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Andrew P. Nowak[a]; Jun Sato[b]; Victor Breedveld[b]; Timothy J. Deming[a]
[a] School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, GA, USA [b] Bioengineering Department, University of California, Los Angeles, CA, USA

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Hydrogel Formation in Amphiphilic Triblock Copolypeptides

ANDREW P. NOWAK[a], JUN SATO[b], VICTOR BREEDVELD[b] and TIMOTHY J. DEMING[a,*]

[a]*School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA;* [b]*Bioengineering Department, University of California, Los Angeles, CA 90049, USA*

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We report the synthesis of a variety of polylysine-*b*-polyleucine-*b*-polylysine, $K_mL_nK_o$ triblock copolypeptides and compare their gel forming properties to diblock copolypeptides. The architecture of the $K_mL_nK_o$ triblock copolypeptides forces the hydrophobic α-helical oligoleucine segments to pack perpendicular to extended fibrils in lamellar structure, and the similarity in properties between diblock and triblock copolymers supports the hypothesis that both systems assemble in a similar manner. The triblock architecture, however, allows preparation of samples with greater gel strength and better salt tolerance than diblock samples at similar concentrations. We have found that block architecture provides a means for fine adjustment of hydrogel properties beyond variation of polypeptide composition, concentration, and chain length.

Keywords: Hydrogel; Copolypeptides; Chain length; Biocompatible

INTRODUCTION

Hydrogel materials are well suited to biotechnology applications such as drug delivery and tissue engineering [1]. While many naturally derived hydrogel materials are biocompatible, they often suffer from inconsistent material properties, and chemical modification to alter their mechanical properties can be difficult. Synthetic hydrogels provide the advantage of tuning desired properties through control over chemical composition, chain length, and polymer concentration [2]. We have used transition metal initiators to synthesize diblock copolypeptides composed of polyelectrolyte segments linked to hydrophobic, α-helical segments that self-assemble to form strong, clear hydrogels [3–5]. Here, we report the synthesis of polylysine-*b*-polyleucine-*b*-polylysine, $K_mL_nK_o$, triblock copolypeptides and compare their gel forming properties to diblock copolypeptides. We have found that block architecture provides a means for fine adjustment of hydrogel properties beyond variation of polypeptide composition, concentration, and chain length.

Characterization of the hydrogels based on polylysine-*b*-polyleucine, K_mL_n, diblock copolypeptides revealed that the chains self-assemble into disorganized fibrillar supramolecular structures where the α-helical oligoleucine segments pack side by side with the helical rods oriented perpendicular to the long axes of fibrils (Fig. 1) [6]. This arrangement places the charged polylysine segments on the outer surface of the fibrils as a dense brush. Defects in fibrillar assembly result in branches and tie-points that give the three-dimensional hydrogel network structure. Based on this model, we reasoned that triblock copolypeptide architectures, with charged polylysine segments on the outside and oligoleucine segments on the inside, should pack well into similar supramolecular assemblies (Fig. 1) [4]. Furthermore, since the triblocks will have two polylysine segments per oligoleucine chain, the density of the polylysine brush should be much greater in triblock assemblies than in the diblock copolymers. This high polyelectrolyte density was expected to enhance formation of smaller diameter fibrils, as opposed to wider fibrils or even membranes, due to increased interchain repulsions in the brush [6]. For this reason, we expected that triblock architectures, with all other parameters being equal, would form stronger hydrogels than their analogous diblock copolypeptide counterparts.

