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Topographies of Organized Monolayer of α-Amylase Observed by Atomic Force Microscopy

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Abstract: In this paper, α-amylase organized monolayer was assembled on the surface of the PET-CO_2^- substrate in different conditions. The different topography of the α-amylase/PET monolayer was obtained by AFM in tapping mode.

Keywords: Topography of monolayer,α-amylase, AFM, self-assembly.

Preparing the organized ultrathin film of proteins is the key to developing of protein-based molecular devices such as biosensors. In the past, the organized molecular layers were fabricated by the means of Langmuir-Blodgett (LB), which utilized the amphipathy of molecules. Molecular deposition (MD) which is based on the electrostatic interaction between the charged substrates and oppositely charged absorbate is a new and versatile method for preparing organized self-assembled ultrathin film. The films of more than ten kinds of bioactive macromolecules including myoglobin, lyzyme, glucoamylase and so on have been successfully prepared[1,2,5]. The topography of surface with atom-level resolution can be directly given by the atomic force microscopy (AFM)[3]. The topography of polyelectrolyte-anti-immunoglobulin (anti-IgG) multilayer on a gold substrate was investigated using AFM by F.Caruso[4]. In our work, the α-amylase organized monolayer was assembled on the surface of the PET-CO_2^- substrate in different conditions. The topographies of monolayer at the surface were studied by AFM.

Experimental

Substrate preparation

The method of preparing negatively charged substrate is the same as that in the reference[5]. Then the PET-CO_2^- substrate was obtained.

The α-amylase molecular deposition assembling.

The α-amylase was dissolved in pH 2 buffer solution, which induce the α-amylase to positive charges. Then the PET-CO_2^- substrate was submerged into the enzyme solution at 30℃ and the α-amylase monolayer is assembled. After about 1 hour, the substrate was taken from solution, rinsed with distilled water twice and dried under reduced pressure.

Atomic Force Microscopy

AFM images were recorded with a Nanoscopy IIIA system (Digited Instrument Inc.) in tapping mode and measured at room temperature in air.

Results and discussion

The α-amylase monolayer assembly

In the processing of assembly, the negatively charged substrate was immersed into the α-amylase solution, in which the α-amylase was induced to positive charges. Driven by the electrostatic interaction, the positive α-amylase was anchored on the negative substrate to form monolayer. The AFM image of the PET-CO_2^- substrate is shown in **Figure 1**. And the α-amylase/PET monolayer is shown in **Figure 2**. The 0.5μm× 0.5μm area is well coverd with α-amylase and the film of α-amylase/PET has a root-mean-square roughness of 2.1 nm.

Figure 1 AFM image of PET-CO_2^- substrate

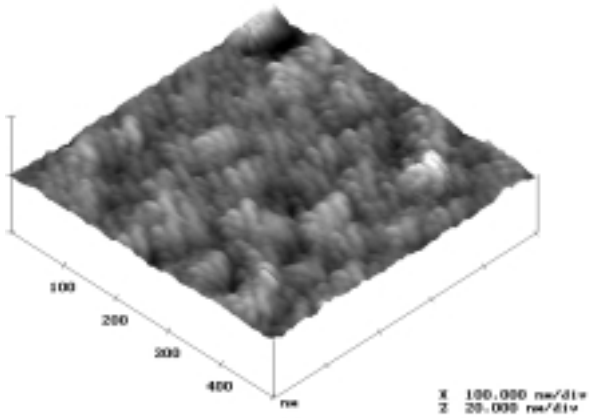


Figure 2 AFM image of α-amylase/PET monolayer (pH=2,higher charged density)

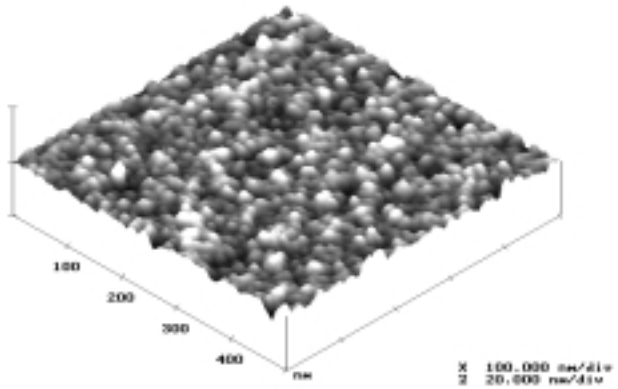


Figure 3 AFM image of α-amylase/PET monolayer (pH=4)

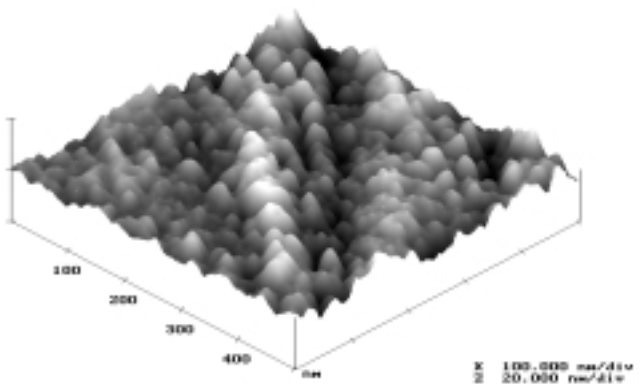
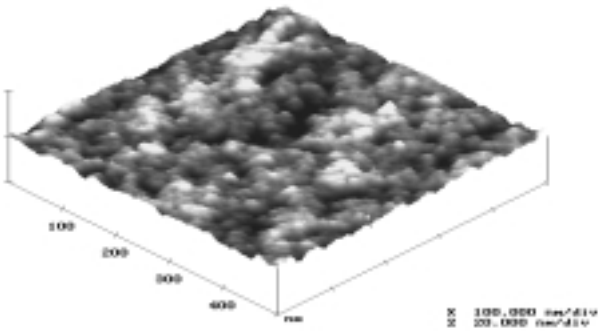


Figure 4 AFM image of α-amylase/PET monolayer (the lower charged substrate)



Comparing the images of **Figure 1** and **Figure 2**, there is an obvious difference between the PET-CO_2^- substrate and the α-amylase/PET monolayer, which confirms the occurrence of foregoing processing of assembling. From the α-amylase/PET monolayer AFM image, we can find that the α-amylase still maintains its spherical shape and the distribution of molecules is uniform and organized in this area.

The topography of α-amylase/PET monolayer at different pH values

There are two major factors including pH value of buffer solution and different charged density PET-CO2$^-$ substrate which can change the topography of α-amylase/PET film in our experiments. The topography of α-amylase/PET monolayer at different pH values are shown in **Figure 2** and **Figure 3**. It is found that the lower is the pH value of buffer solution, the sharper is the shape of α-amylase molecules. The α-amylase/PET monolayer is uniform in wider area up to $0.5 \times 0.5 \mu m^2$. The fluctuation of the topography of the α-amylase/PET monolayer is less than 5 nm.

The topography of α-amylase/PET monolayer in different charged substrate

The PET-$CO2^-$ films were prepared by introducing clean PET films to NaOH solution at some temperature to get the different charged PET-$CO2^-$ substrate[5]. The topography of α-amylase/PET monolayer in different charged density substrate are shown in **Figure 2** and **Figure 4**. It is found that the larger is the charged density substrate, the sharper is the shape of α-amylase molecules. The topography of α-amylase/PET monolayer may influence the microstructure and activity of α-amylase molecules. More detailed studies are working now.

Referrences

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