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Experimental measurement of coil-rod-coil block copolymer tracer diffusion through entangled coil homopolymers

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Abstract

The diffusion of coil-rod-coil triblock copolymers in entangled coil homopolymers is experimentally measured and demonstrated to be significantly slower than rod or coil homopolymers of the same molecular weight. A model coil-rod-coil triblock was prepared by expressing rodlike alanine-rich α -helical polypeptides in *E. coli* and conjugating coillike poly(ethylene oxide) (PEO) to both ends to form coil-rod-coil triblock copolymers. Tracer diffusion through entangled PEO homopolymer melts was measured using forced Rayleigh scattering at various rod lengths, coil molecular weights, and coil homopolymer concentrations. For rod lengths, L , that are close to the entanglement length, a , the ratio between triblock diffusivity and coil homopolymer diffusivity decreases monotonically and is only a function of L/a , in quantitative agreement with previous simulation results. For large rod lengths, diffusion follows an arm retraction scaling, which is also consistent with previous theoretical predictions. These experimental results support the key predictions of theory and simulation, suggesting that the mismatch in curvature between rod and coil entanglement tubes leads to the observed diffusional slowing.

INTRODUCTION

Rod-coil block copolymers have attracted extensive interest as functional nanostructured materials for organic electronics^{1–2} and biomaterials.^{2–4} The self-assembly behavior of these materials is fundamentally different from coil-coil block copolymers due to the mismatch between rod and coil chain topology and anisotropic interactions between the rod blocks.^{2, 5–7} Although the equilibrium thermodynamics of rod-coil block copolymers continues to be widely investigated, few studies have measured dynamic phenomena in these systems. Rheological measurements have been used to identify order-disorder transitions^{8–9} and to measure intrinsic viscosities.¹⁰ Borsali, et al. also provided analytical

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ASSOCIATED CONTENT

Supporting Information. Synthesis details of photochromic ONS dye, B_n oligomer genes, gel permeation chromatography, details of SANS data analysis, and power law fitting of PEO homopolymer diffusion constants. This material is available free of charge via the Internet at <http://pubs.acs.org>.

expressions for dynamic structure factors in dilute solution.¹¹ An understanding of the molecular mechanisms of motion in this important class of molecules is necessary for innovations in mechanics, processing pathways, and self-assembly kinetics. Further studies are necessary to develop the required fundamental knowledge of rod-coil block copolymer dynamics.

Just as the rod's rigidity leads to liquid crystalline and packing geometry effects that have profound implications for rod-coil self-assembly,^{5-7, 12} the difference in rod and coil chain topologies leads to scientifically rich dynamic phenomena. For entangled homopolymers, this difference has been shown to create divergent scaling behaviors for rods¹³⁻¹⁴ (diffusivity $D \sim M^{-1}$, relaxation time $\tau_r \sim M^9$) and coils¹⁴⁻¹⁶ ($D \sim M^{-2.3}$, $\tau_r \sim M^{3.4}$). Molecular dynamics simulations have shown that when rod and coil blocks are combined in the same molecule, the diffusion of the resulting copolymers is slower than both rod and coil homopolymers of the same molecular weight.¹⁷ This slowing is hypothesized to result from curvature mismatch, where the entanglement tubes of rods are perfectly straight while the entanglement tubes of coils have a characteristic curvature of order a , the entanglement length. In the small rod limit where the rod length L is of order a , reptation of the coil block is hindered as the rod moves through curved sections of the entanglement tube. This reptation is increasingly hindered as L/a increases, and hence diffusion of rod-coils is slower than coil homopolymers. In the large rod limit where $L \gg a$, rotation of the rod is severely hindered, so the coil block is forced into configurations parallel to the rod by an arm retraction mechanism before diffusion can occur. This causes diffusion to decrease exponentially as the coil size increases; hence diffusion of rod-coils is slower than rod homopolymers. We refer to the molecular origin of the diffusion of rod-coils as the curvature mismatch hypothesis.