*Corresponding author. E-mail: demingt@seas.ucla.edu

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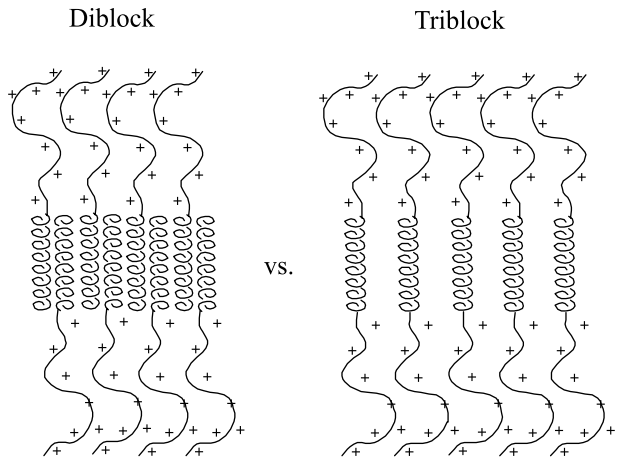

FIGURE 1 Drawings showing predicted packing arrangement of helical segments in block copolypeptide hydrogels. Diblock (K_nL_m) and triblock ($K_nL_mK_o$) architectures are shown for comparison.

When a triblock sample (e.g. $K_{90}L_{20}K_{90}$) was synthesized with a total degree of polymerization (DP) of 200, it was found to behave as a thin fluid at 3 wt% in DI water, while the diblock copolymer of identical DP and composition (e.g. $K_{180}L_{20}$) formed a hydrogel (Table I). The failure of the $K_{90}L_{20}K_{90}$ sample to gel must be tied to the different chain architecture and we suspected that the decrease in polylysine brush length was the key difference. From earlier studies, we had observed that lower DP diblock copolypeptides (e.g. $K_{80}L_{20}$) were also unable to form hydrogels, most likely since the polylysine brush was too short to distort the supramolecular assemblies from flat membranes into fibrillar networks [4]. To investigate the influence of polylysine length on gel formation, a series of diblock copolypeptides was synthesized using a constant 4:1 molar ratio of lysine and leucine (Table II). A total of 6 samples ranging from $K_{160}L_{40}$ to $K_{80}L_{20}$ were synthesized. As overall chain length was reduced from 200 to 100, the gel strengths of the materials steadily decreased until an abrupt transition in the mechanical properties of the materials occurred between the DPs of 160 and 140. This result showed that a critical polyelectrolyte brush length extending from the hydrophobic segments is required to induce gel network formation. Consequently, when the polylysine lengths in the triblock were increased to give $K_{190}L_{20}K_{190}$, a hydrogel was formed at low concentrations (Table I).

To better compare hydrogel properties of diblock and triblock copolypeptides, we chose to use samples with similar hydrophobe and polylelectrolyte segment lengths, where the triblock samples consequently had higher overall DP. When polymers with equivalent hydrophobic lengths were compared (i.e. $K_{160}L_{40}$ and $K_{180}L_{40}K_{180}$) it was found that the strength of the triblocks (G') was consistently higher (Table I). Since the triblock assemblies must pack twice as many polyelectrolyte chains than the diblocks at the block interface, a different supramolecular structure (i.e. narrower fibrils) may form that would explain the higher gel strengths [6]. It is noteworthy that the triblock samples achieve significantly higher gel strengths with only half the weight fraction of leucine content as compared to the diblocks. However, the increased modulus of the triblocks may be due solely to differences in overall DP. In effort to eliminate molecular weight effects, diblocks and triblocks of equal overall chain length, such as $K_{360}L_{40}$ and $K_{180}L_{40}K_{180}$, were also compared to determine how block architecture alone affected hydrogel properties. In this case both diblock and triblock copolymers formed gels, but the $K_{360}L_{40}$ samples gave highly irreproducible rheological data, which prevented any meaningful comparison. Visual observation of the diblock samples revealed inhomogeneities where a dense layer of solvated copolymer was found at the surfaces of the glass containers, likely due to associations between the long polylysine segments and the glass.

TABLE I Hydrogel properties for a variety of K_mL_n and $K_mL_nK_o$ copolypeptide samples. The gel point concentration was determined in DI water and is defined as the lowest concentration for which the storage modulus G' exceeds the loss modulus G'' at an angular frequency $\omega = 1$ rad/s. The G' values were all measured at 1 rad/s for 3.0 wt% samples in both DI water and 100 mM NaCl. NA = experiments not applicable or not performed.

