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Linear poly(ethylenimine)-*graft*-poly(ethylene glycol) copolymers: Their micellization and secondary assembly

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Abstract

Linear poly(ethylenimine)-*graft*-poly(ethylene glycol)s (LPEI-*g*-PEG) with various degrees of grafting and molecular weights were synthesized by Michael addition reaction of LPEI with methoxy poly(ethylene glycol) acrylate. The graft copolymers display pH-sensitive behaviors, and pH variation of the copolymer solution induced the aggregation of graft copolymers, forming the micelles with different sizes. The dynamic light scattering method was used to study the effects of ionic strength, chain composition and pH on hydrodynamic radius. With solvent evaporation of the micelles solution, various morphologies were formed.

Keywords: Self-assembly; Secondary assembly; pH-sensitive; Poly(ethylenimine)

1. Introduction

Linear and branched poly(ethylenimine)s (PEIs) are attractive polymers due to their wide applications in paper production, water purification, and shampoo manufacturing. Recently, PEIs have received particular attention in gene delivery because they combine excellent transfection efficiency with high complexes stability under in vitro and in vivo conditions [1–4]. However, high-molecular-weight PEI is cytotoxic and a major problem associated with PEI-DNA complexes is their tendency to particle aggregation, forming larger particles with broad size distribution. To solve the problems, two main strategies have been reported. One is preparation of monodisperse, amphiphilic core–shell nanoparticles with a PEI shell and a poly(methyl methacrylate) core [5]. The other is modification of PEI by grafting hydrophilic or degradable polymers, such as poly(*N*-isopropylacrylamide) (PNIPAM) [6,7], poly(acrylamide-*co*-acrylic acid) [8], poly(oxypropylene) [9], and chitosan [10], to the PEIs. In addition, water-soluble PEG has been widely used to modify PEIs, especially hyperbranched PEIs. The resultant product showed high solubility in water, and the DNA trapped within the PEI core of the micelles with hydrophilic PEG shell displayed high stability against degradation [11–18]. From the aforementioned discussion, both strategies are related to the formation of micelles. A study of the formation of PEI-*g*-poly(γ-benzyl-*L*-glutamate) (PEI-*g*-PBLG) micelles with PBLG cores and PEI shells has been reported [19]. However, the micellization of PEI-*g*-PEG has not been studied systematically.

Linear PEI has different properties in interaction with DNA than hyperbranched PEI, and the linear triblock copolymer PEI-*b*-PEG-*b*-PEI reacted with α-cyclodextrins (α-CDs) formed unique pH-dependent polypseudorotaxane [20–22]. Thus the graft copolymers PEI-*g*-PEGs might be pH-sensitive, and the pH-induced self-assembly of copolymers is one of the most interesting research subjects. Various block copolymers containing a pH-sensitive block, such as poly(4-vinylpyridine) [23–26], poly(amine methacrylate) [27], poly(tertiary amine methacrylate) [28–30], poly(amido amine) [31], or poly(acrylic acid) [32,33], have been synthesized and their pH-induced self-assembly behaviors were investigated. To study pH-induced

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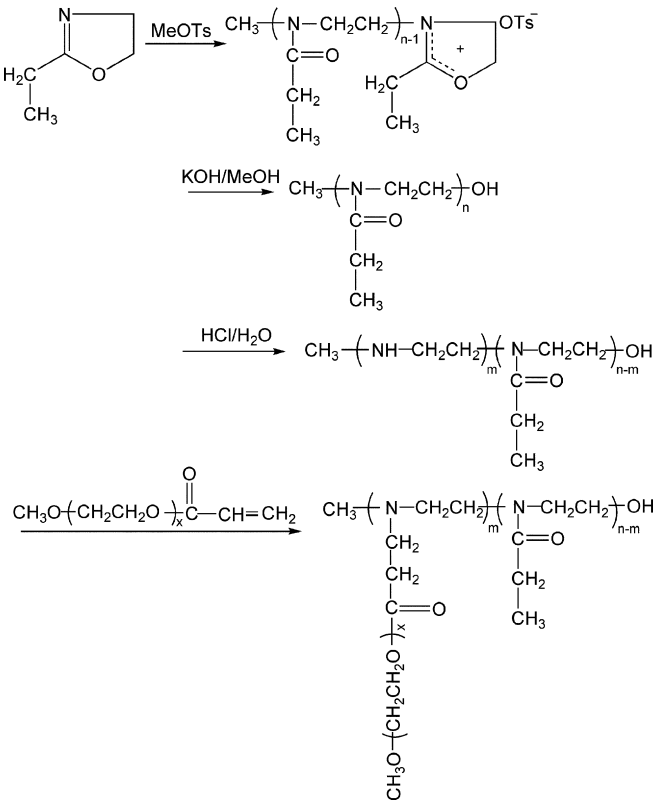
aggregation of graft polymers PEI-*g*-PEGs, the linear PEI was selected, and several synthetic routes for PEI-*g*-PEG have been described in the literature. In most cases, well-defined homopolymers are linked together. For examples, the graft copolymers were synthesized by grafting PEG monomethyl ether with one terminal isocyanate group onto linear or branched PEIs via reaction of amines with the isocyanate group [11,13]; another method is the reaction of amines in PEI with epoxy groups in methoxy PEG glycidyl ether [15,34]. In addition, acyl chloride groups were also used to conjugate with secondary amines in PEI [35]. For easy preparation in the laboratory, we developed a new synthetic route for PEI-*g*-PEGs by the Michael addition reaction of amines in PEI with acryl groups in methoxy poly(ethylene glycol) acrylate, by which adjustment of the chain length and graft density of PEG can be achieved.

