Proceedings of the 8th World Congress on Recent Advances in Nanotechnology (RAN'23)
Lisbon, Portugal – March 23 – 25, 2023
Paper No. ICNNFC 109
DOI: 10.11159/icnnfc23.109

Alternative Carriers in Drug Delivery Systems – Peptide Foldamers

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Abstract - In this paper, we report the obtaining of foldameric peptides by incorporation of *trans*-(1*S*,2*S*)-2-aminocyclopentanecarboxylic acid residues (*trans*-ACPC) into model coiled-coil structures and preliminary studies on their propensity for the encapsulation of small molecules. The secondary structure of the obtained peptides was elucidated using circular dichroism (CD), and the peptides were tested as nanocarriers for the model ligand - 1,6-diphenylehexatriene (DPH) using fluorescence studies. The experimental results were supported by molecular modelling studies. Two of the obtained peptides proved to be very promising hosting platforms for drug delivery systems, and will be used for further studies.

***Keywords*:** ACPC, coiled-coil, drug delivery, encapsulation, foldamer, peptide

1. Introduction

Foldamers are oligomers that can self-organize into precise nanostructures with properties and functions comparable to, yet distinct from, natural biopolymers. [1] Peptide-based foldamers attract a lot of attention as potential components of bionanomaterials due to their unmatched biocompatibility and tuneability, and quite promising features for use in encapsulation and drug delivery. [2] Much attention has been given to coiled-coil peptides, since their higher-order structure can be controlled at the sequence level thanks to well-established design principles. [3] Coiled-coils represent a highly prevalent motif in proteins and provide unique and versatile building blocks for artificial biomolecular systems. [4] pH- and temperature-sensitivity, and the ability to carry hydrophobic drugs within their hydrophobic cores make coiled-coils promising components for drug-delivery systems. Precise positioning of the polar and hydrophobic side chains plays a key role in the formation of stimuli-responsive nanomaterials. [5] Among the many strategies for the synthesis of macromolecular systems, self-assembly formation is one of the currently preferred, mostly because the preparation of a relatively small monomer is much less challenging than the final complex structure. [6] Since the assembly is stabilized by noncovalent interactions, its formation is potentially controllable. Moreover, using sensibly chosen amino acids may enhance the self-assembly propensity and improve the stability of the final structure. Additionally, because they possess noncanonical residues, peptide foldamers are resistant to naturally occurring proteases, which is highly desirable in the case of bioapplications. [7]

2. Results and Discussion

Two previously described [8] α-helical barrels CC-Hex2 and CC-Hept with channels diameter of 4.7–7.7 and 5.4–10.1 Å, respectively, were point mutated to obtain model coiled-coil peptides En_6 and En_7 (Fig. 1A). The model peptides were foldamerized by introduction of *trans*-(1*S*,2*S*)-2-aminocyclopentanecarboxylic acid residues (*trans*-ACPC, Fig. 1B) into their sequences to obtain two sets of peptide foldamers (Fig. 2, Table 1): 1) En_6_1, En_6_2, En_7_1, and En_7_2, where the *trans*-ACPC residue was introduced in the outer position of the coiled-coil structure, and 2) En_6_3, En_6_4, En_7_3, and En_7_4, where *trans*-ACPC was introduced in the main core of the coiled-coil structure. Repeats of hydrophobic (*h*) and polar (*p*) residues are often denoted as *abcdefg* (Fig. 2). Additionally, Ile was introduced instead of Leu at the position *a* in the peptides En_6_2 and En_7_2 to modify the hydrophobic interactions inside the main core of the coiled-coil.

