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Helix—Coil Transition in Cylindrical Brush Polymers with Poly-L-lysine Side Chains

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Supporting Information

ABSTRACT: Cylindrical brush polymers with poly-L-lysine side chains were prepared by grafting lysine NCA from a macroinitiator via living ring-opening polymerization. The main chain degree of polymerization of the methacrylate main chain was $P_w = 870$, the side chains consisted of 25 and 55 lysine repeat units, respectively. Upon deprotection, the cylindrical brush polymers in 0.005 M NaBr exhibited an almost rodlike conformation with a Kuhn statistical segment length of several hundred nanometers. Cryo-TEM as well as AFM in aqueous solution clearly demonstrated pronounced undulations along the main chain at low ionic strength which could not be detected at higher salt concentrations. With increasing concentration of NaClO₄ the PLL side chains underwent a coil-to-helix transition as revealed by CD measurements. The effect of the side chain coil-to-helix transition on the main chain stiffness could not be followed by light scattering due



to intramolecular attraction ("folding") of the cylindrical brushes at high salt concentration, which is somewhat more pronounced for the helical as compared to the coiled PLL side chain conformation. Comparison with linear PLL revealed the coil-to-helix transition to be hardly affected by the high grafting density of the PLL side chains in the cylindrical brush structures.

INTRODUCTION

After its discovery in 1994,¹ cylindrical brush polymers (sometimes called "bottlebrushes") have received considerable attention,²⁻²⁷ because any flexible main chain can be forced to adopt an expanded cylindrical conformation due to the repulsive interaction of the densely grafted side chains. Thus, a variety of cylindrical brush polymers have been synthesized by "grafting through", "grafting from", and "grafting onto" techniques with various combinations of main and side chain monomers. Also branched or dendritic side chains were introduced as "dendronized polymers", leading to cylindrical objects of similar conformations.²⁸⁻³⁵ Unfortunately, the precise main and side chain conformation is extremely difficult to analyze, because the cylinder length might be shorter than the (all-trans) contour length of the main chain depending on subtle details of solvent quality,⁶⁻⁸ on side chain-side chain interactions in cylindrical brushes with chemically different side chains³⁶ and possibly on main chain-side chain interactions. A combination of light and neutron scattering has been employed to experimentally determine the Kuhn length l_k of the cylinder by application of the Kratky-Porod wormlike chain model and the cross-sectional radius of gyration R_{gc} and the cross-sectional density profiles $\rho(R)$ of the side chains has been determined as a function of different side chain lengths.^{7,37} Although experimental results qualitatively agree that the Kuhn length increases with increasing side chain length and that $R_{\rm gc}$ increases with side chain length quantitative agreement has not been achieved so far due to different experimental techniques and theories employed.^{7,12–16} Rathgeber et al.³⁷ have reported that the chain stiffness does not depend on the

side chain length in contrast to all other experimental data and in contrast to very recent computer simulations.³⁸ The origin of this discrepancy might well originate from finite concentration effects which were neglected in ref 37 as already scrutinized in the literature.⁷

Theories on regular comblike polymers^{16,39–44} do not provide a unique picture. Mean field theory predicts $l_k \sim M_{\rm sc}^{216,39}$ which is close to $l_k \sim M_{\rm sc}^{15/8}$ derived by blob arguments.⁴³ Somewhat smaller exponents were reported by Scheutjens–Fleer self-consistent field calculations for good ($l_k \sim M_{\rm sc}^{1.82}$) and poor solvents for long side chains ($l_k \sim M_{\rm sc}^{1.59}$).⁴⁴ Experimental results by Nakamura et al.¹⁶ on cylindrical brushes with flexible side chains in good and theta solvent are well described by the relation^{16,39}

$$l_{\rm k} = l_{\rm k,0} + k M_{\rm sc}^2$$

with $l_{\rm k,0}$ the intrinsic Kuhn length of the main chain without side chain repulsion, whereas deviating results were reported by Zhang et al.⁷ who obtained an exponent of 1.79 in good and 1.42 in poor solvents. All experiments yielded unusually large values for $l_{\rm k,0}$ in the regime of 5.3 nm < $l_{\rm k,0}$ < 12 nm which is not well understood.^{7,16}

