Full Paper

CSIRO PUBLISHING

Aust. J. Chem. **2006**, *59*, 560–563

www.publish.csiro.au/journals/ajc

AFM Studies of β-Sheet Block Copolymers at Solid Surfaces: High-Resolution Structures and Aggregation Dynamics

Peter Schön,[A] *Jurgen M. Smeenk*,[B] *Sylvia Speller*,[A] *Hans A. Heus*,[C] *and Jan C. M. van Hest*[B,D]

[A] Scanning Probe Microscopy Laboratories, Institute for Molecules and Materials, Radboud University Nijmegen, 6525 ED Nijmegen, The Netherlands.

[B] Organic Chemistry Laboratories, Institute for Molecules and Materials, Radboud University Nijmegen, 6525 ED Nijmegen, The Netherlands.

[C] Laboratory of Biophysical Chemistry, Institute for Molecules and Materials, Radboud University Nijmegen, 6525 ED Nijmegen, The Netherlands.

[D] Corresponding author. Email: j.vanhest@science.ru.nl

Supramolecular fibre-like aggregates of different poly(ethylene glycol) modified β-sheet block copolymers have been adsorbed on a highly ordered pyrolytic graphite surface, and studied by tapping-mode atomic force microscopy. High-resolution images provided detailed information on stability, adaptability, and internal structure of the fibres. Dynamic processes observed in real space and time included fibre rearrangement, and even unprecedented fibre growth.

Manuscript received: 5 May 2006.
Final version: 8 July 2006.

Introduction

In materials science there is an increasing demand to develop smart building blocks which functional molecular devices form through the process of self-assembly. In many cases the design of these smart building blocks is inspired by natural materials such as peptides and proteins. The information stored, for example in the primary amino acid sequence of certain polypeptides, can be used to design a well-defined, predetermined folding pattern, resulting in directed interactions between peptide chains. Since the folding of polypeptides remains sensitive to processing conditions, polymer scientists have investigated methods to create more robust peptide-based materials. For this reason, hybrid materials based on synthetic polymers and peptides have gained much interest in recent years, since they combine the adaptability of synthetic polymers with the structural and functional control of polypeptides.[1–3] Polymers in which β-sheet forming elements are incorporated have been studied extensively.[4–12] The spontaneous assembly process of β-sheet peptides is very attractive for the construction of supramolecular polymeric aggregates. In most cases the incorporated β-sheet peptides were prepared by solid-phase peptide chemistry, and therefore, are relatively small, which limited the amount of information stored in the primary amino acid sequence. We have recently introduced a new class of hybrid block copolymers based on β-sheet polypeptides,[13] which were prepared by protein engineering.[14,15] By connecting poly(ethylene glycol) (PEG) chains to both ends of the well-defined polypeptide, fibres were formed with nanometre-scale control over height, width, and surface functionality. Characterization by transmission electron microscopy and atomic force microscopy (AFM) showed that extraordinarily long fibres were formed, which exhibited surprising assembly behaviour.

In order to better understand the stability and assembly process of these hybrid block-copolymer based fibres, we subsequently studied them in detail by high-resolution AFM, and the results are presented here. Striking dynamic behaviour of the fibres was observed when scanning by AFM, leading to spontaneous alignment. It is clear that these self-assembled structures are very robust and have the ability to self-organize by means of a hierarchical assembly process, starting with the folding of the peptides in β-sheets, followed by stacking of the β-sheet block copolymers into nanometre-sized fibres, and subsequent spontaneous alignment of the fibres to nano-patterned arrays. Therefore, these materials hold much promise for the modification of surfaces where functional control at the nanometre scale is required.

