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Self-aggregates of poly(aspartic acid) grafted with long alkyl chains

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Summary

Biodegradable poly(aspartic acid) (PASP) containing octadecyl chain grafts has been synthesized by thermal polycondensation of aspartic acid, aminolysis by octadecylamine, and followed by hydrolysis of remaining succinimide in polymer backbone. The sonicated suspension formed self-aggregates in aqueous solution. Size exclusion chromatography and dynamic light scattering indicated that self-aggregates became compact and particle size was reduced as increasing the amount of substituted octadecyl chains. In the case of low degree of substitution, self-aggregates assembled loosely due to low hydrophobic interaction. In the concentration range of 0.05-1.0%, the stability of aggregates was maintained due to effective hydrophobic interaction and electrostatic repulsion. Below pH 3.2, macroscopic phase-separation appeared in aqueous solution due to attraction of protonated PASP backbone.

Introduction

Recently, self-assemblies or aggregation of polymer amphiphiles in aqueous media have been studied with respect to biological importance and pharmaceutical applications. Among them, synthetic block copolymers and hydrophobized natural polymer amphiphiles such as polysaccharides have been intensively investigated about theoretical approach of self-aggregate formation and their applications [1-5].

Hydrophobically modified water-soluble polymers can be synthesized by several methods including copolymerization of water-soluble monomer with hydrophobic comonomer which can show amphiphilicity [6], attaching hydrophobic moieties such as long alkyl chains or bulky cholesterol derivatives to water-soluble polymer backbone [7-9], and block copolymerization on reactive polymer end groups [2,4,5].

In this study, we report amphiphilic polyelectrolytes grafted with long alkyl chains, and self-aggregates in aqueous solution. Water-soluble polymer backbone used in the study is poly(aspartic acid) which has amide linkages like proteins. Poly(aspartic acid) (PASP) was synthesized by acid-catalyzed thermal polycondensation of L-aspartic acid to give poly(succinimide) (PSI), followed by hydrolysis [10]. PASP is an attractive candidate for drug delivery due to completely biodegradable and acid-containing water-soluble properties [11,12].

Long alkyl chains were attached to PSI via aminolysis and the resulting polymer was hydrolyzed to give PASP grafted with long alkyl chains. Self-aggregate formation was confirmed by experimental methods including size exclusion chromatography and dynamic light scattering.

Experimental

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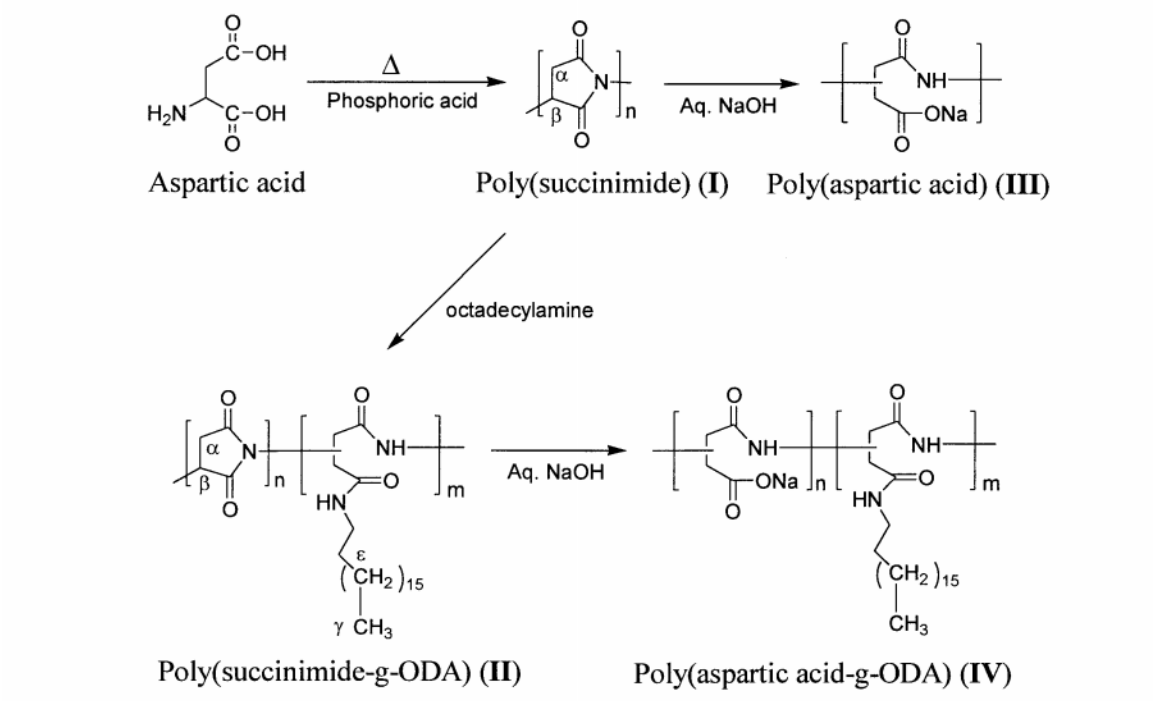

