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Nonionic Block Copolypeptide Micelles Containing a Hydrophobic racemic-Leucine Core

Jarrod A. Hanson^a, Zhibo Li^{a,b}, and Timothy J. Deming^{a,c,*}

^aBioengineering Department, University of California, Los Angeles, CA 90095

^bState Key Laboratory of Polymer Physics and Chemistry, Institute of Chemistry, Chinese Academy of Sciences, Beijing, China 100190

^cChemistry and Biochemistry Department, University of California, Los Angeles CA 90095

Abstract

Block copolymer micelles have been used extensively as carriers for therapeutic drugs and diagnostic molecules. Here, we report the synthesis of nonionic, block copolypeptides, $K^P_x(rac-L)_y$, which have a “reversed” rod-coil structure composed of a hydrophilic, rod-like, α -helical segment attached to a disordered, racemic hydrophobic segment. The self assembly of these block copolypeptides in water was studied, and their compositions were optimized to identify a sample, $K^P_{100}(rac-L)_{10}$, which is able to form well defined micelles that are very stable against dilution, high temperatures, and various media. Micelle structure was determined using a combination of electron microscopy and dynamic light scattering measurements. The potential of these micelles as drug delivery carriers was evaluated by encapsulation of the anticancer drug camptothecin. The drug containing micelles were found to be stable.

Micelles are widely used to disperse materials for a range of food,¹ cosmetic,² and pharmaceutical³ applications. These nanoscale assemblies are composed of amphiphilic molecules that self-assemble in water.³ Block copolymers make up a large class of micelle forming molecules,^{2,4,5} and include those containing polypeptide segments, which can be enzymatically degraded to natural metabolites and possess ordered conformations not found in conventional polymers. Numerous “rod-coil” micelles have been prepared using α -helical hydrophobic polypeptides conjugated to hydrophilic polyethylene glycol (PEG) segments, such as PEG-b-poly(γ -benzyl-L-glutamate)^{6,7} and PEG-b-poly(β -benzyl-L-aspartate).⁸ β -strand polypeptide segments have also been used to facilitate interchain interactions and increase micelle stability.⁹ Here, we report the synthesis of poly(N_ϵ -2-[2-(2-methoxyethoxy)ethoxy]acetyl-L-lysine)_x-b-poly(D,L-leucine)_y, $K^P_x(rac-L)_y$, “reversed” rod-coil block copolymers where the rod-like component is the hydrophilic segment. Use of disordered racemic hydrophobic segments was found to favor formation of spherical micelles in water that can entrap cargos and possess a nonionic, PEG-like corona potentially useful for drug delivery applications (Figure 1A).¹⁰

We previously reported that homopolypeptides of diethylene glycol modified lysine residues, K^P , are nonionic, water soluble, and thermally stable α -helices.¹¹ These segments were used to prepare rod-rod amphiphilic diblock copolypeptides, K^P_x -b-poly(L-leucine), $K^P_xL_y$, which were found to self-assemble into micron-sized vesicles and sheets in water

* demingt@seas.ucla.edu .

Supporting Information Available: Synthesis, experimental procedures, and additional data on the assemblies. This material is available free of charge via the Internet at <http://pubs.acs.org>.

where assembly morphology was strongly directed by the preferred packing of α -helical poly(L-leucine) segments.¹² The $K^P_xL_y$ vesicles were stable under a variety of solution conditions and able to encapsulate small hydrophilic molecules. To disfavor formation of membranes and encourage formation of micelles, we reasoned that use of racemic-leucine would prevent α -helix formation and increase chain flexibility. Disordered racemic polypeptides also present exposed amide groups capable of facilitating H-bonding interactions between hydrophobic chains in the assemblies. Recently, short oligopeptides containing racemic hydrophobic segments were used to show how both intra- and inter-molecular H-bonding can lead to the formation of micelles with a range of diameters and gel-forming properties.¹³

$K^P_x(rac-L)_y$ samples were prepared using two hydrophilic segment lengths ($x = 45$ or 100), and were each combined with a range of racemic-leucine segment lengths ($y = 10, 20, 30, 40$) (Table 1). Aqueous suspensions of these samples were prepared either by direct dissolution in water or by solvent annealing, which was used to fully disperse the chains. For solvent annealing, copolypeptides were initially dissolved in a 3 % (v/v) solution of TFA in THF, which fully solubilizes both segments of $K^P_x(rac-L)_y$. To these solutions were added equal volumes of water followed by exhaustive dialysis against water to remove cosolvents and drive assembly.

Regardless of dissolution method, self-assembled morphology was found to depend primarily on the ratio of hydrophobic to hydrophilic chain lengths. $K^P_{45}(rac-L)_{10}$ samples were found by TEM imaging to give irregular micellar assemblies ranging in diameter from 20 – 150 nm.¹⁴ As the $(rac-L)_y$ length was increased, TEM and fluorescence microscopy imaging showed the formation of large microscale assemblies composed of clusters of irregular nanoscale micelles.¹⁴ Examination of $K^P_{100}(rac-L)_{20}$ and $K^P_{100}(rac-L)_{30}$ samples by fluorescence microscopy and TEM also showed formation of large, irregular microscale aggregates.¹⁴ In these samples, the rod-like hydrophilic segments were not able to efficiently stabilize the micellar cores, leading to extended aggregation into large clusters. In contrast, aqueous suspensions of $K^P_{100}(rac-L)_{10}$ (0.5 % (w/v)) prepared using either method gave well-defined spherical micelles (Table 1), indicating the required hydrophilic to hydrophobic balance to stabilize micelles was obtained in this sample. Negative stain TEM (Figure 2A) and cryogenic TEM (Figure 2B) images of this sample showed spherical micelles with outer diameters of ca. 80 nm and core diameters of ca. 25 nm. Circular dichroism (CD) spectroscopy showed that the $K^P_{100}(rac-L)_{10}$ copolymer is ca. 80 % helical (Table 1),¹⁴ which indicates that the racemic-leucine segment introduces disorder into the hydrophobic domain and part of the hydrophilic segment. In contrast, the CD spectrum of $K^P_{100}L_{10}$ showed an entirely α -helical block copolypeptide (Table 1),¹⁴ which in water yielded only large microscale sheets or clusters (Table 1).¹² These results show the importance of the disordered racemic-leucine segment for spherical micelle formation.