Results and Discussion

The general structure of the β-sheet triblock copolymers investigated in this study is shown in Fig. 1. The polypeptide part consisted of ten repeats of a hairpin-forming sequence $[(\text{Ala-Gly})_3\text{-Glu-Gly}]_{10}$ (Ala = alanine, Gly = glycine, Glu = glutamic acid), in which the glutamic acid moieties are positioned at the turn position. The attachment of PEG chains of 750 and 2000 g mol^{-1} at both ends of the β-sheet induced

10.1071/CH06160 0004-9425/06/080560

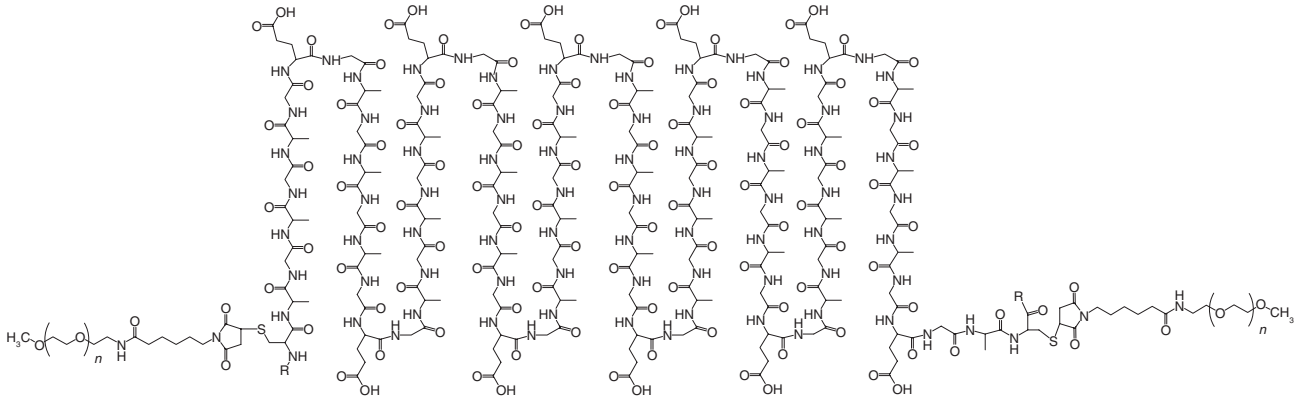

Fig. 1. Structure of poly(ethylene glycol) β-sheet triblock copolymers used in this study. For PEG_{750}-[$(Ala\text{-}Gly)_3$-Glu-Gly]$_{10}$-PEG_{750}, $n = 15$; for PEG_{2000}-[$(Ala\text{-}Gly)_3$-Glu-Gly]$_{10}$-PEG_{2000}, $n = 47$.

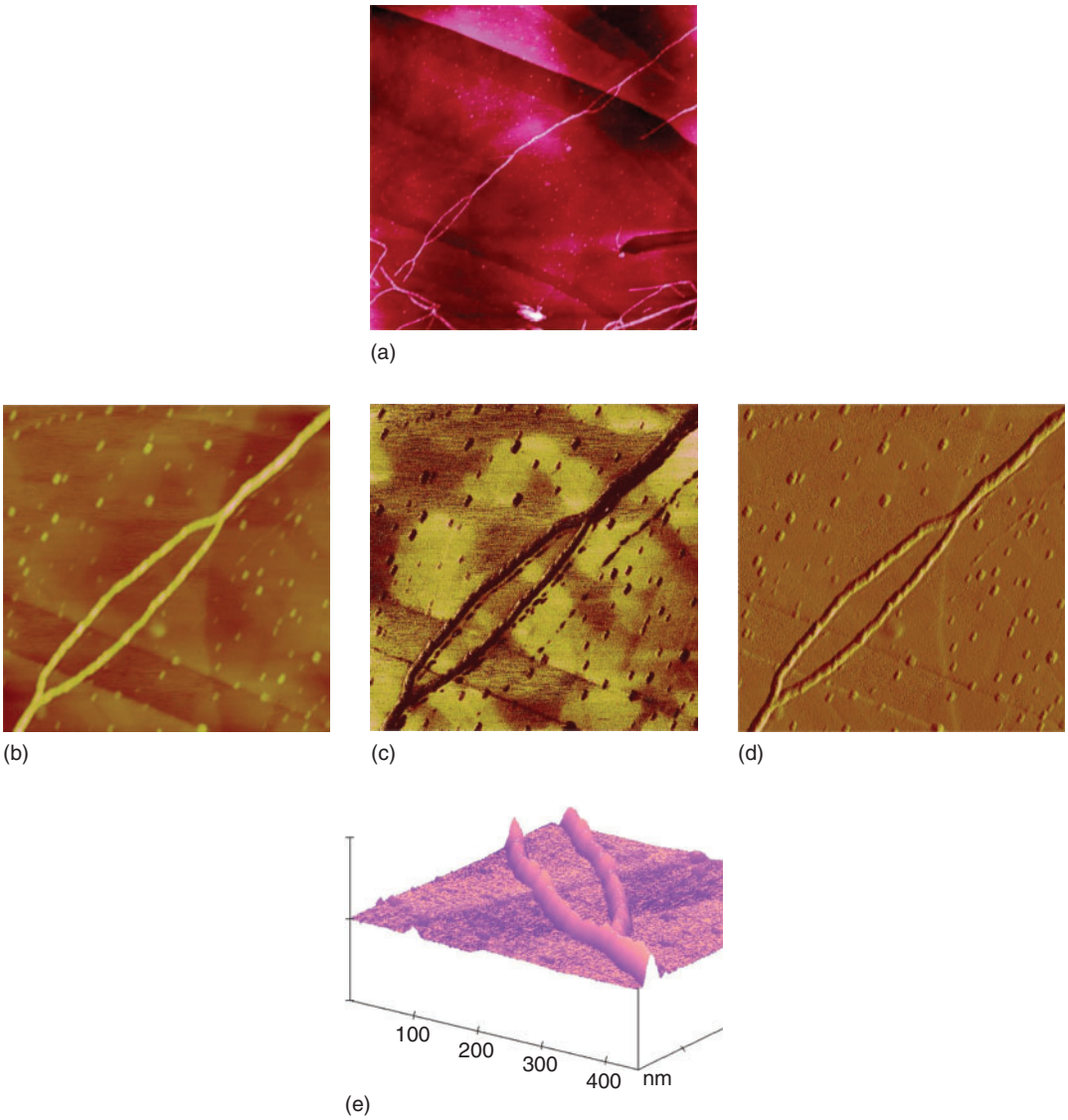

Fig. 2. (a) Tapping-mode AFM topography (2.5 μm × 2.5 μm, vertical scale $z = 7$ nm) of PEG_{2000}-[$(AG)_3EG]_{10}$-PEG_{2000} aggregates on HOPG in air (3.225 μm × 3.225 μm; scan rate 1001 Hz, $z = 10$ nm). (b) Further magnified area (800 nm × 800 nm, scan rate 1.969 Hz) simultaneously recording (b) height ($z = 7$ nm), (c) phase ($z = 5°$), and (d) amplitude ($z = 50$ mV). (e) Three-dimensional view derived from the height topograph in Fig. 3a, revealing fitting/adaptation of the β-sheet filament to the HOPG steps (vertical scale $z = 10$ nm, HOPG step height = 0.5 nm).

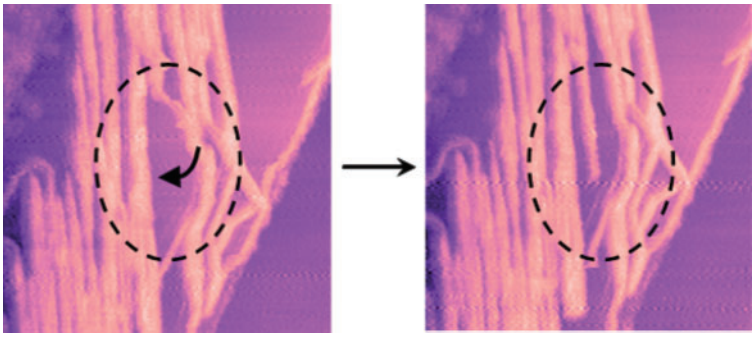

Fig. 3. Tapping-mode AFM topograph (500 nm × 500 nm, vertical scale $z = 4$ nm, scan rate 3.05 Hz) of PEG_{750}-$[(AG)_3EG]_{10}$-PEG_{750} aggregates on HOPG in air. Two consecutive scans indicate rearrangement of a fibre; black arrow indicates the direction of the net movement of the fibre.