Sample	Gel Point (wt%)	G' (3 wt%, Pa)	G' (100 mM NaCl 3 wt%, Pa)
$K_{80}L_{20}$	NA	NA	NA
$K_{60}L_{40}$	NA	NA	NA
$K_{180}L_{20}$	2	12	26
$K_{170}L_{30}$	0.75	590	520
$K_{160}L_{40}$	0.25	4270	300
$K_{90}L_{20}K_{90}$	NA	NA	NA
$K_{190}L_{20}K_{90}$	2.5	24	59
$K_{190}L_{20}K_{190}$	0.5	420	340
$K_{185}L_{30}K_{90}$	0.75	370	460
$K_{185}L_{30}K_{185}$	0.40	1040	230
$K_{80}L_{40}K_{80}$	0.75	700	290
$K_{135}L_{40}K_{135}$	0.25	3670	1200
$K_{180}L_{40}K_{90}$	0.25	4000	880
$K_{180}L_{40}K_{180}$	0.25	8650	1300

TABLE II Hydrogel properties for a series of K_mL_n diblock copolypeptide samples with a constant lysine to leucine molar ratio of 4:1. The gel point concentration was determined in DI water and is defined as the lowest concentration for which the storage modulus G' exceeds the loss modulus G'' at an angular frequency $\omega = 1$ rad/s. The G' values were all measured at 1 rad/s for 3.0 wt% samples in both DI water. NA = experiments not applicable or not performed. M_n are number average molecular weight values for the polylysine domain only determined using size exclusion chromatography.

Sample	$M_n \times 10^{-3}$	Gel Point (wt%)	G' (3 wt%, Pa)
$K_{80}L_{20}$	9.2	>3.0	NA
$K_{96}L_{24}$	11.4	>3.0	NA
$K_{112}L_{28}$	16.6	>3.0	NA
$K_{128}L_{32}$	18.3	0.5–1.0	790
$K_{144}L_{36}$	24.7	0.25–0.5	1180
$K_{160}L_{40}$	25.0	0.25	4270

From our studies of different K–L diblock copolypeptides we were able to identify three main parameters that influence gel formation and gel strength. The clearest trend observed in all copolymers was the increase in gel modulus with increasing leucine segment length (Table I, Fig. 2) [3,4]. The next most significant parameter was the polyelectrolyte brush length, which must be sufficiently large to drive gel network formation, as shown above. The most subtle molecular parameter observed was how increased polyelectrolyte brush density in the triblock samples increased gel modulus. The combined effects of all these parameters must be considered when analyzing the properties of any specific copolymer. For example, these factors can be used to understand the behavior of the triblock $K_{80}L_{40}K_{80}$. This triblock was found to gel even though it contains very short polyelectrolyte segments (Table I). It is worth noting that a similar sample with a shorter leucine domain, $K_{90}L_{20}K_{90}$, and a diblock with similar polyelectrolyte length, $K_{60}L_{40}$, both did not form hydrogels. The combination of a long L_{40} hydrophobic segment and

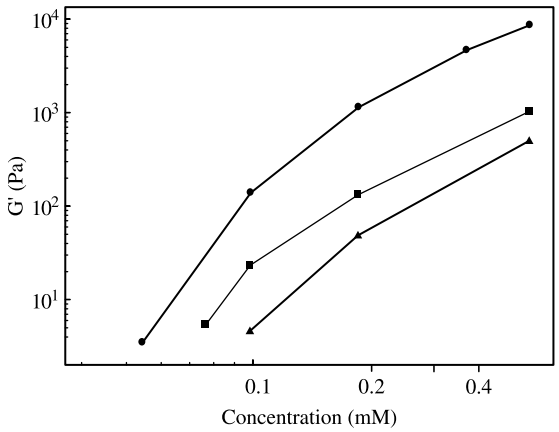

FIGURE 2 Hydrogel strength (G') as a function of molar concentration for symmetric $K_nL_mK_o$ triblock copolypeptides of different oligoleucine length. Gel strengths were measured at 1 rad/s: (●) $K_{180}L_{40}K_{180}$, (■) $K_{185}L_{30}K_{185}$, (▲) $K_{190}L_{20}K_{190}$.

