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Semipermeable Polymer Vesicle (PICsome) Self-Assembled in Aqueous Medium from a Pair of Oppositely Charged Block Copolymers: Physiologically Stable Micro-/Nanocontainers of Water-Soluble Macromolecules

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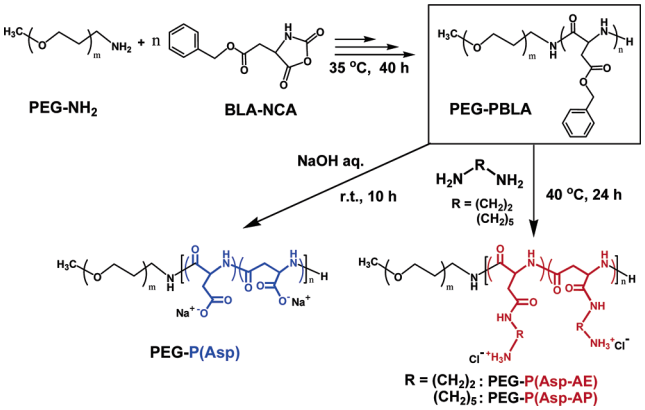
Polymer vesicles enclosing a volume with a molecularly thin membrane, known as "polymersomes", have recently been attracting progressive attention from both fundamental and applied standpoints as carriers or containers for various functionality compounds.[1,2] Particularly, in an aqueous entity, amphiphilic block copolymers have been used for the preparation of polymersomes,[1−3] revealing unique characteristics such as high structural stability compared to conventional liposomes made from low molecular amphiphiles.[1] Very recently, stable encapsulation of biologically relevant substances, including drugs and enzymes, into the polymersomes has been directed to applications as delivery and bioreactor systems.[2] Nevertheless, the hydrophobic nature of the membrane in such amphiphilic polymersomes prevents the penetration of hydrophilic solutes, limiting their functionality as semipermeable container systems. Furthermore, the harsh preparation conditions, including the use of organic solvents, may hamper the encapsulation of fragile compounds such as proteins. Herein, we report for the first time the preparation of stable polymersomes with a semipermeable membrane through a simple mixing of a pair of oppositely charged block copolymers in an aqueous medium. The polymersome formed in this way is a new entity of polymer vesicles with a polyion complex (PIC) membrane and thus may be given a new terminology as a "PICsome".

A PICsome as a hollow sphere needs the formation of a stable layer of PIC lamellae as the partition membrane. In this regard, oppositely charged segments of the block copolymer pair are preferred to have a matched chain length, compensating for the counter charge in a stoichiometric manner and minimizing the phase mixing of the PIC middle layer with the outer and inner shell layers of the hydrophilic segment, in this case, poly(ethylene glycol) (PEG). Here, to satisfy this condition of matched chain length, both anionic and cationic block copolymers were prepared from the same platform polymer, PEG-poly(β-benzyl-L-aspartate) (PEG-PBLA), to have identical molecular weight and composition (Scheme 1). Two types of PEG-PBLA with a different PBLA composition (degree of polymerization (DP) of PBLA; 17 and 100) were prepared by the ring opening polymerization of β-benzyl-L-aspartate *N*-carboxyanhydride initiated from the ω-primary amino group of $CH_3O-PEG-NH_2$ (M_n = 2000, M_w/M_n = 1.05).[4] The anionic component of the PICsome, PEG-poly(α,β-aspartic acid) (PEG-P(Asp)$_{17}$ and PEG-P(Asp)$_{100}$), was obtained from PEG-PBLA by alkali hydrolysis as reported previously.[5] Alternatively, the cationic

