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Short Communication

Examining lysozyme structures on polyzwitterionic brush surfaces

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ABSTRACT

Conformational structures of lysozyme at the interfaces of hydrophilic polymer poly[2-(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl)ammonium hydroxide] (PMEDSAH), are examined to understand the role of protein-polymer interactions on the stability of lysozyme. This work underpins the effect of hydration layer on the structures of physically adsorbed lysozyme on PMEDSAH brushes. Hydrophilic nature and strength of hydration layers around brushes are controlled by varying the brush thickness and temperature. We measured that lysozyme is structurally less stable on 15 nm thick hydrophilic PMEDSAH brushes at 75 °C than at room temperature. To the contrary, 5–8 nm thick brushes stretch in hydrated state by heating, hence yield higher structural stability of lysozyme. These results suggest that short polyzwitterionic brushes can facilitate improved biomaterial interactions that are essential for biosensors performing at elevated temperatures.

1. Introduction

The contact between protein and surface leads to structural changes and bioactivity loss of proteins which influence the performance of enzyme-based biosensors, biofuel cells and enzyme-linked immunosorbent assay [1–4]. Protein-surface interactions governed by physical and chemical properties of both entities and interfacial environment determine protein's fates on a surface. Various applications in food processing and tissue engineering require structurally stable proteins at high temperatures [5–8]. It is, therefore, crucial to tune the interactions between proteins and surfaces for biosensing and anti-fouling applications and retain the structural stability of immobilized proteins. Previous works have shown that the interfacial hydration layer on hydrophilic polymer surface can stabilize structural conformations of adsorbed proteins [9–11]. Poly(ethylene glycol) (PEG) is known for its biological applications, however it decomposes in the presence of oxygen and transition metal ions [12–14] and they lose their protein-resistant property above 35 °C [15]. The hydration layers on PEG are formed through hydrogen bonding with water molecules. Polyzwitterions are promising anti-fouling biomaterials and they present lower hydration free energy and exhibit stronger hydration layer than PEG due to strong electrostatic interactions between ionic groups [14,16–24].

One of the most prominent features of zwitterionic brushes is their reversible self-association behavior that is tuned with an upper critical solution temperature (UCST) [25]. Below the UCST, the strong intra- and inter-chain electrostatic interactions lead to the self-association of brushes and the chains collapse. Interactions between polymer chains are screened by water molecules above the UCST, consequently brushes fully solvate and stretch in this non-associated state [26,27]. Recent studies on polyzwitterions mostly focused on the water structures in hydration layers [28,29], and it was shown that the bioactivity of proteins improved when they were conjugated with polyzwitterions [30–33]. In this study, we investigate the conformational structures of proteins interfacing PMEDSAH brushes as the hydration layer is controlled by the phase transition of zwitterionic brushes. We first discuss the structural changes of lysozyme on PMEDSAH brushes with increasing hydrophilicity. Lysozyme with a high isoelectric point (pI = 11) is physically adsorbed on polymer surfaces and its secondary structural changes are studied in Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR). The collapse of chains by cooling, and swelling of chains by heating will be observed for PMEDSAH brushes at different heights in wet state using Atomic Force Microscopy (AFM) since wettability will be varied by conformational transition of brushes from a collapsed state to a non-associated state. Scheme 1 shows the role of hydration layer effect on the conformational changes of PMEDSAH brushes. Tuning the interactions between protein and polymer controls the bioactivity of proteins, thus it can be used for biochips operating at high temperatures.

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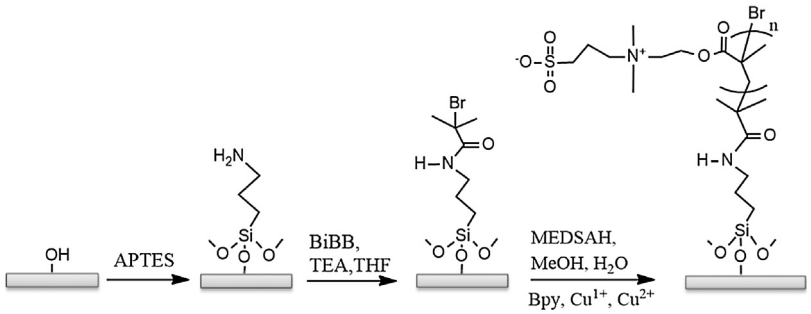
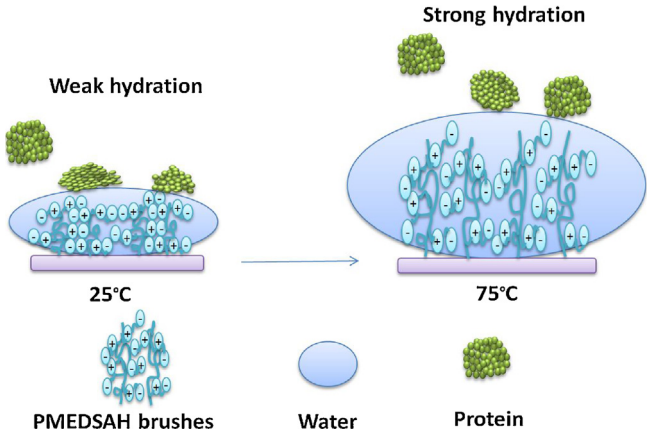


Fig. 1. Grafting PMEDSAH on silicon surface via surface initiated-ATRP.



