

속보

포르피린이 접목된 폴리아미노산 유도체를 이용한 자기조립체에 관한 연구

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Biomimetic Self-assembly of Porphyrin-conjugated Polyaspartamide in Aqueous Solution

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초록: 수용액상에서 다양한 형태를 갖는 자기조립 나노입자는 그 형태적 특이성으로 말미암아 표적지향 약물전달을 포함하여 다양한 응용분야에서 활발하게 연구되고 있다. 본 연구에서는 나노입자의 종횡비(aspect ratio)를 제어하는 새로운 자기조립 형성 기법을 확인하였다. 수용액상에서 자기조립 나노입자를 형성할 수 있는 포르피린이 접목된(grafted) 폴리아미노산 유도체를 합성하였다. 판상구조를 갖는 포르피린 분자의 접목도를 조절하여, 종횡비가 10 이상이 되는 막대형태의 자기조립 나노입자를 제조하였다. 포르피린이 도입된 자기조립 나노입자는 산소분자를 선택적으로 포집할 수 있기 때문에 초음파 및 다양한 영상 진단을 위한 표적지향 전달체로 활용될 수 있다.

Abstract: Nano-sized self-assemblies with various morphologies are being extensively studied to use them in a variety of biological and industrial applications including targeted drug delivery. This study reports a novel strategy to prepare self-assemblies with high aspect ratio by varying packing structure. We synthesized poly(2-hydroxyethyl aspartamide) (PHEA) grafted with porphyrins, which could form self-assemblies in an aqueous solution. Then, increasing the degree of substitution of porphyrins induced the structural transition to rod-like assemblies with an aspect ratio of 10 by inter/intra molecular π - π stacking of porphyrins. Further introduction of metalloporphyrins to PHEA leads to an uptake of oxygen molecules. This strat-

egy to prepare polymer self-assemblies will serve to improve the efficiency of targeted delivery for a molecular optical and ultrasound imaging with various biomedical modalities.

Keywords: polyaspartamide, porphyrin, self-assembly, degree of substitution.

Introduction

Nano-sized self-assemblies formed with amphiphilic block or graft copolymers have been extensively studied for targeted drug delivery of diagnostic and therapeutic molecules.¹⁻³ It is common to modify the self-assemblies' surface by varying the number of targeting ligands for enhancing their targeting efficiency. Recently, several studies have reported that the shape of a particle could be one of the significant factors in modulating the particle's targeting capability. For example, ellipsoidal polymersomes functionalized with RGD peptides were reported to accumulate on a model target tissue, more favorably than spherical polymersomes.⁴ Also, several model inorganic particles with high aspect ratio ($L_{\text{length}}/D_{\text{diameter}}$) were demonstrated to tune their binding affinity to target substrates.⁵ However, there are few successes in preparing polymer self-assemblies with high aspect ratio, because of our limited control over the shape of self-assemblies without instability in an aqueous solution.

Therefore, this study presents a novel strategy to prepare polyaspartamides self-assemblies with high aspect ratio by varying packing structure. First, we synthesized poly(2-hydroxyethyl aspartamide) (PHEA) substituted with porphyrins to form self-assemblies in an aqueous solution (Figure 1(a)). Porphyrin forms a hydrophobic core that provides an extremely versatile nanometer-sized building block with extended π -conjugated macrocyclic ring.^{6,7} Therefore, increasing the degree of substitution (DS) of porphyrins induced the structural transition to rod-like assemblies with an aspect ratio of 10 by inter/intra molecular π - π stacking of porphyrins (Figure 1(b)). Further introduction of metalloporphyrins to PHEA leads to an uptake of oxygen molecules. Overall, this strategy to prepare polymer self-assemblies will serve to improve the efficiency of targeted delivery for a molecular optical and ultrasound imaging with various biomedical modalities.

Experimental

Poly(succinimide) (PSI) was synthesized by acid-catalyzed polycondensation of aspartic acid (Sigma)⁸ and its ring was