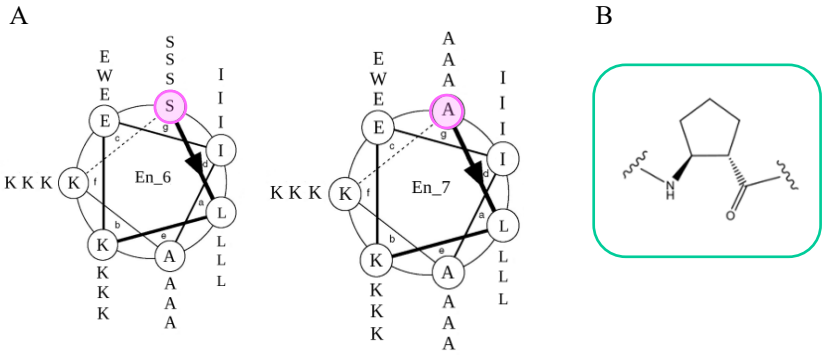

Fig. 1: A) Helical wheels of the model coiled-coil peptides En_6 and En_7. B) The structure of the *trans*-(1*S*,2*S*)-2-aminocyclopentanecarboxylic acid residue (*trans*-ACPC).

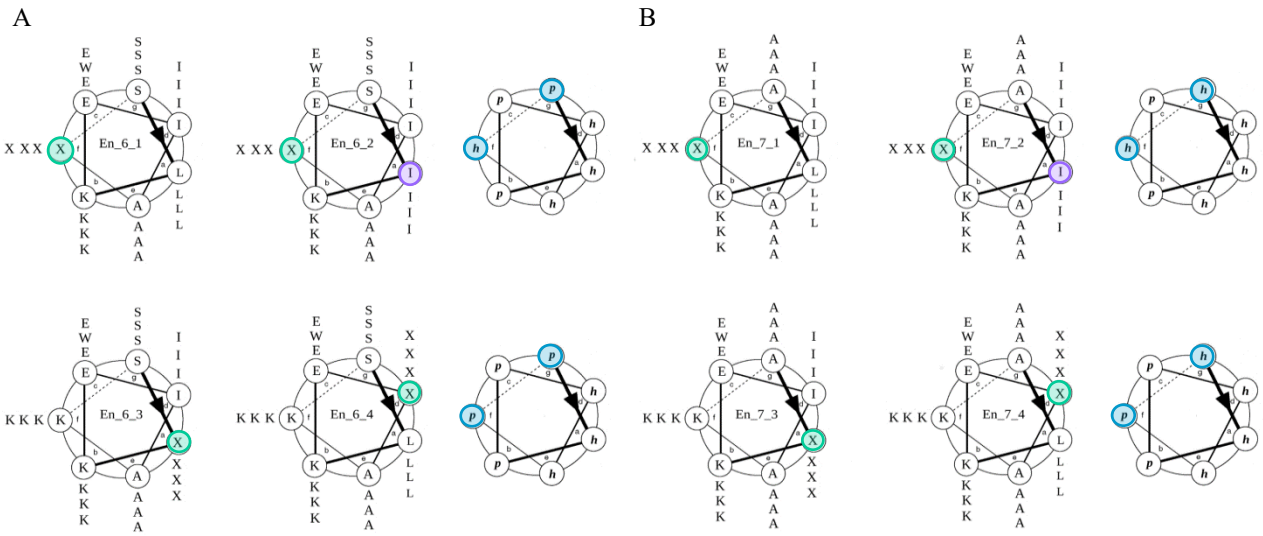

Fig. 2: Helical wheels of A) En_6_x and B) En_7_x groups of peptides.

Table 1: Sequences of the synthesized peptides.

