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Switch-Peptides to Trigger the Peptide Guided Assembly of Poly(ethylene oxide)−Peptide Conjugates into Tape Structures

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Recently, considerable effort was devoted to control structure formation processes in synthetic polymers by peptides.[1−3] This biomimetic route enhances the diversity of polymer assemblies and might allow the rational design of hierarchically ordered nanostructures.[4]

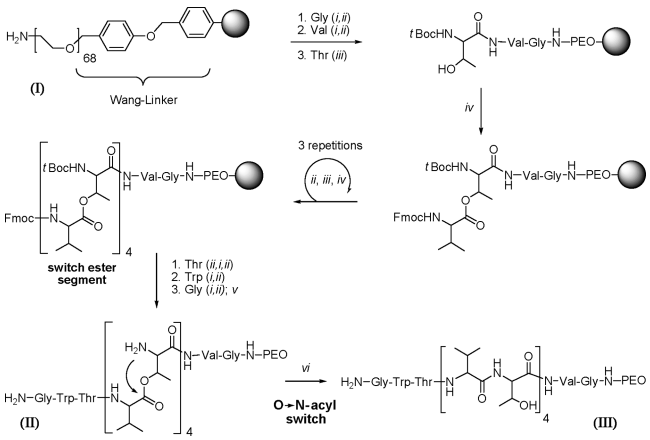
Lynn and co-workers[5] pioneered this route by exploiting the aggregation behavior of a fragment of the β-amyloid peptide, associated with Alzheimer's disease, to organize poly(ethylene oxide) (PEO) into core−shell fibrils. Peptides adopting the β-sheet secondary structure motif are well-suited since anisotropic tape or fibrillar structures can be obtained.[2,3,6] These might be useful as nanowires, fibers for biomedical purposes, scaffolds to position chemical functionalities, or to direct transport (see ref 2 and references cited therein).

However, even if the concept was demonstrated, only short fibers up to 4 μm in length are accessible. To obtain extended and robust nanofibers, fairly stable to mechanical stress and thus more interesting for material science, peptides are required with higher tendency to form stable aggregates. Unfortunately, the solid-phase-supported synthesis (SPPS), purification, and analysis of these peptides is highly challenging.[7]

Recently, a strategy was developed to overcome these difficulties by integrating defined structure defects into peptides.[8−10] These "switch"[9] ester segments (Scheme 1) disrupt the amidic backbone, suppress the aggregation tendency, and thus allow the synthesis of peptides with "difficult sequences", such as the amyloid peptide.[10−12] The peptide backbone can be re-established via a selective rearrangement (O → N acyl switch, Scheme 1), restoring the native peptide function.

In this study, the peptide guided assembly of a PEO−peptide conjugate (**III**) was investigated. The primary structure of the peptide segment shows five diades of alternating threonine and valine residues [(Thr-Val)$_5$]. According to the high β-sheet propensities of valine and threonine as well as the hydrophilic−hydrophobic repeat pattern matching the β-strand periodicity, the (TV)$_5$ domain favors the adoption of highly stable β-sheet secondary structures in water.[13] The functional (TV)$_5$ domain was extended by tryptophan as a spectroscopy marker and at both sides by glycine, contributing to the solubility at the N-terminus and to a decoupling of the aggregator segment from the PEO at the C-terminus. To sufficiently suppress the strong aggregation tendency of the (TV)$_5$ domain, an *all switch* derivative of the peptide segment (**II**) was synthesized by introducing switch defects in each TV segment (O$^{\beta}$ derivative of Thr[5], Thr[7], Thr[9], and Thr[11]; **II**).[15] The peptide was obtained by SPPS as outlined in Scheme 1. The applied Tentagel Wang-PAP resin[2,14] (**I**) had an acid labile ether linker, allowing the liberation of the PEO−peptide conjugate from the support after peptide synthesis.

Scheme 1[a]



[a] Reagents and Conditions: (i) Fmoc-AA, HBTU/DIPEA/NMP, 20 min; (ii) 20 vol % piperidine/NMP; (iii) Boc-Thr-OH, HBTU/DIPEA/NMP, 20 min; (iv) Fmoc-Val-OH, DIC/NMI/DCM, 2 × 2 h; (v) 30 vol % TFA/DCM; (vi) pH= 6.2 (O → N acyl switch).

