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Thermal response of self-organization in an amphiphilic triblock polyelectrolyte and the influence of the globular protein lysozyme

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ABSTRACT

The associative, thermoresponsive and complexation properties of the triblock terpolymer PnBA-b-PNIPAM-b-PAA are explored in aqueous solutions. Using light scattering and small angle neutron scattering PnBA-b-PNIPAM-b-PAA is found to self-organize in aggregates of interconnected chains that are incorporated in larger clusters. Upon temperature increase the aggregates increase in molecular mass and shrink due to the enhancement of the hydrophobic interactions during the hydrophilic-to-hydrophobic transition of PNIPAM. In this state the well-defined aggregate interface gives rise to inter-aggregate spatial correlations. Remarkably the incorporation of lysozyme globules induces spatial correlations even at room temperature and controls the aggregate interconnections and mutual arrangement by tuning their surface charge and roughness. This system may be considered as a multicomponent, multifunctional super-structure with tunable morphology that combines the properties of synthetic and biological macromolecular components.

1. Introduction

Interaction of proteins with synthetic and biological polyelectrolytes is a subject of particular interest in biotechnological applications [1]. The nature of their complexation has been described by electrostatic and hydrophobic interactions. Additionally charge regulation [2] where a protein adjusts its charge to the local electrostatic environment and counterion release [3] where entropy drives the association of polyelectrolytes with proteins have been employed for the explanation of attraction of polyelectrolytes with proteins of net charge of the same sign. Detailed morphological description of the arrangement of globular proteins within spherical polyelectrolyte brushes of linear polyelectrolytes end-grafted on spherical nanoparticles has been established with small angle X-ray scattering [4]. In other works the aggregation state and internal arrangement of protein globules in polyelectrolyte/protein complexes has been revealed by small angle neutron scattering [5]. Recently we have used small angle neutron scattering to investigate self-assembled micelles from amphiphilic block polyelectrolytes as protein nanocarriers [6]. The incorporation of protein globules was detected on the measured form factors and it was also found to lead to organization of the polyelectrolyte micelles in a higher hierarchical level in a tunable manner.

Thermoresponsive character in synthetic macromolecules with interest in biomedical applications is often introduced by poly(N-isopropylacrylamide) (PNIPAM) blocks [7]. PNIPAM [8] has a lower critical solution temperature (LCST) at 32 °C which is between the room and the body temperature with obvious implications in drug delivery [9]. In copolymers based on N-isopropylacrylamide and chargeable groups the thermal transition has been used to control the separation of the enzyme soybean peroxidase from solution [10]. The binding of lysozyme with butyl–modified thermoresponsive polymers poly(allylamine)s was influenced by both the increase in hydrophobicity and change in conformation above the LCST at a pH range where both protein and polymer were positively charged [11]. Encapsulation of bone morphogenetic proteins by elastin-like polymers was proved to relate to the polymer hydrophobic nature above its transition point [12]. In earlier studies thermoresponsive polymers with phase transitions at biologically relevant temperatures controlled hydrophobicity and bioadhesion at surfaces [13]. Grafted thermoresponsive polyelectrolytes have been used in thermoresponsive protein adsorption chromatography for protein purification [14] and thermoresponsive affinity chromatography [15]. Adsorption of fibrinogen on binary PAA/PNIPAM brushes was enhanced above the coil-to-globule transition when PNIPAM was in excess while it decreased when PAA was more than 50% [16].

Complexation of lysozyme with aggregates of the triblock terpolymer poly(ethylene oxide)-b-poly(N-isopropylacrylamide)-b-poly(acrylic acid) (PEO-b-PNIPAM-b-PAA) enhances hydrophobic interactions

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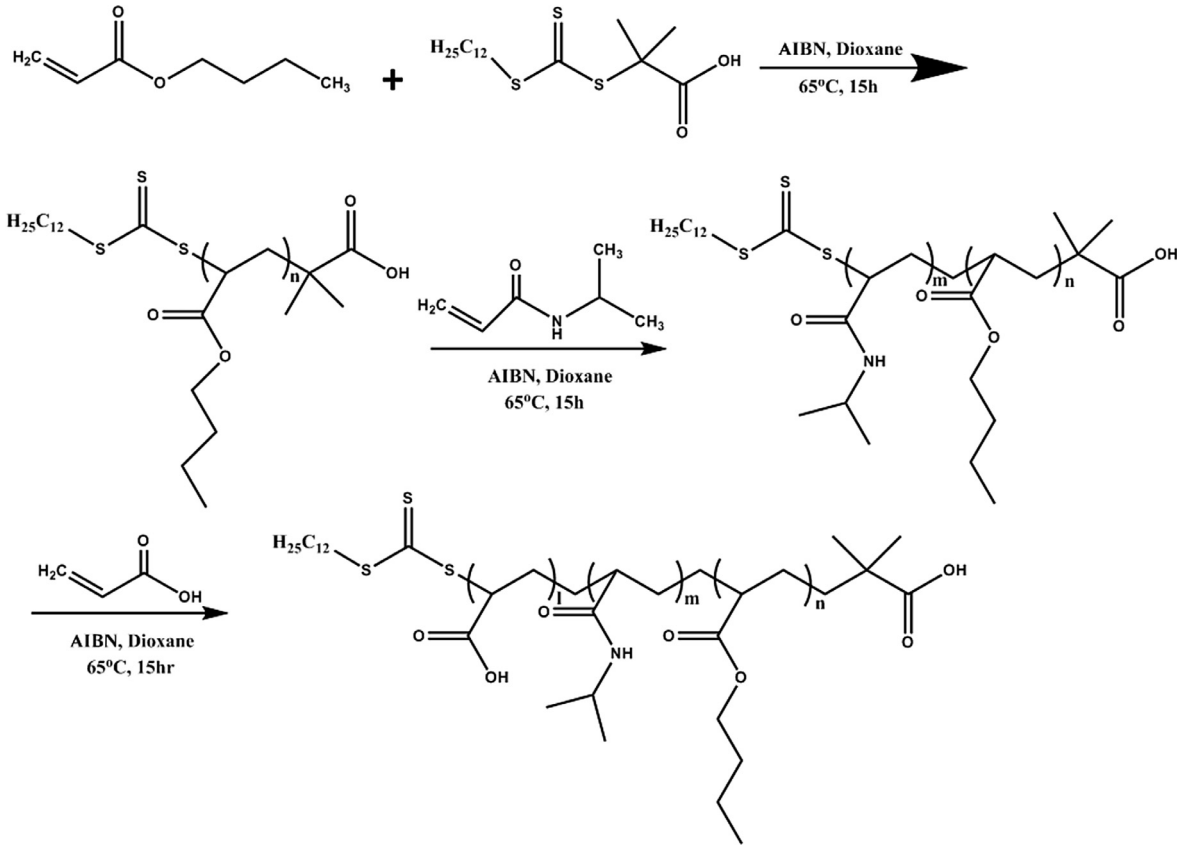

