세포 내 약물 전달을 위한 양친성 폴리 이미다졸 그라프트 아스파트 아마이드 유도체

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Novel amphiphilic polyaspartamide derivatives grafted with imidazole for intracellular drug delivery application

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Introduction

Poly(amino acid)s have become ones of the most important biomaterials owing to their superior biocompatible, biodegradable, and non-toxic properties. In the preparation of synthetic poly(amino acid)s, poly(succinimide) (PSI) can often be used as an intermediate product. PSI can be feasibly converted to hydrophilic biodegradable poly(amino acid)s such as poly(aspartic acid), poly(aspartamide)s, poly(hydroxyethyl aspartamide)s, etc. by simple hydrolysis or aminolysis.[1-3] It has been known that the pH inside the endosome changes from pH 7.0 to pH 5.5 on its way toward lysosome. If some biocompatible polymers have sensitive responses at such small pH changes, they can be employed as intracellular drug delivery carriers with good endosome escaping ability after receptor-mediated endocytosis.[4] The polymers containing imdazole functions like poly(histidine) have been studied for potential applications of tumor targeting drug delivery and non viral gene delivery systems. It has been noticed that the polymers containing imidazole groups whose pKa is near 6.0~6.5 can mediate endosomal escape via the hypothesized proton sponge effect since they have buffering behavior between pH 7 and pH 5.[5-6]

The primary aim of this research was to prepare a series of amphiphlic graft copolymers with varying C18 concentration and investigate their aggregation behavior responding to very small pH change similar to that in endosome. A 1-(3-aminopropyl)imidazole (API) was a very useful reagent to provide pH sensitive imidazole functions to poly(succinimide) (PSI). O-(2-aminoethyl)-O'-methylpolyethylene glycol (MPEG-NH_2) and octadecylamine(C18) were introduced to PSI for reinforceing the hydrophilicity and hydrophobicity, respectively. Hemolytic behavior of the prepared polymer was also studied by RBC hemolysis test.

Experimental

The PSI was synthesized by polycondensation of L-aspartic acid using phosphoric acid as an acid catalyst.[7] C18/MPEG/API-g-polyaspartamide derivatives were prepared by following procedures. C18 (2.0 ~ 5.0g, 0.01 mol) solution in DMf (15ml) was added to a solution of PSI (5.0g, 0.1mol) in the dry DMF (20ml) under N_2 atmosphere. After stirring for 7 hours at 100^{o}C, the reaction mixture was poured into methanol. After filtration, the precipitate was washed with methanol several times, and dried in a vacuum oven for at least one day. MPEG5000-NH_2 (1.5g) was dissolved in 10 ml of DMF. The MPEG-NH_2 solution was slowly added to the prepared C18-g-PSI solution (1 g / 10 ml DMF)at room temperature. The mixture was stirred continuously under N_2 atmosphere at 70 ^{o}C for 48 hours, and then

1-(3-aminopropyl)imidazole was added to polymer solution. After 24 hours, the reaction mixture was dialyzed against the distilled water using a dialysis membrane to get rid of residual MPEG and 1-(3-aminopropyl)imidazole, and then freeze-dried. The structure of C18/MPEG/API-g-polyaspartamide derivatives was represented in Figure 1.

Their pH-sensitive properties of the prepared polymers were studied by light transmittance, dynamic light scattering, and fluorescence spectrometer, and The abilities of these polymers to disrupt RBC membrane was investigated as pH changes.

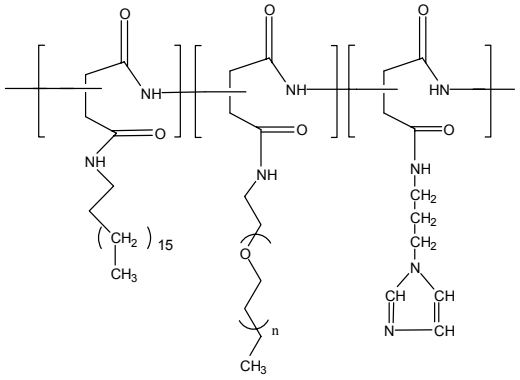

Figure 1. Structure of C18/MPEG/API-g-polyaspartamides

Results and discussion

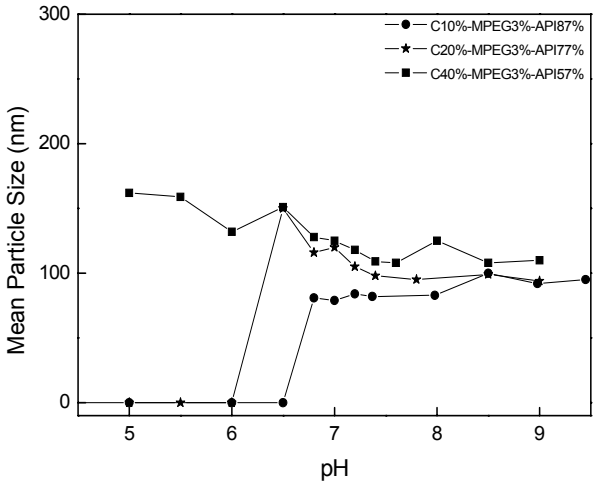

Figure 2. Mean particle size change of C18/MPEG/API-g-polyapartamide derivatives with the function of pH.