Figure 1. Synthesis of PSI and PASP grafted with octadecyl group. The Greek letters refers to ^{1}H-NMR spectra

Materials

L-aspartic acid(Sigma) and octadecylamine(Aldrich) were used as received. Phosphoric acid(85%, Junsei) and sulfolane(Aldrich) were used without further purification. N,N-dimethylformamide(Aldrich) was dried over Molecular Sieve 4A before use.

Synthesis

L-aspartic acid(40g, 0.30 mol) was suspended in sulfolane in the presence of phosphoric acid(15 mmol) and stirred at 170°C under N_2 atmosphere. Water generated in condensation was continuously removed by Dean-Stark trap. After 10h, the reaction mixture was precipitated in excess methanol and successively washed with water until pH of suspension became neutral. The precipitate was washed with methanol and dried at 80°C *in vacuo*. The structure of PSI(**I**) was confirmed by ^{1}H-NMR analysis. ^{1}H-NMR(DMSO-*d6*): δ=2.69 and 3.18(α, 2H, s); 5.25(β, 1H, t).

Synthesized PSI(485mg) was dissolved in DMF(5ml) and octadecylamine(ODA) dissolved in DMF(1ml) was added to the mixture rapidly. The reaction mixture was stirred at 70°C for 24h and cooled to room temperature. Insoluble product was filtered out. The clear solution was added dropwise to 1N NaOH solution to hydrolyze remaining succinimide unit of PSI. After stirring for 3h at room temperature, the reaction mixture was precipitated in excess methanol twice. The precipitate was filtered and then dried *in vacuo* at 60°C. PSI-g-ODA(**II**) ^{1}H-NMR(DMSO-*d6*): δ=0.84(γ, 3H, t); δ=1.22(ε, 30H, s); δ=2.69 and 3.18(α, 2H, s); 5.25(β, 1H, t). PASP(**III**) ^{1}H-NMR(D_2O): δ=2.78 and 2.72 and 2.57(2H, m); δ=4.49(β-opening, 1H, s); δ=4.69(α-opening, 1H, s). The molecular weight of PASP was determined by SEC analysis. Mn=17186, Mw=22803, and PD=1.32. The yields of each product were 85-95%.

Sample preparation

The parent PASP was easily dissolved in water without sonication and resulted in a clear solution. PASP containing octadecyl groups was suspended in deionized water and mixed by vortexing. These milky suspensions were sonicated using a bath type sonifier at room temperature for 10 min to give clear solutions. The solutions were purified by passing through a 0.45μ filter (Whatman) to remove dust. This stock solution of 2 % concentration was diluted with 0.2M of phosphate buffer (pH 7.0) and adjusted to obtain 0.1M buffer concentration for next experiment.

