Organometallic−Polypeptide Block Copolymers: Synthesis and Properties of Poly(ferrocenyldimethylsilane)-*b*-poly(γ-benzyl-L-glutamate)

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Block copolymer self-assembly in solution and the solid state is attracting intense current attention as a route to nanostructured and hierarchical materials with a variety of potential applications.[1] Hybrids of synthetic and biological polymers are of particular interest. Typically, synthetic macromolecules differ with respect to their biological counterparts such as proteins in that their self-assembly is usually driven by largely unspecific hydrophobic interactions. In contrast, the self-assembly of biopolymers such as proteins involves directed hydrogen bonding and both electrostatic and hydrophobic interactions.[2] Block copolymers with synthetic and biological blocks may display interesting self-assembly phenomena and allow the creation of hybrid supramolecular materials.[3,4] To the best of our knowledge, virtually all studies in this field to date have focused on the use of synthetic organic polymers.[5]

The incorporation of metallopolymer segments into well-defined block copolymer structures has received considerable recent interest for the generation of self-assembled supramolecular materials with novel chemical and physical properties. For example, the incorporation of metal centers offers the possibility of introducing redox activity, catalytic behavior, and interesting photophysical properties.[6−9] Well-defined polyferrocenylsilanes (PFSs) are available via the ring-opening polymerization (ROP) of [1]ferrocenophanes.[7,8] Living anionic ROP has provided a route to PFS block copolymers that self-assemble in thin films to yield semiconductive, magnetic, etch-resistant, redox-active, or catalytically active metal-rich nanodomains.[9] Self-assembly of PFS block copolymers in solution yields redox-active and magnetic preceramic micellar aggregates such as spheres, cylinders, and nanotubes.[10] In this communication, we describe the synthesis and structural characterization of the first organometallic−polypeptide block copolymers, poly(ferrocenyldimethylsilane)-*b*-poly(γ-benzyl-L-glutamate) (PFS-*b*-PBLG).[5] We also report the deprotection of this material to form the water-soluble organometallic−polypeptide block copolymers, PFS-*b*-poly(L-glutamic acid) (PFS-*b*-PGA), and some preliminary studies concerning their self-assembly behavior.

Polymerization of an α-amino acid−*N*-carboxyanhydride (α-NCA) is the most common method for preparing block copolypeptides and block copolymers of polypeptides and organic polymers.[11] Although the living metal-catalyzed ROP of α-NCA was discovered recently by Deming,[12] which allows the formation of block copolymers and block copolypeptides, and an elegant method to prepare block copolypeptides by genetic engineering is available,[13] amino-terminated polymers have been widely used as macroinitiators because of the availability of varieties of amino-terminated polymers.[14] Utilizing the benefits of anionic ROP of [1]silaferrocenophanes, we prepared amino-terminated PFS **2** with a narrow molecular weight distribution (Scheme 1).[15]

Dimethyl[1]silaferrocenophane (**1**) was polymerized anionically for 45 min at room temperature in tetrahydrofuran (THF) using *sec*-butyllithium as an initiator. The polymer solution was then cooled to −76 °C, and the living chain end was quenched with a 5-fold excess of 1-(3-bromopropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane. After stirring for 30 min at −76 °C and an additional 1 h at room temperature, the amber solution was precipitated into methanol. Since the tetramethyl-1-aza-2,5-disilacyclopentane protective group is labile toward methanol, complete deprotection was achieved during the precipitation step. Thin-layer chromatography (silica, CH_2Cl_2 eluent) of the resulting amber solid showed two distinct ferrocene-containing bands, indicating that PFS functionalization was not quantitative. The amino-terminated PFS (PFS-NH_2) (**2**) was successfully separated from unfunctionalized PFS (PFS-H) using column chromatography and was isolated in 45% yield. The ^{1}H NMR of the purified PFS-NH_2 in C_6D_6 showed a signal at 2.47 ppm, which corresponds to the methylene protons adjacent to the terminal amino group.[16] By comparison of this signal with the cyclopentadienyl (Cp) signal of the PFS at 4.2 ppm, a number-average degree of polymerization of 38 was obtained for **2**. This value corresponds well to the M_n obtained by gel permeation chromatography (GPC) (M_n = 9330 g/mol, M_w/M_n = 1.10).