Whereas first computer simulations seemed to be compatible with scaling laws⁴⁵ (with deviating exponents as compared to analytical theory, though) newer investigations reveal a considerable gradient of chain stiffness along the contour of

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the "bottlebrush", resulting in a higher flexibility at both ends.^{46–48} Concomitantly, the simulations also show a pronounced nonexponential decay of the bond angle correlation function^{45–49} which additionally questions the applicability of the wormlike chain model to experimental data. Only for very long main and side chains presently outside of the experimentally accessible regime the results of computer simulations and analytical theory are believed to converge.⁴⁸

Very little data exist on cylindrical brush polymers with rodlike side chains, in particular, on how a coil to rod transition of the side chains affects the main chain conformation. Analytical theories do not yield a significantly different scaling behavior of the main chain Kuhn length for flexible and rodlike side chains: For rodlike side chains mean field calculations yield⁵⁰ $l_k \sim M_{\rm sc}^2/\ln M_{\rm sc}$ which is not very different from the various results for flexible side chains. Computer simulations reveal^{51,52} the main chain to become significantly stiffer upon a coil to rod transition of the side chains, but within the limited range of investigated side chain lengths smaller exponents result for both, flexible and stiff side chains ($l_k = 26 + kM_{\rm sc}^{-1.15}$ for flexible and $l_k = 20 + kM_{\rm sc}^{-1.6}$ for stiff side chains) and unusually large values for $l_{\rm k,0}$.⁵³ Kawaguchi et al⁵⁴ have investigated a cylindrical brush with rigid helical poly(*n*-hexyl isocyanate) side chains and obtain $l_k \sim M_{\rm sc}$ and $R_{\rm gc} \sim M_{\rm sc}^{-0.87}$ in clear contradiction to both, computer simulation and scaling results.

Our group has recently published the synthesis and characterization of cylindrical brushes with poly-L-lysine side chains⁵⁵ which were shown to induce a helical conformation of the main chain, if the side chains are forced into a β -sheet conformation.^{56,57} Since poly-L-lysine is known to also exhibit a helical conformation under appropriate solvent conditions,⁵⁸ cylindrical brushes with poly-L-lysine side chains of different lengths were prepared in the present work, in order to monitor the effect of a coil to helix transition of the poly-L-lysine side chains on the main chain conformation.

EXPERIMENTAL SECTION

Synthesis of N-tert-Butoxycarbonyl-1,6-hexanediamine (2). N-tert-Butoxycarbonyl-1,3-hexanediamine (N6NB; 2) was synthesized according to Stahl et al.⁵⁹ Subsequently, monomer 3 (MA-N6NB; 3) was obtained by reaction between N6NB and methacryloyl chloride. In a typical experiment compound N6NB (7.58 g, 0.046 mol) was suspended in methylene chloride (200 mL) and cooled in an ice bath before triethylamine (0.12 mol) was added. To the stirred solution, 5.2 g (0.05 mol) methacryloyl chloride, dissolved in 50 mL methylene chloride, was added dropwise and the solution was kept stirring overnight at 0 °C. After filtration, the solution was washed with aqueous sodium bicarbonate solution (0.1 N, 250 mL), water (3×250 mL), dried over anhydrous sodium sulfate, and evaporated in vacuum. The product 3 (MA-N6NB) was purified two times by column chromatography (ethyl acetate/toluene (50:50). Yield: 80%. ESI(M + Na⁺): 307 g/mol, M = 284 g/mol, FD: M = 284 g/mol. ¹H and ¹³C NMR spectra are shown in the Supporting Information (Figures S1a and S1b) as well as the HPLC elution curve (Figure S1c).

Synthesis of the Macroinitiator 5. 3 was polymerized by RAFT to protected macroinitiator (5) using 4-cyano-4-thiobenzylvaleric acid⁶⁰ (4) as the RAFT-agent and AIBN as the initiator. In a typical polymerization 0.46 mg (2.8×10^{-6} mol) AIBN and 3,93 mg (1.4×10^{-5} mol) 4-cyano-4-thio-benzyl-valeric-acid dissolved in 2.5 mL anisole were placed with 20 g (0.704 mol) MA-N6NB in a Schlenk flask. Oxygen was removed by 4 freeze pump cycles. Subsequently, polymerization was performed at 90 °C for 3 h. Then the solid reaction mixture was diluted with 50 mL THF, precipitated in 700 mL diethyl ether and dried in high vacuum for 24 h. Yield: 6.7 g (30%). For ¹H NMR spectrum see Supporting Information, Figure S2a.

