibility to design and synthesize a wide array of well-defined β -helical polymers and block copolymers.

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