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Biodegradable poly(asparagine) grafted with poly(caprolactone) and the effect of substitution on self-aggregation

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Abstract

Micelle-like aggregates formed with amphiphilic graft copolymers, poly(asparagine)-g-poly(caprolactone) (PAsn-g-PCL) were prepared through 'the precipitation and dialysis method' and their self-aggregation phenomena were investigated by light scattering, fluorescence probing, and TEM. The strong hydrophobic interaction of associated PCL grafts would facilitate the primary aggregate formation in a bimodal size distribution and significantly reduced CAC (critical aggregation concentration) with respect to DS (degree of substitution). Further, the size of aggregates increased as DS reduced apparently. The graft copolymer having a higher DS formed the more rigid or compact hydrophobic core in self-aggregates with polarization as well as smaller aggregates. When introduced in a basic condition at 37 °C, the effective diameter of aggregates increased and the scattering intensity reduced due to the degradation of PCL as time changed. When incubated in aqueous solution at 4 °C, the sample of DS 4.2 maintained without any size change for up to 20 days, while that of DS 1.2 increased up to the equilibrium diameter. But, the rapid growth of turbid state at DS 6.0 was observed within 2 weeks. After 25 days, the effective diameter of aggregates increased irrespective of DS, becoming a little turbid, and attributed to the swelling and degradation of hydrophobic domains with various morphogenic changes.

Keywords: Self-aggregate; Poly(asparagine); Poly(caprolactone); Degree of substitution; Degradation

1. Introduction

Micelle-like aggregates or nanoparticles formed with amphiphilic block or graft copolymers have recently been studied for a possible application of drug carriers [1–4]. Among them, interested are biocompatible block and graft copolymers including hydrophobically modified water-soluble polymers (HMP), which form self-assemblies, nano-sized micelle-like aggregates of various morphologies in an aqueous solution. The hydrophobic microdomains of self-agggregates could be formed through intra- and/or inter-molecular interactions in an aqueous solution and act as host systems for many hydrophobic molecules, while the hydrophilic corona or outer shell plays a role in avoiding the uptake by reticuloendothelial systems (RES). These nano-sized aggregates have the advantages of having a fairy narrow size distribution, a low critical aggregation concentration (CAC), a slow rate of dissociation, and a high drug-loading capacity in biotechnological and pharmaceutical applications.

Poly(amino acid)s grafted with side chains would form water-soluble and biologically acceptable self-aggregates, and construct a hydrophobic core or a hydrophilic shell. Several block and graft copolymers containing a poly(amino acid) core-forming block have been employed as delivery vehicles for anti-cancer drugs [4–6]. In our previous works, nano-sized micelle-like aggregates formed poly(amino acid)s-based amphiphilic copolymers such as poly(aspartic acid)-g-alkyl [3] and poly(hydroxyethylaspartamide)-g-

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dehydroxycholic acid (PHEA-g-DHA) [8], and proteinoid-cholesterol [9] were studied on physiochemical properties and as carriers for hydrophobic drugs. Poly(amino acid)s' derivatives such as poly(aspartic acid) and poly(asparagine) are synthesized by acid-catalyzed thermal polycondensation of L-aspartic via poly(succinimide) (PSI) [8] and has fully biodegradable, water-soluble properties and toxicological suitability and have been proposed as a carrier in a synthesis of prodrug [12,13].

We have recently reported the synthesis of a new aminoacid-based copolymer of poly(asparagine) (PAsn) as a backbone and poly(caprolactone) (PCL), a semi-crystalline biodegradable polymer as grafted hydrophobic chains [9]. Also, the graft copolymers formed self-aggregates in aqueous solution were prepared through 'the precipitation and dialysis method'. In this paper, we investigated the self-aggregation of PAsn-g-PCL graft copolymer that has dependence on the degree of substitution (DS) of PCL groups. The structure of the self-aggregate and the hydrophobic effect of degradable PCL oligomers on the physicochemical properties are discussed.