In order to obtain the free macroinitiator (6) the Boc-protection groups of the precursor were cleaved in a mixture of dichloromethane and trifluoroacetic acid according to R. A. Houghten et al.⁶¹ ¹H NMR analysis of the macroinitiator and its precursor clearly showed that the Boc-protection groups were removed quantitatively (see Supporting Information, Figure S2b).

"Grafting from" Polymerization of the NCA. The NCA of ε benzyloxycarbonyl-L-lysine⁶² (7) was polymerized via ROP from the macroinitiator 6. According to Schlaad,^{63,64} the ionic form of the macroinitiator was deliberately used to suppress the activated monomer mechanism and to prevent the formation of linear contaminations. In a typical experiment 35 mg of the macroinitiator and 2 g of the Z-protected L-lysine NCA⁶² were placed under argon in a 100 mL Schlenk flask and dissolved in 6 mL of purified DMF.⁶⁵ The reaction flask was sealed with a septum, purged with argon for 30 min and placed in an oil bath preheated to 60 °C. The ring-opening polymerization was carried out for 3 days. After precipitation in water, the pure Z-protected poly L-lysine brushes 8 were isolated.

It should be noted that the terminal thiol group of the macroinitiator could also initiate a PLL chain⁶⁶ at the end of the macroinitiator. Given the very many side chains in a cylindrical brush polymer one more PLL chain would not make a significant difference so that this question was not pursued in detail.

Two different monomer-initiator-ratios (25:1; 55:1) were chosen to produce brushes with different side chain length. Both brushes, Z-CB-PLL25 and Z-CB-PLL55 were soluble in HFIP and could be analyzed by static and dynamic light scattering (see Table 1 and Supporting Information for Zimm plots).

Table 1. Light Scattering Results of the Cylindrical Brushes with Z-Protected PLL Side Chains Measured in HFIP with 10^{-3} M LiBr

sample	R _g / nm	$\frac{R_h}{nm}$	ρ- ratio	$M_{ m w}/ m g\cdot mol^{-1}$	${M_{ m w}}^{ m theo}/{ m g\cdot mol^{-1}}$	$\frac{(dn/dc)}{cm^3 g^{-1}}$
Z-CB- PLL55	79.7	47.4	1.68	1.36×10^{7}	1.28×10^{7}	0.228
Z-CB- PLL25	68.9	39.1	1.76	6.06×10^{6}	5.95×10^{6}	0.228

Deprotection of Poly-L-lysine Brushes. In order to obtain the deprotected poly-L-lysine brushes (9), the protected precursors were treated with a mixture of trifluoroacetic acid and hydrogen bromide (33 wt %) in acetic acid according to literature.⁶⁷ Then, 300 mg of the precursor was dissolved in 3 mL of trifluoroacetic acid followed by the addition of 40 mL of hydrogen bromide in acetic acid. The mixture was stirred for 1.5 h and precipitated in 500 mL of diethyl ether. After washing with 3×200 mL of ethanol, the white solid was solved in 30 mL of distilled H₂O and dialyzed for 5 days. Afterward, the polymer 9 was isolated by lyophilization. UV-vis analysis of the precursor and the protected polymer showed that the Z-protection groups were removed quantitatively. The bands at 254 nm, originating from the benzene ring of the Z-group, disappeared in the spectra of the deprotected polymer brushes (see Supporting Information, Figure S5). It should be noted that the hydrobromide of the lysine units is isolated by the procedure described above.

Static and Dynamic light Scattering. Static light scattering (SLS) measurements were performed with an ALV-SP86 goniometer, an ALV-3000 correlator, a Uniphase HeNe Laser (25 mW output power at $\lambda = 632.8$ nm wavelength) and ALV/High QE APD avalanche diode fiber optic detection system. For dynamic light scattering (DLS) an ALV-SP125 goniometer, an ALV-5000 correlator, and a Spectra Physics 2060 Argon ion laser (500 mW output power at $\lambda = 514.5$ nm wavelength) were utilized. The scattered intensity was divided by a beam splitter (approximately 50:50), each portion of which was detected by a photomultiplier. The two signals were cross-correlated in order to eliminate nonrandom electronic noise.