Methods

The dynamic light scattering measurement was performed with an apparatus from Brookhaven Instruments Inc. The scattering angle was fixed at 90° and hydrodynamic diameter and histograms were calculated with NNLS routine and polydispersity, μ_2/Γ^2 was calculated by cumulant method. Turbidity was measured at 500nm using UV/VIS spectrophotometer. The SEC chromatogram was obtained by Waters 626 system equipped with Ultrahydrogel Linear and Ultrahydrogel 120(Waters) column in series. A sample was eluted by 0.1M $NaNO_3$ at the flow rate of 0. 5ml/min. The SEC was calibrated using a standard samples of pullulan (P-82, Shodex)

Results and Discussion

Amphiphilic graft copolymer composed of poly(aspartic acid) as the hydrophilic backbone and octadecyl chain as the hydrophobic segment were successfully synthesized as follows. Octadecyl chain was easily grafted to the PSI backbone via aminolysis reaction. In ^{1}H-NMR spectra, PSI-g-ODA showed the additional absorption at 1.22 ppm due to protons of octadecyl chain, while PSI exhibit 2.69 and 3.18 ppm of methylene protons of the succinimide unit and 5.25 ppm of methine protons. After hydrolysis of PSI with NaOH, PSI was converted to poly(aspartic acid) sodium salt. The FTIR analysis showed the same result that octadecyl group was grafted to PASP backbone. In the case of PASP-g-ODA, C-H stretches of grafted octadecyl chain exhibit additional absorption bands in 2852 and 2925 cm^{-1} compared with PASP that showed broad amide bands at 2800-3680 and 1654 cm^{-1}. In ^{1}H-NMR spectra of PASP, two chemical shifts of methine proton appeared in 4.49 and 4.69 ppm by two different ring-opening manners. As indicated by other studies [10,12], succinimide units were hydrolyzed to give aspartic acid via α- and β- opening. The ratio was 25/75 that was determined by integral value of each methine proton in ^{1}H-NMR spectra. This result shows that β-opening occurred dominantly in hydrolysis than α-opening did.

Degree of substitution	Mole ratio (succinimide unit / ODA)	Elution time (min)	Number-average mean diameter	Polydispersity
0	100/0	28.97	-	-
2%	98/2	28.63	77.4nm	0.258
5%	95/5	29.02	31.1nm	0.262
8%	92/8	29.12	28.0nm	0.246
10%	90/10	29.18	25.7nm	0.323

Table 1. Properties of self-aggregates as a function of degree of substitution in PBS solution (concentration: 1%)

In SEC analysis, the parent PASP eluted earlier than PASP-g-ODA except copolymer of 2% degree of substitution (DS) as shown in Table 1 though molecular weight of PASP-g-ODA increased by attaching octadecyl group with respect to PASP. This result indicates that graft copolymers would form an entity of reduced hydrodynamic dimension such as aggregates in aqueous solution and PASP skeleton in aggregates would be densely packed due to hydrophobic interaction in inner domain comparing to the parent PASP. As DS of ODA increased, the elution time increased slightly. This is due to the facts that hydrodynamic volume is reduced by stronger hydrophobic interaction of octadecyl chain and more compact self-aggregates form as DS increases. In dynamic light scattering, the result was consistent with SEC analysis. The parent PASP did not show any scattering in aqueous solution, while scattering in the case of PASP-g-ODA occurred substantially by aggregate formation. After sonication of solutions, the effective diameter of aggregates of 10% DS maintained constant up to 1 month while that of 2% DS increased to equilibrium diameter. Aggregates of 2% DS were loosely formed and reconstituted to a thermodynamically stable configuration as time changed. Though the size distribution was not monodisperse due to intramolecular and intermolecular interaction between grafted alkyl groups, number-average mean diameters were reduced as DS increased as shown in Table 1. This result should be caused by lower aggregation numbers required for aggregate formation as DS increases.

