Polymersomes from hybrid peptide-based bottlebrush homopolymers

Sameer Dhawan, M.B. Bijesh, V. Haridas[*]

Department of Chemistry, Indian Institute of Technology Delhi, New Delhi 110016, India

ABSTRACT

A series of compounds, in which *exo*-norbornene scaffold appended with amino acids, was synthesized. These compounds underwent ring opening metathesis polymerization (ROMP) catalyzed by Grubbs second generation ruthenium catalyst to form polymers with bottlebrush architecture. Four different polymers **P1-P4** were synthesized, differing in number of lipid units and degree of branching. These polymers were well-characterized by ^{1}H NMR and gel permeation chromatographic (GPC) techniques. These peptide-based homopolymers showed an exceptional ability to self-assemble to form polymersomes. The self-assembled structures were characterized by various ultramicroscopic techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The entrapment capability of polymer vesicles was confirmed by dye-encapsulation studies.

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1. Introduction

Self-assembly is an elegant strategy for the construction of a wide range of supramolecular architectures such as micelles, ribbons, vesicles, helices, rods, toroids, fibres and tubes [1—4]. Among these, vesicles find immense interest due to their potential applications as drug delivery vehicles, nanoscale reaction vessels and stimuli-responsive materials [5—7]. In the early years, Banghem and Horne reported the spontaneous formation of bilayered vesicular structures from an aqueous dispersion of lecithin and cholesterol molecules [8]. After that many research efforts were directed to design and synthesize molecules for vesicular morphology [9,10]. Presently, there exists a wide range of synthetic systems that display vesicular self-assembly in aqueous and in non-aqueous solvents [11].

Polymersomes are vesicles made up of synthetic amphiphilic copolymers [12,13]. Polymersomes or polymer vesicles contain a hollow interior surrounded by polymeric membrane [14,15]. Hammer and Discher et al. coined this term 'polymersomes' for the vesicles obtained from polyethylene glycol-polyethylethylene (PEG-PEE) diblock copolymer due to their resemblance with liposomes [15]. At present, there exist a large number of synthetic amphiphilic block copolymers that have been reported to form polymersomes with wide range of sizes and surface properties

[16,17]. Polymersomes have 10-folds higher membrane strength than those of the liposomes, which imparts better mechanical and chemical stabilities to them [18]. These unique features enabled scientists to explore the potential applications of polymersomes as drug delivery vehicles in order to prevent the premature drug release. Moreover, the hollow interior of polymersomes facilitated the encapsulation of both hydrophilic as well as hydrophobic agents leading to the extension of their applications in the field of drug delivery, diagnostic imaging, DNA-RNA delivery, gene therapy, as artificial organelles, cell mimics, and as nano-reactors for chemical reactions [19—23].

For more than a decade, polymersomes were obtained from the copolymers containing hydrophobic block of various polyesters and polycarbonates, and hydrophilic block of polyethylene glycol (PEG) [24,25]. PEG units were introduced due to their ability to resist plasma protein adsorption which protects them from body's innate immune system [26]. Thus, polymersomes with PEG brushes on their surfaces have stealth character that imparts prolonged blood circulation for targeted drug delivery [27]. Peptide—based polymersomes are more attractive candidates for biomedical applications compared to the conventional synthetic polymers. The reason is not only the presence of metabolizable peptide units but also the unique ability of peptides to form ordered self-assembled structures. Copolymerization of polypeptide blocks with synthetic polymers leads to the formation of ordered nanostructures through intermolecular hydrogen bonding unlike the conventional synthetic copolymers that generally form coil structures [28]. Such

* Corresponding author.
  E-mail address: haridasv@iitd.ac.in (V. Haridas).

hybrid polymers not only provide the proper control over the assembly but also result in the formation of biologically compatible materials [28]. Many of such hybrid peptidic systems have been reported in the literature along with their biomedical applications [29–31].

Mostly, synthetic polymers that can self-assemble to form polymersomes are copolymers [19–23]. Most of the polymersomes forming polypeptide systems are also diblock or triblock copolymers [26–31]. To our knowledge, synthetic peptide based homopolymers that form polymer vesicles are scarce.

2. Experimental section

2.1. Materials

Amino acids used were of L-configuration and were purchased from SRL India. Reagents were purchased from Sigma-Aldrich or Alfa Aesar. All reagents were used without further purification. Solvents employed in the reactions were distilled/dried prior to use. Progress of reactions was monitored by silica gel thin layer chromatography (TLC). Compounds were purified by silica gel column chromatography. Characterizations were done by the ^{1}H NMR, ^{13}C NMR, IR and High Resolution Mass Spectra (HRMS). IR spectra were recorded on a Nicolet, Protégé 460 spectrometer as KBr pellets. Brucker-DPX-300 spectrometer was used for recording ^{1}H NMR spectra. Tetramethylsilane (TMS) was used as an internal standard. Coupling constants are reported in Hz and the data are reported as s (singlet), d (doublet), br (broad), t (triplet) and m (multiplet), dd (double doublet). High Resolution mass spectra (HRMS) were recorded in Bruker MicrO-TOF-QII model using ESI technique. Melting points were recorded on a Fisher-Scientific melting point apparatus.

