The Dominant Role of Side Chains in β-Sheets Aggregates

Emma Fenude

ICB-CNR, Department of Chemical Science and Material Technology, Sassari- Italy

Introduction

It is generally accepted that the proteins folds *via* a hierarchical mechanism in which the folding protein acquires over increasing degree of complexity. Thus folding initiation events occur at the local sequence level and involve residues close together in the amino acid sequence. These nuclei of structure promote interactions between different parts of the sequence leading ultimately to a co-operative rate-limiting step from which the native state emerges. Peptide models have proved extremely valuable in probing the relationship between local sequence information and folded conformation in the absence of the tertiary interactions found in the native state of proteins, allowing intrinsic secondary structure propensity to be investigated in isolation. The rationalization of chemical molecular recognition phenomena frequently relies on our understanding of weak noncovalent interactions, their magnitude, and their cooperative interplay. Quantitative analysis of individual binding contributions is problematic because individual interactions are seldom viewed in isolation but frequently as an incremental component of a stronger interaction. Synergistic effects that link one interaction with other neighboring interactions further complicated the analysis. Refolding of mainly α-helical proteins into largely β-sheet structures has been widely implicated in protein folding-related disease states, including Alzheimer and BSE suggesting that model peptide β-sheets may provide some insight into the underlying molecular basis for β-sheet stabilization, self-association, and prion-like structural transformation. Chemical model systems provide an excellent vehicle with which to explore β-sheets, because they are smaller, simpler and easier to manipulate than proteins. Antiparallel dimeric β-strand formed by D,L-alternating peptides and three stranded β-sheet peptide motifs derived from native protein sequences (or through rational design) may be suitable as vehicle for quantitative analysis of weak non covalent interactions. Here we report about intermolecular interactions and conformational changes between dimeric β-sheet and bioactive peptide that form preferentially a twisted dimeric conformation in solution.

β-Sheets Chemical Model

Alternating D,L-peptides are able to assume specific conformations including, among other, various kinds of single and double stranded β-helix structures, predicted also on theoretical ground. Double stranded β-helix conformation share an important number of structural features with β-sheet ones, as a set of hydrogen bonds between amino and carbonyl backbone groups, and ϕL, ψL, ϕD, and ψD values in poly-L- and poly-D-peptides. Boc-(D-Nle-L-Nle)$_5$-OMe, like to oligonorleucines with number of residues >7, form aggregates which are unsoluble in common organic solvents even at moderate chain lengths. Such supramolecular configuration displays the characteristic ~ 4.7 Å reflections in amyloid X-ray diffraction, which is attributed to the interstrand spacing. A leucine residue in central position of the backbone interrupt the interdigitation of the n.butyl of the norleucine residues that is a determining factor for the stacking. Boc-(D-Nle-L-Nle)$_2$-**D-Leu**-L-Nle-(D-Nle-L-Nle)$_2$-OMe is soluble in organic solvents. 1H-NMR spectra exhibit features, that are characteristics of an antiparallel double-stranded structure. In addition long range connectivities were founds which are compatible whit the existence of a dimer in solution.(Fig.) Furthermore some weak connectivities are found that are compatible with a partial superimposition of a thirty strand. This result demonstrate that side chain interactions is determinant for the stabilization of the aggregate.

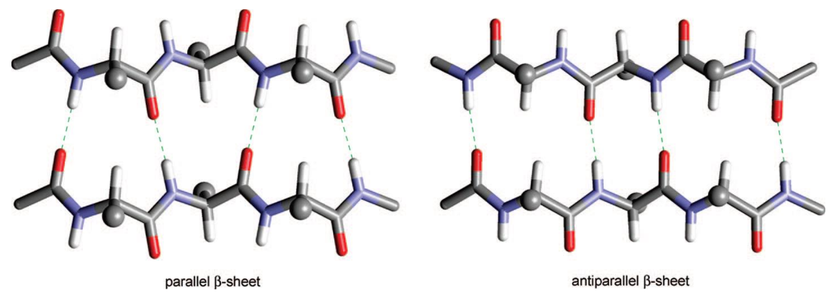

Figure 1. β-Sheet dimers

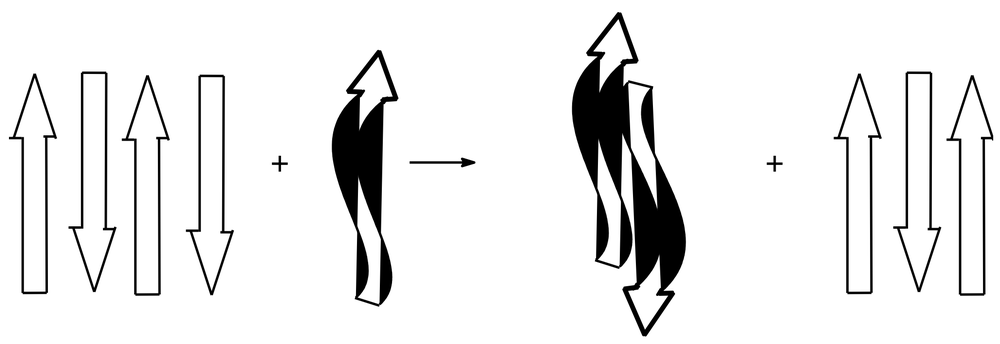

Figure 2. Schematic images of β-sheet aggregate dissociation

With the aim of dissociating the β-sheets aggregate in order to form a β-sheet helix we have synthesized a series of hydrophobic peptides capable of binding the first β-strand of the β-sheet aggregate. 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 element in insect antifreeze proteins.