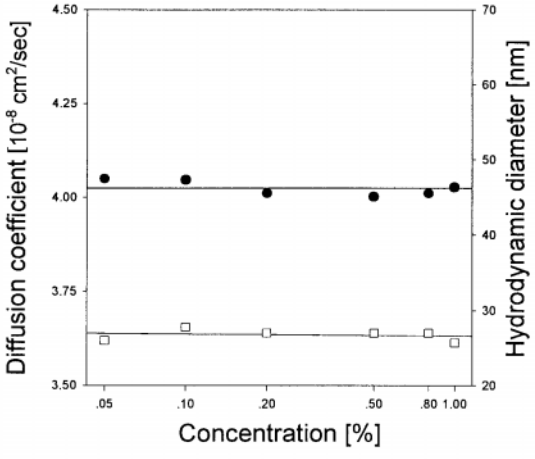

Figure 2. Diffusion coefficient (●) and diameter of primary aggregates (□) of PASP-g-ODA(DS=10%) as a function of the concentration (temperature : 25°C, detection angle : 90°)

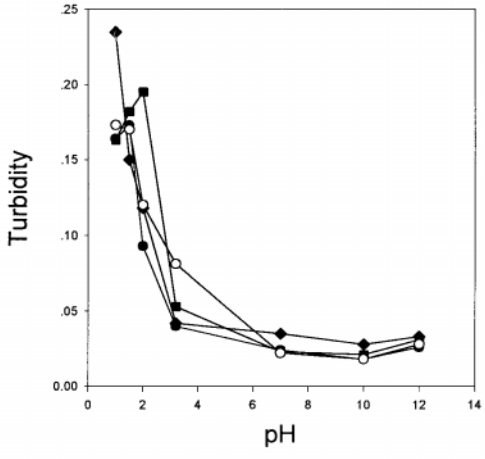

Figure 3. Turbidity change of PASP-g-ODA as a function of pH (●) DS=10%, (◆) DS=8%, (■) DS=5%, (O) DS=2%

The solution behavior of PASP grafted with alkyl chains was investigated as the concentration changed. In general, the hydrophobic groups form the inner hydrophobic domain by self-association and contribute to form a stable aggregate system by playing a role of crosslinker[13]. In the case of 10% DS, substituted alkyl chains played a role of effective hydrophobic groups as stabilizing aggregates in Figure 2. Diffusion coefficients are obviously independent of the concentration, indicating that stable aggregates were preserved without loosening of initial aggregates and the hydrophobic alkyl chains act as crosslinker to stabilize aggregates in this concentration range. Therefore the size of primary aggregates was maintained stably irrespective of the concentration. Also, the

graft copolymers with different DS maintained constant diffusion coefficients irrespective of concentration.

PASP grafted with alkyl chains was charged negatively due to aspartic acid in PASP backbone. The self-aggregates showed the pH-sensitive aggregation behavior as shown in Figure 3. In acidic range, the turbidities of the aqueous solutions were high irrespective of DS due to the formation of large aggregates, while the clear solution was maintained in other ranges and the turbidities were almost same. As a result, the effective diameter below pH 3.2 also increased when determined directly after vortexing the solution. This result should be due to the facts that protonation of aspartic acid in the hydrophilic backbone occurred and large aggregates were formed by the attractive interaction between protonated aspartic acid unit in PASP backbone. In neutral and basic pH ranges, deprotonated aspartic acid units endow each aggregate with negative charge, therefore, self-aggregates can be stabilized by the charge repulsion.

Conclusion

PASP with long alkyl chains were successfully synthesized from PSI as a function of the amount of alkyl chain. PASP grafted with octadecyl group formed self-aggregates in aqueous solutions by the sonication method. As the degree of substitution increased, self-aggregates became compact and the size of primary aggregates was reduced. Hydrophobic interaction of alkyl groups stabilized aggregates effectively irrespective of polymer concentrations. Due to the negative charge of aggregate surface, the turbidities at basic and neutral pH ranges maintained constantly. However, macroscopic phase-separation occurred by the attractive interaction of aggregates in acidic range.

Acknowledgment

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