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Synthesis and Characterization of a Supramolecular DNA-Inspired Nanowire

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Introduction

In Nature, electron transfer (ET) is performed by means of biomolecules. Proteins and DNA are known to effectively mediate ET, but they offer little stability outside their physiological environment. Natural peptides based on sterically hindered, non-coded α-amino acids are biopolymers that possess - even when short - well-defined helical structures, remarkably stable under extreme environmental conditions. We recently reported a bioinspired approach based on nucleobase pairing, which allows helical peptides to self-organize into molecular wires [1]. We used a helical undecapeptide analog of the natural peptide trichogin GA IV, in which four glycines were replaced by four lysines to reduce flexibility, and the *N*- and *C*-termini were functionalized with thymine and adenine, respectively. Through thymine-adenine hydrogen bonds, we assembled the biodevice onto a gold electrode, capping it with Porphyrin(Zn)-Thymine. Under illumination, the peptide-based supramolecular system could generate current [1] and was found to be very stable over time, also in contact with a solution.

We also describe the effect on ET-through-peptide of a pH-controlled, reversible 3_{10}- to α-helix conversion, with a focus on the effect of the pH-induced conformational change on photocurrent efficiency [2]. The biomolecular devices were characterized by electrochemical and spectroscopic techniques, and were able to generate current under illumination, with an efficiency that is the highest recorded so far with biomolecular systems.

Here, we report the synthesis of the peptide Thymine-[$K^{2,5,6,9}$]tric-Adenine (T-tric-A, Figure 1) and the characterization of its self-assembly in several environments. The primary structure of the peptide is: Thymine-CH_2-CO-Aib-Lys-Leu-Aib-Lys-Lys-Leu-Aib-Lys-Ile-Leu-NH-$(CH_2)_2$-NH-CO-CH_2-Adenine.

Results and Discussion

Trichogin is a naturally-occurring peptaibol, with three Aib residues in its sequence, which make it adopt a well-defined helical conformation [3]. On the other hand, Aib is also a sterically-hindered and poorly-reactive amino acid, therefore the synthesis of Aib-containing peptides needs special adjustments, such as double-coupling reactions both for its insertion and - especially - that of the following residue, since the steric hindrance is more felt when Aib is performing the nucleophilic attack. Nonetheless, Aib at position 8 - and Leu at position 7, as well - are successfully coupled by a single coupling reaction mediated by Oxyma pure and DIC (diisopropylcarbodiimide). This has been found effective for Aib residues at an early stage of the synthesis. The remaining Aib-involving steps have been performed by means of double-coupling reactions, with quantitative yields. Thymine-COOH has been coupled to Aib at position 1 by three subsequent coupling reactions. We tried different active agents, observing a better performance in terms of yield and purity with Oxyma/DIC than with HATU.

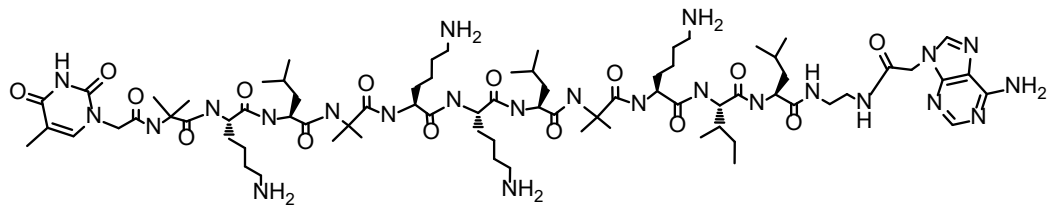

Fig. 1. Chemical structure of peptide T-tric-A.

After cleavage from the resin under mild acidic conditions (1,1,1,3,3,3-hexafluoroisopropanol 30% in CH_2Cl_2), Adenine-COOH was successfully linked to the C-terminal primary amine of the peptide, by reaction in solution, using 1-Hydroxy-7-azabenzotriazole (HOAt) and N-Ethyl-N′-(3-dimethylaminopropyl)carbodiimide (EDC) as active agents. After Boc removal by treatment with a 3M HCl solution in methanol, the peptide was purified by medium pressure liquid chromatography (Biotage Isolera Prime).

We studied the self-assembly of T-Tr(Lys)-A as a function of peptide concentration by circular dichroism. A R value ($\theta_{222}/\theta_{204}$) above 1 is diagnostic of the presence of aggregation. Our CD analysis (Figure 2) registered aggregation (R>1) at concentrations above 0.2 mM. TEM analysis performed on the same solutions analyzed by CD confirmed that at R>1 molecular wires are formed. Our Diffusion-ordered spectroscopy (DOSY) experiment also highlighted the presence of large, self-assembled structures in solution at a high peptide concentration (Figure 3).

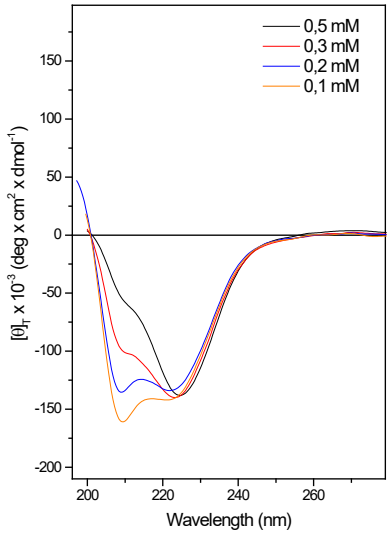

Fig. 2. CD spectra of T-tric-A as a function of increasing peptide concentration in 10:1 v/v acetonitrile/basic water (NaOH 0.1M) solution.