2.2. Methods

2.2.1. Scanning electron microscopy (SEM)

Samples were prepared by dissolving 2 mg of polymer per mL of the chosen solvent system. A 10 μL of the sample solution was drop-casted on a fresh glass coverslip. The coverslip was attached to a stub using carbon tape. The sample was dried and coated with ~10 nm of gold. ZEISS EVO Series Scanning Electron Microscope EVO 50 operating at an accelerating voltage of 0.2–30 kV was used for imaging.

2.2.2. Atomic force microscopy (AFM)

About 10 μL of the sample solution was transferred onto freshly cleaved mica and allowed to dry and imaged using AFM. Bruker Dimension Icon atomic force microscope was used for imaging the samples. Tapping mode was used for imaging. Images were recorded at room temperature and data analysis was performed using nanoscope 5.31r software.

2.2.3. High Resolution transmission electron microscopy (HR-TEM)

Sample solutions of polymers were prepared by dissolving 2 mg of polymer per mL of the chosen solvent system. About 5 μL aliquot of the polymer solution was placed on a copper grid (200 mesh) and allowed to dry at room temperature. Samples were viewed using FEI Tecnai G^2 F20 TWIN transmission electron microscope.

2.2.4. Optical microscopy

Polymer samples were prepared in respective solvent systems. About 10 μL of the polymer solution was mounted on a glass slide and allowed to dry in the open air at room temperature. The sample was viewed using optical microscope (Nikon Eclipse TS100) in bright field.

2.2.5. Fluorescence microscopy

Polymer sample solutions were prepared in chosen solvent systems and were mixed with 0.02 equivalents of 2 mM Rhodamine B dye solution (prepared in the same solvent system). About 10 μL of the sample solution was drop casted on a glass slide and allowed to dry in the open air at room temperature. The unbound Rhodamine B was removed by washing with distilled water. The slide containing the sample was dried by flushing with nitrogen gas and was viewed using optical microscope (Nikon Eclipse TS100) using excitation wavelength λ_{ex} = 510-560 nm.

2.2.6. Dynamic light scattering (DLS) studies

Polymers were dissolved in chosen solvent systems and the solution was filtered by using Nylon syringe filter (pore size = 0.2 μm). Malvern Zetasizer, NANO ZS90 (Malvern Instruments Limited, U.K.) equipped with a 4 mW He–Ne laser operating at a wavelength of 633 nm was used for measuring the particle size. The scattered light from the sample solution was detected at 90° angle. Measurements were carried out in a glass cell at 25 °C.

2.2.7. Gel permeation chromatography (GPC)

Molecular weight distribution and polydispersity index of the polymers were analyzed by Waters gel permeation chromatography (GPC) equipped with L-2414 refractive index detector and Waters styragel HR3 and HR4 columns in series using THF as eluent (flow rate 1 mL/min; polystyrene standards).

2.2.8. X-ray diffraction studies (XRD)

X-ray crystal structure of monomer was carried out on a BRUKER AXS SMART-APEX diffractometer with a CCD area detector (Mo Kα = 0.71073 Å, monochromator: graphite). The measured intensities were reduced to F2 and corrected for absorption with SADABS. Frames were collected at T = 298 by ω, φ and 2θ-rotation at 10 s per frame with SMART. Structure solution, refinement, and data output were carried out with the SHELXTL program. Non-hydrogen atoms were refined anisotropically and C-H hydrogen atoms were placed in geometrically calculated positions by using a riding model.

2.3. Synthesis and characterization

2.3.1. Synthesis of *L1*

To an ice-cooled solution of *tert*-butyloxy carbonyl (Boc) protected alanine **A1** (1.00 g, 5.29 mmol) in dry CH_2Cl_2 (100 mL) was added sequentially, NHS (0.913 g, 7.94 mmol), DCC (1.64 g, 7.94 mmol), hexylamine (0.803 g, 7.94 mmol), NEt$_3$ (1.11 mL, 7.94 mmol) and left stirred for overnight. The precipitate was filtered off and the filtrate was washed sequentially with 0.2 N H_2SO_4, $NaHCO_3$ and water. The organic layer was collected and dried over anhyd. Na_2SO_4 and evaporated under vacuum to yield 1.20 g of the pure compound. Yield: 83%; Appearance: Yellow viscous liquid; ^{1}H NMR (300 MHz, CDCl$_3$): δ 0.88 (t, *J* = 6.3 Hz, 3H, C*H*$_3$-), 1.30 (m, 9H, AlaC*H*$_3$- + CH$_3$(C*H*$_2$)$_3$-), 1.45 (s + m, 11H, -C(C*H*$_3$)$_3$ + -NHCH$_2$C*H*$_2$-), 3.24 (m, 2H, -NHC*H*$_2$-), 4.12 (m, 1H, -NHC*H*C=O), 5.06 (br s, 1H, BocN*H*-), 6.26 (br s, 1H, -N*H*-); ^{13}C NMR (75 MHz, CDCl$_3$): δ 13.95, 18.44, 22.49, 26.47, 28.30, 29.46, 31.43, 39.47, 50.12, 80.01, 155.55, 172.55. IR (KBr): 3302, 3098, 2976, 1716, 1487, 1168, 1062 cm^{-1}. HRMS calcd. for $C_{14}H_{28}N_2O_3Na$, m/z = 295.1992, obtained m/z = 295.1990.

2.3.2. Synthesis of *M1*

To an ice-cooled solution of **L1** (0.200 g, 0.74 mmol) was added HCl in EtOAc (4 mL) and left stirred for 4 h. The reaction mixture was evaporated under vacuum. To the resulting amine was added