This study provides the first experimental evidence in support of the curvature mismatch hypothesis. As in the previous theory and simulation work, the study focuses on tracer diffusion of coil-rod-coil triblock copolymers in entangled coil homopolymer solutions. This system was chosen not only because coil-rod-coils are of interest for their self-assembly behavior,^{7, 18} but also because the presence of coils for both the tracer end blocks and the surrounding obstacles allows maximum use of existing coil reptation theories. Tracer diffusion is measured using forced Rayleigh scattering (FRS),¹⁹⁻²¹ where the tracer coil-rod-coil triblocks are covalently labeled with a photochromic dye and thus detected against the silent background of coil homopolymers. FRS is particularly convenient for polymer science due to the ease of dye labeling and its wide *in situ* measurement range of 10^{-13} – 10^{-19} m²/s, and it has been successfully utilized to measure probe diffusion,²²⁻²⁴ rotational diffusion,²⁵ reptation,²⁶⁻²⁹ and diffusion in self-assembled block copolymers.³⁰⁻³² A model coil-rod-coil triblock copolymer is synthesized by bioconjugation of poly(ethylene oxide) PEO chains to the ends of a perfectly monodisperse alanine-rich α -helix that was isolated by bacterial expression. Using FRS, tracer diffusivities are acquired at various ratios of L , M_c , and a and compared with the key predictions of the curvature mismatch hypothesis, providing insight into the mechanisms of rod-coil block copolymer diffusion.

EXPERIMENTAL

Biosynthesis of rodlike polypeptides

A gene encoding for an alanine-rich polypeptide was kindly provided by K. L. Kiick in the pET19b-RF1-B6 plasmid.³³ This rodlike polypeptide is a hexamer of the peptide sequence AAAQAAQAQAAAEAAAQAAQAQ, denoted as B throughout this work. This repeat unit has a length of 3.3 nm and a molar mass of 1982 Da.

The gene encoding B₆ was amplified directly from the pET19b-RF1-B6 plasmid by polymerase chain reaction (PCR) flanked by *Pst*I and *Hind*III restriction sites, and digested and ligated into the multiple cloning site of the pQE9 expression plasmid. Shorter and longer B_n oligomers were prepared by adapting the methods of Farmer, *et al.* and also incorporated into pQE9 (Supporting Information).³³ A few single amino acid residues were then introduced by site-directed mutagenesis: a tryptophan near the N-terminus to increase the A₂₈₀ signal, a lysine near the C-terminus for polymer conjugation, and cysteines near the N- and C-termini for attachment of a photochromic dye. These mutations are shown in Figure 1a. All final plasmids were confirmed by restriction analysis (Figure 1b) and dideoxy sequencing (GENEWIZ, Cambridge, MA).

pQE9 expression plasmids containing a series genes encoding for B_n oligomers of varying length were transformed into the SG13009(pREP4) expression host and shaken in 1 L of Terrific Broth with ampicillin (200 mg/mL) and kanamycin (50 mg/mL) at 37°C. Expression was induced at OD₆₀₀ = 0.8–1 with isopropyl β-D-1-thiogalactopyranoside (IPTG) at a final concentration of 1 mM. The cells were harvested by centrifugation 5hr after induction and frozen overnight at –80°C. The cells were thawed and resuspended in buffered 8 M urea (pH = 8.0), sonicated, and then centrifuged to clarify the cell lysate. The hexahistidine-tagged polypeptides were isolated from the lysate by metal affinity chromatography under reducing conditions (using 20 mM β-mercaptoethanol in all buffers). The protein solution was dialyzed against Milli-Q water and lyophilized. Each 1 L of culture produced 50–100 mg of protein, and the purity was verified by SDS-PAGE (Figure 1c).

Conjugation of ONS dye to rodlike polypeptides

To label the polypeptides for diffusion measurements, a photochromic dye was conjugated to two cysteine residues near the N- and C-termini using thiol-maleimide coupling (Figure 2). The synthesis of the maleimide-functionalized photochromic 4'-(*N,N*-dimethylamino)-2-nitrostilbene dye (ONS-M) is reported in the Supporting Information. B_n polypeptide was dissolved to 1 mg/mL in a 3:1 DMSO:(10 mM NaH₂PO₄) mixture. After adding tris(2-carboxylethyl)phosphine (TCEP) and ONS-M at a 20:1 molar ratio, the pH of the solution was adjusted to 8.0 and the reaction was stirred overnight in the dark. The ONS-B_n polypeptides were isolated by metal affinity chromatography under reducing conditions, dialyzed against Milli-Q water, and lyophilized. A colorimetric assay of ONS-B₆ showed the dye conjugation efficiency was near 100%.