The presence of four Lys residues makes the peptide pH-sensitive [2,4].

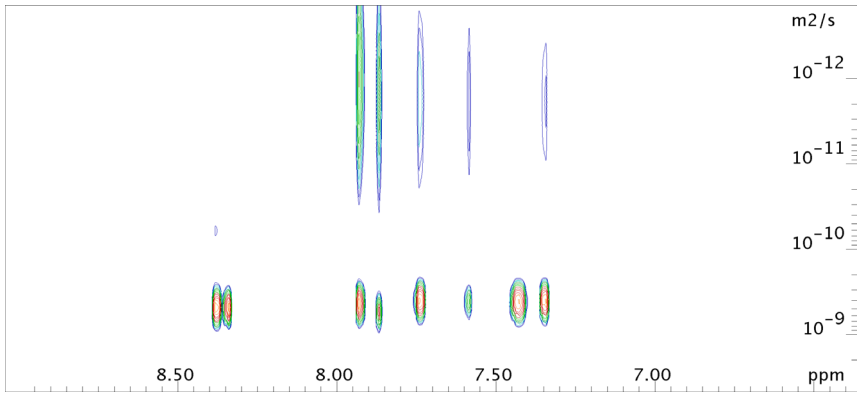

Fig. 3. DOSY map obtained for peptide T-tric-A (2mM in CD_3CN/D_2O 9:1; 400 MHz, 278K).

We previously reported that Lys-containing trichogin analogs were able to switch their conformation reversibly between fully-developed α-helix and 3_{10}-helix in response to pH variation. We thus studied by CD and TEM the self-assembled peptide-based structures at different pH values.

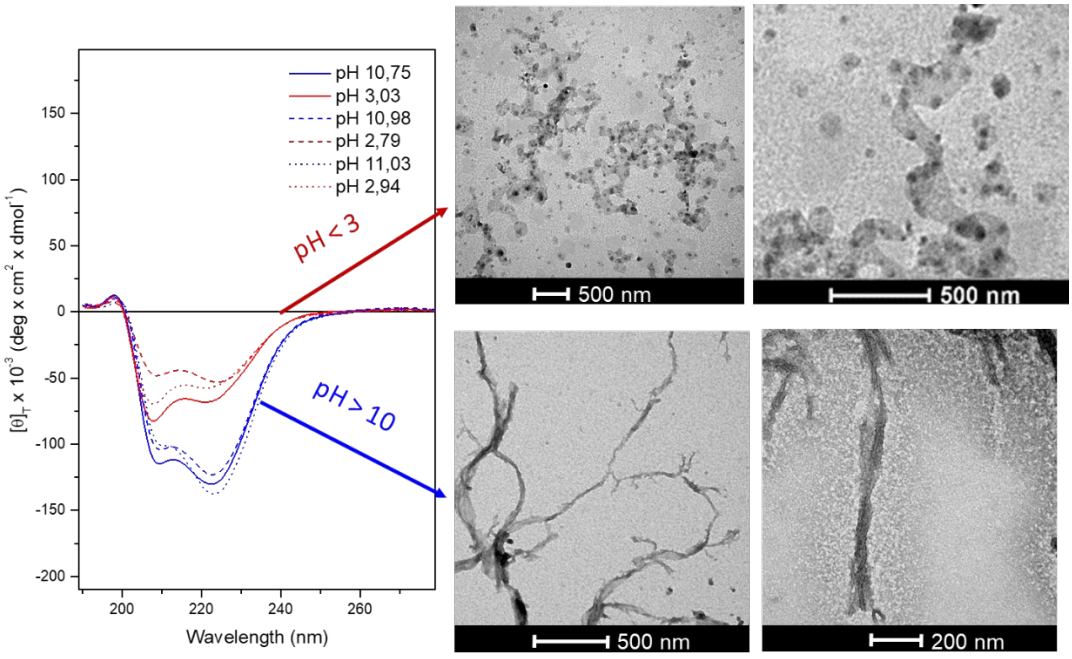

Fig. 4. CD spectra obtained for peptide T-tric-A at different pH values, obtained by switching the pH into the same cuvette [0.3 mM in ACN/NaOH 0.1M 10:1 (starting point)]. TEM images: T-tric-A 0.3 mM in: (i) ACN/(NaOH 0.1mM) 10:1 solution pH 11 (below); (ii) + 2 µl HCl 3M (pH 3) (above).

Figure 4 shows the results from our combined CD and TEM analysis, performed at different pH values, obtained by adding NaOH/HCl alternatively into the CD cuvette and performing both analysis on the same peptide solution. The reversible, conformational transition already detected on the single peptide seems to take place also in the supramolecular assembly, making it a kind of molecular spring. Despite the low peptide concentration - suitable for CD analysis but not for good TEM images - we could unambiguously detect and confirm the presence of the twisted fibers at both pH values tested.

In conclusion, we performed the synthesis of the peptide T-tric-A. The characterization of its self-assembly in several environments highlighted the peptide propensity to form molecular wires, maintaining the ability to reversibly switch its 3D-structure between two helical conformations of different length in response to pH variations.

Acknowledgments

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