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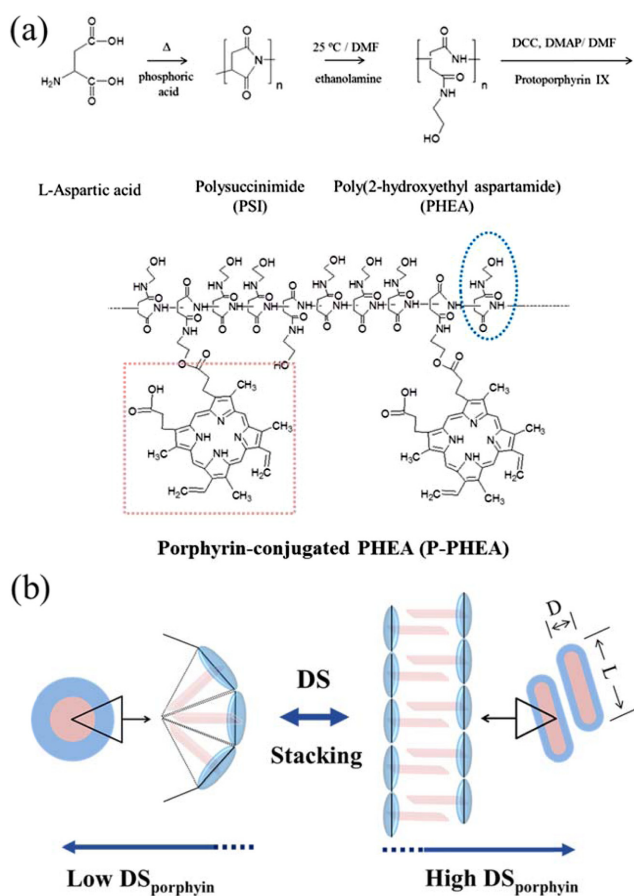


Figure 1. (a) Top-down synthesis scheme of porphyrin-conjugated PHEA *via* nucleophilic substitution of PSI from L-aspartic acid; (b) Schematic description of the polyaspartamide self-assemblies as a function of DS, by inter/intra molecular π - π stacking of porphyrins.

opened with ethanolamine (Sigma) to give poly(2-hydroxyethyl aspartamide) (PHEA).⁹ Then, porphyrin (Protoporphyrin IX, Sigma) was substituted to PHEA by a DCC-mediated reaction through the formation of ester linkages. Dicyclohexylcarbodiimide (DCC, Aldrich), dimethylaminopyridine (DMAP, Aldrich) and synthesized PHEA were added to the solutions of designated amounts of porphyrin in dried *N,N*-dimethylformamide (DMF, Sigma) under stirring at 25 °C. The resulting chemical structures of porphyrin-conjugated PHEA (P-PHEA) were confirmed with ¹H NMR (Avance II, Bruker Biospin). The integrals of characteristic peaks were used to quantify the DS of porphyrins in each sample using eq. (1).

$$DS_{\text{porphyrin}} (\text{mol}\%) = \frac{\text{The integral of the peak in } 3.6\sim 3.7 \text{ ppm}/2}{\text{The integral of the peak in } 3.1\sim 3.2 \text{ ppm}} \times 100\% \quad (1)$$

The self-assemblies of P-PHEAs in an aqueous solution

were prepared by precipitate-dialysis method using dimethyl sulfoxide (DMSO, Sigma), which is a good solvent for both PHEA and porphyrin. Nano-sized self-assemblies are formed during the precipitation process and the formation is completed by removal of DMSO through extensive dialysis against DI water. The size distribution was measured by a dynamic light scattering (DLS, Malvern Inc.) equipped with a He-Ne laser at a scattering angle of 90°. For measuring CAC (critical aggregation concentration), steady-state fluorescence spectra were measured using a photo luminescence QM40 with a bandwidth of 2.0 nm for excitation and emission. The morphology of the self-assemblies was observed using a transmission electron microscopy (FE-TEM, JEOL) at 120 kV.

Results and Discussion

First, we prepared porphyrin-conjugated poly(2-hydroxyethyl aspartamide) (P-PHEA) *via* top-down synthesis with a controlled number of porphyrin molecules, to examine the effect of degree of substitution (DS). For this purpose, designated amount of porphyrin was added to PHEA to prepare P-PHEA as a function of DS (Figure 1(a)). Table 1 shows the molecular characterization of synthesized P-PHEAs. The DS of porphyrins to PHEA, defined as the percentage of succinimide units substituted with porphyrins, was varied from 4.8 to 24.0 mol% by altering the feed mole ratio of porphyrins.

The synthesized P-PHEA forms self-assemblies in an aqueous solution, as do the other amphiphilic copolymers, due to intra- and/or intermolecular hydrophobic interactions of hydrophobic parts. Room temperature emission spectra ($\lambda_{\text{exc}}=405 \text{ nm}$) of the polymers as shown in Figure 2(a) display typical porphyrin emission and are very similar with the exception of the extra band at 580 nm for P5-PHEA. These results demonstrate that the P-PHEA with a high DS was more strongly stacked than that with a low DS by a plane-by-plane interaction of the neighboring molecules. Generally, the hydrodynamic diameter of spherical self-assemblies of various graft polymer systems decreased with increasing DS of the hydrophobic grafts.^{8,10} However, the hydrodynamic diameter of self-assemblies of P-PHEAs, measured by DLS (Table 1), does not show the inverse dependency with DS.