Mean particle size change of polymers with varying pH was presented in pH Figure 2. As shown in Figure 2, polyaspartamides with high concentration of C18 in polymers formed stable nano-aggregates by strong hydrophobic association of C18 in aqueous solution in whole physiological pH range, while the other polymer with low concentration of C18 showed pH

dependent aggregation behavior. They assembled to form nano-aggregates at above pH 7, but dissociated at below pH 7. It is because repulsive effect between charged imidazole groups was stronger than association interaction by hydrophobic C18 chains.

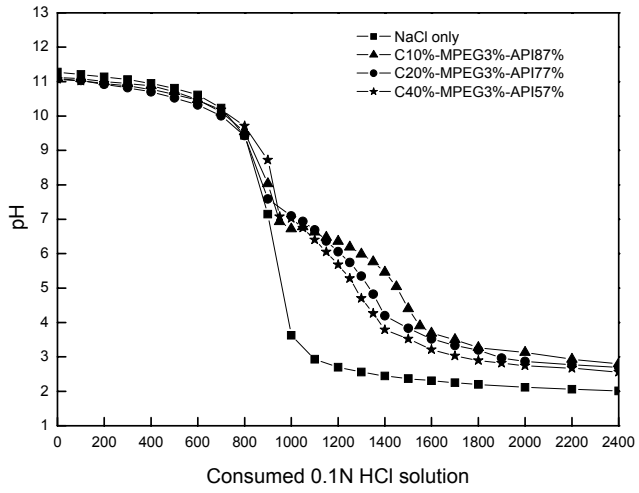

Figure 3. Acid-base titration curves of C18/MPEG/API-g-polyaspartamides.

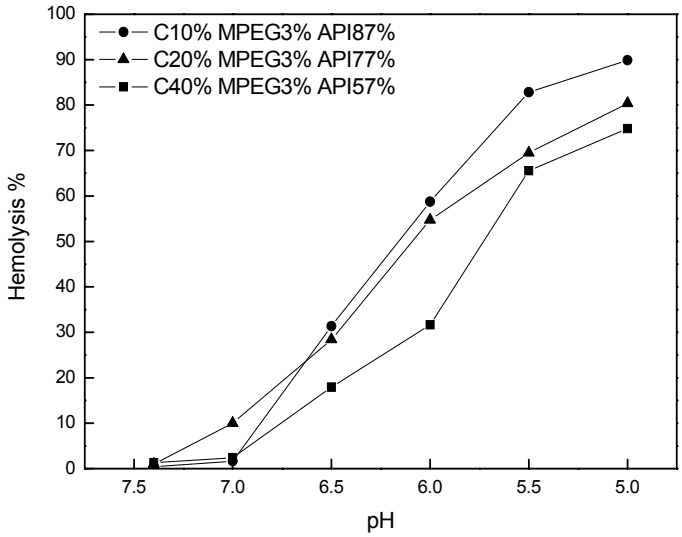

Figure 4. pH dependence of RBC hemolysis by C18/MPEG/API-g-polyaspartamide derivatives at the polymer concentration of 1mg/ml.

To investigate the buffering property of the prepared C18/MPEGAPI-g-polyaspartamides, their

acid-base titration curves are represented in Figure 3. A buffering region appeared between pH 5 and 7.5 for all the polymers. The midpoint of the pH buffering range was determined as the pKa of the polymers, and that of the prepared polyaspartamides was approximately 6.25. The buffering capacity increased with the concentration of API in polymers, and the polymers with high API concentration showed considerable buffering capacity.

As shown in Figure 3, C18/MPEGAPI-g-polyaspartamides show pH-dependent hemolysis behavior. All polymers has little hemolytic activity at above pH 7. Hemolysis by polymers started from pH 6.5, and drastically increased with decreasing pH. This means the protonation of imidazole groups cause the fusion of RBC membrane. The hemolysis efficiency increased with imidazole concentration in polymers.

Conclusion

All C18/MPEG/API-g-polyaspartamide derivatives showed sharp transmittance transition and buffering behavior in endosomal pH range (pH 5 ~7) by protonation of imidazole rings. pH-dependent aggregation and deaggregation behavior was observed in the polymers with low concentration of C18, while polymers with high concentration of C18 formed stable nano-aggregates in all pH range. CAC (critical aggregation concetration) of polymers increased with increasing the concentration of hydrophobic octadecylamine in polymer. C18/MPEG/API-g-polyasprtamide derivatives are little hemolytic at above pH 7.0, while they displayed very high hemolysis % at below pH 5.5. These prepared pH-sensitive polyaspartamide derivatives are worth initial basis to design the drug delivery carrier for intracellular drug delivery.

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