In this article, we report the synthesis of graft copolymers, LPEI-*g*-PEGs, and their micellization in aqueous solution was induced by pH variation. Evaporation-induced aggregates of LPEI-*g*-PEG micelles were also studied.

2. Experimental

2.1. Synthesis of LPEI-g-PEGs

The graft copolymers, LPEI-*g*-PEGs, were synthesized according to Scheme 1. The synthesis includes three steps: ring-opening polymerization of 2-ethyl-2-oxazoline (EOz) to form poly(2-ethyl-2-oxazoline) (PEOz), hydrolysis of PEOz to form LPEI, and Michael addition of A-MPEG with LPEI. The materials used in the experiment, the synthesis and characterization of A-MPEG and LPEI-*g*-PEGs were described in Supplementary material.



Scheme 1. Synthesis of PEI-*g*-PEG.

2.2. Preparation of micelles

The micelles were prepared according to the reported method [36]. The LPEI-*g*-PEG was dissolved in water at pH 2.0, and then the solution was adjusted to pH 8.5 by slow addition of NaOH aqueous solution (0.1 N) to induce the formation of micelles. The final polymer concentration was 0.2 mg/ml. The samples for ESEM and AFM tests were prepared by spin-coating a drop of the micelles solution onto silicon wafer.

2.3. Characterization

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded on a Bruker DMX300 spectrometer using tetramethylsilane (TMS) as an internal reference. ^{13}C NMR was performed using an inverse-gated broadband decoupled (INVGATE) program. Gel permeation chromatograph (GPC) measurements were carried out at 25 °C on a Waters instrument (515 HPLC pump) equipped with Ultrastyragel columns (HT3 and HT4) and a Wyatt interferometer refractometer. Chloroform was used as eluent, and polystyrene standards were used in the calibration of molecular weights and molecular weight distributions. The morphologies of microspheres were observed by an environmental scanning electron microscope (ESEM, Model XL 30 ESEM FEG from Micro FEI Philips). The surface morphologies of the films were characterized with an atomic force microscope (SPA300HV/SPI3800N, Seiko Instruments Inc., Japan) in tapping mode in ambient atmosphere. A silicon cantilever (spring constant ∼2 N/m and resonant frequency ∼70 kHz, Olympus Co., Japan) with an etched conical tip (radius of curvature ∼40 nm, as characterized by scanning over a very sharp needle array, NT-MDT, Russia) was used for scanning. Size distribution of the micelles was measured by dynamic light scattering with a vertically polarized He–Ne laser (DAWN EOS, Wyatt Technology). The scattering angle was fixed at 90° and the measurement was carried out with constant temperature 25 °C.

The pH-induced phase transition of the aqueous solution was measured with a UV/vis recording spectrophotometer (UV-2401PC, SHIMADZU) by monitoring the transmittance of a 500-nm light beam through the aqueous solution. The solution pH was monitored using a HANNA211 Microprocessor pH meter and measured 30 min after adding NaOH for adjustment of pH.

3. Results and discussion

3.1. Synthesis of LPEI-g-PEGs via Michael addition reaction

The cationic ring-opening polymerization of EOz was carried out with methyl *p*-toluenesulfonate as initiator, as shown in Scheme 1. The conditions and the results are listed in Table 1. The results show that the molecular weights of the resultant polymers increased with the increased molar ratio of monomer