Therefore, we hypothesized that grafting of porphyrins with macrocyclic ring to a hydrophilic backbone polymer would drive the structural transition of the self-assemblies as a function of DS. In order to examine the structures of self-assembled P-PHEA with DS, TEM measurements were performed.

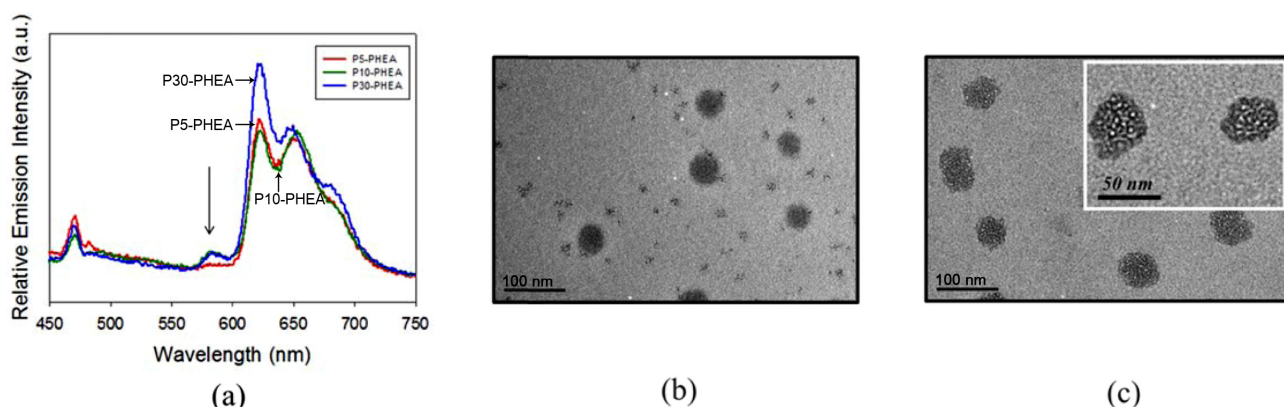


Figure 2. (a) Emission spectra of P-PHEAs ($\lambda_{\text{exc}}=405$ nm) in an aqueous solution. TEM images of self-assemblies of P5-PHEA (DS: 4.8 mol%) with porphyrin (b); metalloporphyrin (c) in aqueous solution. Inset represents the magnified self-assemblies in TEM image.

Table 1. Molecular Characterization of Porphyrin-conjugated PHEA

Sample	Feed ^a	DS ^b	Number ^c	Diameter ^d	CAC ^e	$f(w)$ ^f
PHEA	100/0	-	-	Highly soluble	-	100
P5-PHEA	95/5	4.8	27	54	4.3×10^{-2}	85
P10-PHEA	90/10	9.4	54	105	2.5×10^{-2}	75
P30-PHEA	70/30	24.0	137	79	3.2×10^{-2}	54

^aFeed mole ratio (succinimide unit/porphyrin). ^bDegree of substitution (mol%) determined based on ¹H NMR of graft copolymers. ^cNumber of porphyrins per one polymer chain. ^dEffective diameter (nm) obtained by DLS. ^eCritical aggregation concentration (mg/mL). ^fHydrophilic mass ratio to total mass, $f(w) = \frac{a_w}{a_w + b_w} \times 100$, where a_w is the mass of hydrophilic part, and b_w is the mass of hydrophobic part.

Figure 2(b) shows the image of P5-PHEA with spherical micelle-like structures. TEM images are almost consistent with DLS data in terms of size, around 50 nm. P5-PHEA conjugated with metalloporphyrins (Fe) shows still spherical assemblies after exposure to dissolved oxygen (Figure 2(c)). The two images in Figure 2(b) and (c) are clearly distinct by several voids visualized with the contrast difference as shown in the magnified image in Figure 2(c). In addition, the self-assemblies remained stable in PBS buffer solution with a minimal change in the diameter of assemblies.