Scheme 1. Synthetic route for PnBA-b-PNIPAM-b-PAA triblock terpolymer.

and inter-aggregate associations in PNIPAM-containing triblock polyelectrolytes while it makes thermoresponsive transitions totally irreversible [17]. In poly(n-butyl acrylate)-b-poly(N-isopropylacrylamide)-carboxylic acid (PnBA-b-PNIPAM-COOH) self-assembled core-shell micelles a length-scale and concentration dependent thermal transition was observed and was affected by interactions with lysozyme [18]. The charged carboxylic end-group stabilized the system during the hydrophilic-to-hydrophobic transition of PNIPAM while lysozyme globules accumulated near the micellar cores. In the case of poly(n-butyl acrylate)-b-poly(acrylic acid) (PnBA-b-PAA) the hydrated charged outer shell of PAA was responsible for the interactions with the oppositely charged lysozyme and this led to intermicellar bridging associations [6].

In this article we investigate the self-assembling and thermoresponsive behavior of poly(n-butyl acrylate)-b-poly(N-isopropylacrylamide)-b-poly(acrylic acid) (PnBA-b-PNIPAM-b-PAA) and the effects upon addition of lysozyme in aqueous solutions. This triblock terpolymer is expected to have tunable hierarchical morphology at aggregate and inter-aggregate level and thermoresponsive behavior that should be controlled by the incorporation of oppositely charged protein globules. We use light scattering methods to extract the size and the surface charge of the aggregates and complexes with lysozyme and to map their thermoresponsive transition. Small angle neutron scattering is performed to resolve the response of the internal organization of PnBA-b-PNIPAM-b-PAA aggregates in the presence and absence of lysozyme. This work illustrates the ability of multi-block stimuli-responsive copolymers to self-organize and respond at several length scales and the possibility to alter these properties by interactions with biological components.

2. Experimental

2.1. Materials

2.1.1. Synthesis and characterization of PnBA-b-PNIPAM-b-PAA

The synthesis of the PnBA-b-PNIPAM-b-PAA triblock terpolymer was performed using the reversible addition fragmentation chain transfer radical polymerization technique (RAFT) [19]. Polymerizations were run typically at c.a. 20%wt/v monomer concentrations. Firstly, the PnBA block was synthesized in one step polymerization of nBA monomer (0.5 g) using 2-dodecylsulfanylthiocarbonylsulfanyl-2-methylpropionic acid (0.25 mmol), as the RAFT CTA agent, in the presence of AIBN (as a 3×10^{-5} mol/mL solution in dioxane) as the polymerization initiator (moles AIBN: moles CTA = 1:5), at 65 °C over 15 h in 1,4-dioxane. The resulting homopolymer was isolated and purified by precipitation into excess diethyl ether twice (90% yield, $M_{w,\ SEC}$ = 2.000, M_w/M_n = 1.09). Then, the PnBA-b-PNIPAM block copolymer was synthesized by using the PnBA block (0.45 g, 0.22 mmol) as the macro-RAFT agent and N-isopropyl acrylamide monomer (5 g). The synthesis occurred at 65 °C for 15 h in the presence of AIBN (moles AIBN: moles macroCTA = 1:10), using 1,4-dioxane as the solvent. The desired block copolymer was precipitated twice into diethyl ether and was left to dry under vacuum prior to use (95% yield, $M_{w,\ SEC}$ = 21,800, M_w/M_n = 1.23). Finally, PnBA-b-PNIPAM-b-PAA triblock terpolymer was synthesized by the copolymerization of acrylic acid (AA, 6.2 g) with the PnBA-b-PNIPAM block copolymer (5.1 g). In this case PnBA-b-PNIPAM played the role of the macro-RAFT agent. The polymerization conditions were the same as those described for the synthesis of the PnBA-b-PNIPAM block copolymer, i.e. 65 °C for 15 h in the presence of AIBN, using 1,4-dioxane as the solvent. The resulting triblock terpolymer PnBA-b-PNIPAM-b-PAA, after precipitation in diethyl ether and drying under vaccum (95% yield), was characterized by SEC, ^{1}H NMR and FTIR and it was found to have the expected structure and chemical