Conjugation of ONS dye to PEO homopolymers

Dihydroxy terminated PEO (Polymer Source, Montreal, Canada) was dissolved in DCM with a 10-fold molar excess of ONS-COOH and a 20-fold molar excess of 4-dimethylaminopyridine and *N,N*-dicyclohexylcarbodiimide. The reaction was refluxed at 55°C for 2 days in the dark. The polymer was then concentrated, precipitated in ether, and dried. The polymer was further purified by size exclusion chromatography using a Superdex 200 column with Milli-Q water as the mobile phase.

Conjugation of PEO coil blocks to ONS-labeled polypeptides

Triblock copolymers were prepared by conjugating PEO to the amines on the N-terminus and the C-terminal lysine on the polypeptides (Figure 2). ONS-B_n polypeptide was dissolved to 1 mg/mL in 8 M urea with 100 mM NaH₂PO₄ and the pH of the solution was adjusted to 9.0. A 10-fold molar excess of *N*-hydroxysuccinimide functionalized PEO (Creative PEGworks, Winston Salem, NC) was dissolved in a minimal amount of Milli-Q water (~30–40% final concentration) and immediately added to the ONS-B_n solution while stirring vigorously. After 1 hour, the pH was adjusted to 9.0 and another 10-fold molar excess of PEO was added. After an additional hour, the solution was filtered through a 0.2 µm syringe filter and exchanged into 20 mM ethanolamine (pH = 10.0) and concentrated using a centrifugal filter with a 10 kDa molecular weight cutoff. The PEO-B_n-PEO triblock copolymer was isolated from the other reaction products by anion exchange chromatography using a HiTrap Q Sepharose HP 5 mL column (GE Healthcare, Waukesha, WI), eluting with a gradient of 0 mM to 100 mM NaCl (Figure 3). The start and elution buffers included 6 M urea for PEO-B₉-PEO to enhance separation. Fractions containing pure triblock copolymer were combined, dialyzed against Milli-Q water, and lyophilized. The SDS-PAGE shows that the final product is almost quantitatively free of diblock contaminants with a purity of >98%, and only a single peak is observed in the aqueous gel permeation chromatograph (Supporting Information). The overall synthesis is summarized in Figure 2.

Forced Rayleigh scattering measurements

Tracer diffusion measurements of various molecules listed in Table 1 in entangled PEO homopolymer solutions were performed using forced Rayleigh scattering. The PEO homopolymer ($M_n = 387$ kDa, PDI = 2.52) from Sigma (St. Louis, MO) initially contained insoluble stabilizing agents that were removed by dissolving it in water, centrifuging, and then lyophilizing the supernatant. Samples were prepared by dissolving lyophilized tracers in 10 mM NaH₂PO₄ (pH = 7.0), and then adding PEO to the desired matrix concentration. The matrix molecular weight was chosen to be significantly higher than the tracer molar mass to minimize the effect of constraint release, and previous studies have shown that tracer diffusion is independent of matrix molecular weight when the matrix is 3 times larger than the tracer.³⁴ The final tracer concentration was such that the number density of rods was less than one per (40 nm)⁻³ volume. Since the longest rod investigated was $L = 30$ nm, the tracer concentrations were sufficiently dilute to ensure minimal rod-rod interactions. The entangled polymer solutions were pressed between two circular quartz crystal slides with a 1 mm thick Teflon spacer in a brass holder¹⁹ and allowed to equilibrate for at least 24 hours before measurement. To promote more uniform mixing, in some cases both the tracers and

PEO were first dissolved in excess Milli-Q water, lyophilized, and re-dissolved in the 10 mM NaH₂PO₄ (pH = 7.0). Diffusion measurements performed on samples prepared by both methods gave identical results.