2. Experiments

2.1. Materials

PSI, PAsn and PAsn grafted with PCL-diol (PAsn-g-PCL) were synthesized in our previous studies [9–11]. L-aspartic acid (Sigma), PCL-diol (mol. wt. 1250, Polyscience, Inc), phosphotungstic acid (TAAB), and sodium hydroxide (Junsei Chemical) were used as purchased. Fluorescent probe, pyrene (99%, optical grade) and diphenylhexatriene (DPH) were both purchased from Aldrich. *N*,*N*-dimethylformamide (DMF; Aldrich) and tetrahydrofuran (THF; Aldrich) were dried over molecular sieve 4 Å before use.

2.2. Sample preparation

The stable solution of self-aggregates of PAsn-g-PCL was prepared when water was added to the DMSO solution of PAsn-g-PCL [9]. First, the purified copolymer (10 mg) was dissolved in a common solvent (DMSO, 1.0 ml) for both segments and then water (10 ml), which is a precipitant for PCL segments but a good solvent for PAsn segments, was added to induce the aggregation of graft copolymers. The mixed solution of graft copolymer was stirred and then dialyzed for 2 days against distilled water using a dialysis membrane (MWCO = 8000–12000 g/mol).

2.3. Dynamic light scattering measurements

The sample solutions were filtered with 0.45 μm pore size membrane filters to remove dust prior to measurement. Light scattering measurement was performed for determining the size distribution with an apparatus from Brookhaven Instruments Inc. equipped with a diode laser of 523 nm. The scattering angle was fixed at 90° for dynamic light scattering (DLS), the effective diameter was obtained by the Stokes–Einstein relationship, and histograms were calculated with the NNLS routine. When the difference between the measured and calculated baselines was less than 0.2%, the correlation function was accepted.

2.4. Transmission electron microscopy

The morphology of self-aggregates was observed using a transmission electron microscopy (TEM) with the negative staining technique. A drop of self-aggregates solution (Conc.: 1.0 mg/ml, pH 6.8) containing 0.1% phosphotungstic acid (PTA) was placed on a copper grid coated with a carbon film. The grid was held horizontally for 30 s to allow aggregates to settle down and then vertically to allow excess fluid to drain. Observation was carried out at 80 kV with Philips CM200.

2.5. Fluorescence measurements

Critical aggregation concentration (CAC) was determined from steady-state fluorescence spectra using a Perkin–Elmer luminescence spectrometer with a bandwidth of 2.5 and 5.0 nm for excitation and emission. The excitation spectra were obtained using emission wavelength of 390 nm. The stock solution of pyrene (6.0×10^{-4} M) was prepared in acetone and dropped into the polymer solution. Then, the solution was sonicated with a bath-type sonifier (Branson) for 10 min, and induced equilibrium of pyrene and polymers. The mixture was incubated at room temperature for at least 24 h. The polarization of hydrophobic domain was determined with DPH as a fluorescent probe. The stock solution of DPH (2.0×10^{-3} M) was prepared in tetrahydrofuran (THF) and dropped into the polymer dispersion. The DPH concentration was adjusted to 2.0×10^{-6} M. Then, the polymer series with DPH was mixed by vortex and bubbled with nitrogen and incubated at 50 °C for at least 5 h. As the fluorescence quantum yield of DPH depends on the solvent polarity, DPH can be used as a sensitive probe to monitor the formation of hydrophobic domains. The fluorescence polarization of DPH was determined as the following equation.

$$\text{Fluorescence polarization} = \frac{(I_{VV} - I_{VH})}{(I_{VV} + I_{VH})}$$

where I_{VV} and I_{VH} are emission intensities when an emission polarizer is oriented parallel and perpendicular to an excitation polarizer, respectively. The emission intensities were recorded at 430 nm with excitation of 360 nm, varying the temperature of an aggregate solution from 25 to 60 °C.

2.6. Collapse behavior of self-aggregates

The collapse procedure of self-aggregates prepared by the simple hydrolysis of grafted PCL oligomers was monitored by a dynamic light scattering (DLS) and pH meter (Orion