Next, the morphology and diameter of self-assemblies formed with P10-PHEA and P30-PHEA were further examined using TEM. Interestingly, the porphyrin-conjugated PHEAs with high DS (10 mol%) of substituted porphyrins were formed rod-like assemblies with an average diameter (D) of 6 nm (± 0.5 nm) and length (L) of 60 nm (± 5 nm) as shown in Figure 3(a), estimated using ImageJ. Figure 3(b) shows the image of P30-PHEA with rod-like structures with 5 nm (± 0.4 nm) of D and 50 nm (± 6 nm) of L . Therefore, the high aspect ratio (L/D), around 10, is obtained from 9.4 mol% of DS at which $f(w)$ becomes 75, would be the structural transition point. Actually, this value is lower than the critical value

at which the micelle-to-bilayer transition of general graft copolymer systems, estimated based on the hydrophilic mass ratio to total mass proposed by Discher and Eisenberg.^{1,11} The a_w is the mass of hydrophilic PHEA backbone units occupied by one porphyrin molecule at a given DS of P-PHEA, and b_w is the mass of one porphyrin molecule as shown in Figure 3(c). This indicates that grafting of porphyrins with macrocyclic ring to a hydrophilic backbone polymer drives the structural transition of the self-assemblies by a plane-to-plane stacking. Furthermore, porphyrin provides the versatile building block for tailored materials properties, and oxygen binding capability for sensing and delivery.¹²⁻¹⁴ Therefore, we envisage that the strategy to prepare polymer self-assemblies would serve to improve the efficiency of targeted delivery for a molecular optical and ultrasound imaging with various biomedical modalities.

Conclusions

Overall, this study presents a novel strategy to prepare polyaspartamides self-assemblies with high aspect ratio by varying packing structure. First, we synthesized poly(2-

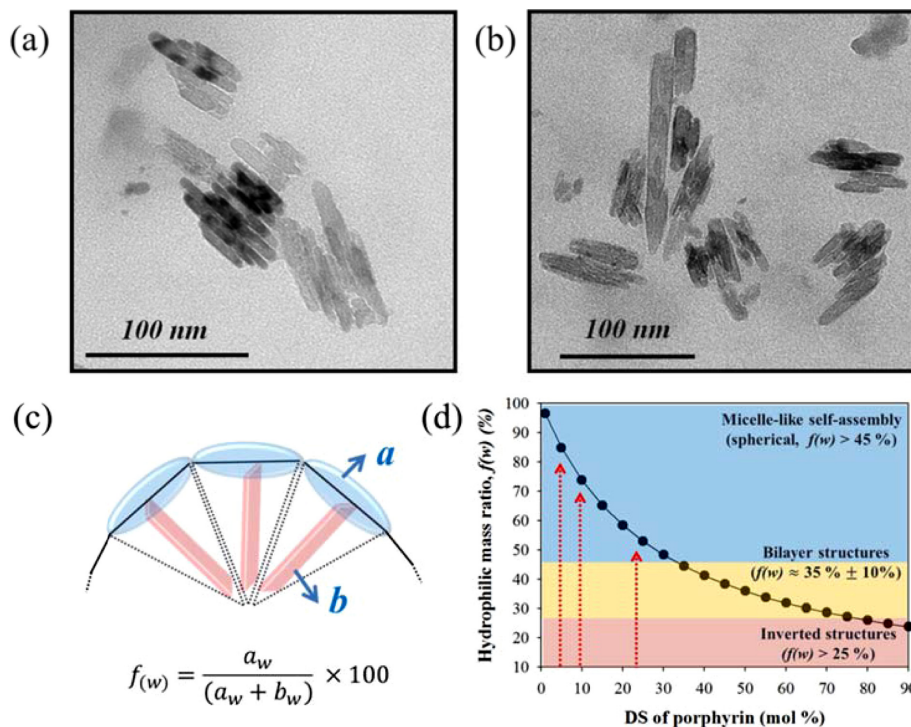


Figure 3. TEM images of self-assemblies of (a) P10-PHEA (DS: 9.4 mol%); (b) P30-PHEA (DS: 24.0 mol%). (c) Scheme for estimating the $f(w)$ of porphyrin-conjugated graft polymer system. (d) Estimation of the $f(w)$ of porphyrin-conjugated PHEAs.¹⁵ The solid curve represents a calibration curve to relate $f(w)$ to the self-assemblies' morphology of P-PHEAs with varying DS. Dashed lines show the DS points, 4.8, 9.4, and 24.0 mol%.

hydroxyethyl aspartamide) (PHEA) substituted with porphyrins to form self-assemblies in an aqueous solution. Increasing the degree of substitution (DS) of porphyrins induced the structural transition to rod-like assemblies with an aspect ratio of 10 by inter/intra molecular π - π stacking of porphyrins. We expect that further modification of intrinsic structure of porphyrin-conjugated PHEA (e.g., molecular weight and DSs) and extrinsic condition of the self-assembly (e.g., temperature, concentration and pH of aqueous media) would further tune the size and morphology.

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