Solutions were typically measured from 30 to 150° in steps of 5° (SLS) or in steps of 20° (DLS). The static scattering intensities were analyzed according to Zimm in order to yield the weight-average molar





mass, M_{w} , the square root of *z*-average mean square radius of gyration, $R_g \equiv \langle R_g^2 \rangle_{z'}$ and the second viral coefficient A_2 . The experimental uncertainties are estimated to $\pm 5\%$ for M_w and R_g . The correlation functions showed a monomodal decay and were fitted by a sum of two exponentials, from which the first cumulant Γ was calculated. The *z*average diffusion coefficient D_z was obtained by extrapolation of Γ/q^2 for to q = 0 leading to the inverse *z*-average hydrodynamic radius $R_h \equiv$ $\langle 1/R_h \rangle_z^{-1}$ by formal application of Stokes law. The experimental uncertainties are estimated to $\pm 2\%$ for R_h .

Stock solutions of each sample were prepared at c = 0.1 g/L and filtered through 0.45 μ m pore size Millipore GHP filters into 20 mm diameter quartz cuvettes (Hellma). Further dilutions were made by subsequent addition of filtered solvent into the LS cuvette and the respective concentrations were obtained by weighing.

The refractive index increments at $\lambda = 632.8$ nm wavelength were measured by a home-built Michelson interferometer as described

elsewhere.⁶⁸ The experimental uncertainties are estimated to $\pm 2\%$ for dn/dc.

Gel Permeation Chromatography (GPC). GPC was performed with a Hitachi L7100 pump and a L-2490 refractive index detector, a Waters 486 UV detector operating at 280 nm wavelength and a Waters AF degasser. The flow rate of the solvent DMF was set to 1 mL/min at a temperature of 333 K. Three columns filled with polystyrene beads (nominal pore sizes 10^4 , 10^5 , and 10^6 Å pore size, 5 μ m size, MZ-Analysentechnik) were utilized as separation medium.

Circular Dichroism (CD). CD measurements were performed with a JASCO J-815 instrument equipped with a solid-state detector. Measurements were done in the step-scan mode. One mm thick quarz cuvettes were used and filled with solutions of c = 0.1 g/L polymer concentration.

Atomic Force Microscopy (AFM). Measurements were performed with a Veeco MultiMode Scanning Probe Microscope and a

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Nanoscope IIIa Controller. All micrographs were taken in the Tapping mode. For measurements on the dried samples Olympus OMCL-AC160-W2 cantilevers with a resonance frequency of 300 kHz (spring constant 42 N/m) were utilized, for measurements in solution Veeco SNL-10 cantilevers with a resonance frequency of 40–75 kHz, (spring constant 0.58 N/m).

Cryo-TEM. The sample was vitrified in liquid propane using the climate chamber of a Vitrobot (FEI, Eindhoven, Netherlands) at room temperature and 80% humidity. The cryo-TEM measurements were performed at 120 kV by a Tecnai 12 (FEI, Eindhoven, Netherlands) equipped with a BIO-TWIN-lens and a 4k CCD (Tietz Video and Image Processing Systems GmbH, Gauting, Germany).

RESULTS AND DISCUSSION

The synthesis of the poly-L-lysine brushes was performed by ring-opening polymerization of the Z-protected L-lysine NCA from a macroinitiator as described by Zhang et al.⁵⁵ (Figure 1).



Figure 2. Zimm plot of the Boc-protected macroinitiator **5**: $M_w = 2.59 \times 10^5$ g/mol, $A_2 = 1.6 \times 10^{-7}$ mol·dm³·g⁻², $\langle R_g^2 \rangle_z^{1/2} = 15.7$ nm with (dn/dc) = 0.04 cm³/g.



Figure 3. AFM-picture of Z-CB-PLL55 (8) spin-cast from HFIP solution on graphite (HOPG).

The absolute molar mass of the protected macroinitiator **5** was determined by light scattering to $M_{\rm W}$ = 259000 g/mol (Figure 2) which corresponds to 870 monomer units (weight-average). The polydispersity was estimated to $M_{\rm w}/M_{\rm n}$ = 1.5 by GPC (polystyrene calibration).

