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pH-responsive micelles and vesicles formed from a water-soluble schizophrenic diblock copolymer

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ABSTRACT

A pH-responsive diblock polymer (pDEA-*b*-pAaH) composed of poly(*N*,*N*-diethylaminoethyl methacrylate) (pDEA) and poly(6-acrylamidohexanoic acid) (pAaH) was synthesized *via* reversible addition-fragmentation chain transfer (RAFT) controlled/living radical polymerization and characterized by NMR. pDEA-*b*-pAaH in water and salt solution self-assembled to schizophrenic micelles in acidic and basic conditions. NMR, light scattering, TEM, and fluorescence measurements provided clear evidence of core–shell micelles and inverted micelles under acidic and basic pH conditions. The micelle size was determined by dynamic light scattering (DLS) measurement at different pH values. pDEA-*b*-pAaH does not dissolve in water at around pH 6, because the anionic pAaH and cationic pDEA blocks were subject to electrostatic interactions; however, the precipitate formed in pure water at pH 6 dissolved in 1.1 M NaCl because the electrostatic interactions were screened by the large excess of NaCl. When pDEA-*b*-pAaH in 1.1 M NaCl aqueous solution was dialyzed against pure water to remove NaCl, the polymer formed a vesicle structure that could incorporate water-soluble polymer guest molecules into the interior water phase.

1. Introduction

Block copolymers containing incompatible blocks show interfacial activity and form micelles in solutions [1–4]. AB diblock copolymers self-assemble to core–shell nano-aggregates selectively (A-core/B-shell and B-core/A-shell) depending on the solvent's selectivity toward the different blocks. Micelles with interchangeable cores and shells can be formed. Block copolymers that form "schizophrenic" nano-scale structures in solvents, varying with changing external conditions, have been of interest in last few years [5].

Stimuli-responsive water-soluble polymers have been the subject of much interest in recent years [6–8]. The aqueous solution behavior of such "smart" polymers can be tuned based on pH [9–11], ionic strength [12,13], temperature [14–16], and the presence of additives [17,18]. Such stimuli-responsive polymers are important from the points of view of industry [19,20] and pharmaceutical research [21–23]. Double hydrophilic block copolymers (DHBCs) can show stimulus-triggered self-assembly; any one of the blocks can become hydrophobic, and this makes DHBCs behave like amphiphilic polymers. The various DHBCs investigated have been neutral–neutral [24], neutral–ionic [14,25–28], and cationic–anionic [29]. While studies report normal and inverted core–shell nanostructures – micelles, vesicles, and so on – among neutral–ionic block copolymers depending on the stimulus response and the molecular characteristics of the copolymer, for oppositely charged blocks (the so called zwitter ionic block copolymers) both normal and inverted morphologies can be achieved by altering the pH of the solution.

Polyzwitterions are (co)polymers that contain both cationic and anionic groups, which may be located on the same monomer unit (polybetaines) or on different monomer units (polyampholytes). Increasingly, attention has been given to polyampholytes as protein models. When the ratio of cationic to anionic groups is close to unity, the polymer coil collapses to a globule-like conformation due to attractive electrostatic or dipolar interactions, and may form a precipitate/coacervate. The addition of small amount of salt screens out these interactions; thus, the polymer chain expands and the polymer remains soluble. This behavior of polyzwitterions is the opposite to that exhibited in general by dilute aqueous solutions of polyelectrolytes [30]. Tuzar et al. [31] synthesized amphiphilic poly(sulfobetaine) block copolymers by sulfonation of poly(*N*,*N*-dimethylaminoethyl methacrylate)-block-poly(methyl methacrylate) (pDMA-*b*-pMMA) and studied the self-assembly behavior of

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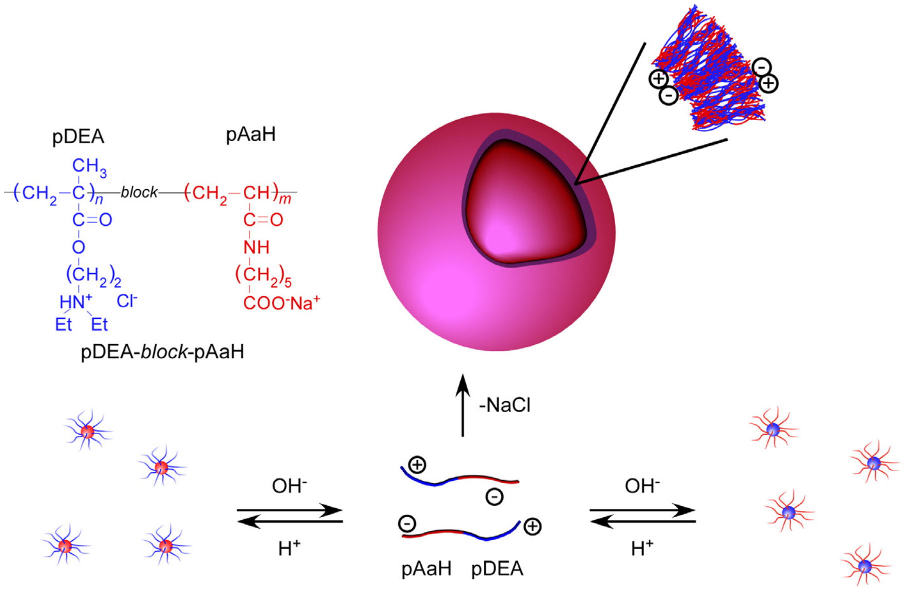

