Topography of self-assembled zein structures on hydrophilic and hydrophobic surfaces

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Introduction

Zein, a major protein of corn, is conspicuous for its ability to form films. It is relatively hydrophobic, soluble in alcohol-water mixtures (40% - 85%) but insoluble in pure water or alcohols. Current structural models of the zein molecule (Argos et al 1982, Matsushima et al 1997) consider zein is formed by nine to ten tandem repeats of α-helical segments aligned in anti-parallel fashion joined at each end by glutamine rich bridges. In Matsushima's model, helical segments are aligned in a row forming a rectangular prism measuring 160Å x 46Å x 12Å. The front and back faces of the prism (160Å x 46Å) corresponding to the helix surfaces are considered hydrophobic, while the top and bottom faces (160Å x 12Å) containing the glutamine bridges are considered hydrophilic. This regular geometry allows zein to self-assemble into chains and layers or films. The structure of zein aggregates is affected by the environment polarity (Wang et al 2003).

Zein adsorption to hydrophilic and hydrophobic surfaces was investigated by surface plasmon resonance (SPR) (Wang et al 2004). SPR is an optical technique that allows the measurement of thickness changes, at the nm scale, when an adsorbate is deposited on a surface. In 75% isopropanol, zein showed a higher initial rate of adsorption and higher maximum adsorption on hydrophilic than on hydrophobic surfaces. Flushing the SPR cell to remove loose binding allowed the evaluation of apparent monolayer values. The hydrophilic monolayer was 4-5 times higher than the hydrophobic. Monolayer differences were attributed to zein footprint considering that zein maintained its prismatic geometry after adsorption. It was proposed that zein adsorbed to hydrophilic surfaces through its glutamine rich face thus giving a small footprint and high pile. When it adsorbed to hydrophobic surfaces zein would use its hydrophobic face, giving a large footprint and low pile. In the present work, the morphology of zein deposits was examined by atomic force microscopy (AFM) to investigate the effect of environment polarity on structure of zein aggregates.

Experimental

Chemicals. Commercial zein, regular grade F4000 (Freeman Industries Inc., Tuckahoe, NY); ethyl alcohol, 200 proof (AAPER Alcohol, Shelbyville, KY); 2-propanol, electronic use; chloroacetic acid, certified; sulfuric acid, certified; and hydrogen peroxide 30 %, ACS certified (Fisher Scientific, Fair Lawn, NJ). 11-mercaptoundecanoic acid ($COOH(CH_2)_{10}SH$), and 1-octanethiol ($CH_3(CH_2)_7SH$) were purchased from Aldrich Co., Inc., Milwaukee, WI.

Sample preparation. SPR cell slides were prepared following the method used in the surface plasmon resonance study by Wang et al (2004). Glass microscope slides were washed with sulfuric acid and coated with a 50 nm thick gold layer. Slides were then immersed in either 2mM 11-mercaptoundecanoic acid or 2mM 1-octanethiol to allow the formation of self-assembled monolayers (SAMs) on the gold surfaces. 11-mercaptoundecanoic acid generated a hydrophilic surface while 1-octanethiol formed a hydrophobic surface. Zein solutions, 3mg/ml in 75% 2-propanol, were forced to flow on the SAMs to allow zein adsorption. Slides were examined by AFM after zein deposition and after rinsing the slides with distilled water or 75% 2-propanol. Slides were kept in desiccators and allowed to dry for 24 hours before scanning.

AFM experiments were carried out with a Nanoscope IIIa (Dimension 3100, Digital Instruments Inc., Santa Barbara, CA) where the tip motion was followed by deflection of a laser beam reflected off the rear side of the cantilever. The deflection was monitored with a position-sensitive detector. AFM images were taken with an A-type scan head. Silicon nitride (Si_3N_4) probes attached to a microfabricated cantilever (200 μm, U shape base) were used. Measurements were carried out in the contact force mode. Images were recorded in the repulsive region of the force-distance curve, where the probing tip is brought into repulsive contact with the sample and the static deflections of the cantilever are measured. Scanning line frequencies were 1 Hz for large-scale scans.

Results and Discussion

AFM images were taken from the center of the slides to avoid regions of abnormal adsorption at the sides. Roughness was calculated using the root-mean-square (R_{ms}) deviation of the heights of the various features imaged (Huang and Gupta 2001). Gold-coated substrates were imaged to characterize the native surface. Gold substrates were rather smooth and uniform. The calculated diameter of gold grains was 200 – 300 nm with a roughness (R_{ms}) of 1.84 nm. AFM images of hydrophilic and hydrophobic SAM surfaces on gold-coated slides appeared uniformly smooth with grain diameter 200-280 nm. SAM surfaces showed no major features when compared to gold deposits.