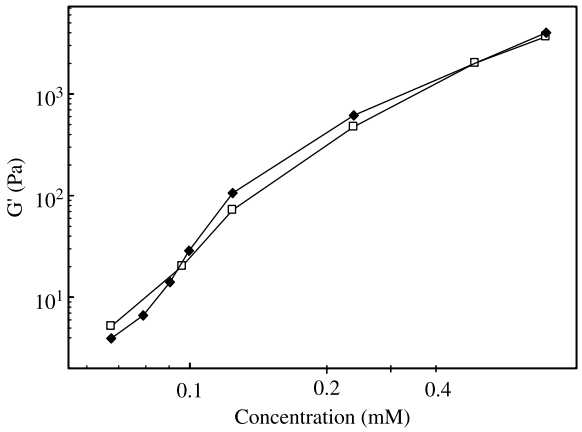

FIGURE 3 Hydrogel strength (G') as a function of molar concentration for symmetric and asymmetric $K_nL_mK_o$ triblock copolypeptides with overall identical polylysine content. Gel strengths were measured at 1 rad/s: (◆) $K_{180}L_{40}K_{90}$, (□) $K_{135}L_{40}K_{135}$.

increased polyelectrolyte density of the triblock architecture in $K_{80}L_{40}K_{80}$ must act to overcome the drawback of a short polyelectrolyte segment to yield a modest hydrogel.

Since polyelectrolyte length and density play a complex role in formation of these hydrogels, we further analyzed the effects of polyelectrolyte length by preparation of triblocks with short and long polyelectrolyte segments in the same polymer. These asymmetric copolymers ensure mixing of dissimilar polyelectrolyte segments in the assemblies, which permits comparison of samples with identical polylysine contents but different individual chain lengths. Comparison of an asymmetric triblock ($K_{180}L_{40}K_{90}$) to a compositionally identical symmetric triblock ($K_{135}L_{40}K_{135}$) at the same molar concentration revealed that they have near indistinguishable mechanical properties (Fig. 3). This result shows that the total polyelectrolyte content of a copolymer is the most important parameter in determining gel strength when hydrophobic segment length is held constant. This trend is shown by comparison of $K_{80}L_{40}K_{80}$, $K_{135}L_{40}K_{135}$, and $K_{180}L_{40}K_{180}$, where G' increases steadily with total polylysine content (Fig. 4).

The triblock copolypeptide gels were found to respond to added salts in a manner similar to the diblock copolypeptide gels [7]. When salt was introduced into a hydrogel solution, triblock gels with large hydrophobic domains (e.g. $K_{180}L_{40}K_{180}$) began to precipitate from solution and lose mechanical integrity. However, samples with shorter hydrophobic segments (e.g. $K_{190}L_{20}K_{190}$, and $K_{185}L_{30}K_{185}$) are inherently weaker gels, but were stable in salt solutions, and even showed an increase in G' for the asymmetric triblock samples. While the diblock and triblock systems are qualitatively similar in their behavior in salt solutions, the triblocks form gels that are in general more stable in the presence of

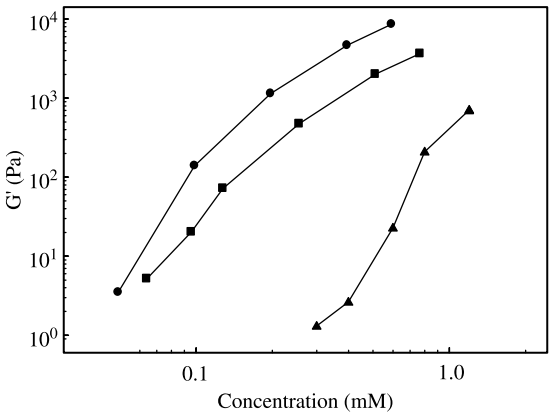

FIGURE 4 Hydrogel strength (G') as a function of molar concentration for symmetric $K_nL_mK_o$ triblock copolypeptides of different polylysine length. Gel strengths were measured at 1 rad/s: (●) $K_{180}L_{40}K_{180}$, (■) $K_{135}L_{40}K_{135}$, (▲) $K_{80}L_{40}K_{80}$.