The assembly of amino acids linked with native amide bonds was accomplished by automated standard Fmoc protocols. The switch ester segments were obtained by subsequent attachment of a *t*Boc-protected threonine derivative, having an unprotected hydroxyl side chain functionality (Boc-Thr-OH), followed by the coupling of Fmoc-Val-OH to this β-hydroxyl group (Scheme 1). For the latter, enforced coupling protocols using transesterfication conditions were necessary.[10]

The nonswitched PEO−peptide conjugate (**II**) was liberated from the support, isolated, and the molecular structure was confirmed by ^{1}H NMR spectroscopy. This reveals a ratio of the number average degree of polymerization of 72 EO units to 5 Val and 4 switch $^{O\beta}$Thr units, meeting the theoretical value of **II** within the experimental error (GWT-(VT)$_4^{switch}$-VG-PEO$_{68}$). MALDI-TOF mass spectrometry supports this since a mass distribution centered at *m*/*z* 3787 was observed with a spacing of *m*/*z* 44, characteristic for PEO, and mass peaks that are assignable with *m*/*z* ±1 accuracy (cf. Supporting Information, SI). The presence of an additional distribution centered at *m*/*z* 2806 and a distinct peak at *m*/*z* 1064 can be conclusively explained by in-source fragmentation during the ionization step in the MALDI process. The observable mass pattern suggests the fragmentation via a β-hydrogen transfer rearrangement at the first weak switch ester link (T^{11}V^{10}), leading to the corresponding GWT-(VT)$_3^{switch}$V and T-VG-PEO$_{68}$ fragments (cf. SI).

After the synthesis, **II** was readily dissolvable in water and the switch segments remained stable at a pH ≈ 5. Moreover, under these conditions, no aggregation or β-sheet formation could be observed. The increase of the pH leads to the deprotonation of the α-ammonium groups in the switch segments, triggering the O →

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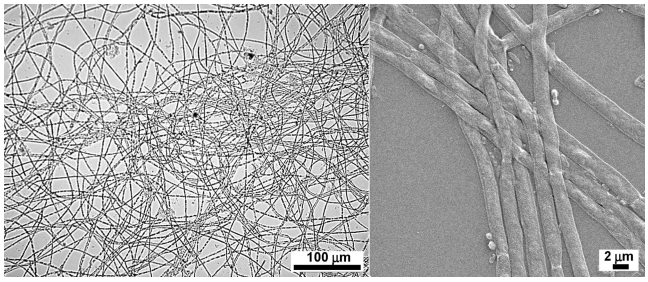

Figure 1. Spontaneously formed aggregates by peptide guided assembly of **III**. Light microscopy (left) and SEM micrograph (right).

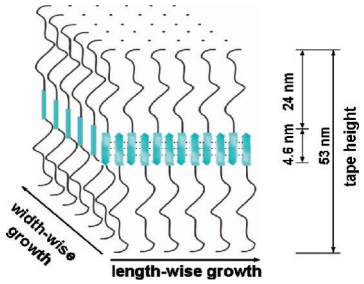

Figure 2. Idealized structure proposal for the aggregation of **III**.

N acyl switch, restoring the native peptide backbone and thus the aggregation tendency of the peptide (**III**). The rearrangement proceeds highly selective via a five-membered ring, and side reactions, such as intermolecular acyl transfer, were not reported.[9−11,15]

By adjusting the pH to 6.2 a slow, well-controlled O → N acyl switch occurred over a period of about 7 days. This was indicated by the disappearance of the absorption maximum in UV circular dichroism spectroscopy (CD) that is characteristic for the switch segment (210 nm). During this time, the spontaneous formation of long, nonbranched fibers was observed with light microscopy (Figure 1, left). These self-assembled fibers exhibit a width of about 2 μm and lengths up to several millimeters. The comparatively small mean curvature adapted by the fibers indicates a persistent behavior. The structures were isolated by filtration, washed with water, and characterized by FT-IR spectroscopy, verifying the absence of switch ester carbonyl bands ($\nu = 1745$ nm^{-1}) and the presence of amide I and amide II band, characteristic for β-sheet secondary structures (cf. SI). Moreover, ^{1}H NMR spectroscopy of **III** in DMSO-d_6 confirms conclusively the formation of 5 native threonine residues compared to the 72 PEO repeat units (cf. SI).

Scanning electron microscopy (SEM) revealed the formation of homogeneous tapes with an average width of 2.0 ± 0.5 μm (Figure 1, right). The crossing of two tapes in the SEM micrograph apparently suggests a small height, which was confirmed by AFM measurements with about 50 ± 5 nm (cf. SI).

Occasionally, a secondary growth process was observed, forming on top of the primary tapes additional layers with much smaller widths. The nucleation and growth processes of these secondary structures are currently under investigation.

Considering the observations summarized above and the literature describing peptide self-assembly,[2,16] a preliminary model is suggested. As outlined in Figure 2, the tape structure is formed by the aggregation of antiparallel β-sheets. Due to polarized FT-IR spectroscopy (data not shown), these are positioned parallel to the tape growth axis and perpendicular to the tape face. Thus the length of the tapes is determined by β-strand aggregation forming β-sheets (length-wise growth) and the width by stacking of these sheets as suggested for congener peptide tape structures (width-wise growth).[16] The β-sheet stacking is probably driven by the hydrophobic effect, minimizing the energy of both the hydrophobic valine and the fairly hydrophobic threonine sheet faces. The latter might be additionally stabilized by the formation of hydrogen bonds between the hydroxyl groups of threonine. The suggested dense packing of the peptide−PEO building blocks restricts the space available for the PEO coils, forcing the PEO in a brush-like configuration that covers the upper and the lower face of the tapes. This assumption is supported by the average height of the tapes of about 50 ± 5 nm (cf. SI). Considering 3.5 Å per amino acid in a β-strand and per repeat unit of an all-trans PEO chain, a tape height of 53 nm can be calculated.

Due to the prohibition of the twist, typically occurring in β-sheets, residual dipole moments that are compensated by the rotation of the β-strands in the β-sheet can build up. This overall dipole moment probably limits the growth of the tape width to a rather uniform 2 μm. By restricting the width, the system gains a degree of freedom to propagate in tape length (Figure 2).

In conclusion, a strong β-sheet-forming peptide was conjugated to PEO and utilized to guide the organization into tape structures. The aggregation tendency of the peptide was temporarily suppressed for ease of synthesis by the integration of multiple switch-peptide backbone defects. Rearrangement of the defects re-establishes the native peptide backbone, thus triggering the assembly by switching the aggregation properties on. Due to the slow generation of the peptide aggregator segments, assembly of the conjugates occurs in a highly controlled manner.

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Supporting Information Available: Materials, synthesis procedures, and characterization data for compounds **II** and **III** as well as characterization data of the aggregates (IR, AFM). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Rathore, O.; Sogah, D. Y. *J. Am. Chem. Soc.* **2001**, *123*, 5231. (b) Vandermeulen, G. W. M.; Tziatzios, C.; Klok, H. A. *Macromolecules* **2003**, *36*, 4107. (c) Ayres, L.; Vos, M. R. J.; Adams, P.; Shklyarevskiy, I. O.; van Hest, J. C. M. *Macromolecules* **2003**, *36*, 5967.
(2) Eckhardt, D.; Groenewolt, M.; Krause, E.; Börner, H. G. *Chem. Commun.* **2005**, 2814.
(3) Smeenk, J. M.; Otten, M. B. J.; Thies, J.; Tirrell, D. A.; Stunnenberg, H. G.; van Hest, J. C. M. *Angew. Chem., Int. Ed.* **2005**, *44*, 1968.
(4) Whitesides, G. M. *Small* **2005**, *1*, 172.
(5) Burkoth, T. S.; Benzinger, T. L. S.; Urban, V.; Lynn, D. G.; Meredith, S. C.; Thiyagarajan, P. *J. Am. Chem. Soc.* **1999**, *121*, 7429.
(6) (a) Aggeli, A.; Boden, N.; Zhang, S. *Self-Assembling Peptide Systems in Biology, Medicine and Engineering*; Kluwer Academic Publishers: Dordrecht, The Netherlands,, 2001; p 364. (b) Collier, J. H.; Messersmith, P. B. *Adv. Mater.* **2004**, *16*, 907. (c) Hamley, I. W.; Ansari, I. A.; Castelletto, V.; Nuhn, H.; Rosler, A.; Klok, H. A. *Biomacromolecules* **2005**, *6*, 1310.
(7) Quibbel, M.; Johnson, T. *Difficult Peptides. Fmoc Solid Phase Peptide Synthesis. A Practical Approach*. Oxford University Press: Oxford, 2000; pp 115−136.
(8) Sohma, Y.; Sasaki, M.; Hayashi, Y.; Kimura, T.; Kiso, Y. *Chem. Commun.* **2004**, 124.
(9) Mutter, M.; Chandravarkar, A.; Boyat, C.; Lopez, J.; Dos Santos, S.; Mandal, B.; Mimna, R.; Murat, K.; Patiny, L.; Saucede, L.; Tuchscherer, G. *Angew. Chem., Int. Ed.* **2004**, *43*, 4172.
(10) Carpino, L. A.; Krause, E.; Sferdean, C. D.; Schuemann, M.; Fabian, H.; Bienert, M.; Beyermann, M. *Tetrahedron Lett.* **2004**, *45*, 7519.
(11) Dos Santos, S.; Chandravarkar, A.; Mandal, B.; Mimna, R.; Murat, K.; Saucede, L.; Tella, P.; Tuchscherer, G.; Mutter, M. *J. Am. Chem. Soc.* **2005**, *127*, 11888.
(12) Sohma, Y.; Sasaki, M.; Hayashi, Y.; Kimura, T.; Kiso, Y. *Tetrahedron Lett.* **2004**, *45*, 5965.
(13) Janek, K.; Behlke, J.; Zipper, J.; Fabian, H.; Georgalis, Y.; Beyermann, M.; Bienert, M.; Krause, E. *Biochemistry* **1999**, *38*, 8246.
(14) Hartmann, L.; Krause, E.; Antonietti, M.; Boerner, H. G. *Biomacromolecules* **2006**, *7*, 1239.
(15) Beyermann, M.; et al. Manuscript submitted.
(16) Lamm, M. S.; Rajagopal, K.; Schneider, J. P.; Pochan, D. J. *J. Am. Chem. Soc.* **2005**, *127*, 16692.

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