Secondary Structure-Driven Self-Assembly of Thiol-Reactive Polypept(o)ides

Tobias A. Bauer, Jan Imschweiler, Christian Muhl, Benjamin Weber, and Matthias Barz*

ABSTRACT: Secondary structure formation differentiates polypeptides from most of the other synthetic polymers, and the transitions from random coils to rod-like α-helices or β-sheets represent an additional parameter to direct self-assembly and the morphology of nanostructures. We investigated the influence of distinct secondary structures on the self-assembly of reactive amphiphilic polypept(o)ides. The individual morphologies can be preserved by core cross-linking via chemoselective disulfide bond formation. A series of thiol-responsive copolymers of racemic polysarcosine-block-poly(S-ethylsulfonyl-DL-cysteine) (pSar-b-p-(DL)Cys), enantiopure polysarcosine-block-poly(S-ethylsulfonyl- DL-cysteine) (pSar-b-p(l)Cys), and polysarcosine-block-poly(S-ethylsulfonyl-DL-homocysteine) (pSar-b-p(l)Hcy) was prepared by N-carboxyanhydride polymerization. The secondary structure of the peptide segment varies from α-helices (pSar-b-p(l)Hcy) to antiparallel β-sheets (pSar-b-p(l)Cys) and disrupted β-sheets (pSar-b-p(DL)Cys). When subjected to nanoprecipitation, copolymers with antiparallel β-sheets display the strongest tendency to self-assemble, whereas disrupted β-sheets hardly induce aggregation. This translates to worm-like micelles, solely spherical micelles, or ellipsoidal structures, as analyzed by atomic force microscopy and cryogenic transmission electron microscopy, which underlines the potential of secondary structure-driven self-assembly of synthetic polypeptides.

INTRODUCTION

Secondary structure formation as a feature of polypeptides is the fundamental basis for the higher ordered structural diversity and specific function of proteins and other biomaterials.1–3 As a prominent example, the polyalanine domains of spider silk undergo a transition from α-helices in solution to highly ordered β-sheets in the solid state, which, among other factors, accounts for feasible processing paired with exceptional elasticity and toughness.4–7 Consequently, not only from a polymer chemistry perspective, secondary structure displays an additional parameter influencing the preparation and application of peptide materials.8–11 For polypeptides derived from single amino acids, primarily, α-helices or β-sheets are obtained, with exceptions for proline-type amino acids.11,12 Upon secondary structure formation, the induced coil-to-helix or coil-to-sheet transitions refer to a rigidification of the polymer segment (rod-like structures) and provide a driving force for self-assembly.12,13 In particular for synthetic polypeptides of polylglutamic acid and polyllysine, the stimuli that induce α-helix formation, such as pH or temperature, have been the subject of multiple investigations.14–20 Furthermore, Deming and co-workers intensively elucidated the thermo- and redox-responsiveness of α-helical polyhomocysteine derivatives.21,22 On the other hand, for polycysteine or polycysteine, research has been more dedicated to resolving the strong induction of β-sheet formation, e.g., by side chain functionalization with oligo ethylene glycol moieties.23–25

In contrast to polypeptides, polypeptoids, with the branching on the nitrogen atom instead of the α-position, do not show classical secondary structure formation as no hydrogen bond donor is present if no specific modification was introduced.26–28 As such, polysarcosine (pSar, poly(N-methyl glycine)) adopts random coil conformation in aqueous solution, is highly water-soluble (A2 parameter of 3.50–4.50 mol·dm⁻³·g⁻² for pSarAC at 20 °C), and can be considered a stealth-like material.29–33 For medical applications, polysarcosine is considered the most promising alternative to polyethylene glycol since substantial advantages like reduced proinflammatory cytokine secretion and reduced complement activation as well as evasion of the accelerated blood clearance (ABC) phenomenon have been reported.34–36 The combination of polypeptides and polysarcosine in polypept(o)ides thus represent a promising class of biobased or even

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endogenous materials. While the concept of polypeptide-oligomers extends from linear block copolymers to cylindrical bottle-brush and star-shaped polymers including miktoarm stars, polysarcosine itself has further been successfully combined with various other classes of synthetic polymers.