The macroinitiator PFS-NH_2 (**2**) was used for the ROP of γ-benzyl-L-glutamate NCA (γ-Bn-Glu NCA) in order to obtain the organometallic−peptide block copolymers. NCA polymerization is usually carried out in dimethylformamide (DMF), but as PFS **2** is insoluble in this solvent, polymerization was carried out by adding an equal volume of a solution of **2** in THF to a solution of γ-Bn-Glu NCA in DMF. The resulting mixture was stirred at room temperature for 48 h, after which the resulting viscous amber solution was precipitated into methanol. After vacuum-drying, the amber block copolymers were obtained as glassy solids in good yields. All block copolymers were easily soluble in common organic solvents such as THF, CH_2Cl_2, and chloroform, but only in hot DMF. The block ratio of PBLG block copolymers is usually determined by ^{1}H NMR spectroscopy in chloroform containing 1% trifluoroacetic acid (TFA), since TFA is well-known to prevent aggregation of PBLG.[17] However, because of the sensitivity of PFS to acid, the ^{1}H NMR spectrum of PFS-*b*-PBLG (**5**) was obtained in $CDCl_3$.[16] The block ratio between the PFS and PBLG segments was determined by comparison of the ^{1}H NMR signals for the benzyl protons of PBLG at 5.03 ppm and the Cp protons of PFS at 4.20 ppm (Table 1).

GPC traces of the block copolymers, with THF containing 0.003 M tetrabutylammonium bromide ([^{n}Bu$_4$N]-Br) as an eluent, were monomodal and showed narrow molecular weight distributions (<1.2). The molecular

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Scheme 1. Synthesis of Polyferrocenylsilanes (PFSs) with a Terminal Amino Group and Poly(ferrocenyldimethylsilane)-*b*-poly(γ-benzyl-L-glutamate) (PFS-*b*-PBLG) Block Copolymers

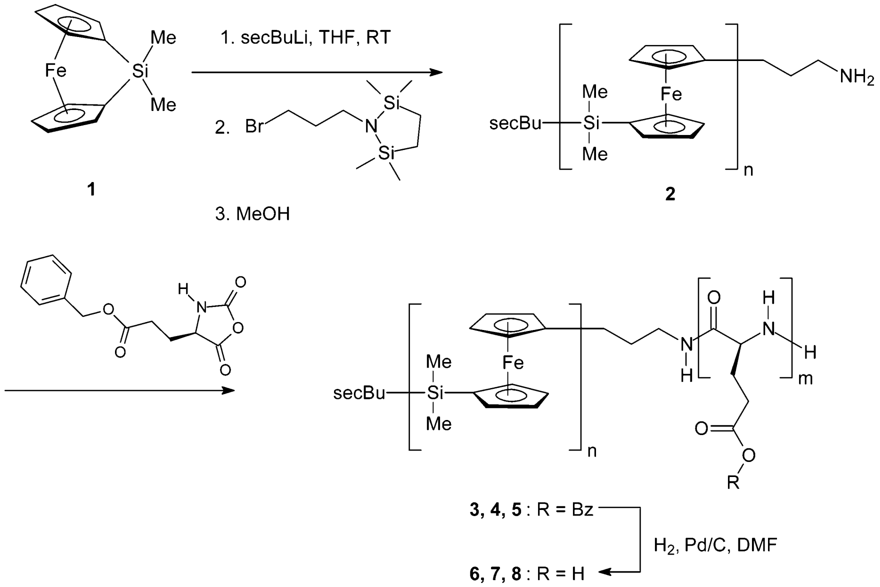

Table 1. Characterization of Poly(ferrocenyldimethylsilane)-*b*-poly(γ-benzyl-L-glutamate) (PFS-*b*-PBLG) 3–5

polymer	DP_n (PFS)[a]	DP_n (PBLG)[b]	M_n	PDI (M_w/M_n)	block ratio (PFS:PBLG)[h]
PFS-NH_2 **2**	38	–	9330[c]	1.10[f]	–
PFS-PBLG **3**	38	69	94200[d] (30200)[e]	1.14[g]	1:1.8
PFS-PBLG **4**	38	95	104400[d] (26900)[e]	1.13[g]	1:2.5
PFS-PBLG **5**	38	133	121800[d] (38500)[e]	1.16[g]	1:3.5

[a] Degree of polymerization of PFS obtained by ^1H NMR. [b] Degree of polymerization of PBLG obtained by ^1H NMR. [c] Number-average molecular weight of PFS macroinitiator obtained by gel permeation chromatography (GPC) with tetrahydrofuran (THF) and polystyrene standards. [d] Number-average molecular weight of PFS-*b*-PBLG obtained by GPC (0.003 M tetrabutylammonium bromide ([^{n}Bu$_4$N]Br) in THF) with polystyrene standards. [e] Molecular weight of PFS-*b*-PBLG calculated by ^1H NMR. [f] Molecular weight polydispersity index (PDI) of PFS macroinitiator obtained by GPC with THF. [g] PDI of PFS-*b*-PBLG obtained by GPC (0.003 M [^{n}Bu$_4$N]Br in THF). [h] Number-average block ratio between PFS and PBLG obtained by ^1H NMR.

