Organogel Formation by Hierarchical Self-Assembly of β-Helix Forming Peptides

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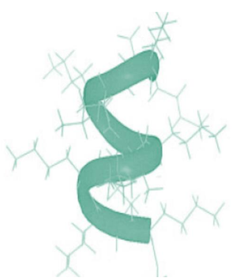
Introduction

Self-assembling peptides have been used to form a variety of novel nanostructures. The large number of amino acids available, both natural and synthetic, allow the utilization of a wide variety of interactions to drive the self-assembly process. Many of these nanostructures result in the formation of organogel which can be used for important industrial applications. Organogels are a three-dimensional network of peptide chains swollen with organic solvent. To fully utilize these biomaterials for applications, the design principles that drive self-assembly and determine their material properties need to be better understood. Several of design variables that affect self-assembly and material properties of different families of peptides has been investigated previously. For example, for α-peptides it has been shown that tuning hydrophobic and π-π interactions through the amino acid sequence was critical to determining whether a gel phase could form. Changing the amino acid sequence has also been found to change the secondary structures formed, as the peptide self-assemble in the case of a short eight-residue peptide, GV8, which causes transitions between α-helical and different β-sheet structures, and results in differences in the rheological properties. Herein a series of D,L-oligonorleucines was studied for they gelation properties in organic solvent at low concentrations uncovering key parameters driving organogel formation.

Method

Hydrogen-bonding vs Hydrophobic interactions: there are many occasions in the literature where conformational propensity is varied through hydrophobic interactions, even at the expense of hydrogen-bonds. 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 antiparallel β-helix, form instead an 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. Supramolecular gels are formed by the self-assembly of small molecules under the influence of various non-covalent interactions. As the interactions are individually weak and reversible it is possible to perturb the gels easily, which in turn enables fine tuning of their properties. Synthetic supramolecular gels are kinetically trapped and usually do not show time variable changes in material properties after formation.



Boc-(D-Nle-L-Nle-)$_3$-D-NMeNle-L-Nle-D-Nle-L-Nle-OMe An N-methylated residue at the n-3 position of the chain was used to reduce the maximum number of H-bonds realizable. This peptide forms **single stranded** β-helix in $CDCl_3$ solution[2].

Boc-(D-Nle-L-Nle)$_5$-OMe (X) forms aggregates which are unsoluble in common organic solvents even at moderate chain lengths [1].

Boc-(D-Nle-L-Nle)$_2$-D-Leu-L-Nle-(D-Nle-L-Nle)$_2$-OMe Insertion of a leucine residue in central position (5 or 6) of decapeptide backbone reduce hydrophobic interaction between norleucine side chains (interdigitation). This product forms **double stranded, antiparallel** β helix in $CDCl_3$ solution[2]

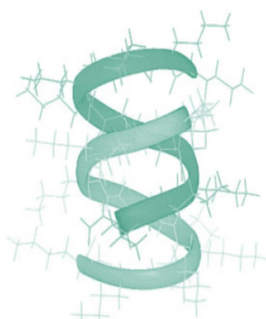


Figure 1. Boc-(D-Nle-L-Nle-)$_3$-D-NMeNle-L-Nle-D-Nle-L-Nle-OMe, single $\beta^{4.4}$-helix

Figure 2. Boc-(D-Nle-L-Nle)$_2$-D-Leu-L-Nle-(D-Nle-L-Nle)$_2$-OMe, ↓↑ $\beta^{5.6}$-helix

Boc-*n*-OMe

The lower members of the series Boc-*n*-OMe display good solubility in various organic solvents, but the solubility decreases with increasing *n*. The members with n ≥ 8 are insoluble even in highly polar solvents. All peptides were synthesized using coupling reaction in chloroform solution. D,L-oligonorleucines having n=8, 9, 10, 12 form as gel, already in the reaction medium. This behaviour clearly manifests a preference of the norleucines to form supramolecular aggregates. In chloroform solution, the lower Boc-oligomers exhibit NMR NH-signals which (*i*) move in all cases to lower field on increasing the concentration and (*ii*)tend to be the more downfield the longer the chain. It appears therefore than the decrease of the solubility in chloroform upon increasing n is mainly due to an increasing tendency of the oligomers to self-associate through intermolecular H bonds. In the solid state the D,L-oligonorleucines with n ≥ 8 exhibit IR absorption spectra with the amide A band at relatively low frequencies (3280-3285 cm^{-1}), as expected for structures with H-bonded NH-groups, and X-ray powder diagrams with a strong reflection at 2Θ ≈ 18.8° (d=0.47) which is attributed to the interstrand spacing and is characteristic in amyloid aggregates of β-sheets with the chains oriented in parallel. These observations suggest that the structures that the oligonorleucines prefer to form, both in solution and in the solid state, is an extended chain structure that self-assembly to form a supramolecular structure with parallel orientation of the strands.

Boc-(D-Nle-L-Nle)$_3$-OMe CDCl3 19.6 mg/mL solution NMR spectra exhibit NMR NH-signals which move in all cases to lower field on increasing the concentration. Boc-(D-Nle-L-Nle)$_3$-OMe, like Boc-(L-Ile)$_6$ [3], with increasing concentration form soluble β-aggregates with antiparallel orientation of strands. The assembly is driven by hydrogen bond interactions.