With its ability to form bioreversible disulfide bonds, the non-essential amino acid cysteine plays a unique role within nature. In living cells, catalyzing proteins and small molecules orchestrate disulfide bond formation among specific cysteines generating functional proteins. To transfer chemo-selective disulfide bond formation to functional polymers, our group reported on the reactive S-alkylsulfonyl-protecting group for cysteine and homocysteine. This reactive group differentiates hard and soft nucleophiles and grants living amine-initiated ring-opening polymerization of S-alkylsulfonyl-protected N-carboxyanhydrides (NCAs). The S-alkylsulfonyl group can then be addressed by post polymerization protection.

MATERIALS AND METHODS

Unless stated otherwise, reagents and solvents were purchased from Sigma-Aldrich and used as received. N,N-Dimethylformamide (DMF) was bought from Acros (99.8%, extra dry over molecular sieves) and purified by repetitive freeze–pump thaw cycles prior to use (water content, <50 ppm). N,N-Tert-Butoxy carbonyl (Boc)-1,2-diaminoethane was obtained from Sigma-Aldrich (>98%) and stored over activated molecular sieves before further use. Milli-Q water was prepared using a MILLI-Q Reference A+ system. Water was used at a resistivity of 18.2 MΩ·cm and a total organic carbon (TOC) of <5 ppm. Hexafluoroisopropanol (HFIP) and potassium trifluoracetate were purchased from Fluorochem. Deuterated solvents were obtained from Deutero GmbH and were used as received.

1H NMR spectra were recorded on a Bruker Avance III 400 at room temperature at a frequency of 400 MHz. DOSY spectra were recorded on a Bruker Avance III HD 400 (400 MHz). Calibration of the spectra was achieved using the solvent signals. NMR spectra were analyzed with MestReNova version 12.0.4 from Mestrelab Research S.L. Degrees of polymerization (D) by 1H NMR were calculated by comparing the integral of the initiator peak and the integrals of the α-protons. For block copolymers containing polysarcosine, the chain lengths of the second block were calculated from the integral of the α-protons and the signals of the polysarcosine backbone, as derived from HFIP GPC relative to polysarcosine standards.

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy was performed on a FT/IR-4100 (JASCO Corporation) with an ATR sampling accessory (MIRacle, Pike Technologies). IR spectra were analyzed using Spectra Manager 2.0 (JASCO Corporation). NCA polymerization was monitored by FT-IR spectroscopy, and the polymerization was judged to be completed if the carbonyl peaks at 1858 and 1788 cm−1 had vanished.

Analytical gel permeation chromatography (GPC) was performed on a Jasco system at a flow rate of 0.8 mL min−1 and a temperature of 40 °C. The eluent was HFIP equipped with 3 g L−1 potassium trifluoracetate. The column material was a modified silica gel (PF Gaussian columns; particle size, 7 μm; porosity, 100 and 400 Å), purchased from PSS Polymer Standards Service GmbH. For polymer detection, a UV detector (Jasco UV-2075+) at a wavelength of λ = 230 nm was employed. Molecular weights were determined by using a calibration with PMMA (PSS Polymer Standards Services GmbH) with toluene as an internal standard. The elution diagram was evaluated with PSS WinGPC (PSS Polymer Standards Service GmbH).

Circular dichroism (CD) spectroscopy was performed on a Jasco J-815 spectrometer at a temperature of 20 °C using Spectra Manager 1.53.04 (Jasco). The spectra were recorded in HFIP at a concentration of 0.1 g L−1 using a quartz cell with a path length of 1 mm. θ20 was calculated with the equation below: θ20 = 0.1 g L−1·l = 0.1 cm, and M̅(repeating Unit) = 195.26 g mol−1 for S-ethylsulfonyl-cysteine and S-ethylsulfonyl-υ-cysteine, M̅(repeating Unit) = 209.26 g mol−1 for S-ethylsulfonyl-υ-homocysteine, and M̅(repeating Unit) = 71.08 g mol−1 for sarcosine.