100 mM NaCl. This stability is likely due to the increased solubility of the triblocks since the assembled hydrophobic segments are better stabilized by the presence of hydrophilic polylysine on both ends.

The architecture of the $K_mL_nK_o$ triblock copolypeptides forces the hydrophobic α-helical oligoleucine segments to pack perpendicular to extended fibrils in lamellar structure, and the similarity in properties between diblock and triblock copolymers supports the hypothesis that both systems assemble in a similar manner [6]. The triblock architecture, however, allows preparation of samples with greater gel strength and better salt tolerance than diblock samples at similar concentrations. This versatility makes the triblock copolypeptides more attractive than diblocks for many practical hydrogel applications in ionic media, including personal care products and as biomedical materials.

SUPPORTING INFORMATION

Samples

All block copolypeptides were synthesized, purified and characterized as previously described [4]. Isolated yields of the final copolymers ranged between 75% and 90%. Amino acid compositions of the copolymers were found to be within 3% of predicted values. Chain lengths of the copolymers were found to be within 8% of predicted lengths with chain length distributions (weight average length/number average length) ranging between 1.1 and 1.3. We have found that block copolymers with chain length distributions greater than *ca*. 1.5 are unable to form hydrogels [4].

Rheology

Rheological measurements were performed on an ARES-LS1 controlled strain rheometer from Rheometric Scientific (now TA Instruments). Additional studies of the dynamic moduli were carried out on an MCR300 controlled stress rheometer from Paar-Physica. Both instruments were equipped with cone–plate configurations. Two different geometries were used on the ARES: a 50 mm diameter cone with a 2° angle and a 25 mm cone with a 4° angle. The latter geometry is less stress-sensitive because of the smaller diameter but requires a smaller sample volume. The choice between the two geometries was based on a qualitative judgment of the sample properties and the amount of material available. Concentrated, gel-like samples could be measured with the 25 mm cone, whereas dilute solutions required the sensitivity of the 50 mm cone. For a number of samples both geometries were employed to ensure reproducibility, and excellent agreement was always observed. The MCR300 was operated with a 50 mm diameter 1° cone or with a 25 mm 2° cone.

The rheological properties of all samples were determined through the following measurement protocol. First, an oscillatory strain amplitude sweep (strain amplitude $\gamma_o = 0.001–10$) at fixed frequency ($\omega = 6$ rad/s) was performed to establish the linear regime. Having established the maximum strain permitted for linear viscoelastic response, an oscillatory frequency sweep (frequency $\omega = 0.01–100$ rad/s) was performed to measure $G'(\omega)$ and $G''(\omega)$, the linear viscoelastic storage and loss moduli, respectively. The strain and frequency sweeps were used to characterize well-rested samples in "equilibrium" as well as to monitor the recovery of gels after their breakdown by large-amplitude oscillatory strain. Between strain amplitude sweeps and frequency sweeps, samples were allowed to rest for several minutes to facilitate sample recovery from the large nonlinear oscillatory deformations. In addition to oscillatory measurements, steady–shear flow curves were also measured.

Finally, we note that the rheological measurements were not sensitive to the methods used to prepare the solutions. In general, solutions were prepared by dissolving freeze–dried block copolypeptide samples in deionized water, followed by enhancing the mixing and dissolution process with a vortex mixer. However, identical mechanical properties were obtained by allowing the copolypeptides to dissolve without agitation overnight. Samples were left overnight before performing rheological experiments, which was sufficiently long to ensure reproducibility. All rheological measurements were carried out with Peltier thermostating units at 23.5°C in order to minimize evaporation of the aqueous samples.

Acknowledgements

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