Forced Rayleigh scattering measurements were performed using an instrument and methodology adapted from previous designs.^{19–21} A SpectraPhysics Cyan 100 mW laser at 488 nm in single-longitudinal mode operation was used as the light source. The beam was split, focused, and recombined at an angle θ and a spot size of $\sim 1 \text{ mm}^2$ on the sample, which was held at 25°C for all measurements using a recirculating water bath. A holographic grating was created by exposing the ONS-labeled sample for 500 ms, and the subsequent diffusive decay was monitored by Bragg diffraction of a 10^4 -fold attenuated beam at the same wavelength. Each sample was measured 5 times for at least 3 angles.

Circular dichroism

To prevent dimerization during measurement, the thiol residues of the polypeptides were alkylated with a 10-fold molar excess of tris(2-carboxyethyl)phosphine and iodoacetamide in 8M urea + 20 mM ethanolamine (pH = 10.0), overnight. Samples were then dialyzed against Milli-Q water and lyophilized. The polypeptides were further purified by anion exchange chromatography using a Mono Q 4.6/100 1.7 mL column (GE Healthcare, Waukesha, WI) in 8M urea + 20 mM ethanolamine (pH = 10.0), eluting with a gradient of 0 mM to 200 mM NaCl, and then dialyzed against Milli-Q water and lyophilized. The polypeptides were dissolved to a 20 μM concentration (determined gravimetrically) in 10 mM NaH₂PO₄ (pH = 7.0). Spectra were recorded using an Aviv 202 spectrometer in a quartz cuvette with 1 mm path length. Background scans of the buffer were subtracted from the sample spectra. Data was recorded at 25°C, at every 1 nm and averaged over 10 seconds.

Small angle neutron scattering (SANS)

SANS data was collected on the Low Q Diffractometer at the Lujan Center in Los Alamos National Laboratory. Samples were prepared as described for the FRS measurements, except the tracers were blended with a solution of deuterated PEO homopolymer ($M_n = 165.2 \text{ kDa}$, PDI = 1.35 determined by GPC using the specific refractive index of hydrogenated PEO of 0.044 mL/g, Polymer Source, Montreal, Canada) in deuterated phosphate buffer (Cambridge Isotope, Andover, MA). Background samples without tracers were also measured. Scattering patterns were collected until 500,000 events above background with an collimating aperture of 6 mm diameter. 1D reductions were executed and the background-subtracted data was converted to absolute intensities.³⁵ The reduction procedure accounted for data smearing due the finite collimation and pixel size.

RESULTS AND DISCUSSION

Characterization of polypeptide helicity and coil-rod-coil aggregation state

In order to perform forced Rayleigh scattering measurements of tracer diffusion, model dye-labeled coil-rod-coil triblock copolymers were synthesized with highly rodlike midblocks and minimal aggregation between tracer molecules. These triblock copolymers were composed of PEO as the coil block and polypeptide α -helices as the rod block. α -helices are

convenient model rods since their persistence lengths are ~ 100 nm,³⁶ and they can be synthesized with perfect monodispersity through bacterial expression. The polypeptides used in this study are from a class of well-studied helices,^{33, 37–38} composed mostly of alanine for its high helix propensity^{39–40} and glutamine and glutamic acid for enhancing water solubility through hydrogen bonding and charge. PEO was chosen for the coil blocks and the surrounding homopolymer matrix since it is known to interact weakly with proteins,⁴¹ which minimizes chemical interactions with the rod blocks. For this study of entangled dynamics, PEO is also convenient because its melt entanglement molecular weight of 2 kDa is low compared to other water-soluble polymers.⁴²