Atomic force microscopy (AFM) was measured on mica using Cypher AFM (Asylum Research) using tapping mode at a scan rate of 1 Hz. Samples were prepared by drop-casting of a particle solution (V = 5 μL and ϕ = 50 mg mL−1 in Milli-Q water) onto freshly cleaned mica. The sample was dried overnight at room temperature. Images were evaluated using Gwyddion 2.49.

For cryogenic transmission electron microscopy (CryoTEM), 5 μL of CCPM solution (50 mg L−1, in Milli-Q water) was applied to freshly glow-discharged carbon grids with a copper 200 mesh (Quantifoil Micro Tools GmbH). The excess fluid was removed by direct blotting (2.5 s), and the grids were individually plunge-frozen in liquid ethane. Grids were cryo transferred in liquid nitrogen using a Gatan cryoholder (model 626 DH) to a Tecnai T12 transmission electron microscope equipped with a field emission electron source and operating at 120 kV accelerating voltage. Images were recorded using a TemCam-F416 (TVIPS, Gauting, Germany). Software ImageJ 1.52h (National Institutes of Health, USA) was used for image evaluation.

Polycation Synthesis. Polymers were prepared by amine-initiated ring-opening N-carboxyanhydride (NCA) polymerization in dry DMF under Schlenk conditions. The respective monomers, sarcosine-NCA, S-ethylsulfonyl-υ-cysteine-NCA, S-ethylsulfonyl-υ-cysteine-NCA, and S-ethylsulfonyl-υ-homocysteine-NCA, were synthesized and characterized as reported previously.

Polysarcosine (pSar)17 (P1 and P2). Sarcosine-NCA (5.98 g, 52.0 mmol, and 200 equiv) was transferred into a predried Schlenk tube and dissolved in 60 mL of dry DMF, and N-(ter-butoxycarbonyl)-1,2-
diaminoethane (41.6 mg, 0.26 mmol, and 1.0 equiv) was added as a stock solution in dry DMF. The clear, colorless solution was stirred at 10 °C in the absence of light until the reaction was completed after six days, as monitored by IR spectroscopy. The sarcosine amino terminal was quenched by addition perfluorophenyl-4-azidobutanoate (153 mg, 0.52 mmol, and 2.0 equiv) and N,N-diisopropylethylamine (221 µL, 1.30 mmol, and 5.0 equiv). The reaction mixture was stirred overnight followed by addition of acetic anhydride (248 µL, 2.60 mmol, and 10 equiv) and N,N-diisopropylethylamine (884 µL, 5.20 mmol, and 20 equiv) to react with residual end groups. The slightly yellow solution was stirred for one additional day at room temperature before the polymer was precipitated in 600 mL of diethyl ether, collected on a Buchner funnel, washed with acetone and diethyl ether, and dried in vacuo. Next, the Boc-protecting group was removed. The polymer (3.4 g) was dissolved in 50 mL of water and stirred at room temperature for 18 h and protected from light. The sarcosine amino terminus was quenched by addition perfluorophenyl-4-azidobutanoate (153 mg, 0.52 mmol, and 2.0 equiv) and N,N-diisopropylethylamine (221 µL, 1.30 mmol, and 5.0 equiv). The reaction mixture was stirred overnight followed by addition of acetic anhydride (248 µL, 2.60 mmol, and 10 equiv) and N,N-diisopropylethylamine (884 µL, 5.20 mmol, and 20 equiv) to react with residual end groups. The slightly yellow solution was stirred for one additional day at room temperature before the polymer was precipitated in 600 mL of diethyl ether, collected on a Buchner funnel, washed with acetone and diethyl ether, and dried in vacuo. Next, the Boc-protecting group was removed. The polymer (3.4 g) was dissolved in 50 mL of water and cooled to 0 °C, and 50 mL of trifluoroacetic acid was added in one portion. After 4 h at 0 °C, the solution was transferred into dialysis bags (MWCO, 3.5 kDa) and dialyzed against Milli-Q water, saturated sodium hydrogen carbonate solution, and Milli-Q water. The aqueous solution was lyophilized, and the polysarcosine (P1) was obtained as a colorless powder (3.08 g, 82%).

