Generation of Multiblock Copolymers by PCR
Alemdaroglu, Fikri E.; Zhuang, Wei; Zöphel, Lukas; Wang, Jie; Berger, Rüdiger; Rabe, Jürgen P.; Herrmann, Andreas
Published in: Nano Letters

DOI: 10.1021/nl901899t

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date: 2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 04-05-2019
Generation of Multiblock Copolymers by PCR: Synthesis, Visualization and Nanomechanical Properties

Fikri E. Alemdaroglu,†,§ Wei Zhuang,‡ Lukas Zöphel,† Jie Wang,† Rüdiger Berger,† Jürgen P. Rabe,*,‡ and Andreas Herrmann*,†,|,

Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany, and Humboldt University Berlin, Newtonstrasse 15, 12489 Berlin, Germany

Received June 13, 2009

ABSTRACT

PCR was successfully implemented into polymer chemistry to produce linear multiblock structures up to pentablock architectures. Salient features of the generated DNA polymer hybrids were the ultrahigh molecular weights and their structural accuracy. Besides pushing the limits in block copolymer synthesis, a highly sophisticated characterization of the DNA/synthetic polymer hybrids was carried out by scanning force microscopy (SFM). Direct visualization revealed single polymer chains with the expected contour lengths for the DNA blocks and a characteristic kink at the central organic polymer unit bridging them. Furthermore, DNA triblock copolymers were manipulated by SFM, which so far has only been demonstrated for neat DNA and dendronized polymers. Upon blowing circular topologies, the DNA and the organic polymer chain have been extended and the contours of the three blocks could thereby be imaged separately.

Block copolymers are attractive materials due to their variable and predictable morphologies and broad range of applications in the field of nanostructured materials.1–3 Although the first block copolymer has been synthesized nearly half a century ago, the development of new synthetic strategies of highly defined and complex block copolymer topologies is still progressing.4,5 Recently, a novel class of linear block copolymers has been introduced that contains DNA as a biological segment covalently linked to synthetic polymer units.6 As a consequence of connecting these two classes of materials, DNA block copolymers (DBC) originate that are outfitted with engineered material properties that cannot be realized with polymers or nucleic acids alone. Therefore, DBCs have rapidly found remarkable applications ranging from gene or drug delivery,7 sensitive DNA detection,9 to biomaterial purification,10 and as programmable nanoreactors.11 Several synthetic routes and coupling strategies were established to produce single stranded (ss) DBCs allowing to vary the nature of the organic polymer and the sequence composition of the oligodeoxynucleotide (ODN) segment.6 For the preparation of DBCs with an extended double stranded (ds) nucleic acid block, the polymerase chain reaction (PCR) in combination with ss DBCs was employed yielding di- and triblock architectures of the type A-DNA and A-DNA-A(B), respectively (A and B denote different organic polymer units).12 In this contribution, we tackled a different synthetic challenge, namely the extension of multiple primers coupled to a macromolecular non-nucleic acid moiety to generate more complex block copolymer architectures. This is an important synthetic task because it might allow one to employ DNA-side chain polymers and DNA-nanoparticles that are comprised of several ODN sequences as primers in PCR.13–15 In particular, we demonstrated that two primers connected via a flexible polymer can both be extended successfully employing PCR. As a result DNA multiblock copolymer structures with extended ds DNA segments of type DNA-A-DNA and A-DNA-A-DNA-A were obtained. Moreover, the significant molecular weight increase of the nucleic acid units allowed direct visualization of single block copolymers by scanning force microscopy (SFM), and even the nanomechanical properties of single bioorganic hybrids could be investigated by SFM.

In molecular biology PCR is an efficient technique to produce a specific DNA sequence in vitro by employing a DNA-template, two oligonucleotide primers, the four deoxynucleotide triphosphates (dNTPs), and a thermostable DNA polymerase in a three-step amplification process over several cycles.16 Because of its extreme sensitivity and specificity, it is commonly used in medical and biological research for
a variety of tasks, such as the detection of hereditary and infectious diseases, paternity testing, and the cloning of genes. We assumed that this technique could be transferred to polymer chemistry for the generation of well-defined multiblock copolymers with monodisperse, high molecular weight nucleic acid blocks. It was postulated that a triblock copolymer of type ss DNA-A-ss DNA as one primer and a conventional ODN as a second primer would lead to triblock copolymers of type ds DNA-A-ds DNA with extended nucleic acid segments. When instead of the ODN a ss DNA diblock copolymer is employed as a second primer, pentablock copolymers are generated. The lengths of the nucleic acid segments are determined by the annealing sites of the primers on the template (Figure 1).