The helical nature of the polypeptides was confirmed by the double minima at 208 nm and 222 nm in the circular dichroism (CD) spectra (Figure 4). The mean residue ellipticities at these minima, $[\theta]_{208}$ and $[\theta]_{222}$, have been used in previous studies to estimate the α -helical content of polypeptides. These studies provide various formulas to calculate the fractional helicity, based on theoretical ellipticities of 100% helical poly-L-lysines or alanine-rich polypeptides.^{43–45} Applied to this system, these formulas average to obtain estimates ranging from 64% α -helix for the shortest $L = 10$ nm (B_3) peptide to 83% α -helix for the longest $L = 30$ nm (B_9) peptide (Table 2). The data was also analyzed using the CDSSTR algorithm in the CDPro package with a basis set of 43 soluble and 13 membrane proteins.^{46–47} Resulting α -helical contents were 60% for $L = 10$ nm and 78% for $L = 30$ nm, in agreement with single-wavelength helicity estimators. In order to account for the residues at the N- and C-termini that are not part of the rodlike alanine-rich regions, the helicities were recalculated in terms of only the B_n helical domain residues (Table 2). The helicity increases slightly with the peptide length as expected,^{33, 37, 48} but they are always greater than 80%, suggesting that the alanine-rich segments of the B_n polypeptides are excellent model rods.

Accurate diffusion measurements require minimal chemical interactions between the tracers, specifically that the labeled molecules do not aggregate under the experimental conditions. Previous work has shown that the α -helical polypeptides used in this study are monomeric in dilute aqueous buffers.³⁸ Using analytical ultracentrifugation and native polyacrylamide gel electrophoresis, previous researchers detected no aggregation for concentrations up to 140 μ M, which is far higher than the maximum tracer concentration of 25 μ M for the diffusion measurements in this study.

After bioconjugation, dynamic light scattering was used to estimate the hydrodynamic radius (R_H) of the tracers in phosphate buffer at the diffusion measurement concentration of 25 μ M to confirm that the block copolymers also do not aggregate. $R_H = 9.1$ nm for ONS-labeled PEO (132 kDa) agrees well with $R_g = 13.3$ nm from literature estimates⁴² and $R_g/R_H = 1.5$ for random coils, confirming that the coil homopolymer is highly soluble and does not aggregate, as expected. $R_H = 8.7$ nm for ONS-labeled PEO- B_6 -PEO (40 kDa coils, total 95 kDa) is quite similar to the coil homopolymer, suggesting the coil-rod-coil triblock copolymers also do not aggregate in dilute solution.

Small angle neutron scattering (SANS) demonstrates that the coil-rod-coil molecules also retain a monomeric state in concentrated PEO solutions. ONS-labeled PEO and ONS-

labeled PEO-B₆-PEO in deuterated 50% PEO solutions showed similar scattering profiles (Figure 5). The low- q scattering of both the tracer coil homopolymer and coil-rod-coil triblocks is confounded by anomalously high scattering that has been attributed to end-group clustering effects in PEO solutions.^{49–51} The coil homopolymer data for $q > 0.01 \text{ \AA}^{-1}$ fits excellently to a Debye function with a radius of gyration of $R_g = 13.3 \text{ nm}$. An additional power-law term may be added to the Debye function to capture the low- q effects;⁴⁹ a fit to this function provides an estimate of $R_g = 9.6 \text{ nm}$ and a low- q power law exponent of -3.5 (Supporting Information). These estimates are comparable to the literature value of $R_g = 13.3 \text{ nm}$ for PEO melts at 80°C .⁴² Despite this agreement, quantitative estimation of R_g is difficult due to low- q scattering from end-group clustering in PEO solutions. However, the high q region shows power law scaling indicative of the chain configuration; this exponent depends strongly upon the aggregation state of the molecules. Using chain dimensions estimated from the PEO homopolymer, the form factor for PEO-B₆-PEO was calculated under various rod block aggregation states (Supporting Information). Monomeric coil-rod-coil triblocks produce a Porod exponent for $0.02 \text{ \AA}^{-1} < q < 0.1 \text{ \AA}^{-1}$ of -1.78 , in between the rod and coil exponents of -1 and -2 , respectively. As the aggregation number increases, the fractal nature of the aggregates becomes closer to a dense sphere, so the calculated Porod exponent decreases monotonically toward the limit of -4 . The high- q exponent measured by SANS was -1.65 ± 0.02 , which is consistent with coil-rod-coils that did not aggregate under these experimental conditions.