Scheme 1. Synthesis of a Pair of Oppositely Charged Block Copolymers



component was prepared from PEG-PBLA by aminolysis of flanking benzyl ester groups with an excess amount of diamine. A notable property of PBLA is that the benzyl ester groups can easily undergo quantitative aminolysis reactions with various diamines at ambient temperature via the formation of a succinimidyl ring structure as an intermediate, allowing the preparation of cationic poly(aspartamide)s with different amine functionalities. Indeed, quantitative aminolysis was confirmed from ^{1}H NMR spectra.[4] Two types of diamines with a different number of methylene units, 1,2-diaminoethane and 1,5-diaminopentane, were used in the aminolysis to obtain PEG-poly([2-aminoethyl]-α,β-aspartamide) (PEG-P(Asp-AE)$_{17}$ and PEG-P(Asp-AE)$_{100}$) and PEG-poly([5-aminopentyl]-α,β-aspartamide) (PEG-P(Asp-AP)$_{17}$ and PEG-P(Asp-AP)$_{100}$), respectively, to explore the effect of the alkyl-spacer length on the self-assembly behavior.

The anionic and cationic block copolymers were separately dissolved in 10 mM Tris-HCl buffer (pH 7.4) with a physiological salt concentration of 150 mM NaCl. Both solutions were then mixed in an equal ratio of $-COO-$ and $-NH_3^+$ units to form PIC and subsequently subjected to sonication.[4] Flow particle image analysis[4] and dark-field microscopic (DFM) observation suggested the formation of spherical particles with the diameter up to 10 μm in the PEG-P(Asp)$_{100}$/PEG-P(Asp-AP)$_{100}$ system (Figure 1), which is obviously with a larger size range than that of the well-documented PIC micelles with a core−shell architecture.[6] The DFM image was more fascinating, showing characteristic ringlike scatterings (Figure 1), suggesting the hollow structure of the particles. Note that the scattering light intensity in DFM correlates with the density of the objects, giving a ringlike image for hollow particles with a large density difference between the inner and peripheral regions.[7] The

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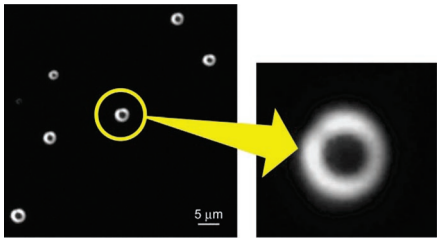

Figure 1. Dark-field microscopic images of PICsomes prepared from a PEG-P(Asp)$_{100}$/PEG-P(Asp-AP)$_{100}$ system.

balance of the segment length in the block copolymer is expected to play a substantial role in the self-assembly process. Indeed, the combination of the block copolymers with a shorter charged segment, PEG-P(Asp)$_{17}$/PEG-P(Asp-AP)$_{17}$ system, gave a DFM image with only few ring-scattering objects dispersed in the major part of the small dot scatterings presumably from micelles. Block copolymers with shorter charged segments compared to the PEG segment adopt a cone-shaped conformation preferring the micelle architecture.[6] The molecular shape gradually changes from cone to rod with the increased length of the charged segments relative to the PEG segments, and eventually, the assembly should adopt the vesicular structure with a smaller curvature than the spherical micelle.[8] The observations here are consistent with this general rule of vesicular formation through molecular assembly. Another factor influencing the vesicular formation seems to be the alkyl-spacer length of the cationic side chain of the poly(aspartamide) segment, which may be related to the flexibility of the ion pair formed in the PIC structure. A decrease in the alkyl-spacer length from pentyl to ethyl in the side chain of the cationic poly(aspartamide) segment, viz. the PEG-P(Asp)$_{100}$/PEG-P(Asp-AE)$_{100}$ system, resulted in a significant decrease in the size (<1 μm) of the ring scatterings observed in DFM. It is likely that the length of the alkyl spacer may be a critical factor in determining the stable PICsome size, yet a further detailed study should be needed to confirm this assumption.

