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Synthesis, characterization, conformation and self-assembly behavior of polypeptide-based brush with oligo (ethylene glycol) side chains

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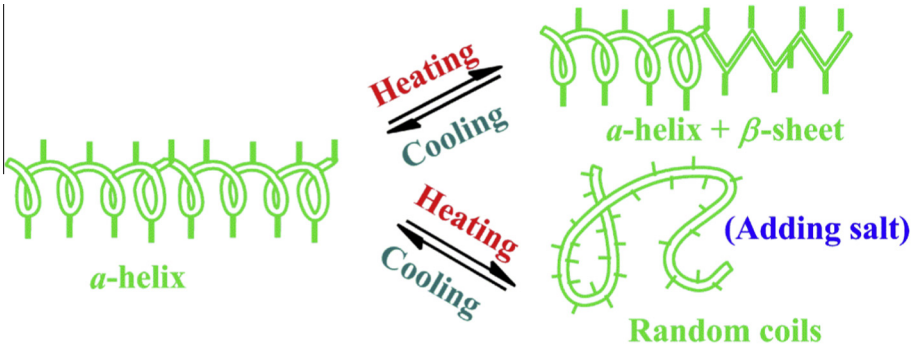
HIGHLIGHTS

- A polypeptide-based brush composed of polypeptide backbone and oligo (ethylene glycol) side chains was effectively synthesized.
- The α-helix of the polypeptides showed reversible changes upon heating in water.
- The self-assembly micelles formed by the brush is thermo-sensitive in water.

GRAPHICAL ABSTRACT

The conformation dependance of polypeptide blocks on temperature.



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ABSTRACT

A new polypeptide-based copolymer brush composed of poly (γ-propargyl-*L*-glutamate)-*block*-poly (propylene oxide)-*block*-poly (γ-propargyl-*L*-glutamate) backbone (PPLG-*b*-PPO-*b*-PPLG) and oligo (ethylene glycol) (PEG) side-chain was synthesized by combination of *N*-carboxyanhydride ring-opening polymerization and click chemistry. Nearly 100% grafting efficiency was achieved by copper-catalyzed azide-alkyne Huisgen 1,3-dipolar cycloaddition (CuAAc) reaction. The α-helical conformation adopted by the grafted polypeptide blocks in water was relatively stable and showed a reversible change in a heating–cooling circle from 5 to 70 °C. It displayed weak stability against elevated temperature but still reversible changes in the presence of 0.47 M NaCl. The brushes were amphiphilic and could self-assemble into thermo-sensitive micelles in water. Big micelles could break into small micelles upon heating due to the improved solubility.

Introduction

Secondary structure of polypeptides forms cornerstone of their three-dimensional assemblies and regulates numerous biological activities and functions. Inspiration from this nature for the design and synthesis of functional materials has led to many achievements for biomedical and biomimetic applications [1–5]. Polypeptide-synthetic polymer bioconjugates attract increasing interests in drug or gene delivery and biological mineralization, because these materials provide unique opportunities to combine the best of both materials [2,3]. Ring-opening polymerization (ROP) of N-carboxyanhydrides (NCAs) is one of the most important tools to prepare such materials [6,7]. Although the obtained polypeptide

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segments are much simpler than natural peptides, they still adopt the α-helical or β-sheet conformation. Block copolymerization using selective primary amine end-functionalized synthetic polymer as macroinitiators to initiate the ROP of NCA monomers is a convenient mean to tailor the physicochemical properties of the polypeptide backbone. Following this strategy many AB or ABA type polypeptide-synthetic polymer block copolymers were prepared [3–7]. Hence, during the last 30 years, bioconjugates from synthetic polymers and polypeptides prepared by ROP of NCAs have been recognized as attractive building blocks for biomimetic materials, drug or gene delivery carriers, and even broad-spectrum antimicrobial peptides (AMPs) for mimics of natural AMPs [4,5,8]. However, one important goal of these materials is to mimic the complex biomacromolecules on mechanically, chemically, and architecturally. Thus, the AB or ABA type copolymers are still insufficient in their structures.

An alternative route that allows one to add the chemical complexity of the polypeptide segments is to associate the polypeptide backbone with another polymer in brush architecture. Such brush copolymers offer, indeed, the unique self-assembly in nanostructures [9,10]. Conventional approaches for the side-chain functionalization of these polypeptides involve aminolysis or transesterification of a well-defined polypeptide precursor, which requires the use of harsh deprotection chemistry and may have low grafting efficiency [11,12]. Very recently, "thiol" photo-click chemistry has found its convenience and high efficiency to associate the polypeptide backbone with other functional molecules [4,13]. However, nearly all these functional molecules have low molecular weights, because the efficiency of "thiol" click reaction depends on concentration of thiyl radical [14,15]. Obviously, higher molecular weight thiols produce thiyl radicals with lower concentration, leading to lower efficiency. Therefore, the click reaction, copper-catalyzed azide-alkyne Huisgen 1,3-dipolar cycloaddition (CuAAc) has been a good way to prepare polypeptide-polymer brushes [4], even though concerns about the cytotoxicity of copper salts for the use of biomaterials are always being mentioned.