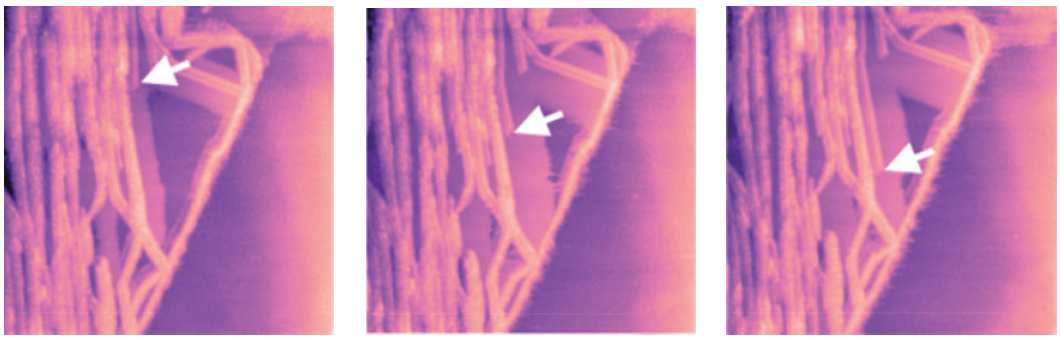

Fig. 4. Tapping-mode AFM topograph (600 nm × 600 nm, vertical scale $z = 4$ nm, scan rate 2.65 Hz) of PEG_{750}-$[(AG)_3EG]_{10}$-PEG_{750} aggregates on HOPG in air. Images after (a) 31, (b) 45, and (c) 60 scans; white arrow indicates the front of the growing fibre.

stable fibre formation.[15] Therefore, these two constructs were chosen for more detailed AFM investigation.

Solutions of β-sheet block copolymers in methanol were drop-cast on HOPG, and evaporated to dryness. Morphologies of formed aggregates were visualized by tapping-mode AFM in air.

High-resolution images were obtained for aggregates derived from PEG_{2000}-$[(Ala\text{-}Gly)_3\text{-}Glu\text{-}Gly]_{10}$-$PEG_{2000}$. In Fig. 2, a filamentous aggregate several micrometers in length is shown. The aggregate may comprise two single linear filaments sticking parallel/longitudinal to each other. Measured fibre heights within the presumed double strand ranged from 2.8 to 3.7 nm (full-width half-maximum, FWHM 20–22 nm), while the single filament showed heights from 2 to 2.8 nm (FWHM 15 nm). Further magnification revealed periodicities within the aggregate in all three signals (Figs 2b–2d) recorded simultaneously within the double (periodicity ∼50 nm) and single filaments (periodicity ∼25–35 nm). We think that this fine structure is not an AFM artefact, but could possibly be explained by the existence of preorganized ensemblages of block copolymers that subsequently are assembled into fibres. Fig. 2e shows a three-dimensional view derived from the height topography. The smooth adaptation of the filament to the HOPG steps can be seen, giving evidence for flexibility within the filamentous structure. These high-resolution images are in line with our previous measurements, and show that the β-sheet block copolymers are able to form long and stable fibres, with a strong tendency to align and stack. The propensity to align is considered to be a result of the interaction between the PEG polymers of different fibres. Similar supramolecular aggregates comprised of PEGylated oligothiophene have also been observed by Leclère et al.[16] The periodic internal structure is an indication that the fibres are built from smaller aggregates.

Figs 3 and 4 show topographies obtained of a sample of PEG_{750}-$[(Ala\text{-}Gly)_3\text{-}Glu\text{-}Gly]_{10}$-$PEG_{750}$. Densely packed domains and individual filaments with fibre-like signatures were observed, one of them aligning along a graphite step. Some non-ordered material deposits were seen.