weights obtained were highly overestimated compared to the molecular weight obtained from ^1H NMR, presumably due to the stiff rodlike conformation of the PBLG block in the GPC eluent. The possibility of aggregation of block copolymers in THF/[^{n}Bu$_4$N]Br was ruled out from dynamic light scattering (DLS) experiments. Preliminary DLS experiments on solutions of **5** (2 mg/mL, 90° acquisition angle), the block copolymer with the longest PBLG block, were carried out to examine whether the molecules were significantly aggregated under the GPC elution conditions. The small value of the apparent hydrodynamic radii in THF (5.7 nm) and in 0.003 M [^{n}Bu$_4$N]Br in THF solution (4.1 nm) were consistent with individual molecules of low polydispersity.[16] At number-average degree of polymerizations above about 14, PBLG adopts an α-helix conformation in both the solid state and solutions.[18] FT-IR spectra (NaCl, Nujol) of the block copolymers showed the amide I and amide II bands at 1650 and 1550 cm^{-1}, respectively, which are characteristic of the α-helix conformation.[19]

The thermal transition behavior of the block copolymers was studied by differential scanning calorimetry (DSC). All block copolymers showed a glass transition at ca. 29 °C for the PFS block and a 7/2 to 18/5 α-helix transition[20] for the PBLG block at ca. 110 °C. In addition, weak first-order transitions were observed in both the second heating scan and cooling scans, which indicates that the block copolymers have crystallization and melting transitions (Figure 1). Polarized optical microscopy (POM) revealed that strong birefringence appeared at 90 °C upon cooling the block copolymers from the isotropic melt at 170 °C to near the first-order transition. The birefringence of the block copolymers disappeared on reheating the sample above the first-order transition temperature. On the basis of the POM observations, the first-order transition in the DSC cooling scan at ca. 79 °C was assigned to an isotropic-to-liquid crystalline phase transition, and the first-order transition in the heating scan at ca. 92 °C was attributed to the liquid crystalline-to-isotropic phase transition. This suggests that the PFS-*b*-PBLG block copolymers are in a thermotropic liquid crystalline state at 90 °C.[21] Exploiting the advantage of the high electron density of the PFS block, direct observation of the liquid crystalline phase was carried out with transmission electron microscopy (TEM) (Figure 1, right). The specimen was prepared by cooling the isotropic melt of **4** to 90 °C. The TEM image showed a lamella phase with alternating PBLG and PFS layers with ca. 20 nm periodicity, which nearly coincides with the molecular dimensions of **4**.

Amphiphlic block copolymers, poly(ferrocenyldimethylsilane)-*b*-poly(L-glutamic acid) (PFS-*b*-PGA), were obtained by hydrogenation of the PBLG block with H_2/Pd in DMF. The deprotected block copolymers (**6**–**8**) were completely soluble in aqueous base (0.1 M NaOH). The complete removal of benzyl groups was confirmed by the absence of benzyl protons in the ^1H NMR spectrum taken in D_2O. Only signals of the PGA block were observed because the PFS in the micelle core is too immobile for its proton signals to be resolved in the ^1H