The hollow structure of the large PIC assembly from PEG-P(Asp)$_{100}$/PEG-P(Asp-AP)$_{100}$ system as "PICsome" was further directly evidenced from the encapsulation of the water-soluble macromolecule labeled with fluorescein isothiocyanate, FITC-dextran (FITC-Dex, M_n = 40 000), into the PICsome. Cross-sectional observation by the confocal laser scanning microscope (CLSM) clearly confirmed the successful inclusion of FITC-Dex into the PICsome by a simple mixing of PEG-P(Asp-AP)$_{100}$ (1 mg/mL) with PEG-P(Asp)$_{100}$ (1 mg/mL) containing FITC-Dex (1 mg/mL) (Figure 2).[4] The PICsome with encapsulating FITC-Dex was appreciably stable in physiological buffer as observed by CLSM even after 3 months standing at ambient temperature.

The semipermeability of the PICsome membrane was then investigated using fluorescent molecules with different molecular weights. The fluorescence collected through the objective lens was resolved by the diffraction grating and monitored by a 32-channel arrayed detector. Upon addition of dextran labeled with tetramethylrhodamine isothiocyanate (TRITC-Dex, M_n = 70 000) to the solution of PICsome with encapsulated FITC-Dex, a green fluorescence of FITC inside the PICsome was clearly observed, sharply discriminated from the red fluorescence of TRITC-Dex in the outer medium, in the merged image of CLSM taken at the excitation wavelength for FITC and TRITC (488 and 543 nm) (Figure 2a). On the other hand, upon addition of free TRITC (MW = 443.5) to the solution of the FITC-Dex encapsulating PICsome, a yellow color was observed inside the PICsome (Figure 2b).[4] An emission spectrum of the region of interest (ROI) in Figure 2b shows the

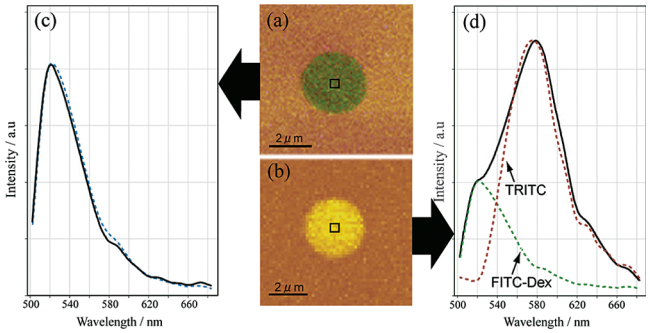

Figure 2. CLSM images and emission spectra of PICsome encapsulating FITC-Dex. Images after the addition of (a) TRITC-Dex or (b) TRITC. (c) Spectra of the ROI in (a) after (solid line) and before (blue dotted line) the addition of TRITC-Dex. (d) A spectrum of the ROI in (b) (solid line) with reference spectra of FITC-Dex (green dotted line) and TRITC (red dotted line).

intense fluorescence with the maximum at 580 nm and the shoulder at 520 nm (Figure 2d). The profile was reasonably fitted with both references of FITC-Dex and TRITC, indicating the penetration of TRITC into the PICsome interior. In contrast, the spectrum of the ROI in Figure 2a corresponds to the spectrum of FITC-Dex before the addition of TRITC-Dex (Figure 2c), indicating the segregation of TRITC-Dex from the PICsome interior. These results visually demonstrated the semipermeable character of the PIC membrane. Notably, the PICsome was able to retain its vesicular structure in the presence of a colloidal osmotic pressure of approximately 10 μOsm from the encapsulated FITC-Dex and was stable even in the medium containing 10% fetal bovine serum at 37 °C,[4] being feasible for biomedical applications.

In summary, a novel entity of a polymer vesicle, a PICsome, was prepared here by a simple mixing of a pair of oppositely charged block copolymers composed of biocompatible PEG and poly(amino acid)s in an aqueous medium. The PICsome is stable in proteinous medium and has a partition membrane with a unique three-layered structure. These biocompatible composition and biologically relevant characteristics of the PICsomes may open their future utility in biomedical fields such as carriers of therapeutic compounds and compartments for diagnostic enzymes.

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Supporting Information Available: Syntheses, characterizations, and preparations of PICsomes. This material is available free of charge via the Internet at http://pubs.acs.org.

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