Peptide Organogels

DOI: 10.1002/anie.200801056

Organogelation of Sheet–Helix Diblock Copolypeptides**

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Polypeptide-based materials have recently attracted much attention for the development of stimuli-responsive, soft materials.[1] This is due to their chemical and structural similarities to natural biomolecules such as silk fibers, catalytic enzymes, and neuro-debilitating amyloid plaques.[2] Noncovalent hierarchical assembly of peptides and proteins leads to precisely defined nano- and microstructures with significantly higher levels of complexity than could be obtained from nonpeptide block copolymers or low-molecular-weight amphiphiles. Hybrid copolymers where one block is a rigid α-helical or β-sheet motif and the other a flexible coil are commonly employed to create nanoscale objects based upon phase separation and stacking interactions.[3] Herein we report the discovery of thermoreversible gelation of well-defined diblock copolypeptides, which contain both an α-helical and a β-sheet motif. We also propose a new mechanism for gelation based upon the self-assembly of the polymers as probed by SEM, IR, and X-ray diffraction.

Our initial studies involved the synthesis of well-defined block copolypeptides of poly(*O*-benzyl-L-threonine) (PBnT) with either poly(γ-benzyl-L-glutamate) (PBnE) or poly(ε-*N*-Boc-L-lysine) (PBocK) by the sequential ring-opening polymerization of *N*-carboxyanhydrides (Table 1 and Figure 1 a).[4] Homopolymers of the constituent blocks displayed excellent solubility in $CHCl_3$ (> 100 mg mL^{-1} at room temperature) whereas the block copolymers required either heating to > 50 °C or sonication to induce dissolution. To our surprise,

Table 1: Molecular parameters and critical gelation concentrations of the block copolymers.

Block copolymer	M_n[a]	M_w/M_n	CGC [wt %][b]
$PBnT_{47}$-*b*-BnE_{28}	15 500	1.60	4
$PBnT_{26}$-*b*-BnE_{22}	10 200	1.47	4
$PBnT_{50}$-*b*-BnE_{25}	14 500	1.71	3
$PBnT_{16}$-*b*-BnE_{16}	6800	1.54	–
$PBnT_{16}$-*b*-$BocK_{19}$	7300	1.47	–
$PBnT_{46}$-*b*-$BocK_{18}$	12 800	1.54	5
$PBnT_{20}$-*b*-$BocK_{22}$	8900	1.60	–
$PBnT_{20}$-*b*-$BocK_{46}$	14 300	1.60	6

[a] Determined by SEC and ^{1}H NMR spectroscopy (see the Supporting Information). [b] Critical gelation concentration by inversion tests at room temperature in $CHCl_3$.

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[**] This work was supported by a doctoral training account award from the Engineering and Physical Sciences Research Council (EPSRC) (UK).

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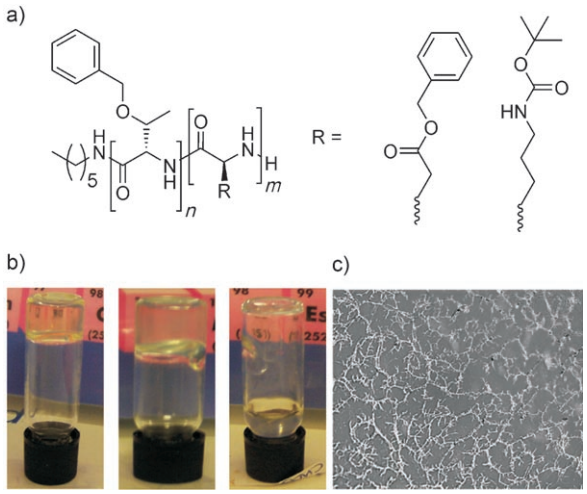

Figure 1. a) Polymers used in this study. Subscripts denote the number average degree of polymerization. R = γ-benzyl-L-glutamate (left), ε-Boc-L-lysine (right). b) Chloroform solutions of $PBnT_{25}$-*b*-$BocK_{18}$. From left to right: 6, 5, 4 wt %. c) Optical micrograph (image 250 μm across) of $PBnT_{26}$-*b*-BnE_{22} cast from CH_2Cl_2 solution.

$PBnT_{47}$-*b*-BnE_{28} formed transparent, thermoreversible gels in chloroform upon standing at room temperature (gel structure was lost on heating to > 35 °C). Gelation was also observed in dichloromethane (DCM) and tetrachloroethane, but not in benzyl alcohol, *N*,*N*-dimethylacetamide (DMAc), or *N*,*N*-dimethylformamide (DMF). Subsequently, the solution properties of a range of block copolymers were examined. The critical gelation concentration (Table 1) appeared to be related to the PBnT length; < 20 units did not give rise to gelation at concentrations up to 8 wt %, whereas $PBnT_{46}$-*b*-BnE_{28} resulted in gelation at a concentration as low as 3 wt %. Changing the second block from PBnE to PBocK also resulted in polymers that produced organogelation (Figure 1 b). Binary mixtures of the homopolymers in varying ratios did not result in any gelation.