**Scheme 1. Synthesis of Block Copoly promotides with Varying Secondary Structures**

**Block Copolymer Syntheses.** All block copolymers were prepared from pSar macroinitiators, following the procedure described below for pSar-b-p(DL)Cys using the respective NCAs.

**Polysarcosine-b-block-poly(S-ethylsulfonyl-L-cysteine)**

Polysarcosine-b-block-poly(S-ethylsulfonyl-L-cysteine)ₙ (pSarₙ-b-p(3εCysₙ)) (P3–P6). The polysarcosine macroinitiator (111.9 mg, 9.2 µmol, and 1.0 equiv) was weighed into a predried Schlenk tube and dried by azotropic distillation with toluene in vacuo twice. Next, the macroinitiator was dissolved in freshly degassed dry DMF (1.45 mL) and cooled to −10 °C, and S-ethylsulfonyl-L-cysteine NCA (33.0 mg, 138 µmol, and 15 equiv) was added as a stock solution in dry DMF. The polymerization was performed at an overall mass concentration of [β] = 100 g L⁻¹ and monitored by IR spectroscopy. After 5 days, full conversion was observed, and the polymer was precipitated in THF. The suspension was centrifuged (4500 rpm, 15 min, and 4 °C) and decanted. This procedure was repeated twice concluding with pure diethyl ether. The product was dried in vacuo yielding pSarₙ-b-p(3εCysₙ) (P3) as a colorless solid (130 mg, 93%).

**Polysarcosine-b-block-poly(S-ethylsulfonyl-L-homocysteine)**

Polysarcosine-b-block-poly(S-ethylsulfonyl-L-homocysteine)ₙ (pSarₙ-b-p(3ε2S-DLHcyₙ)) (P7–P10). The polymerization was performed at an overall mass concentration of [β] = 100 g L⁻¹ and monitored by IR spectroscopy. After 5 days, full conversion was observed, and the polymer was precipitated in THF. The suspension was centrifuged (4500 rpm, 15 min, and 4 °C) and decanted. This procedure was repeated twice concluding with pure diethyl ether. The product was dried in vacuo yielding polysarcosine standards.29

**Cross-Linker Synthesis.** The synthesis of (R)-lipoic acid hydrazide (3) was performed in a two-step synthesis via (R)-methyl lipoate (2) starting from (R)-lipoic acid (1). The respective protocols were adapted and modified from Hassan and Malmén and Koufaki et al.51,52

(R)-Lipoic acid (2.0 g, 9.69 mmol, and 1.0 equiv) was dissolved in 20 mL of methanol, and a catalytic amount of sulfuric acid (0.20 mmol, and 0.01 equiv) was added. The reaction mixture was stirred at room temperature for 18 h and protected from light. The solvent was removed under reduced pressure, and the crude solid was redissolved in dichloromethane. The organic phase was washed with...
saturated NaHCO₃ solution and brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. (R)-Methyl lipoate (2) was obtained as a yellow oil (1.90 g, 94%) and used without further purification. ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 6.92 (b s, 1H, NHCO), 4.14 (b s, 2H, −NH), 3.60 (m, 1H, −CH=O), 3.14 (m, 2H, −SCH₂), 2.40 (m, 1H, −SCH₂CH₂−), 2.00 (t, J = 7.3 Hz, 2H, −COCH₂), 1.85 (m, 1H, −SCH₂CH₂−), 1.70−1.43 (m, 4H, −β-CH₂−δ-CH₂), 1.38−1.27 (m, 2H, −γ-CH₂−).

(R)-Methyl lipoate (2) (1.9 g, 8.6 mmol, and 1.0 equiv) was dissolved in 10 mL of methanol, and hydrazine hydrate (1.3 mL, 25.8 M (calc.), 243.06 g/mol). ESI-MS (m/z = 243.20): [M + Na]^+. 

RESULTS AND DISCUSSION

To investigate the influence of secondary structure on the self-assembly of thiol-reactive polypept(o)ides, amphiphilic block copolymers of racemic polysarcosine-block-poly(S-ethylsulfonyl-DL-cysteine) (pSar-b-p(DL)Cys, P3−P6), enantiopure polysarcosine-block-poly(S-ethylsulfonyl-L-cysteine) (pSar-b-p(L)-Cys, P7−P10), and polysarcosine-block-poly(S-ethylsulfonyl-L-homocysteine) (pSar-b-p(L)Hcy, P11−P14) have been prepared.

As shown in Scheme 1, all polymers were synthesized by living ring-opening NCA polymerization. The preparation of block copolymers, when reacted at −10 °C in DMF, the reactive S-ethylsulfonyl-protecting group remains intact, and a shift compared to the pSar macroinitiator can be detected by GPC in hexafluoroisopropanol (HFIP) (Figure 1), which indicates successful chain extension. 

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Self-Assembly and Core Cross-Linking. The preparation of core cross-linked polymeric micelles was adapted and modified from Klincker et al. Each polypept(o)ide was assembled at the respective turning point. 

pSar−b−p(x)Cys (x = 1, 3, 10) were obtained as a yellow oil (1.22 g, 64%) and used without further purification. ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 3.25−3.03 (s + m, 4H, −CH₂=O−CHOH), 3.15 (m, 2H, −SCH₂), 2.42 (m, 1H, −SCH₂CH₂−), 2.31 (t, J = 7.3 Hz, 2H, −COCH₂), 1.87 (m, 1H, −SCH₂CH₂−), 1.73−1.48 (m, 4H, −β-CH₂−δ-CH₂), 1.42−1.30 (m, 2H, −γ-CH₂−).

Figure 1. Analytical gel permeation chromatography in HFIP. (A) pSar−b−p(x)Cys (P3−P6), (B) pSar−b−p(L)Cys (P7−P10), and (C) pSar−b−p(L)Hcy (P11−P14), with the respective pSar macroinitiators (P1 and P2).
shown in Figure 2A, predominant signals of the amide bands at 1546 and 1317 cm⁻¹ refer to the α-helical p(L)HCy block despite the coexistence with the random coil of polysarcosine, which overlaps for the amide I peak. Furthermore, CD spectroscopy in HFIP showed an increasing concision of the characteristic signals (minima at 212 and 226 nm, maximum at 194 nm) for growing p(L)HCy chain lengths confirming the α-helical structure (Figure S2).62 For enantiopure pSar-b-p(L)Cys copolymers, the shoulder at 1703 cm⁻¹ (red arrow) accounts for the antiparallel orientation of the β-sheets, while no characteristic peaks can be distinguished for racemic pSar-b-p(L)Cys copolymers. When analyzed by NMR spectroscopy (Figure 2B), block copolymers of pSar-b-p(L)Cys show much broader peaks compared to enantiopure pSar-b-p(L)Cys, suggesting a rather atactic polymer structure similar to observations for the racemic p(DL)Cys homopolymers.48 Taken together and referring to the secondary structures detected for the respective homo- and copolymers in solution by circular dichroism spectroscopy,47-49 GPC, IR, and NMR spectroscopy confirm the successful preparation of reactive block copoly(e)ptide(s) with secondary structures varying from α-helices to disrupted or antiparallel β-sheets.

The influence of secondary structure on solution self-assembly was investigated by a titration experiment. As illustrated in Figure 3A, the reactive polypeptide(s) were dissolved in a good solvent, N,N-dimethylacetamide (DMAc), and water, equipped with 1 mM sodium acetate (pH 4.75), was added stepwise. When a certain buffer concentration is reached, polymers start to assemble, and the scattering
Sigmoidal shapes were obtained for all aggregation curves; the three copolymers of comparable chain lengths showed significant variations in their aggregation behavior (Figure 3B). Sigmoidal shapes were obtained for all aggregation curves; however, the sigmoidal fit revealed a turning point at 27.3 ± 0.8% for the enantiopure pSar200−b-p(1)Cys10, while turning points of 51.2 ± 0.2 and 32.3 ± 1.0% were derived for racemic pSar170−b-p(DL)Cys11 and pSar170−b-p(1)Hcy10, respectively. Moreover, the final plateau level of pSar200−b-p(1)Cys810 was found to be ~3-fold higher compared to racemic pSar170−b-p(DL)Cys11 and ~2-fold higher compared to pSar170−b-p(1)Hcy10. As the primary structure does not differ significantly among the selected copolymers, the observed effects can be attributed to the variation of the secondary structure, which confirms antiparallel β-sheets as an assembly-promoting character stronger than α-helices, likely caused by attractive interactions between individual β-sheets.