Since polyethylene glycol (PEG) is known to function as an enhancer in PCR, PEG was selected as the organic component of the ss DBCs. The triblock copolymer primer ssTB1 was synthesized using a DNA synthesizer with a bisphosphoramidite PEG polymer as the key intermediate. Onto the central PEG domain (Mw = 2000 g/mol, PDI = 1.1), two identical ss ODNs were attached (22mer, sequence, 5′-CCTCGCTCTGCTAATC-3′; Mw = 6670 g/mol). Employing ssTB1 as backward primer, conventional ODNs as forward primers, and the plasmid (pBR322) as the template in the PCR process resulted in triblock copolymers of type ds DNA-b-PEG-b-ds DNA exhibiting nucleic acid units with lengths of 167, 225, and 500 base pairs (bp). To achieve effective amplification, an optimized PCR procedure was developed with an annealing time of 4 min, in contrast to a period of 30 s for denaturation and extension. Otherwise standard conditions for the thermal cycling protocol were employed. The triblock copolymers were analyzed by 1% agarose gel electrophoresis followed by ethidium bromide staining (Figure 2). The nonpolymer functionalized primers were selected to hybridize in increasing distance from ssTB1 on the template leading to increasing molecular weights of the nucleic acid segments. As controls, amplicons were generated that have the same sequence as the nucleic acid block present in the triblock structures. As expected, the triblock copolymers showed lower electrophoretic mobilities than the pristine DNA. In the case of DNA(167 bp)-b-PEG(2K)-b-DNA(167 bp) (Figure 2, lane 3) the largest shift was detected whereas DNA(500 bp)-b-PEG(2K)-b-DNA(500 bp) (Figure 2, lane 9) exhibited the lowest mobility. For DNA(225 bp)-b-PEG(2K)-b-DNA(225 bp) (Figure 2, lane 6) an intermediate mobility was observed. Beside characterization with gel electrophoresis, DNA-b-PEG-b-DNA triblock copolymers were characterized by restriction analysis with a sequence specific endonuclease to confirm the resulting triblock copolymer structures (see Supporting Information). Furthermore, since SFM has proven to be a powerful tool for visualizing single DNA molecules and isolated micelles of DBCs, the triblock copolymers were thus verified by direct visualization of single DBC molecules employing SFM. The samples were scanned in soft tapping mode in buffer on mica (Figure 3). A mean contour length of 344 ± 22 nm was measured for DNA(500 bp)-b-PEG(2K)-b-DNA(500 bp) as an average from 100 polymer molecules. This yields a rise per bp of 0.34 ± 0.02 nm which is in good agreement with the expected value for ds DNA in the B-form. Frequently a kink of the polymer chain was observed at half contour length, which can be explained by the presence of a short flexible polymer bridging the equally sized DNA blocks. In the case of triblock copolymers with a nucleic acid block of 225 bp, a mean length of 171 ± 13 nm was calculated that results in a rise per bp of 0.35 ± 0.03 nm. For the triblock copolymer with DNA blocks of 167 bp, a contour length of 123 ± 11 nm was determined that is slightly higher than the theoretically expected value. Control experiments with pristine ds DNA of 167, 225, and 500 bp showed only single DNA fragments of stretched polymer chains as expected for a semiflexible polymer with a persistence length of 50 nm. Kinks within these structures were not observed (see Supporting Information).

In order to realize pentablock architectures, ssTB1 and several ss DNA diblock copolymers (ssDB1, ssDB2, and ssDB3) were employed in the PCR process. In contrast to

**Figure 1.** Schematic representation of the generation of DNA pentablock copolymers by PCR.

**Figure 2.** Gel electrophoretic analysis of the DNA multiblock copolymers. Lane 1 shows DNA ladder (10000—100 bp). Lanes 2, 5, and 8 are the pristine DNA controls with 167, 225, and 500 bp, respectively. Lanes 3, 6, and 9 represent the triblock copolymers with 167, 225, and 500 bp nucleic acid blocks, respectively. Lanes 4, 7, and 10 show DNA pentablock copolymers with 167, 225, and 500 bp DNA segments, respectively.
modified ODNs as reported previously. From the combination of ssTB1 and ssDB1, ssDB2, or ssDB3 as set of primers with a dotted arrow. Figure 4c shows the resulting structures after dragging by the SFM tip along the moving trace marked in Figure 4b, the triblock polymer was elongated to 432 nm, which means that compared to the original length of 356 nm the triblock polymer was 1.4 times elongated upon dragging across the surface.

In Figure 4a–c, the PEG polymer incorporated in the middle of the triblock cannot be distinguished from ds DNA. This can be attributed to the fact that the polymer may relax into a densely packed globule after manipulation and before imaging, due to restoring forces on the one hand and low surface friction on the other. For the case of the short and flexible PEG block without specific interactions with the dodecyamine-coated HOPG surface, assuming a bulk density of PEG of 1.2 g/cm³, the 2000 g/mol PEG block may condense into a cylinder with 2 nm diameter (same as ds DNA) and only 1 nm length, which is so small that it cannot be distinguished from the ds DNA in the SFM images.