Table 2. Light Scattering Results of Cylindrical Brushes with Deprotected PLL Side Chains (9) Measured in 0.005 M NaBr Solution

sample	R _g / nm	$R_h/$ nm	ρ- ratio	$M_{ m w}/ m g\cdot mol^{-1}$	${M_{ m w}}^{ m theo}/{ m g\cdot mol^{-1}}$	$\frac{(dn/dc)}{cm^3 g^{-1}}$
CB- PLL55	84.1	56.7	1.48	9.95×10^{6}	1.08×10^{7}	0.164
CB- PLL25	75.9	42.0	1.80	8.14×10^{6}	4.81×10^{6}	0.164

The light scattering results of the cylindrical brushes with protected poly-L-lysine side chains 8 with nominal side chain degrees of polymerization 25 and 55, Z-CB-PLL25 and Z-CB-PLL55, are summarized in Table 1.

The theoretical molar mass $M_{\rm w}^{\rm theo}$ in Table 1 is calculated by

$$M_{\rm w}^{\rm theo} = M_{\rm w}^{\rm MI} + ([M]/[I]) P_{\rm w}^{\rm MI} M_0^{\rm Z-lys}$$
(1)

with M_w^{MI} and P_w^{MI} the molar mass and degree of polymerization of the macroinitiator, respectively, [M]/[I] the monomer to initiator ratio and $M_0^{Z^2}$ the molar mass of the Z-protected lysine repeat unit. The agreement of the theoretical and the experimentally determined molar masses demonstrates a near to complete conversion of the protected lysine monomers and that no significant amount of linear PLL has formed. The latter could be confirmed by capillary electrophoresis in aqueous solution on the deprotected samples.⁶⁹ It should be noted, that these results do not prove that each of the macroinitiator sites has started a PLL side chain. Since the side chains cannot be cleaved off the main chain for an independent molar mass determination without degradation of the PLL side chains the length of the side chains given above is derived assuming 100% grafting density. If the true grafting density were less the length of the side chains would increase accordingly. Thus, the precise length of the side chains cannot be determined.

AFM analysis on a mica surface shows the typical wormlike chain conformation expected for cylindrical brush polymers (Figure 3).

Upon deprotection both cylindrical brush polymers were soluble in aqueous solution. Static and dynamic light scattering results in 0.005 M NaBr solution are summarized in Table 2 (see Figure 7 below and Supporting Information for Zimm plots and angular dependent DLS measurements, Figures S3 and S4).

Upon deprotection, the molar mass of the protected cylindrical brushes, $M_{\rm w}^{\rm Z-CB}$, is expected to decrease due to the loss of the Z-protection groups according to

$$M_{\rm w}^{\rm CB,cal} = M_{\rm w}^{\rm Z-CB} M_{\rm o}^{\rm HBr-Lys} / M_{\rm o}^{\rm Z-lys}$$
(2)

with $M_o^{Z-lys} = 262$ g/mol and $M_o^{HBr-Lys} = 208$ g/mol the molar masses of the Z-protected lysine and deprotected lysine hydrobromide repeat units. Whereas the experimentally determined molar mass of CB-PLL55 agrees well with the calculated one, the molar mass of CB-PLL25 is too large by 70%, which is well outside the experimental uncertainty. We have no explanation for this discrepancy except for a subtle aggregation tendency. The short PLL side chains might not be long enough in order to completely shield the hydrophobic main chain. As shown below the calculated molar mass is indeed found if CB-PLL25 is dissolved in a helicogenic solvent for the PLL side chains.



Figure 4. AFM micrographs in aqueous solution (left, height; middle, amplitude) and cryo-TEM picture (right) of sample CB-PLL55 in 0.005 M NaBr solution.