When the chain lengths of the hydrophobic polypeptide blocks are increased, turning points generally shift to aggregation at lower buffer concentrations for all copolymers (Figure 4). In addition, the dynamic range, thus the area between the start of the aggregation and the primary plateau, becomes narrower by increasing the hydrophobic segment. As such, the dynamic range extends from 20 to 50 vol % buffer for pSar170−b-p(1)Hcy44 but from 15 to 30 vol % buffer for pSar170−b-p(DL)Hcy44. Furthermore, the trend of antiparallel β-sheets as being the strongest promoter of self-assembly can be confirmed by the shift of the turning point from 27.3 ± 0.8 vol % for pSar200−b-p(1)Cys10 to concentrations as low as 8.7 ± 0.3 vol % buffer for pSar200−b-p(1)Cys810. Interestingly, for pSar200−b-p(1)Cys810 and pSar200−b-p(1)Cys82, the primary aggregation seems to be accompanied by a secondary aggregation, as the sigmoidal fit cannot be applied over the entire range, indicating further rearrangement of the initially formed micelles.68,69

Finally, the findings on the aggregation behavior were transferred to the preparation of core cross-linked polymeric micelles (CCPMs) from the reactive polypept(o)ides, as illustrated in Figure 5A. The copolymers were dissolved in DMAC and equilibrated by addition of buffer at their respective turning points, before solvent exchange was completed by dialysis. When analyzed by DLS (Figure 5B), both α-helical pSar-b-p(1)Hcy and racemic pSar-b-p(1)Cys yielded small polymeric micelles of similar size (Dh 36–44 nm) and a narrow PDI (0.04–0.15), despite increasing chain lengths of the respective hydrophobic blocks. In contrast, an increased size was detected for enantiopure pSar-b-p(1)Cys (Dh 55–76 nm) directing to larger structures for polymers with longer hydrophobic blocks. For further analysis, micelles were cross-linked by reaction of the S-ethylsulfonyl group with dihydrolipoic acid hydrazide (3), and the morphology of the

Figure 3. Influence of the secondary structure on the aggregation behavior. (A) Schematic illustration of the dynamic range. (B) Aggregation curve of polypept(o)ides (P3, P7, and P11) with sigmoidal fit.

Figure 4. Aggregation curves for copolymers P3–P14. (A) pSar-b-p(DL)Cys, (B) pSar-b-p(1)Cys, and (C) pSar-b-p(1)Hcy.
obtained disulfide cross-linked micelles was investigated by AFM (Figure 5C–E and Figures S5–S8). Of note, the cross-linking reaction did not influence the overall size distribution (Figure S3). Analysis by AFM revealed spherical structures with diameters well below 50 nm for CCPMs from racemic pSar-b-p(DL)Cys containing 15 wt % p(DL)Cys, while spherical to slightly elongated particles could be observed for 26 wt % p(DL)Cys (Figure 5C and Figure S5). As shown by AFM and cryoTEM (Figure 5D and Figures S6 and S7), solely worm-like micelles were obtained for pSar-b-p(L)Cys containing both 12 and 29 wt % enantiopure p(L)Cys, which corresponds well with previous findings as well as with the larger size determined by DLS.50 Spherical CCPMs only were obtained from α-helical pSar-b-p(L)Hcy of 15 or 27 wt % p(L)Hcy, as shown in Figure 5E and Figure S8.70

Taken together, secondary structure formation is a major parameter affecting the self-assembly of polypept(o)ides. In direct comparison, the reactive amphiphilic block copolymer

Figure 5. Self-assembly and core cross-linking. (A) Schematic illustration of secondary structure-driven self-assembly. (B) Single-angle DLS analysis of polymeric micelles (left: pSar-b-p(\(\alpha\))Cys and pSar-b-p(l)Cys, right: pSar-b-p(l)Hcy). Morphological analysis of core cross-linked polymeric micelles by AFM and cryoTEM: (C) pSar-b-p(\(\alpha\))Cys, (D) pSar-b-p(l)Cys, and (E) pSar-b-p(l)Hcy.