Forced Rayleigh scattering

Forced Rayleigh scattering measurements were used to measure diffusion of coil-rod-coil tracers through entangled PEO solutions, demonstrating slowed diffusion relative to PEO homopolymers. The real-time diffracted intensities of the various ONS-labeled polymer samples were fit to a stretched exponential function,

$$I = \left(A e^{-(t/\tau)^\beta} \right)^2 + B, \quad (1)$$

which provided better fits than single exponentials by reflecting a narrow spectrum of relaxation times from the polydispersity of the tracers.^{19, 31} The function contains an amplitude A , an incoherent baseline B , a decay time τ , and a stretching parameter β . Fits to each scattering signal were excellent with R^2 near unity (Figure 6a). The mean relaxation time $\langle \tau \rangle = (\tau/\beta)\Gamma(1/\beta)$ is related to the diffusivity D by $\langle \tau \rangle = \frac{d^2}{4\pi^2 D}$, where Γ is the gamma function and d is the characteristic spacing of the holographic grating, which is related to the laser wavelength λ and the angle θ by,

$$d = \frac{\lambda}{2 \sin(\theta/2)}. \quad (2)$$

These angles were selected such that the decay times were significantly longer than the 500 ms exposure time, ensuring that appreciable diffusion does not occur during the writing of the holographic gratings. Diffusivity and the associated error of each sample was then

determined by linear regression of $\langle \tau \rangle$ vs. d^2 over many measurements at many angles (Figure 6b).

Diffusion measurements

Tracer diffusion measurements of ONS-tagged PEO homopolymers confirm that FRS measurements reproduce well-known reptation behavior (Figure 7 and Supporting Information), showing a scaling of $D_0 \sim M_n^a c^b$ with exponents $a = -2.36 \pm 0.14$ and $b = -3.47 \pm 0.27$, where c is the weight fraction of the PEO homopolymer matrix and M_n is the number-average molecular weight of the tracer as determined by size exclusion chromatography. The M_n exponent is in excellent agreement with both reptation theory¹⁶ and previous experimental results;¹⁵ however, the dependence on matrix concentration is different from deGennes' $D_0 \sim c^{-1.75}$ prediction⁵² for semi-dilute polymers in good solvents that has also been verified by experiment.^{15, 27} A stronger $D_0 \sim c^{-3}$ has been previously observed for self and tracer diffusion measurements in concentrated solutions above ~10–20 wt%.^{28–29, 34} This was explained by DLS measurements and scaling predictions in the θ -condition, which may apply at these high concentrations.⁵³ These tracer diffusion measurements of PEO homopolymers are in good agreement with this previous work on concentrated solutions.

Tracer diffusion measurements of coil-rod-coil triblock copolymers in an entangled coil homopolymer matrix show that the presence of the rod block causes substantial slowing of diffusion as a function of rod length and matrix concentration. For convenient comparison with the theory and simulation results from the curvature mismatch hypothesis,¹⁷ results are presented with respect to reduced variables. Diffusivity is normalized by the coil homopolymer diffusivity D_0 , which can be calculated using the data and scaling laws in Figure 7. Rod length is normalized by the entanglement length of the surrounding homopolymer matrix a . Entanglement lengths are estimated using $a \sim c^{-2/3}$ for semi-dilute theta solutions from reptation theory,^{14, 54} and $a = 3.5$ nm for the melt state at $T = 25^\circ\text{C}$ as measured previously by linear rheology.^{42, 55}

The rapid decrease of tracer diffusivity with increasing matrix concentration verifies key predictions of the curvature mismatch hypothesis (Figure 8a). In the dilute matrix limit the behavior of the block copolymers follows the $D \sim c^{-3}$ behavior observed for coil homopolymer tracers. As concentration increases, the diffusivity departs from this behavior and decreases even more rapidly with increasing matrix concentration. This departure is explained by replotting the data against entanglement length a (Figure 8b), using the scaling above. According to the curvature mismatch hypothesis, the diffusion of coil-rod-coils slows down with rod length L , particularly as L approaches a . The experimental data shows the departure of coil-rod-coils from coil homopolymer behavior occurs at a critical a , and this critical value is larger for larger rod lengths. For $L = 20$ nm and $L = 30$ nm, a departure from coil homopolymer diffusion behavior is observed at even the lowest matrix concentrations. This slowing of diffusion with increasing L and decreasing a is consistent with the curvature mismatch hypothesis.