Table 3. Contour Lengths Determined by AFM in Different Solvents

sample	solvent	$L_{\rm n}^{\rm AFM}/$ nm	$L_{ m w}^{ m AFM}/$ nm	$L_{ m w}^{ m AFM}_{ m AFM}/L_{ m n}^{ m AFM}$	counts
CB-PLL25	0.005 M NaBr	149	200	1.34	276
CBPLL-25	0.5 M NaClO ₄	149	209	1.40	359
Z-CB-PLL55	spin-cast HFIP	186	226	1.22	321
CB-PLL55	0.005 M NaBr	181	244	1.35	309
CB-PLL55	0.5 M NaClO ₄	160	214	1.34	254

The dimensions R_g and R_h of the deprotected PLL brushes increase somewhat, which is to be expected by the introduction of ionic charges. Application of the wormlike chain model^{70–72}



Figure 5. CD spectra of (a) CBN-PLL55 and (b) CB-PLL25 in aqueous solution for different concentrations of NaClO₄. The solid lines represent the fits to the data by the program Dichroweb.⁷⁸⁻⁸⁰ (\bullet , H₂O; red \bullet , 0.1 N NaClO₄; green \bullet , 0.25 N NaClO₄; blue \bullet , 0.5 N NaClO₄; purple \bullet , 1.0 N NaClO₄). Note that θ is given in millidegrees and not in terms of molar ellipticity.



Figure 6. Content of α -helix versus NaClO₄ concentration for CB-PLL25 (black), Cb-PLL55 (red), and linear PLL with $P_w = 50$ (green) and linear PLL with $P_w = 1500$ (blue).

and accounting for polydispersity as well as for the contribution of the side chains at both chain ends to the contour length of the cylinders⁷³ (see below), the Kuhn statistical segment length was calculated to increase from $l_{\rm k}$ = 300 nm for the protected side chain sample Z-CBB-PLL55 in HFIP (for comparison: $l_{\rm k}$ = 170 nm for CBB-Z-PLL25 in HFIP) to $l_{\rm k}$ = 400 nm of the deprotected sample in aqueous 0.005 M NaBr solution. These values are among the largest reported for cylindrical brush polymers and reveal a particularly strong repulsion between the polypeptide side chains. It should be noted, however, that the determination of the Kuhn length becomes quite inaccurate if the Kuhn length becomes comparable to or even exceeds the contour length because R_g is getting too close to the rod limit $(R_g^{rod} = 103 \text{ nm}, l_k = \infty)$. Moreover, the application of the Kratky-Porod wormlike chain model to cylindrical brush polymers might not be justified, because recent computer simulations have demonstrated the main chain persistence to significantly decrease from the middle of the cylindrical brushes toward the ends.^{46–49}

In order to qualitatively confirm the large persistence of the cylindrical brushes, AFM in aqueous 0.005 M NaBr solution as well as cryo-TEM were performed on sample CB-PLL55. The Cryo-TEM picture shown in Figure 4 (right) clearly shows almost rodlike structures whereas the AFM-pictures (left) show significantly curved cylinders. Obviously, the interaction with the negatively charged mica surface disturbs the conformation of the cylindrical brushes during the adsorption process. Both, AFM and Cryo-TEM pictures reveal a peculiar shape of the cross-section reminiscent of "pinned clusters" postulated by scaling arguments.^{74,75} It should be noted, however, that scaling



Figure 7. (a) Zimm plot for sample CB-PLL55 in 0.005 M NaBr: $M_w = 9.95 \times 10^6 \text{ g/mol}$, $A_2 = 4.87 \times 10^{-8} \text{ mol} \cdot \text{dm}^3 \cdot \text{g}^{-2}$, $\langle R_g^2 \rangle_z^{1/2} = 84.1 \text{ nm}$ with $(dn/dc) = 0.164 \text{ cm}^3/\text{g}$. (b) Zimm plot for sample CB-PLL55 in 0.5 M NaCl: $M_w = 8.68 \times 10^6 \text{ g/mol}$, $A_2 = 7.73 \times 10^{-9} \text{ mol} \cdot \text{dm}^3 \cdot \text{g}^{-2}$, $\langle R_g^2 \rangle_z^{1/2} = 86.4 \text{ nm}$ with $(dn/dc) = 0.164 \text{ cm}^3/\text{g}$. (c) Zimm plot for sample CB-PLL55 in 0.5 M NaCl: $M_w = 8.68 \times 10^6 \text{ g/mol}$, $A_2 = 7.73 \times 10^{-9} \text{ mol} \cdot \text{dm}^3 \cdot \text{g}^{-2}$, $\langle R_g^2 \rangle_z^{1/2} = 86.4 \text{ nm}$ with $(dn/dc) = 0.164 \text{ cm}^3/\text{g}$. (c) Zimm plot for sample CB-PLL55 in 0.5 M NaClO₄: $M_w = 8.13 \times 10^6 \text{ g/mol}$, $A_2 = 1.29 \times 10^{-8} \text{ mol} \cdot \text{dm}^3 \cdot \text{g}^{-2}$, $\langle R_g^2 \rangle_z^{1/2} = 49 \text{ nm}$ with $(dn/dc) = 0.164 \text{ cm}^3/\text{g}$. (d) Apparent diffusion coefficient D_{app} versus q^2 for CB-PLL55 in 0.005 M NaBr (triangles), 0.5 M NaCl (circles), and 0.5 M NaClO₄ (squares).