Herein, using a new clickable NCA monomer of γ-propargyl-*L*-glutamate (PLG-NCA), we described the synthesis of polypeptide-based brushes by the combination of ROP of NCAs and CuAAc click reaction. Firstly, using poly (propylene oxide) bis(2-aminopropyl ether) (NH_2-PPO-NH_2) as an initiator, ABA triblock copolymer of poly (γ-propargyl-*L*-glutamate)-*block*-poly (propylene oxide)-*block*-poly (γ-propargyl-*L*-glutamate) (PPLG-*b*-PPO-*b*-PPLG) was prepared by the ROP of PLG-NCA. Then, azide-terminated oligo (ethylene glycol) (mPEG-N_3) was coupled with the side chain alkyne group of PPLG to generate the targeted brush. Typically, route to the brush is illustrated in Scheme 1, where it was designated as PPLG*g*PEO-*b*-PPO-PPLG*g*PEO. Further, the secondary structure of the polypeptide block and its stability in water under variable conditions are investigated by circular dichroism (CD) spectra. Self-assembly behaviors of the brush in water are explored by angle-dependant dynamic light scattering (DLS), temperature-dependent ^{1}H NMR and UV–visible spectra.

Experimental

Materials

NH_2-PPO-NH_2 (M_n = 4000 g/mol, PDI = 1.17) and Poly (ethylene glycol) monomethyl ether (PEO-OH, M_n = 750 g/mol) were purchased from Sigma–Aldrich and used as received. $CuSO4{\cdot}5H_2O$ and sodium ascorbate were purchased from Aladdin (China). *N,N*-dimethyl formamide (DMF) was dried over CaH_2 and then distilled under vacuum. Azide terminated oligo (ethylene glycol) (mPEO-N_3) was synthesized according to literature procedure [16].

Methods

^{1}H NMR spectra were recorded with a Varian 300 MHz spectrometer, $CDCl_3$, D_2O and CF_3COOD were used as solvents; FTIR spectra were recorded on a Thermo Nicolet/Nexus 670 spectrophotometer and obtained from 32 scans with a resolution of 4 cm^{-1}. Samples were cast onto a KBr plate; Size exclusion chromatography (SEC) was performed on a Waters system in DMF with 0.05 M LiBr at 60 °C against polystyrene standards, operating at flow rate of 1.0 ml/min; Roman spectra were obtained on a Renishaw inVia microscope with laser wavelength of 541.5 nm, and the polymer samples were cast onto a silicon wafer and scanned in the range of 500–3500 cm^{-1}; CD spectra were performed on a JASCO J-810 spectrometer using water or acetonitrile as solvents, with a path length of 1.0 cm. Five scans were conducted and averaged between 180 and 300 nm at a scanning rate of 200 nm/min with a resolution of 0.5 nm. For temperature-dependent CD studies, the solution was allowed to equilibrate for 5 min prior to data collection. For pH-dependent CD studies, pH of the sample solution was diluted to 2.0 or 9.0 from a mother solution of 0.1 M HCl or NaOH. The CD spectra were expressed as the mean residue ellipticity (MRE) (unit: deg cm^2/dmol), which was calculated by equation $[\theta]_\lambda = MRW \times \theta_\lambda/10 \times d \times c$ [17]; where MRW (g/mol) is the mean residue weight of peptide block, θ_λ is the observed ellipticity (deg) at the wavelength of λ, d is the path length (cm) and c is the concentration (g/ml). Zeta potential analysis of the micelles was performed on a Malvern Zetasizer. pH of micelle solution with concentration of 0.54 mg/ml was adjusted to 2.0, 7.0 or 9.2 with 0.1 M HCl or NaOH. DLS experiments were made using a Brookhaven Instruments BI-200SM goniometer with incident light at 532 nm. Apparent Diffusion Coefficients (D_{app}) was calculated following $\Gamma = D_{app}q^2$, where Γ is the decay rate; q is the scattering vector and given by $q^2 = 4\pi n/\lambda \sin(\theta/2)$, where λ is the wavelength of the incident laser, θ is the scattering angle and n is the refractive index of the media. The hydrodynamic radius (R_h) was then calculated from Stokes–Einstein Equation: $k_BT/6\pi\eta D_{app}$, where k_B is Boltzmann constant, T is temperature and η is the viscosity of the medium. Angular-dependent DLS experiments were performed in the angle range 40–120 °C. For temperature-dependent DLS studies, the solution was allowed to equilibrate for at least one hour prior to data collection. UV–visible spectra was conducted on a UV9000s visible spectrophotometer to determine the cloudy point of as-prepared polymers, which was obtained according to the transmittance of polymer solution at 500 nm.

Synthesis of γ-propargyl-L-glutamate N-carboxyanhydride (PLG-NCA)

L-glutamic acid (5.0 g, 0.035 mol) was suspended in propargyl alcohol (0.25 mol) at 0 °C. Then sulfuric acid (4.0 g, 98%) was added dropwise over 20 min. After stirring at room temperature overnight, triethylamine (4.5 g) and 200 ml ether were added into the mixture. The obtained precipitate was filtrated, washed with acetone and dryed under vacum. γ-Propargyl-*L*-glutamate (PLG) was finally obtained as a white power. PLG (4.1 g, 0.022 mol) was suspended in anhydrous THF (100 ml) in a reaction flask fitted with a reflux condenser and N_2 bubbler. After heating to 50 °C, triphosgene (2.2 g, 7.33×10^{-3} mol) was added and the reaction solution became clear at once. After 30 min the reaction solution was cooled to room temperature and then poured into *n*-hexane (300 ml). The mixture was placed at −20 °C overnight, and then a slightly brown liquid appeared at the bottom of the flask. After removing the supernatant, the brown oil was collected and redissolved in 100 ml of ethyl acetate, followed by washing with 100 ml ice-cold water and 50 ml of 0.5% $NaHCO_3$ ice-cold aqueous solution. The organic phase was then dried over anhydrous $MgSO_4$ and evaporated to give 1.80 g of PLG-NCA as a viscous liquid. Yield: