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Morphological variation of stimuli-responsive polypeptide at air–water interface

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ABSTRACT

The morphological variation of stimuli-responsive polypeptide molecules at the air–water interface as a function of temperature and compression was described. The surface pressure–area (π–A) isotherms of an elastin-like polypeptide (ELP) monolayer were obtained under variable external conditions, and Langmuir–Blodgett (LB) monolayers were deposited onto a mica substrate for characterization. As the compression of the ELP monolayer increased, the surface pressure increased gradually, indicating that the ELP monolayer could be prepared with high stability at the air–water interface. The temperature in the subphase of the ELP monolayer was critical in the preparation of LB monolayers. The change in temperature induced a shift in the π–A isotherms as well as a change in ELP secondary structures. Surprisingly, the compression of the ELP monolayer influenced the ELP secondary structure due to the reduction in the phase transition temperature with decreasing temperature. The change in the ELP secondary structure formed at the air–water interface was investigated by surface-enhanced Raman scattering. Moreover, the morphology of the ELP monolayer was subsequently imaged using atomic force microscopy. The temperature responsive behavior resulted in changes in surface morphology from relatively flat structures to rugged labyrinth structures, which suggested conformational changes in the ELP monolayers.

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1. Introduction

Elastin-like polypeptides (ELPs), one of the most widely studied temperature-responsive biopolymers, display low critical solution temperature behavior. ELPs are protein-based polymers composed of repeating units of amino acids and have recently emerged as a promising new class of stimuli-responsive materials. ELPs exhibit inverse phase transition behavior accompanied by a conformational change from a disordered structure to an ordered structure [1]. These structural changes lead to intermolecular aggregation, which rapidly increases the turbidity of the ELP solution [2–4]. Due to these thermo-responsive characteristics and excellent biocompatibility, ELPs have been identified as an important biomaterial in various fields such as the separation and purification of proteins, biosensors, drug and gene delivery systems, or tissue engineering [5–10].

Though the conformational behavior of the polypeptides has been studied extensively in solution under different conditions [11–13], there have been few reports on the interfacial properties of ELP monolayers formed at the air–water interface. It is critical to consider the interfacial properties, rather than the bulk properties of ELPs when they are applied to the solid surface as a thin layer or fabricated using emulsion systems. The phase transition behavior in monolayers differs significantly from that in bulk due to both the interaction of polymer chains with the water subphase and their spatial confinement at the air–water interface [14,15]. By the Langmuir–Blodgett (LB) method, it is possible to obtain information about interfacial properties and to develop stable monolayer films of amphiphilic molecules at the interface [16].

We described for the first time the compressive behavior of thermo-sensitive ELP monolayers at the air–water interface. We focused on how temperature and compression affect the

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secondary structure of ELPs and the morphological changes in ELP monolayers. The two-dimensional properties of the aggregates and the conformational changes in the polymer chains induced by temperature and compression could be derived from surface pressure–area (π–A) measurements, surface enhanced Raman scattering (SERS) spectra and topographical images.

2. Experimental

The ELP gene $[(VPGVG)_{14}(VPGKG)]_8[VPGVG]_{40}$ was oligomerized inside pGEM-T easy vector (Promega, USA) by a recursive directional ligation method known as a controlled stepwise oligomerization process [17]. ELP gene-containing expression vectors were transformed into an *Escherichia coli* strain $BLR(DE)_3$ (Novagen, USA) for expression. $BLR(DE)_3$ cells transformed with ELP expression vectors were first inoculated into 50 mL of Circle-Grow medium (Q-biogene, USA) supplemented with 100 μg mL^{-1} ampicillin and incubated for 12 h at 37 °C with shaking (150 rpm). After culture, the ELPs were purified by an inverse transition cycling (ITC) method [6].

The inverse transition temperature (T_t) of ELP was characterized by the change in optical density (turbidity) at 350 nm using UV–vis spectroscopy in the temperature range from 10 to 55 °C. 0.5, 1.0, 2.5, 5.0 and 10 mg mL^{-1} ELP solutions were prepared with deionized (DI) water for the measurement of transition temperature. The inverse phase transition temperature was determined as the temperature at which the turbidity of the ELP solution was 50% of its maximum.

The π–A isotherms were recorded at different temperatures using an LB system (KSV-mini, KSV Instruments Ltd., Finland). The ELP solution in DI water (40 μL, 1 mg mL^{-1}) was deployed dropwise on the water surface. The ELPs adsorbed at the air–water interface were compressed using two mobile barriers at a speed of 1 mm min^{-1}. The subphase temperature was maintained by a circulating water bath and the working temperatures were 10, 20, 30 and 40 °C. The ELP monolayers formed at the interface were transferred onto either an AuNPs-assembled Si wafer or freshly cleaved mica substrates by a vertical dipping method. Following a relaxation period of 10 min, the deposition was performed at constant surface pressure (2 mN m^{-1}, 21 mN m^{-1}, 25 mN m^{-1}) at different temperatures at a dipping speed of 1 mm min^{-1}.