Table 4. Light Scattering Results of Samples CB-PLL25 and CB-PLL55 in Aqueous Salt Solutions

sample	solvent	R _g /nm	R_h/nm	$A_2/\text{mol}\cdot\text{dm}^3\cdot\text{g}^{-2}$	$M_{ m w}/{ m g}{ m \cdot mol}^{-1}$	ho-ratio	structure
CB-PLL 25	0.5 N NaClO ₄	56.4	37.1	-6.53×10^{-8}	5.01×10^{6}	1.52	helix
CB-PLL 25	0.5 N NaBr	64.8	42	5.85×10^{-8}	6.90×10^{6}	1.54	coil
CB-PLL 55	0.5 N NaClO ₄	49.0	38.4	1.29×10^{-8}	8.13×10^{6}	1.28	helix
CB-PLL 55	0.5 N NaCl	60.4	44.8	7.73×10^{-9}	8.86×10^{6}	1.35	coil

arguments were derived for flexible main chains with a Gaussian segment distribution between the branch points. Thus, the coincidence with the "pinned cluster" scenario might be accidental.

In addition the contour length of the deprotected cylindrical PLL brushes was determined by AFM in aqueous solution at a mica surface. The number and weight-average contour lengths L_n and L_w for CB-PLL 25 and CB-PLL 55 could be measured and are given in Table 3 along with the calculated contour lengths L_w based on the number-average degree of polymerization $P_w^{\rm MI} = 870$, the contour length of the polymethacrylate main chain repeat unit, l = 0.25 nm, and an estimate of the side chain contribution at both ends to the total length of the cylinder

$$L_{\rm w} = P_{\rm w}^{\rm MI} l + 2L^{\rm chain\,end} \tag{3a}$$

and

$$L^{\text{chain end}} = \langle R^2 \rangle^{1/2} = (L^{\text{PLL}} l_k^{\text{PLL}})^{1/2}$$
(3b)

with L^{PLL} and l_k^{PLL} the contour and the Kuhn statistical segment lengths, respectively, of the PLL side chains. Utilizing $l_k^{\text{PLL}} = 4 \text{ nm}$ (due to some stretching of the densely grafted side chains) the contour lengths are calculated to be $L_w = 223 \text{ nm}$ for CB-PLL25 and $L_w = 226 \text{ nm}$ for CB-PLL55.

The contour lengths agree within experimental uncertainty (approximately $\pm 10\%$ comprising the statistical error due to the limited number of molecules counted and the reliability of the software for length determination) and compare well with the values deducted from the light scattering and GPC results of the macroinitiator. The polydispersity $M_{\rm w}/M_{\rm n} = 1.35$ is somewhat lower than determined by GPC via polystyrene calibration.

Since it is well-known, that linear PLL adopts a coillike conformation in aqueous NaBr solutions and an α -helical structure in aqueous NaClO₄ solution (>0.25 M),^{76,77} the conformation of the PLL side chains in the cylindrical brush polymers was investigated by CD spectroscopy in the respective solvents. The results are shown in Figure 5 for CB-PLL55 (a) and for CB-PLL25 (b).

The typical minima at 208 and 222 nm clearly reveal a major fraction of the PLL side chains to adopt a helical conformation. Quantitative analysis of the spectra was performed by the program Dichroweb (routine CDSSTR)^{78–80} in terms of coil, α -helix and β -sheet content. The α -helix contents as a function of NaClO₄ concentration are shown in Figure 6 for CB-PLL25 and CB-PLL55 cylindrical brushes as well as for a short (P_w = 50) and long (P_w = 1500) chain linear PLL for comparison. As expected the α -helix content is higher for the longer PLL chain since the stability of the helix decreases for shorter chains.



