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Nonionic Block Copolypeptide Micelles Containing a Hydrophobic *rac*-Leucine Core

Jarrod A. Hanson,[†] Zhibo Li,[†,‡] and Timothy J. Deming*[,†,§]

[†]*Bioengineering Department, University of California, Los Angeles, California 90095,* [‡]*State Key Laboratory of Polymer Physics and Chemistry Institute of Chemistry, Chinese Academy of Sciences, Beijing, China 100190, and* [§]*Chemistry and Biochemistry Department, University of California, Los Angeles, California 90095*

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Micelles are widely used to disperse materials for a range of food,[1] cosmetic,[2] and pharmaceutical[3] 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 poly(ethylene 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(DL-leucine)$_y$, K^P_x(*rac*-L)$_y$, "reversed" rod−coil block copolymers where the rodlike 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)$_y$, $K^P_xL_y$, which were found to self-assemble into micrometer-sized vesicles and sheets in water 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 *rac*-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 intermolecular 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 *rac*-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.

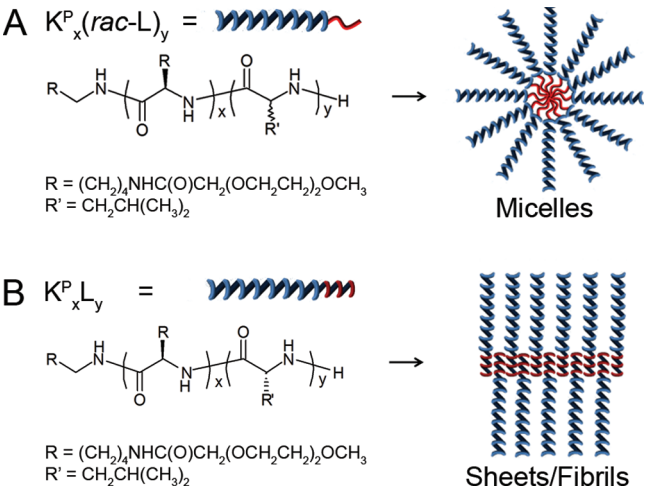

Figure 1. Schematic showing block copolypeptides and self-assembled structures: (A) K^P_x(*rac*-L)$_y$ micelles; (B) $K^P_xL_y$ sheets/fibrils.

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 to 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 rodlike 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 *rac*-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 *rac*-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]

*To whom correspondence should be addressed. E-mail: demingt@seas.ucla.edu.

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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] solvent annealing	aggregate size (nm)[d] direct dissolution	cmc (M)[e]	% helicity[f]
$K^P_{100}L_{10}$	28	1.1	$K^P_{97}L_{11}$	90	C/S	100, 450	>1000	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	10−1000			
K^P_{100}(*rac*-L)$_{30}$	28	1.1	K^P_{97}(*rac*-L)$_{31}$	90	C	500−3000			
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	500−1000			
K^P_{45}(*rac*-L)$_{30}$	13	1.2	K^P_{45}(*rac*-L)$_{29}$	86	C	500−1000			
K^P_{45}(*rac*-L)$_{40}$	13	1.2	K^P_{45}(*rac*-L)$_{38}$	94	C	1000−25000			

[a] K^P segment length and polydispersity (M_w/M_n) determined using gel permeation chromatography. [b] Calculated using ^1H {^{13}C} NMR integrations. [c] C = micellar clusters, S = sheets, M = micelles. [d] Determined from DLS data using CONTIN algorithm and/or analysis of DIC optical microscopy images. [e] Measured using pyrene fluorescence.[15] [f] Determined using intensity of minimum at 222 nm in CD spectra.[16]

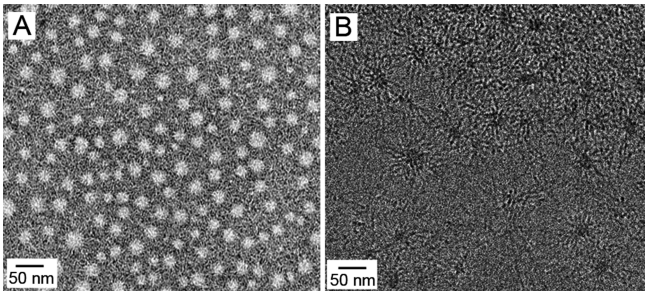

Figure 2. (A) Negative stain TEM image showing nanostructure of K^P_{100}(*rac*-L)$_{10}$ micelles. (B) Cryogenic TEM image of a 0.50% (w/v) aqueous suspension of K^P_{100}(*rac*-L)$_{10}$. Scale bars = 50 nm.

Table 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] In (g cargo encapsulated/g cargo). [b] In (g cargo encapsulated/(g polymer + g cargo)). [c] Determined from DLS data using CONTIN algorithm. Standard deviations are given in parentheses.

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}(*rac*-L)$_{10}$, which possesses an uncommon rodlike 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}(*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.

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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.

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