For SERS measurements of ELP, gold nanoparticles attached to Si wafers were prepared as a SERS substrate. For the synthesis of gold nanoparticles (AuNPs), 50 mg of $HAuCl_4$ was dissolved in deionized (DI) water (50 mL) followed by the addition of 1 mL of citrate aqueous solution (1 w/w%). The reaction temperature was maintained at 100 °C for 30 min, and then the colloidal solution was kept at room temperature.8

For fabrication of AuNP-assembled Si wafers, Si wafers were ultrasonicated for 20 min in acetone and in DI water serially to clean the surface. The surface of the Si wafer was activated by soaking in piranha solution (a mixture of H_2SO_4 and 30 v/v% H_2O_2 (3:1)) for 30 min, and then rinsed with DI water several times, and then dried by blowing nitrogen gas. The positively charged Si wafer surface was formed by dipping the pretreated Si wafers in a mixed solution of 3-aminopropyltriethoxysilane in anhydrous ethanol (total 5 v/v%) for an hour. The excess chemicals were removed by ultrasonicating the Si wafers in ethanol followed by rinsing with ethanol and DI water several times, and then the Si wafers were dried with nitrogen gas. Surface-modified Si wafers were submerged in a AuNP colloidal solution to bind AuNPs to the surface for an hour at room temperature followed by rinsing with DI water several times.

For Raman scattering measurement, a micro-Raman system (JY-Horiba, LabRam 300) was utilized. The signal was collected using a ×100 objective lens (Olympus, 0.90 NA) with back-scattering geometry equipped with a thermoelectrically cooled (−70 °C) CCD detector. The 647-nm laser line from a Kr ion laser (Coherent, Innova 300C) was used as an excitation source. At each measurement, acquisition time was 20 s, and the sample power was about 0.5 mW. The amide bands in SERS spectra were deconvoluted with Origin 8 (OriginLab, Northampton, MA) using Gaussian curve fitting.

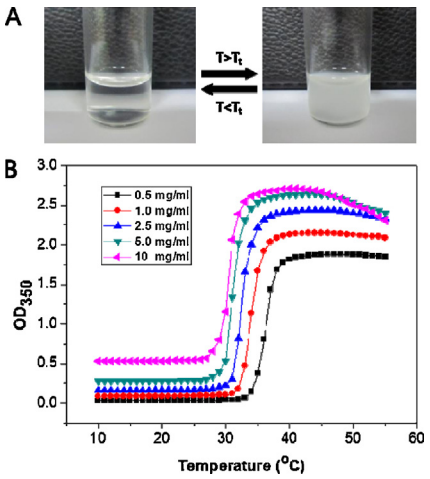

Fig. 1. Inverse phase transition of ELP. (A) Temperature-triggered reversible phase transition of ELP and (B) turbidity of the ELP solution at different concentrations as a function of temperature.

The surface morphology of ELP monolayers deposited on the freshly cleaved mica substrates was obtained with atomic force microscopy (AFM, XE-100, Park Systems) in non-contact mode using non-contact cantilevers (spring constant 42 N m^{-1}, resonance frequency of 330 kHz). The scans were conducted at 0.5 Hz for surface areas of 0.5 × 0.5 and 1 × 1 μm^2. The images were analyzed by an XEI program (Park Systems) for flattening and measuring RMS roughness.

3. Results and discussion

Temperature-triggered aggregation of ELPs resulted in transition of ELP solution from a transparent liquid to a turbid solution (Fig. 1A). ELP is a temperature-responsive polymer that undergoes a hydrophilic–hydrophobic phase transition at T_t. The T_t of ELP is affected by several factors such as the salt concentration, molecular weight of ELP molecules and an additional hydrophobic amino acid. It also depends on the concentration of ELP. The T_t was determined to be 35 °C at 0.5 mg mL^{-1} of ELP concentration and decreased as the ELP concentration increased (Fig. 1B).

Fig. 2A shows the surface pressure–area (π–A) isotherms of ELPs adsorbed at the air–water interface over a temperature range of 10–40 °C. The compression of a monolayer caused the surface pressure to increase gradually in the expanded regime up to 2 mN m^{-1}, followed by a steep increase before the plateau regime (25 mN m^{-1}) where the surface pressure slowly increased over a large variation of the interfacial area. For ELPs, the increase in subphase temperature was translated into an overall shift in the isotherms toward larger interfacial areas.

The limiting interfacial area of ELP molecules could be obtained by extrapolating the linear region (5–20 mN m^{-1}) to zero surface pressure as a parameter for the shift of isotherm curves. The variation in the limiting interfacial area and gradient of the π–A isotherm were plotted as a function of temperature in Fig. 2B. The limiting interfacial area increased with temperature (69.57 cm^2 at 10 °C and 168.94 cm^2 at 40 °C) because the increased hydrophobicity of ELPs caused strong anchoring at the air–water interface.

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