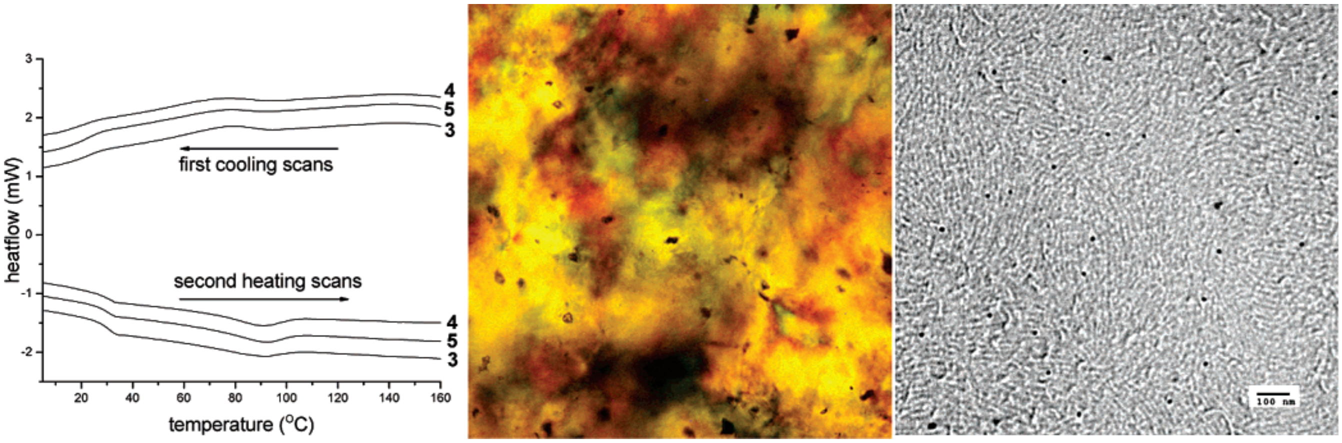

Figure 1. Differential scanning calorimetry (DSC) thermograms of block copolymers **3**−**5** (left) and representative polarized optical microscopy (POM) (middle) and transmission electron microscopy (TEM) images (right) for the block copolymer **4**. The dark lines in the TEM micrograph indicate polyferrocenylsilane rich domains. Scale bar: 100 nm.

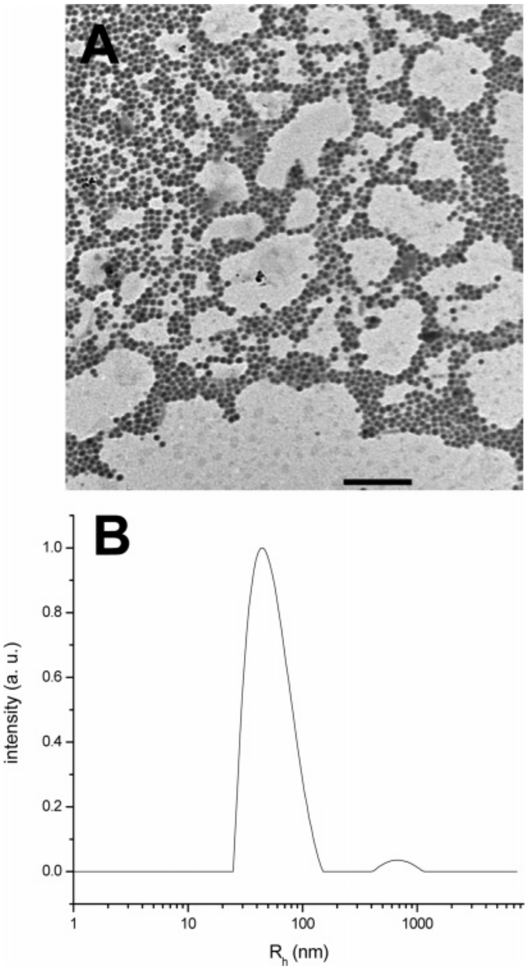

Figure 2. (A) Transmission electron microscopy (TEM) image of micelles of poly(ferrocenyldimethylsilane)-*b*-poly(L-sodium glutamate) (**8**) in water. Scale bar represents 500 nm. (B) Dynamic light scattering result of an aqueous micelle solution of **8** (1 mg/mL).

NMR. Micellar solutions of PFS-*b*-poly(L-sodium glutamate) in water (pH = 6.6) were analyzed by TEM and DLS (Figure 2). DLS studies of the micelle solution showed a mean apparent hydrodynamic radius ($R_{h,app}$) of 48.7 nm with a polydispersity index of 0.154. The unstained TEM images showed PFS domains (ca. 40 nm) with near spherical shapes, which correspond to the cores of the micelles. All PFS domains are segregated due to the surrounding PGA corona. The micelle solution was stable at neutral or basic pH, but upon decreasing the pH to 4, substantial aggregation of micelles resulting in precipitation was observed, presumably due to intermicellar hydrogen bonding.

In summary, we synthesized a series of new organometallic−polypeptide block copolymers, PFS-*b*-PBLG, by using amino-PFS (**2**) as a macroinitiator for the ROP of γ-Bn-Glu NCA. In bulk, the resulting block copolymers showed thermotropic liquid crystalline behavior. Deprotection of the PBLG block resulted in amphiphilic block copolymers, PFS-*b*-PGAs, which form spherical micelles in aqueous solution with an organometallic core and a polypeptide shell. The solid and solution state self-assembly behavior of the block copolymers discussed in this contribution are interesting for the development of stimuli-sensitive materials due to the redox-active ferrocene moieties in the organometallic block. A detailed study of their self-assembly behavior will be presented elsewhere.

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Supporting Information Available: Experimental details for the synthesis of PFS-NH_2 (**2**) and block copolymers **3**−**5**, hydrogenation of **5**, and GPC and DLS results. This material is available free of charge via the Internet at http://pubs.acs.org.

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