In order to distinguish different blocks in a single copolymer chain or in a copolymer thin film, the key is to control the conformations of the different blocks on the surface, which in our case for single molecule imaging is limited by the compact PEG globule. However, this limit has been overcome by SFM imaging after a “blowing manipulation”, in which the PEG moiety could be clearly distinguished from ds DNA by SFM (Figure 5a). In this experiment, a thin film of chloroform was spin-coated on the same surface in order to generate a surface pressure inside a topological crossover triblock loop by a tapping SFM tip, which can stretch and overstretch the triblock chain and even stabilize the overstretched chain. Figure 5a displays a blown triblock hybrid loop, where a thin polymer chain with about 18 nm length bridges two thick chains aside. The length of the thin part corresponds to the stretched contour length of the PEG exhibiting about 48 repeat units (3.77 Å per PEG repeat unit) and about 2110 g/mol molecular weight. Figure 5b shows a raw height profile of the down part of the blown chain, indicating a clear height dip in the middle. According to a height profile along the whole contour after correction for the background height, the thin chain part has an average height of 0.46 ± 0.08 nm, while the two thick chain parts exhibit an average height of 0.80 ± 0.17 nm. Therefore we attribute the thin chain in the middle to the PEG block, and the thick chains aside to ds DNA.

The elongation of the single triblock molecule reveals the unique mechanical properties of ds-DNA, that is, B-form ds DNA can be overstretched to the S-form by a factor of 1.7 times in solution or 2 times on a surface. In the triblock dragging experiment, we noticed that both ends of the triblock were almost immobilized on the surface, which may be due to high surface friction resulting from high concentration of dodecyamine underneath. The final elonga-
tion of the triblock chain of 1.4 times is therefore the average elongation of different pieces along the whole chain. On the other hand, the manipulation of ds DNA by an SFM tip has shown that the maximum force acting on the molecule is at the position where the SFM tip contacts the molecular chain.34 Interestingly, the scission of the triblock does not occur at the position loaded by the maximum force but almost at the center region of the triblock. It is consistent with the fact that ss DNA has a much smaller Young’s modulus than ds DNA under the same force loading conditions.31 SFM cantilever pulling experiments have also proven that the covalent bond in polysaccharide can be ruptured at about 1000 pN while ds DNA remains unbroken at the same force.35 Similarly, in our horizontal manipulation of triblock molecules the single PEG backbone breaks at a lower force (about 1500 pN at 100 nm/s horizontal pulling velocity) than ds DNA, which is consistent with a much stronger break force.34

In summary, PCR was successfully implemented into polymer chemistry to produce complex linear multiblock structures up to pentablock architectures. Concurrently, it was demonstrated for the first time that PCR extension of DNA triblock copolymer primers is feasible. Salient characteristics of the DNA polymer hybrids were the high molecular weights exceeding 600 000 g/mol and their structural accuracy. Noteworthy are the modularity of the approach and the ease of controlling the molecular weights of the biological blocks that can be adjusted by the annealing sites of the polymer functionalized or conventional ODN primers on the template. Besides gel electrophoresis and restriction analysis, the DNA multiblock architectures were characterized by SFM. Direct visualization revealed single polymer chains on mica with the expected contour lengths for the DNA blocks and a characteristic kink at the central organic polymer unit bridging them. Furthermore, in order to investigate their nanomechanical properties the triblock hybrids were manipulated by an SFM tip on alkylamine precoated graphite, which so far has only been demonstrated for neat DNA and dendronized polymers. In addition, a recently developed blowing manipulation was applied to blow circular topologies, so that the ds DNA and the organic polymer chain have been extended and the contours of the three blocks could thereby be imaged separately on the surface. To the best of our knowledge, this experiment afforded for the first time to visualize the three blocks of a single linear triblock copolymer chain with recognizable contours by SFM. Moreover, dragging-breaking experiments revealed that the
single PEG backbone breaks at a force at which the ds DNA backbones keep unbroken, thereby identifying the mechanical weak point of the DNA-polymer hybrids.

Acknowledgment. This work, as part of the European Science Foundation EUROCORES Program BIONICS, was supported by funds from the EC Sixth Framework Program. Additional funding was provided by the ERA-CHEMISTRY and the DFG through Sfb 625 “From Single Molecules to Nanostructured Materials” and Sfb 448 ”Mesoscopically Organized Composites.

Supporting Information Available: Preparation of tri-block and pentablock DNA copolymers, restriction analysis, details about SFM visualization and manipulation. This material is available free of charge via the Internet at http://pubs.acs.org.

References
(23) Saenger, W. Principles of nucleic acid structure; Springer-Verlag: New York, 1984; p 556.

NL90189NT