Polymeric gelators (distinct from the more common situation where micelles or vesicles are formed)[5] support the solvent by the formation of a network structure by the self-assembly of rigid insoluble blocks, chain-end entanglements, or association of protein motifs (such as α helices,[6] β sheets,[7] and leucine zippers[8]). Given the highly soluble nature of the individual homopolymers in chlorinated solvents, the occurrence of gelation is surprising. Scanning electron microscopy (SEM) indicated a range of morpholo-

gies including those of a highly porous nature (Figure 2a) and some which were more dendritic in nature (Figure 2b). The fiber diameters as measured by SEM were between 0.37 to 1.31 μm: several orders of magnitude larger than the dimensions of the constituent polymers.

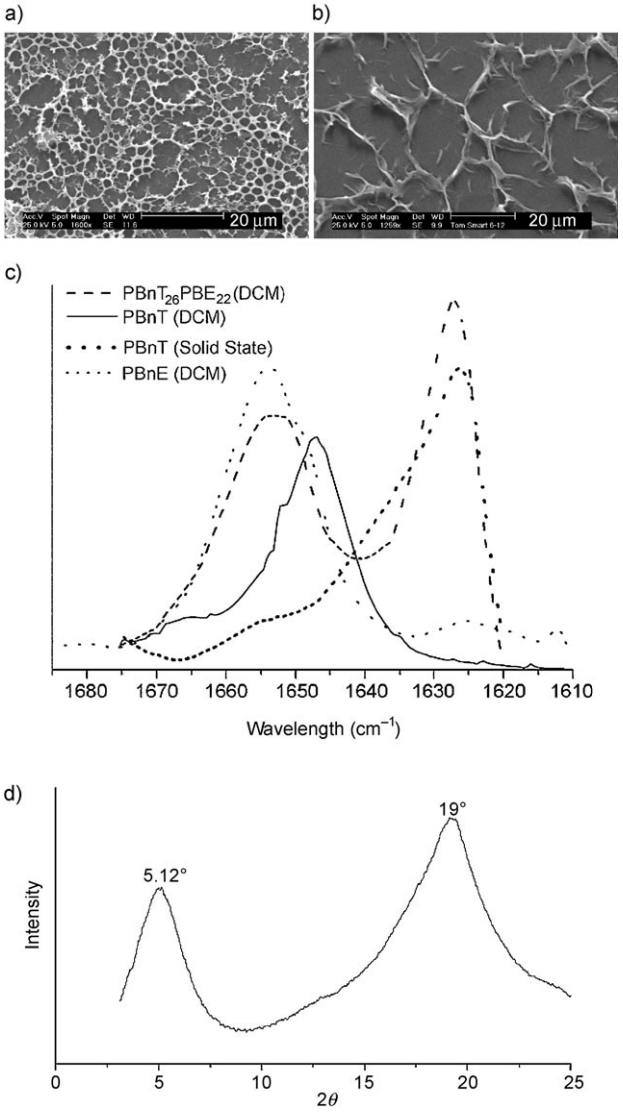

Figure 2. SEM images of dried-down samples of a) $PBnT_{16}$-*b*-$BocK_{19}$ and b) $PBnT_{26}$-*b*-BnE_{22}. c) Infrared spectra of the amide I region of $PBnT_{26}$-*b*-BnE_{22}, PBnT, and PBnE in dichloromethane (DCM) and PBnT in the solid state. d) WAXS spectrum of $PBnT_{45}$-*b*-$BocK_{18}$.

To investigate the origin of the gelation behavior, the secondary structure of the block copolymers in dilute CH_2Cl_2 was investigated by FTIR (Figure 2c). The amide I region of the IR spectrum of PBnE shows a peak at 1652 cm^{-1} which is characteristic of its well-known α helix. PBnT displays a peak at 1647 cm^{-1}, which is presumed to be a random coil rather than an α-helical conformation (threonine is a helix-breaking residue[9]). The solid-state spectrum of PBnT has a peak at 1630 cm^{-1}, indicating the presence of β sheets. For $PBnT_{26}$-*b*-BnE_{22}, the characteristic α-helix peak of PBnE is present, but there is also a peak at 1627 cm^{-1} which suggests that PBnT retains its solid-state, β-sheet structure when conjugated to PBnE. The formation of β sheets in solution from β-sheet-forming peptides has previously been shown to be promoted by conjugation to poly(ethylene glycol) (PEG),[10] but not to our knowledge by conjugation to α-helical sequences. Changing the helical block to PBocK also resulted in macroscopic gelation and the formation of assembled networks, indicating that the structural reorganization occurs upon conjugation to a different helix-forming second block. The wide-angle X-ray scattering (WAXS) pattern for $PBnT_{46}$-*b*-$BocK_{18}$ (Figure 2d) gave *d* spacings of 16.9 and 4.55 Å. Similar results were obtained for the PBnE-containing polymer. A *d* spacing of 4.55 Å has previously been attributed to antiparallel β-sheet formation in poly(L-alanylglycine)[11] and has also been observed in the self-assembled PEG-*b*-β-sheet-peptides described by Klok and co-workers.[12] In the latter case, the sheets aligned into lamella with an insulating PEG layer. The separation between lamella was 12.7 Å, compared to 16.9 Å in the present system. The increased separation can be attributed to the steric bulk of the benzyl protecting groups on the threonine residues, together with the helices which are aligned in an unfavorable parallel fashion, rather than the preferred antiparallel alignment. A schematic of this is shown in Figure 3. A possible explanation for the morphologies