AFM images showing typical surface topography of zein deposits on hydrophilic surfaces are presented in Figure 1. Figures 1a and 1b show zein deposits after rinsing with distilled water or 75% 2-propanol, respectively. Distinct circular and relatively tall structures, 50 nm high, were observed after rinsing with distilled water. Surface roughness was calculated at 6.96 nm. A possible explanation of the formation of zein cylinders is that when zein was first attached to the carboxylic heads of 11-mercaptoundecanoic acid it formed worm-like string structures which provided the base structure. After surface saturation, excess zein molecules attached themselves to the worm-like base piling up to form a high rise. When surfaces were flushed with water, the polarity of the solution sharply increased prompting hydrophobic aggregation into closed structures to minimize surface exposure to water. Rinsing with 75% 2-propanol washed away any structures leaving a uniform surface of zein molecules attached to SAMs (Figure 1b).

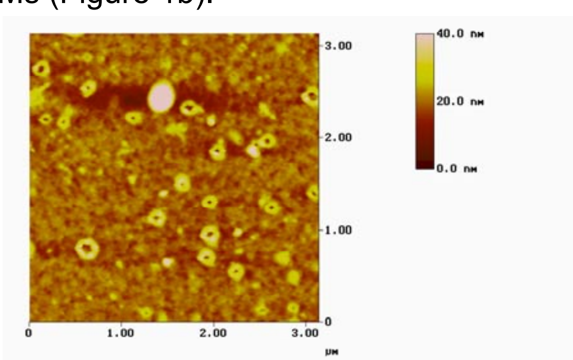

Figure 2a. AFM image of zein deposited on a hydrophobic SAMs washed with distilled water

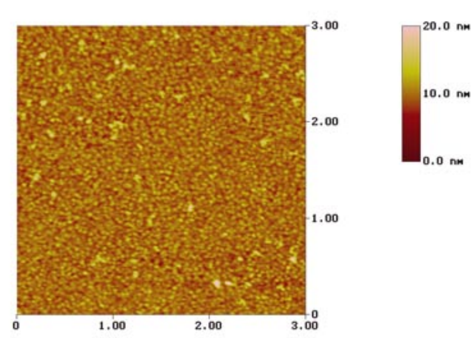

Figure 2b. AFM image of zein deposited on a hydrophobic SAMs washed with buffer

AFM images showing typical surface topography of zein deposits on hydrophobic surfaces are presented in Figure 2. Figures 2a and 2b show zein deposits after rinsing with distilled water and 75% 2-propanol, respectively. Both surfaces seem uniformly covered with zein deposits. Roughness was estimated at ~1.26 nm and average height was ~8.5 nm. It is possible that increasing the environment polarity promoted surface uniformity by inducing hydrophobic interactions between zein and the underlying SAMs. Rinsing with 75% 2-propanol washed away excess zein leaving a uniform surface of zein molecules attached to the SAMs. Further research is needed to investigate the effect of environment polarity, surface and medium, on the morphology of zein deposits. This information would be useful in the design of novel biobased polymeric materials.

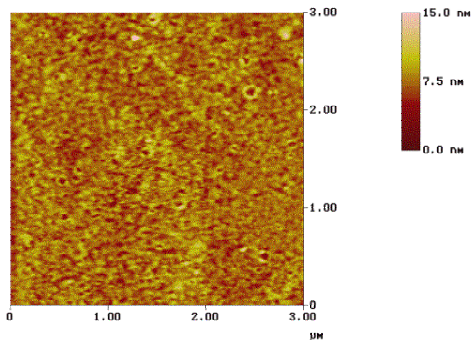

Figure 2a. AFM image of zein deposited on a hydrophobic SAMs washed with distilled water

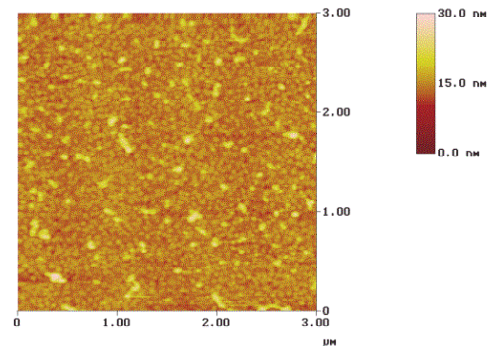

Figure 2b. AFM image of zein deposited on a hydrophobic SAMs washed with buffer

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