A more detailed investigation over longer time revealed striking AFM topographies of reorganization processes within the two-dimensional aggregate, in specific rearrangements (Fig. 3), and filament growth (Fig. 4). In Fig. 3, the release process of a curved filament (indicated with an arrow) from the aggregate bundle to the right is displayed: the subsequent shift over a distance of approximately 50 nm to the left enables the fibre to straighten and to align with the fibre stack. Even more remarkable was the observation of growth of a single filament by approximately 300 nm within 120 min, as indicated by the arrows in the three selected AFM images of Fig. 4.

Both processes were observed using AFM. It is likely that the AFM imaging also influenced the observed dynamics by accelerating the equilibration process. The use of AFM

to manipulate matter has been shown.[17–19] Further, AFM studies on the dynamic processes of synthetic polymer molecules revealed dependencies on either vapour or solvent environments.[20] In this case, AFM was used for imaging, and the AFM tip could supply enough energy to induce reorganization only because of the tendency of the fibres to align. The latter process is more difficult to explain, and to our knowledge has not been observed before. Fibre growth could be followed over a longer period up to 120 min, after which no further rearrangement events could be observed. The continuous growth of the fibre rules out the possibility of artefacts induced by the presence of a double AFM tip. We conclude that what is observed is a crystallization process of the filaments/aggregates. For instance, non-ordered block copolymers surrounding the fibres could be picked up by the AFM tip and transferred to the growing filament. We are currently studying this phenomenon in more detail, for example, by changing scanning rate and direction. Full measurements revealing dynamic processes in more detail can be found as a movie in the Accessory Publication.

Conclusions

We have presented high-resolution images of β-sheet triblock copolymers. Important features were the fibre stability and tendency to align. High-resolution AFM also enabled us to study unusual processes of fibre alignment and even growth. This last observation is striking and is a subject of further studies. In the future, a better understanding of fibre behaviour and alignment will allow us to apply these fibres as scaffolds for the attachment of bioactive moieties at the β-turn positions.

Accessory Publication

A movie of AFM images showing the dynamic processes is available from the author or, until August 2011, the *Australian Journal of Chemistry*.

Experimental

Atomic Force Microscopy

For AFM imaging a Nanoscope IV multimode instrument (Veeco/Digital Instruments, Santa Barbara, CA), equipped with a 12-μm scanner (E scanner), was used.

Tapping in air was performed with 100-μm long standard silicon tips (NSG 10, ND-MDT, Moscow) with average nominal resonant frequency of 255 kHz, and average nominal force constant of 11.5 $nN\,nm^{-1}$. For high-resolution images, cantilever frequencies between 270 and 290 kHz were applied. Scanning speeds of 1–3 lines s^{-1} were applied, and height, phase, and amplitude signals were recorded. Amplitude during scanning was between 0.7 and 0.9 V.

Image processing (flattening) and data analysis were performed with the *Nanoscope ver. 5.12r5* software.

Sample Preparation

Commercially available highly ordered pyrolytic graphite (HOPG) plates were cut into small squares (~2 mm × 2 mm), glued to steel discs with cyanuracrylate and cleaved with tape immediately before use. PEG-modified β-sheet block copolymers were synthesized and crystallized into fibres according to Smeenk et al.[15] Crystallization of the conjugates was induced by vapour diffusion of methanol (Merck) into a solution of either 1 or 10 $mg\,mL^{-1}$ of conjugate in 70% formic acid (Merck). An Eppendorf tube containing the conjugate solution was placed in a Duran bottle containing 20 mL of methanol. The bottle was closed and incubation was carried out for two days. The fibres were dissolved in methanol to final concentrations of 10–100 $\mu g\,mL^{-1}$.

Samples (1–10-μL volumes) were placed on freshly cleaved HOPG surfaces and evaporated to dryness in air. Samples were mounted on the AFM sample stage and imaged in dynamic (tapping) mode in air.