Name	Sequence
En_6	Ac-GEIAKSLKEIAKSLKWIAKSLKEIAKSLKG-NH_2
En_6_1	Ac-GEIAXSLKEIAXSLKWIAXSLKEIAXSLKG-NH_2
En_6_2	Ac-GEIAXSIKEIAXSIKWIAXSIKEIAXSIKG-NH_2
En_6_3	Ac-GEIAKSXKEIAKSXKWIAKSXKEIAKSXKG-NH_2
En_6_4	Ac-GEXAKSLKEXAKSLKWXAKSLKEXAKSLKG-NH_2
En_7	Ac-GEIAKALKEIAKALKWIAKALKEIAKALKG-NH_2
En_7_1	Ac-GEIAXALKEIAXALKWIAXALKEIAXALKG-NH_2
En_7_2	Ac-GEIAXAIKEIAXAIKWIAXAIKEIAXAIKG-NH_2
En_7_3	Ac-GEIAKAXKEIAKAXKWIAKAXKEIAKAXKG-NH_2
En_7_4	Ac-GEXAKALKEXAKALKWXAKALKEXAKALKG-NH_2

The peptides were synthesized on the solid support, purified by HPLC, and confirmed by mass spectrometry. Circular dichroism (CD) was used to elucidate the secondary structure of the obtained peptides (Fig. 3A). The model peptides En_6 and En_7 possess two minima at λ=208 and 222 nm with the ratio of $R(\theta_{222}/\theta_{208})$ close to 1, which is typical for α-helix structure. [9] Introduction of *trans*-β residue to the structure of the model coiled-coils resulted in the formation of eight peptides with foldameric helix structures [10], each with a characteristic minimum in the range between 206 and 208 nm.

Subsequently, fluorescence spectroscopy was used to probe the binding of the ligand to the synthesized helical peptides (Fig. 3B). 1,6-diphenylehexatriene (DPH, Fig. 3C) shows fluorescence (λ_{max} = 455 nm) only when in a hydrophobic environment, so it was chosen as a model ligand for the encapsulation tests. The conditions of DPH encapsulation in the synthesized peptide cavities were optimized and systematic studies were carried out for the 500 µM µM peptide concentration and for the 5 µM ligand concentration in the phosphate buffer (C= 0.05 M, pH = 7) at T=20 ºC. The obtained results indicate a significant difference in the propensity for encapsulation of the obtained peptides. In general, the En_6_x group of peptides showed a better propensity for model ligand encapsulation compared to the En_7_x group. The most promising candidates for drug delivery platforms proved to be the peptides En_6 and En_6_3.

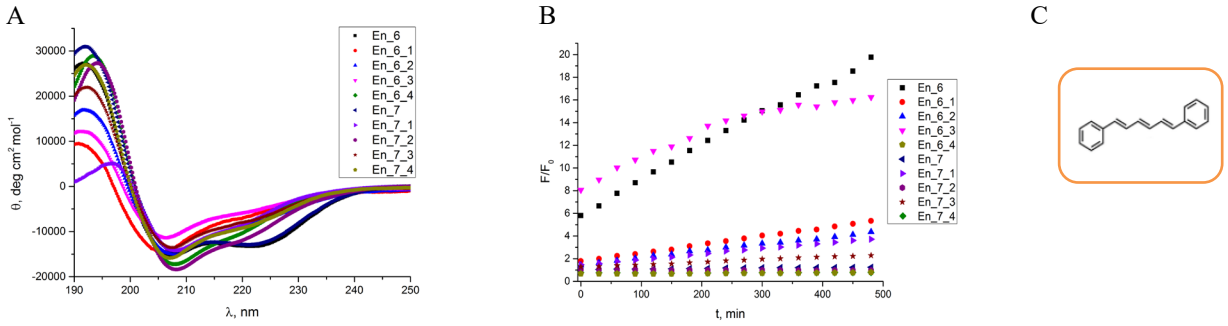

Fig. 3: A) CD spectra of the studied peptides in phosphate buffer. C_{pep} = 80 µM, C_{buffer} = 0.05 M, pH = 7, T = 20 ºC. B) Fluorescence intensity changes followed for 8 h. C_{pep} = 500 µM, C_{lig} = 5 µM, C_{buffer} = 0.05 M, pH = 7, T=20 ºC. C) The structure of 1,6-diphenylehexatriene (DPH).