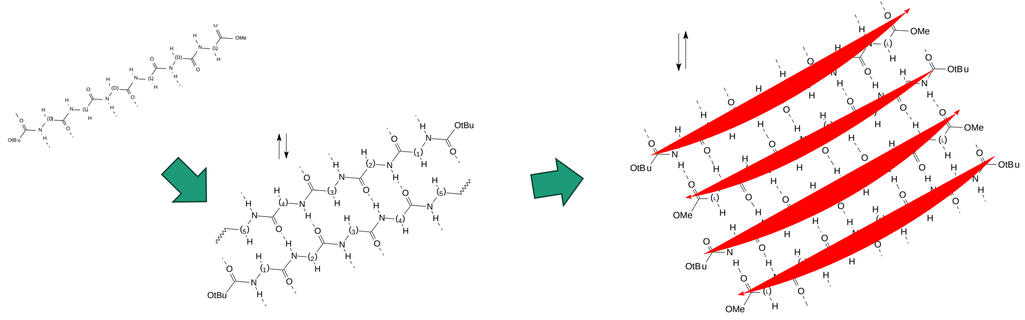


Figure 3. Conformational behavior of Boc-(D-Nle-L-Nle)$_3$-OMe in CDCl3 solution at high concentration [3,4].

Boc-(D-Nle-L-Nle)$_3$-OMe CDCl3 3.4 mg/mL T=263-333°K, solution NMR spectra; display good solubility and exhibit NH-signals characteristic of the monomeric peptide chain. The sample is allowed to slowly return to room temperature and after 24 hours a clear organogel is formed. The assembly is driven by 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.

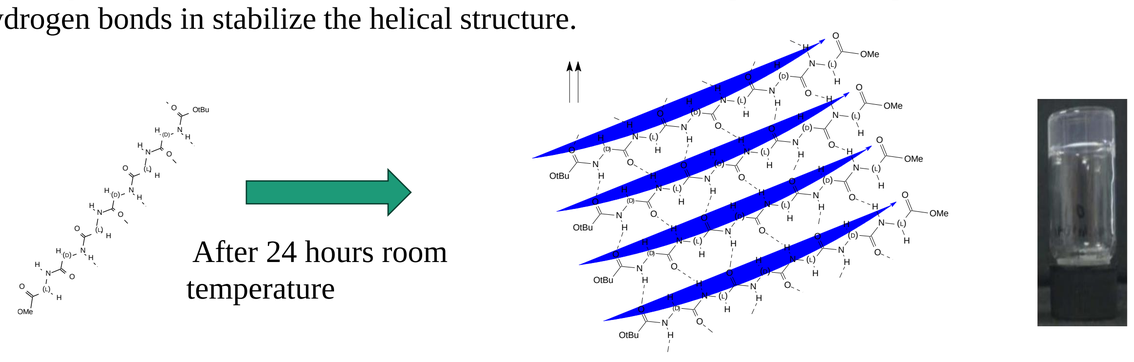


Figure 4. Conformational behavior of Boc-(D-Nle-L-Nle)$_3$-OMe in CDCl3 solution at high temperatures.

Boc-(D-Nle-L-Nle)$_5$-Me (**X**) The pure product appears as a fine white powder insoluble in CDCl3, the NMR spectrum shows low intensity peaks for the protons of the side chains which indicate the presence of small quantities of product in solution. A milky gel is formed during the experiment. According to the X-ray and IR data the chains of the higher oligonorleucines, including the Boc-protected hexapeptide, in gel phase are assembled in β-structures. Interestingly the IR spectra of the pure products show no apparent band around 1690 cm-1. The absence of a band at, or near, this frequency, suggests a parallel arrangement of the peptide chains in the β-structures[5].

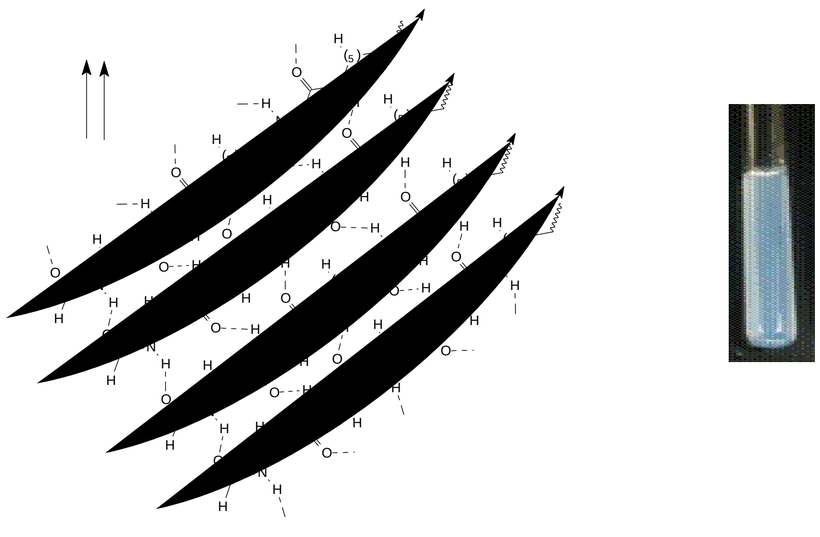


Figure 5. β-Strands alignment and interstrand hydrogen bonds in Boc-(D-Nle-L-Nle)$_5$-OMe

Conclusions

In the design of self-assembling peptide systems, changes to the amino acid sequence alter the non-covalent interactions that drive self-assembly. For D,L-oligonorleucines two fundamental important interactions in their self-assembly into β-structure organogel are the hydrogen bonding between the peptide backbones and the hydrophobic interactions between the side chains of core amino acids in the sequence. The non-covalent interactions can be controlled between these peptides to further modulate their self-assembly and tune the properties of the composite materials. Physical gels are unique self-healing materials in which the existence of equilibrium ensures their eventual recovery.

References

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