Fig. 1. Schematic illustration of pH-induced aggregation behavior of pDEA-*b*-pAaH in 1.1 M NaCl aqueous solution under varying pH conditions, and formation of vesicle upon removal of NaCl using a dialysis method.

the amphiphilic poly(sulfobetaine) block copolymers. Arotçaréna et al. [16] prepared a double thermo-responsive block copolymer using the nonionic monomer *N*-isopropylacrylamide (NIPA) and the zwitterionic monomer 3-(*N*-(3-methacrylamidopropyl)-*N*,*N*-dimethyl) ammoniopropane sulfonate (SPP) *via* reversible addition-fragmentation chain transfer (RAFT), and examined micelles with interchangeable cores and shells. Liu et al. [32] prepared a pH-responsive schizophrenic diblock copolymer (VBA-*b*-MEMA) composed of poly(4-vinylbenzoic acid) and poly(2-(*N*-morpholino)ethylmethacrylate) *via* a combination of atom transfer radical polymerization (ATRP) and protecting techniques. The MEMA block showed lower critical solution temperature (LCST) behavior in water. In basic conditions VBA-*b*-MEMA forms micelles composed of a VBA core and MEMA shells; however, under acidic conditions above the LCST, VBA-*b*-MEMA forms micelles composed of a MEMA core and VBA shells. Rodríguez-Hernández et al. [33] prepared poly(L-glutamic acid)-*b*-poly(L-lysine) (PGA-*b*-PLys) *via* ring-opening polymerization. PGA-*b*-PLys forms schizophrenic vesicles, which can reversibly be produced in moderately acidic or basic aqueous solutions. The polyampholyte may precipitate around the isoelectric point due to electrostatic interactions between the cationic and anionic blocks. The polyion complex precipitates formed from polyampholytes around the isoelectric point have not been much researched.

In this study, we report the synthesis of a pH-responsive diblock polymer (pDEA-*b*-pAaH) composed of poly(*N*,*N*-diethylaminoethyl methacrylate) (pDEA) and poly(6-acrylamidehexanoic acid) (pAaH). The diblock copolymer was characterized by NMR spectroscopy. Schizophrenic micelles under acidic and basic pH conditions were studied using different spectral and scattering methods. The pDEA block in pDEA-*b*-pAaH dissolved in water at acidic pH, while the pAaH block was soluble in water at basic pH. Therefore, both acidic and basic water behaved as a selective solvent, and thus pDEA-*b*-pAaH formed micelles. In a basic medium, these micelles were composed of the pDEA core and ionized pAaH shells, while under acidic conditions the same polymer formed micelles with a pAaH core and ionized pDEA shells (Fig. 1). pDEA-*b*-pAaH could not dissolve in pure water at around pH 6 because of electrostatic interactions between the cationic pDEA and anionic pAaH blocks. However, the precipitate dissolved in 1.1 M NaCl because the electrostatic interaction was screened by the large excess of NaCl. When the pDEA-*b*-pAaH aqueous solution containing 1.1 M NaCl was dialyzed against pure water to remove NaCl, the polymer formed a vesicle structure. We confirmed that the vesicle could incorporate water-soluble guest polymer molecules into the interior water phase.

2. Experimental

2.1. Materials

N,*N*-Diethylaminoethyl methacrylate (DEA) was passed through basic alumina columns to remove inhibitors. 6-Acrylamidohexanoic acid (AaH) was prepared as reported previously [9]. 4-Cyanopentanoic acid dithiobenzoate (CPD) was synthesized according to the literature [34]. Methanol was dried over 4 Å molecular sieves and distilled. *N*-Phenyl-1-naphthylamine (PNA, 98%) purchased from Tokyo Chemical Industry was recrystallized from methanol. 4,4′-Azobis (4-cyanovaleric acid) (V-501, 98%) from Wako Pure Chemical Industries, Texas Red labeled dextran (Texas Red-dex, molecular weight = 3000) from Life Technologies Japan Ltd., and 9-aminoacridine hydrochloride monohydrate (9-AA, 98%,) from Aldrich were used as received. Water was purified with a Millipore Milli-Q system. Other reagents were used as received.

2.2. Synthesis of poly(N,N-diethylaminoethyl methacrylate) (pDEA)

A methanol solution (28 mL) of V-501 (65.4 mg, 0.233 mmol) and CPD (164 mg, 0.585 mmol) was added to an aqueous solution (65 mL, pH 6.2) of DEA (12.0 g, 64.8 mmol). The mixture was stirred at 60 °C for 8 h under Ar. After the reaction, the conversion estimated based on ^{1}H NMR spectroscopy was 84.2%. The reaction mixture was dialyzed against pure water for two days using a dialysis membrane with a molecular weight cutoff (MWCO) of 14 kDa. Poly(*N*,*N*-diethylaminoethyl methacrylate) (pDEA) was recovered by freeze-drying (9.93 g, 82.7%). The values of number-average molecular weight (M_n(GPC)) and molecular weight distribution (M_w/M_n), estimated by gel-permeation chromatography (GPC), were 1.55×10^4 and 1.14, respectively. The number-average degree of polymerization (DP) was 102 as estimated by ^{1}H NMR.