References

[1] G. W. M. Vandermeulen, H. A. Klok, *Macromol. Biosci.* **2004**, *4*, 383. doi:10.1002/MABI.200300079

[2] C. D. H. Alarcon, S. Pennadam, C. Alexander, *Chem. Soc. Rev.* **2005**, *34*, 276. doi:10.1039/B406727D

[3] J. M. Smeenk, D. W. P. M. Löwik, J. C. M. van Hest, *Curr. Org. Chem.* **2005**, *9*, 1115. doi:10.2174/1385272054553622

[4] T. S. Burkoth, T. L. S. Benzinger, V. Urban, D. G. Lynn, S. C. Meredith, P. Thiyagarajan, *J. Am. Chem. Soc.* **1999**, *121*, 7429. doi:10.1021/JA991233X

[5] T. S. Burkoth, T. L. S. Benzinger, D. N. M. Jones, K. Hallenga, S. C. Meredith, D. G. Lynn, *J. Am. Chem. Soc.* **1998**, *120*, 7655. doi:10.1021/JA980566B

[6] A. Rosler, H. A. Klok, I. W. Hamley, V. Castelletto, O. O. Mykhaylyk, *Biomacromolecules* **2003**, *4*, 859. doi:10.1021/BM034058S

[7] H. A. Klok, A. Rösler, G. Götz, E. Mena-Osteritz, P. Bäuerle, *Org. Biomol. Chem.* **2004**, *2*, 3541. doi:10.1039/B415454A

[8] O. Rathore, D. Y. Sogah, *J. Am. Chem. Soc.* **2001**, *123*, 5231. doi:10.1021/JA004030D

[9] O. Rathore, D. Y. Sogah, *Macromolecules* **2001**, *34*, 1477. doi:10.1021/MA001553X

[10] J. Yao, D. Xiao, X. Chen, P. Zhou, T. Yu, Z. Shao, *Macromolecules* **2003**, *36*, 7508. doi:10.1021/MA034573I

[11] J. H. Collier, P. B. Messersmith, *Adv. Mater.* **2004**, *16*, 907. doi:10.1002/ADMA.200306379

[12] D. Eckhardt, M. Groenewolt, E. Krause, H. G. Börner, *Chem. Commun.* **2005**, 2814. doi:10.1039/B503275J

[13] M. T. Krejchi, E. D. Atkins, A. J. Waddon, M. J. Fournier, T. L. Mason, D. A. Tirrell, *Science* **1994**, *265*, 1427.

[14] J. M. Smeenk, M. B. J. Otten, J. Thies, D. A. Tirrell, H. G. Stunnenberg, J. C. M. van Hest, *Angew. Chem. Int. Ed.* **2005**, *44*, 1968. doi:10.1002/ANIE.200462415

[15] J. M. Smeenk, P. Schön, M. B. J. Otten, S. Speller, H. G. Stunnenberg, J. C. M. van Hest, *Macromolecules* **2006**, *39*, 2989. doi:10.1021/MA0521654

[16] P. Leclère, E. Hennebicq, A. Calderone, P. Brocorens, A. C. Grimsdale, K. Müllen, J. L. Brédas, R. Lazzaroni, *Prog. Polym. Sci.* **2003**, *28*, 55. doi:10.1016/S0079-6700(02)00033-3

[17] R. Garcia, R. V. Martinez, J. Martinez, *Chem. Soc. Rev.* **2006**, *35*, 29. doi:10.1039/B501599P

[18] L. B. Zhang, J. X. Shi, J. L. Yuan, S. M. Ji, M. Chang, *Mater. Sci. Forum* **2004**, *471*, 353.

[19] M. Guthold, W. Liu, B. Stephens, S. T. Lord, R. R. Hantgan, D. A. Erie, R. M. Taylor, R. Superfine, *Biophys. J.* **2004**, *87*, 4226. doi:10.1529/BIOPHYSJ.104.042333

[20] M. O. Gallyamov, B. Tartsch, A. R. Khokhlov, S. S. Sheiko, H. G. Borner, K. Matyjaszewski, M. Möller, *J. Microsc.-Oxford* **2004**, *215*, 245. doi:10.1111/J.0022-2720.2004.01381.X