Molecular modelling was used to support the obtained experimental results. Models were generated from the crystal structure of CC_Hex2 (PDB: 6EIZ) by mutation and superimposition of the helices in Biovia Discovery Studio Visualizer followed by minimization in Amber with AMBER ff15ipq-m force field. [11] The minimized structures were then docked with DPH using AutdockVina. The results of the modelling and the docking are in agreement with the experimental results. The comparison of the electron density maps and the size of the inner diameter of the barrels, fundamental for encapsulation, shows continuity and agreement between En_6 and En_6_3 (Fig. 4 A, B), after minimization of the structure. The docking results of these two alpha barrels showed higher affinity energies of all the presented models, -7.6 and -7.3 kcal/mol, respectively. However, some of the sequence mutations of the helix, as for En_6_4, show a poor packing between helices after the substitution of the Ile positions by *trans*-ACPC, as compared to En_6_3 (Fig. C, D). The lack of compactness between the helices and modification of the inner diameter of the barrel can induce different conformations or oligomerization states of the barrel and in consequence show a poor capacity for encapsulation of molecules such as DPH. These disturbances of the barrel are directly reflected on its capacity to dock DPH leading to incongruent results and low affinities. Such results might be an explanation for the significant differences in the encapsulation capacities for each barrel.

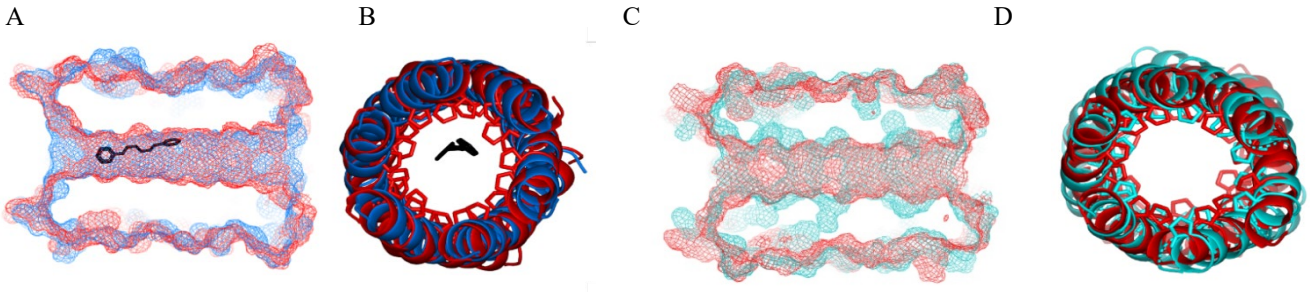

Fig. 4: A) Electron density map of En_6_3 (red) with docked DPH (black) superimposed over En_6 (blue). B) Top view of the superimposed alpha barrels En_6_3 (red) and En_6 (blue). C) Electron density map of En_6_3 (red) superimposed over En_6_4 (cyan). D) Top view of the superimposed alpha barrels En_6_3 (red) and En_6_4 (cyan).

3. Conclusions

In conclusion, we have designed eight peptide foldamers, based on two model coiled-coils, with *trans*-ACPC residues located in different positions in the structure. We have shown that proper positioning of polar and hydrophobic residues in the coiled-coil structure allows the incorporation of *trans*-ACPC into the inner position of the main core (positions *a* or *d*) without loss of helical structures, as was proven by CD. In fluorescent studies, the obtained peptides showed a very different propensity for encapsulation of the model ligand. The experimental results are in agreement with the molecular modelling studies and show that only distinct positioning of specific amino acid residues permits the proper incorporation of the ligand into the cavities. The En_6 and En_6_3 peptides will be used for further investigation, as they have proven to be promising hosting platforms for drug delivery systems.

Acknowledgements

The work was financially supported by the National Science Centre, Poland, Grant No. 2017/26/D/ST5/00341 (to M. S.). The project is co-financed by the Polish National Agency for Academic Exchange (to M. S.)

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