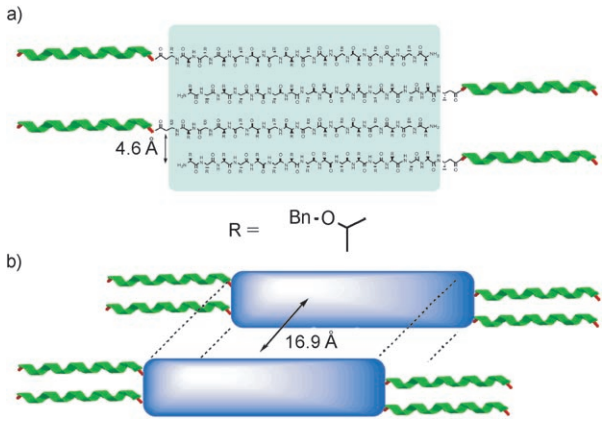

Figure 3. Proposed mechanism of self-assembly. a) Antiparallel β sheet with protruding helices ($PBnT_{16}$-*b*-BnE_{16}). b) Stacking of lamella structures into tapes for $PBnT_{46}$-*b*-$BocK_{18}$.

observed by SEM (Figure 2a and b) is that the tapes produced by lamella stacking twist to minimize unfavorable interactions between parallel α helices. Connections between curved tapes can then result in a porous morphology, as observed in diblock polypeptide hydrogels.[13]

Addition of 5 wt% trifluoroacetic acid (TFA) (PBnE) or MeOH (PBocK) results in complete loss of gelation, indicating that hydrogen bonding is involved in the macroscopic gelation process. This is in contrast to the system described by Manners et al.[14] in which the stacking of α helices in rod–coil block copolymers was insensitive to the addition of H-bond-disrupting solvents. However, the rather low temperature at which our gels lose structure (around 35 °C) is unexpected for an H-bonded network and may indicate denaturation of the

β-sheet assembly. A thorough circular dichroism study would be required to confirm this.

In summary, this investigation shows for the first time that combining the two most ubiquitous secondary structure motifs found in natural polypeptides is possible by the well-known polymerization of *N*-carboxyanhydrides. Uniquely, the β-sheet-forming block (poly(benzyl-L-threonine)) is individually soluble, but undergoes a conformational change upon growth of the second block. Self-assembly is driven by the onset of β-sheet formation in PBnT, which itself is an interesting phenomenon. We believe this is the first time that macroscopic organogelation has been reported from entirely peptide-based polymers and may lead to new insights into the mechanisms of macroscopic fiber formation. The gelation of organic solvents by degradable polymers could have applications in art restoration,[15] whereas the polypeptides themselves could serve as templates for nanoscale engineering[16] or as degradable, porous structures.

Experimental Section

Synthesis and characterization of block copolymers: L-Threonine(*O*-benzyl), L-lysine(ε-*N*-Boc), and γ-benzyl-L-glutamate *N*-carboxyanhydrides were synthesized from their corresponding *N*-Boc amino acids as previously reported.[17] Polymerization was undertaken using *N*,*N*-dimethyl acetamide as the solvent and *n*-hexylamine as the initiator, by the sequential addition of monomers.

For the organogels: A sample of the block copolymer was sonicated in a sealed vial containing the desired solvent. It was then allowed to stand at room temperature for 2 h after which the vial was inverted for 1 h then photographed. The absence of flow after 1 h of inversion was used to assign the gel state. All measurements were repeated twice.

Optical microscopy and SEM: The samples were prepared by casting a dilute (< 0.1 mg mL^{-1}) CH_2Cl_2 solution of the polymer onto a glass substrate and dried under a flow of N_2 overnight. Where appropriate, SEM samples were sputter-coated with a thin layer of gold to improve resolution. SEM was performed on a FEI XL30 ESEM operating between 20–25 kV. WAXS was undertaken using a Bruker D8 operating at 40 kV using a copper-alpha source with a wavelength of 1.54 Å with a 2-D CCD detector.

Received: March 4, 2008
Revised: April 2, 2008
Published online: June 4, 2008

Keywords: carboxyanhydrides · gels · peptides · polymerization · self-assembly

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