Another key prediction of the curvature mismatch hypothesis is that normalized diffusivity D/D_0 is independent of the coil size in the small rod regime, where $L \sim a$. This was tested experimentally by synthesizing B₃ polypeptides ($L = 10$ nm) with both 28 kDa and the typical 40 kDa PEO end blocks. The 28 kDa end block tracers show a higher diffusivity as expected because their total molecular weight is different, i.e. the D_0 is different between the two tracers (Figure 9a). Diffusivities of the two tracers can be normalized using D_0 calculated from Figure 7, showing that D/D_0 is indeed independent of the coil size as predicted (Figure 9b). The larger uncertainty in a few of the data points arises as the diffusivity approaches the upper limit of the instrument in its current configuration, where fewer scattering angles are available for the faster exponential decays.

Since D/D_0 is independent of the coil size, direct comparison with the curvature mismatch hypothesis is obtained by replotting the data from Figure 8 against L/a , the key independent variable in the small rod regime. This master curve of the experimental data (Figure 10a) demonstrates that incorporation of a rod into a block copolymer can decrease diffusivity by over an order of magnitude. This may have profound implications for optimal processing conditions for rod-coil block copolymers, and provides a potential explanation for previous reports of kinetically trapped nanostructures in rod-coil systems.⁵ Diffusion measurements from the various matrix concentrations can then be compared to the previous molecular dynamics (MD) simulation results.¹⁷ The agreement is good at the lower values of L/a , which supports the curvature mismatch predictions in the small rod regime. For the higher values of L/a , D/D_0 becomes highly dependent on the matrix concentration and no longer follows a universal curve as predicted in the small rod regime. This phenomenon occurs especially for the two longest rod lengths $L = 20$ nm.

As $L \gg a$, block copolymer diffusion is predicted to occur by an arm retraction of the coil blocks, and $D \sim D_0^r \exp(-\nu M_c/a^2)$ where ν is a prefactor, M_c is coil molecular weight, and D_0^r is the diffusivity of a rod homopolymer of the same total degree of polymerization.^{56–57} At a constant M_c and a (i.e., constant matrix concentration), the diffusivity depends on L only through the D_0^r term. Since the rod is only small mass fraction of the block copolymers in this study, it is thus expected that the dependence of D on L should be weak in this regime. This is verified by the experimental data (Figure 10a), where at constant matrix concentrations D/D_0 becomes approximately constant with respect to L/a at the highest rod lengths. The arm retraction mechanism is further verified by the linear relationship between $\log D$ and a^{-2} for the longest rod length tracers PEO-B₆-PEO and PEO-B₉-PEO where $L = 20$ nm (Figure 10b).

While the qualitative slowing observed in the MD simulation has been verified by the experiment, the experimental data for the smaller rod lengths do not perfectly collapse onto a single master curve. This spread in the data occurs because the matrix concentration is varied in the experimental measurements but were held constant in the MD simulation. Collapsing all concentrations onto a single curve in order to map D/D_0 vs. L/a introduces two challenges. First, the entanglement length at different concentrations was derived from literature values and scaling relationships. Error in the a vs. c relationship (for example, related to the solvent quality for PEO chains) may confound the mapping against L/a .

Second, curvature mismatch predicts that the confining potential of the surrounding entanglements on the rod governs the dependence of D/D_0 vs. L/a in the small rod regime.¹⁷ The dependence of this confining potential on matrix concentration has not been fully characterized,^{58–60} so collapsing concentrations may depend on additional factors. Nevertheless, the quantitative agreement in the short rod regime provides support for the curvature mismatch hypothesis.

CONCLUSIONS

Forced Rayleigh scattering measurements of coil-rod-coil block copolymers in entangled coil homopolymer solutions confirmed the predictions of theory and simulations that led to the curvature mismatch hypothesis. In the short rod regime where $L \sim a$, the predicted slowing of diffusion with increasing L/a was confirmed and reasonable quantitative agreement between molecular dynamics simulation and experimental data was obtained. For the longest rods investigated, the observed behavior as a function of L/a and $1/a^2$ both confirm the arm retraction diffusion mechanism proposed by the theory. Incorporation of a rod into a block copolymer was shown to decrease diffusivity by over an order of magnitude in some cases, with profound implications on optimal processing conditions for rod-coil block copolymers. The same hindered relaxation mechanisms could potentially translate to other dynamical phenomena such as self-assembly kinetics and polymer rheology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

a) Amino acid sequence of rodlike polypeptides. b) DNA agarose gel electrophoresis result of pQE9 expression plasmids encoding the polypeptides digested with *EcoRI* and *HindIII*. c) SDS-PAGE of the polypeptides stained with Coomassie blue.