Figure 8. (a) AFM micrographs of CB-PLL25 in 0.5 M NaClO₄ solution, where the side chains adopt a helical conformation. (b) AFM micrographs of CB-PLL25 in 0.5 M NaBr solution, where the side chains are coiled. Left: height. Right: amplitude.

Unfortunately, a shorter linear PLL with $P_w = 25$ was not available in order to elucidate the chain length dependence of the coil-to-helix transition of linear PLL for very short chains. On the basis of the comparison of CB-PLL55 with the linear PLL50 the topological constraint of the cylindrical brush seems to favor a somewhat higher helix content, but does not significantly affect the PLL secondary structure within experimental uncertainty in terms of PLL side chain length. It should be noted that the PLL side chain length could not be independently determined but was calculated with the assumption of 100% grafting density. If the actual grafting density is smaller, the side chains become larger and could get closer to the long chain limit of the helix content. This small effect is in contrast to cylindrical brush polymers with polyproline side chains which were observed to undergo a transition of proline II to proline I helices in contrast to linear polyproline chains.⁸¹ This observation was explained by the steric overcrowding of the polyproline side chains in the cylindrical brush which favors the "slimmer" proline II conformation.

Since the overall conformation of the cylindrical brushes may be expected to depend on both, the secondary structure of the side chains and on the ionic strength, static and dynamic light scattering experiments were performed on both samples in 10^{-3} M and 0.5 M NaCl/0.5 M NaBr solution as well as in 0.5 M NaClO₄ solution. Representative measurements are shown in Figure 7 for CB-PLL55. A summary of all measurements is given in Table 4 (also see Supporting Information, Figures S6 – S9).

The light scattering results qualitatively show that the dimensions of the cylindrical brushes shrink with increasing

ionic strength as expected. At constant ionic strength the dimensions are consistently smaller for the helical conformation of the side chains as compared to coiled side chains. However, a quantitative analysis in terms of the wormlike chain model does not seem to be meaningful for the following reasons: The radii of gyration of the short side chain polymer CB-PLL25 are consistently larger as compared to the larger side chain polymer CB-PLL55. This is not at all expected since the extension of the cylindrical brush polymers should increase with increasing side chain length. At the same time the hydrodynamic radii of CB-PLL25 and CB-PLL55 do not change significantly. This inconsistency may be partly caused by the aggregation tendency of CB-PLL25 in aqueous solution. Irrespective of molar mass, the ratio R_{o}/R_{h} of 1.35 and 1.28 of CB-PLL55 in 0.5 M NaBr and 0.5 M NaClO₄, respectively, indicates a partial collapse of the brushes. This is actually confirmed by the AFM measurements shown in Figure 8. It is seen that the cylindrical brushes predominantly fold back on themselves apparently by some attractive interaction between the side chains. This behavior is expected for cylindrical brushes in poor solvents as predicted theoretically $^{82-84}$ as well as observed experimentally on dried cylindrical brushes with PMMA side chains.^{71,85} Here it is demonstrated that the folding phenomenon also occurs in solution and does not constitute a drying artifact.

CONCLUSION

Cylindrical brushes with polypeptide side chains have advanced to interesting research subjects, because the main chain exhibits a large directional persistence and the conformation may be tuned from coillike to helical or to β -sheet as demonstrated before.⁵⁷ Whereas the twist of the side chain β -sheets forced the

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main chain into a helix with a pitch of 20 nm, the influence of the coil-to-helix side chain conformation on the main chain conformation is more subtle and less spectacular. The theoretically interesting influence of the coil-to-rod transition of the side chains onto the main chain stiffness could not be detected due to the decreasing solvent quality of the helical side chains which caused the cylindrical brushes to adopt a more compact "folded" conformation due to side chain-side chain attraction.

At low ionic strength the main chains were observed to show regular undulations in the cross-section, which may be interpreted in terms of "pinned cluster" or "string of spheres" scenarios. However, to the best of our knowledge, a profound theory on the conformation of cylindrical brushes with ionic side chains as a function of added salt is still missing.

ASSOCIATED CONTENT

S Supporting Information

NMR and UV spectra, Zimm plots and q^2 -dependence of the reduced first cumulant, Γ/q^2 . This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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