Scheme 1. Lysozyme denatures at the interface of collapsed brushes at 25 °C in its weak hydrated state (on the left). At 75 °C, lysozyme retains its native nature on extended polyzwitterionic chains (on the right).

2. Material and methods

Acetone, methanol, toluene, tetrahydrofuran (THF) and sulfuric acid were purchased from PHARMCO-AAPER. Phosphate Buffered Saline (PBS), lysozyme was purchased from Thermo Scientific. All other chemicals were purchased from Sigma-Aldrich. Silicon wafers were purchased from University Wafer Inc.

2.1. Initiator coating

A 1 cm × 1 cm silicon surface was cleaned with acetone and ethanol, then dried with nitrogen gas. All surfaces were treated with UV before cleaning with piranha solution for 1 h. The surface was dried at 80 °C for 0.5 h. To prepare PMEDSAH grafted surfaces, cleaned silicon substrate was immersed in a 5 mM APTES solution (6 μL APTES in 5 mL toluene) for 6 h at 70 °C. After rinsing with toluene to remove free APTES molecules, and drying under nitrogen gas, the APTES modified substrate was immersed into 10 mL THF solution, then 0.1 mL 2-bromoisobutyryl bromide (BIBB) and 0.5 mL triethylamine (TEA) was added. The reaction proceeded for 2 h at room temperature. The substrate was then rinsed with THF and DI water.

2.2. PMEDSAH polymerization by surface initiated (SI) – ATRP

CuBr (0.034 g), $CuBr_2$ (0.0017 g) and 2,2-dipyridyl (Bpy, 0.077 g) were dissolved in mixture of DI water (3 mL) and methanol (12 mL). The solution was deoxygenated by three cycles of freeze-pump-thaw. Then the solution was heated to 60 °C and MEDSAH monomer (3 g) was added to the mixture under nitrogen flow. The solution was deoxygenated by a continuous stream of nitrogen for 15 min. Initiator coated substrate was immersed in the mixture and allowed to polymerize at room temperature at varying times. Following polymerization, substrate was rinsed and sonicated in DI water, methanol and acetone, and immersed in warm DI water overnight to remove copper ions and free monomer on the surfaces. Surface was dried under nitrogen. Fig. 1 illustrates the procedure of SI-ATRP of PMEDSAH on silicon substrate.

The preparation of PMMA grafted silicon substrate has been described previously [34]. Briefly, the substrate was silanized with (3-mercaptopropyl) trimethoxysilane (MPTMS). PMMA brushes were grafted to the surface via "click-ene" reaction: The alkene functionalized PMMA (100 mg) and photo initiator DMPA (5 mg) were dissolved in 2 mL acetone. Solution mixture was spin coated on the MPTMS modified surface at 2000 rpm for 1 min. Then the surface was exposed to 365 nm UV irradiation for 60 min. Finally, the surface was thoroughly rinsed with toluene and acetone to remove physically adsorbed PMMA and DMPA.

2.3. Protein adsorption and ATR-FTIR spectroscopy

Polymer grafted substrates were treated with a 100 μg/mL solution of lysozyme in D_2O for 2 h at different temperature, then was air-dried. IR spectra of protein films were measured in a Bruker Tensor 27 FTIR spectrometer, coupled with VeeMax III ATR accessory and a Germanium flat crystal at 60° of incidence. P-polarizer was used to enhance the signal. An unmodified silicon surface was used as background. All spectra were collected at 4 cm^{-1} resolution and data was processed by the OPUS software. Six runs were performed on each sample to confirm the reproducibility. IR spectra were smoothed by 25-point Savitzky–Golay smoothing algorithm, followed by subtracting the PMMA spectrum from that of protein-adsorbed surface. The secondary derivative analysis and curve fitting procedure were carried out to locate the position of overlapped components in the amide I band (1600–1700 cm^{-1}). Peak positions were assigned to different secondary structures of attached proteins. Structural contents were obtained by an area integration of each peak. Water contact angle measurements were performed in a goniometer (Ramé-hart Model 500-F1) equipped with a real time heating stage. Contact angles from 9 different measurements were averaged for each sample. AFM imaging on films in solution was performed by Asylum Bio-3D AFM in tapping mode, a sharp silicon probe with aluminum or gold coated cantilever was used (tip radius R = 6 nm, AppNano, Inc.). Spring constants were determined by thermo noise method.

3. Results and discussion

3.1. PMEDSAH brushes

Fig. 2 displays the surface IR spectra after each step of functionalization. -NH group from APTES based initiator at 1590 cm^{-1} shifts to a higher frequency after PMEDSAH grafting and merges