Figure 2.
Synthesis of dye-labeled coil-rod-coil block copolymers.

Figure 3.

SDS-PAGE of anion exchange chromatography fractions following the reaction of 40 kDa N-hydroxysuccinimide functionalized PEO with ONS-B₃. The elution shows a clear separation between PEO-B₃-PEO triblocks (lanes 1–6) from PEO-B₃ diblocks (lanes 11–14).

Figure 4. Circular dichroism spectra of the polypeptides. Minima at 208 nm and 222 nm confirm helicity.

Figure 5. Small-angle neutron scattering of ONS-labeled PEO (132 kDa) and ONS-labeled PEO-B₆-PEO in 50% PEO solutions. To provide contrast, the tracers were protonated while the PEO and buffer in the surrounding solutions were deuterated.

Figure 6.

a) Decays of diffracted scattering intensity for ONS-labeled PEO132k and PEO-B₆-PEO, both in entangled solutions of 40% PEO homopolymer. The black lines are fits to equation (1). b) $\langle r^2 \rangle$ vs. d^2 for PEO-B₆-PEO in various concentrations of PEO homopolymer. Lines are linear regressions of slope 1 that determine the tracer diffusivity in each sample.

Figure 7. Diffusivities of PEO homopolymers of various molecular weights in entangled PEO solutions of various concentrations. The data follows $D_0 \sim M_n^{-2.36} c^{-3.47}$, which is consistent with reptation scaling predictions.

Figure 8.

a) Diffusivities of coil-rod-coil triblock copolymers of various rod lengths in entangled PEO solutions of various concentrations, compared to the diffusivity of a PEO homopolymer. The coil block was 40 kDa for all triblock tracers, so the total molecular weight of the triblocks ranged from 88kDa to 100kDa. b) Diffusivities plotted against entanglement length as estimated by scaling relationships and linear rheology.

Figure 9.

a) Diffusivities of PEO-B₃-PEO triblock copolymers ($L = 10$ nm) for coil blocks of 28kDa and 40kDa. b) Diffusivities normalized by coil homopolymer diffusivity D_0 .

Figure 10.

a) Normalized diffusivity of coil-rod-coil triblocks as a function of normalized rod length. Experimental results are directly compared with the previous molecular dynamics simulation results. Data at the other matrix concentrations follow the same trends but were omitted for clarity. b) Diffusivity of the longest rod length tracers PEO- B_6 -PEO and PEO- B_9 -PEO vs. $1/a^2$. The linear fits support the arm retraction mechanism in the long rod regime.

Table 1

Coil homopolymer and coil-rod-coil triblock copolymer tracers.

Name	Rod M_n (kg/mol)	Rod length L (nm)	Coil M_n (kg/mol)	Coil PDI	Total M_n (kg/mol)
PEO48k	-	-	48	1.15	48
PEO102k	-	-	102	1.09	102
PEO132k	-	-	132	1.04	132
PEO150k	-	-	150	1.25	150
PEO-B ₃ -PEO	8.1	9.9	40	1.02	88
PEO-B ₄ -PEO	10.1	13.2	40	1.02	90
PEO-B ₆ -PEO	14.4	19.8	40	1.02	94
PEO-B ₉ -PEO	19.9	29.7	40	1.02	100
PEO28-B ₃ -PEO28	8.1	9.9	28	1.09	63

Table 2

Fractional helicities of the B_n polypeptides determined by circular dichroism.

Polypeptide	Length (nm)	Helicity	Estimated Helicity of Alanine-rich Region
B ₃	10	64%	82%
B ₄	13	71%	85%
B ₆	20	74%	86%
B ₉	30	83%	90%