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with an \( \alpha \)-helical segment, pSar-b-p(L)Hcy, only yielded spherical structures, whereas \( \beta \)-sheets in enantiopure pSar-b-p(L)Cys strongly direct self-assembly to worm-like particles and even affect racemic pSar-b-p(DL)Cys with longer hydrophobic segments.

**CONCLUSIONS**

In conclusion, our head-to-head comparison underlines the influence of secondary structure formation on the aggregation and self-assembly of block copolypeptides. Herein, we reported the successful synthesis of reactive, S-ethylsulfonyl-protected pSar-b-p(DL)Cys, pSar-b-p(L)Cys, and pSar-b-p(DL)Hcy. Despite comparable primary structures, the secondary structure of these block copolymers varies from disrupted \( \beta \)-sheets to antiparallel \( \beta \)-sheets and \( \alpha \)-helices, which impacts their tendency to self-assemble during the solvent switch. The antiparallel \( \beta \)-sheets of enantiopure pSar-b-p(L)Cys strongly induce early aggregation compared to the racemic pSar-b-p(DL)Cys counterpart and narrow the dynamic range. Moreover, the \( \alpha \)-helix in pSar-b-p(L)Hcy favors the formation of spherical structures, whereas \( \beta \)-sheets direct self-assembly into worm-like micelles for enantiopure pSar-b-p(L)Cys and even influence the self-assembly of pSar-b-p(DL)Cys in which the antiparallel orientation is disrupted by the racemic hydrophobic block. A detailed understanding of the secondary structure would provide powerful control over the morphology of micellar assemblies and fine-tuning of particle properties beyond the hydrophilic–hydrophobic ratio. This implies a major significance for polypeptides as drug delivery systems and in materials science.

**ASSOCIATED CONTENT**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.biomac.1c00253.

HFIP GPC of polymerizations in the presence of thiourea; CD spectra, DLS, GPC, and additional AFM/cryoTEM images of core cross-linked polymeric micelles; NMR data of polymer and cross-linker synthesis; DOSY NMR spectroscopy of (co-)polymers (PDF)

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**AUTHOR CONTRIBUTIONS**

Experiment design, polymer synthesis, nanoparticle preparation, AFM, and CD measurements were performed by T.A.B., J.L. contributed to polymer synthesis and nanoparticle formation, and C.M. contributed to polymer synthesis. B.W. performed cryoTEM analysis. The manuscript was written by T.A.B. and M.B. The project was supervised by M.B. All authors have given approval to the final version of the manuscript.

**Notes**

The authors declare the following competing financial interest(s): Matthias Barz holds the patent Thiol-protected amino acid derivatives and uses thereof WO2015169908A1.

**ABBREVIATIONS**

AFM; atomic force microscopy; Boc; tert-butyloxycarbonyl; CCPM; core cross-linked polymeric micelle; CD; circular dichroism; DLS; dynamic light scattering; DMAc; N,N-dimethylacetamide; DMF; N,N-dimethylformamide; DMSO; dimethylsulfoxide; GPC; gel permeation chromatography; FT-IR spectroscopy; Fourier transform infrared spectroscopy; HFIP; 1,1,1,3,3,3-hexafluoropropan-2-ol; MWCO; molecular weight cutoff; NCA; N-carboxyanhydride; pSar; polysarcosine; p(L)Cys; poly(S-ethylsulfonyl-L-cysteine); p(DL)Cys; poly(S-ethylsulfonyl-DL-cysteine); p(L)Hcy; poly(S-ethylsulfonyl-L-homocysteine); cryoTEM; cryogenic transmission electron microscopy

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