Micelle stability was also investigated. The $K^P_{100}(rac-L)_{10}$ micelles possess a low critical micelle concentration, CMC (Table 1), relative to other block copolymer micelles such as those based on Pluronics®.¹⁷ They also showed excellent stability at elevated temperatures and under a variety of solution conditions.¹⁴ This stability is surprising since the micelles form by direct dissolution in water, and may be due to interchain H-bonding interactions that occur once the micelle cores are formed. These features may be important for drug delivery uses where micelles are often subject to variations in media and high dilution.³

Also important for drug delivery is the encapsulation of hydrophobic cargos. The encapsulation ability of $K^P_{100}(rac-L)_{10}$ micelles was determined using model cargo pyrene as well as an anticancer drug camptothecin, CPT. These cargos were incorporated into micelles using a modified-emulsion method.¹⁸ We found that pyrene could be loaded into

micelles, but at the cost of increasing the micelle diameters, possibly due to poor packing within the micelle core (Table 2). However, CPT was more promising as it could be encapsulated at a higher level with a smaller increase in micelle diameter (Table 2).

In summary, we found that $K^P_{100}(\text{rac-L})_{10}$, which possesses an uncommon rod-like hydrophilic segment and a disordered hydrophobic segment, is able to form highly stable, nanoscale polypeptide micelles. This optimized composition was essential for formation of spherical micelles rather than extended assemblies typically observed with polypeptides. The $K^P_{100}(\text{rac-L})_{10}$ micelles were also able to efficiently encapsulate cargos such as the anticancer drug CPT without destabilization and have promise for use as drug carriers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Schematic showing block copolypeptides and self-assembled structures. (A) $K_x^P(rac-L)_y$ micelles. (B) $K_x^P L_y$ sheets/fibrils.

Figure 2.

A) Negative stain TEM image showing nanostructure of $K^P_{100}(rac-L)_{10}$ micelles. B) Cryogenic TEM image of a 0.50 % (w/v) aqueous suspension of $K^P_{100}(rac-L)_{10}$. Scale bars = 50 nm.

Table 1

Block copolypeptide synthesis and assembly data

Block Copolypeptide	M_n ($\times 10^{-3}$) ^a	M_w/M_n	Found Composition ^b	Yield (%)	Self-Assembled Structure ^c	Aggregate Size (nm) ^d		CMC (M) ^e	% Helicity ^f
						Solvent Annealing	Direct Dissolution		
K ^P ₁₀₀ L ₁₀	28	1.1	K ^P ₉₇ L ₁₁	90	C/S	100, 450	> 1,000	3.8×10^{-7}	99
K ^P ₁₀₀ (rac-L) ₁₀	28	1.1	K ^P ₉₇ (rac-L) ₁₁	91	M	80	80	8.1×10^{-7}	80
K ^P ₁₀₀ (rac-L) ₂₀	28	1.1	K ^P ₉₇ (rac-L) ₂₂	87	C	10 – 1,000	-	-	-
K ^P ₁₀₀ (rac-L) ₃₀	28	1.1	K ^P ₉₇ (rac-L) ₃₁	90	C	500 – 3,000	-	-	-
K ^P ₄₅ (rac-L) ₁₀	13	1.2	K ^P ₄₅ (rac-L) ₉	92	C	20 – 150	-	-	-
K ^P ₄₅ (rac-L) ₂₀	13	1.2	K ^P ₄₅ (rac-L) ₂₁	87	C	500 – 1,000	-	-	-
K ^P ₄₅ (rac-L) ₃₀	13	1.2	K ^P ₄₅ (rac-L) ₂₉	86	C	500 – 1,000	-	-	-
K ^P ₄₅ (rac-L) ₄₀	13	1.2	K ^P ₄₅ (rac-L) ₃₈	94	C	1,000 – 25,000	-	-	-

^aK^P segment length and polydispersity (M_w/M_n) determined using gel permeation chromatography.

^bCalculated using ¹H {¹³C} NMR integrations.

^cC = micellar clusters, S = sheets, M = micelles.

^dDetermined from DLS data using CONTIN algorithm and/or analysis of DIC optical microscopy images.

^eMeasured using pyrene fluorescence.¹⁵

^fDetermined using intensity of minimum at 222 nm in CD spectra.¹⁶

Table 2

Cargo encapsulation data

Cargo	% Encapsulation ^a	% Loading ^b	Micelle diameter (nm) ^c
pyrene	47 (5)	2.3 (0.2)	280 (130)
camptothecin	76 (5)	3.8 (0.2)	118 (50)

^a (g cargo encapsulated/g cargo).

^b (g cargo encapsulated/(g polymer + g cargo)).

^c Determined from DLS data using CONTIN algorithm. Standard deviations given in parentheses.