Twisted and Coiled β-Sheet

Most β-sheets have twisted, not flat structures, which resembles the right propeller (if one is looking along the β-strand. The twisting degree of β-sheets in proteins is different, but the average dihedral angle between neighbouring β-strands is close to 20°. In strongly twisted β-sheets, β-strands should be twisted as well as coiled in order to generate a large contact surface without damaging the hydrogen bond system (Fig.2). Chaperone domains convert prolyl isomerase into generic catalyst of protein folding. The catalytic performance of the folding enzymes is determined by generic substrate recognition at the chaperone domain and efficient transfer to the active site in the prolyl isomerase domain.

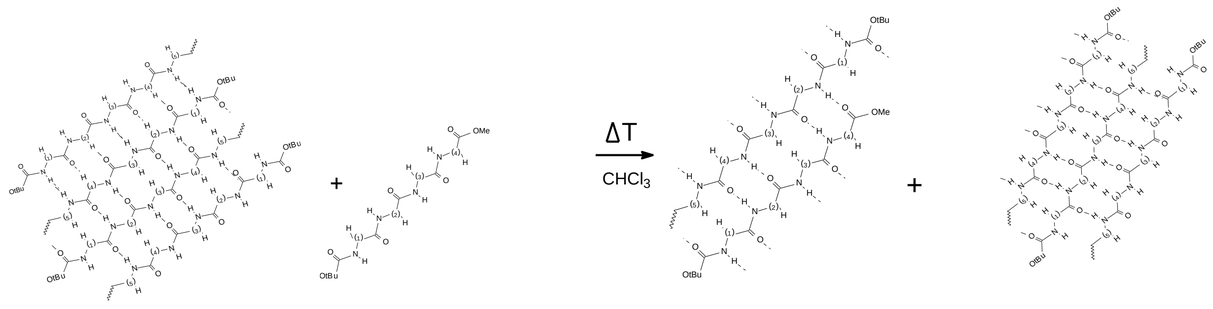

Figure 3. HCO-Nle-Leu-Leu-Leu-Phe-Leu-Nva-Nva-OMe conformational changes on raising the temperature

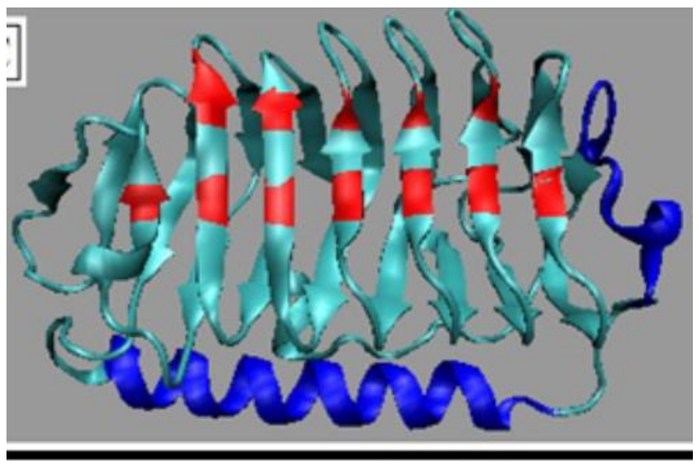

Figure 1. Fungal anti-freeze protein (PDB ID: 3V N3).

Structural features of β-sheets aggregates: HCO-Nle-Leu-Leu-Leu-Phe-Leu-Nva-Nva-OMe
Structural features of β-sheets "breaker": Ac-Leu-Leu-Leu-Phe Leu-OMe, Ac-Leu-Leu-Phe-Phe-Pro-OMe
NMR studies of synthetic β-sheet have elucidated the importance of hydrophobic interaction in the stabilization of β-sheet aggregates. The observed β-sheet aggregate formed by HCO-Nle-Leu-Leu-Leu-Phe-Leu-Nva-Nva-OMe is totally unsoluble in $CDCl_3$, then not detectable at the NMR spectra in solution. At reverse the 8-residue peptide (a strand of aggregate) and the 5-residue peptide (the breaker) are identified (Fig.3) with NMR spectra in $CDCl_3$ solution. The presence of Hydrogen bonds reveal that the two peptides interact with each other. From NMR spectra we can also calculate that the dissociation of β-sheet aggregate is 5%. Synthetic β-sheet aggregates that presents hydrogen bonding edges can form well-defined hydrogen-bonded dimers. Dimerization occurs readily in chloroform solutions. Interactions among the side chains, as well as hydrogen bonding among the main chain are important in dimer formation. These studies help illustrate the importance of intermolecular edge-to-edge interaction between β-sheets in peptides. and may lead to new structure.

Conclusions

It is already reported that thrombin destabilizes β-sheet aggregates in a concentration-dependent manner. Small peptides synthesized using thrombin as a template exhibit functional similarity. These information encouraged the synthesis of small peptides that can be used together with sequences containing the catalytic residues of a folding enzyme in order to obtain a double strand β-sheet helix.

References

[1] Fenude Schoch E., Römer U.D., Lorenzi G.P., Int. J. Peptide Protein Res.(1994) 44, 10-18
[2] Navarro E., Fenude E., Celda B., Biopolymers (2004) 73, 229-241
[3] Tsuyoshi Takahashi, Hisakazu Mihara; Acc. Chem. Res. (2008) 41 (10); 1309-1318
[4] Nowick S. James; Acc. Chem. Res. (2008) 41 (10); 1319-1330