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Pepsin nanofilm self-assembly on the positively charged poly (ethylene terephthalate) substrate

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Abstract: Pepsin was assembled on the surface of prepared poly (ethylene terephthalate) (PET-NH_3^+) substrates. The composition and structure of the pepsin / PET-NH_3^+ assembling films in different condition were characterized by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM).

Key words: Pepsin, Self-assembly, PET-NH_3^+ substrate, XPS and AFM
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INTRODUCTION

Molecular deposition (Decher et al., 1992) is a popular method for biomolecules self-assembly with preservation of their native state without influencing their structure. It plays an important role in the study of molecular devices and provides suitable model surfaces to be used for researching biomolecular interaction. Films (Lvov et al., 1999) of many bioactive macromolecules such as myoglobin, lyzyme, glucoamylase were successfully prepared with Si/SiO_2 as substrates, and biosensors (Sun et al., 1996; Li et al., 2000) based on self-assembled biomolecular films were studied. Self-assembling films (Lin et al., 2000; He et al., 2001) of enzymes such as lipase and α-amylase on the PET-CO_2^- substrates by molecular deposition were reported previously. This paper deals with the assembly of pepsin on PET-NH_3^+ substrate and the use of XPS and AFM to characterize the assembling films.

EXPERIMENTAL

Materials

Pepsin from porcine mucosa (E.C. 3.4.23.1, M_w 35000, pI = 1.0) and hemoglobin from bovine erythrocytes were obtained from Lizhu Co. Ltd., Shanghai; poly (allylamine hydrochloride) (PAH) (M_w circa. 15000) was purchased from Aldrich Chem. Co.; all other chemicals and solvents were from commercial sources.

Substrate preparation

The clean PET films were prepared (Lin, 2000). PET-NH_3^+ films made by immersing clean PET film in PAH solution (1mg/ml, pH = 11.5) for 1 hour at 30℃. The film was removed from the solution, rinsed thrice with water and introduced to pH 2.0 water for 30 min. After rinsing thrice with water, the substrate was kept in distilled water.

Pepsin molecular-deposition assembly and activity measurement

The pepsin was dissolved in buffer (pH > pI) and induced to produce negative charges. The pepsin deposition was carried at 30℃. The PET-NH_3^+ film and pepsin solution were prepared fresh every day. Pepsin activity was measured in DAB9 by the method of Stellmach (Stellmach et al., 1992).

X-ray photoeletron spectroscopy

XPS was performed with an ESCALABMKII instrument (VG Co., British), using a take-off angle of 30°. The atomic percentages

for the self-assembled monolayers were obtained by correcting the data using XPS sensitivity factors given by the instrument manufacturer (C, 1.00; N, 1.77; O, 2.85).

Atomic force microscopy

AFM images were recorded with a Nanascopy IIIA system (Digital Instruments, Inc.) in tapping mode and in air.

RESULTS AND DISCUSSION

PET-NH_3^+ film was prepared by using Wei Chen's method (1997). The composition on the surface of the PET-NH_3^+ film was determined by XPS. The result indicates that there was 4.5% nitrogen on the PET-NH_3^+ surface. There were two kinds of nitrogen in C-NH_3^+ (the peak at 402eV) and -CONH- (the peak at 400eV) as shown in Fig. 1. The amount of C-NH_3^+ was about 70% and that of -CONH- was about 30%, which indicates about one C-NH_3^+ group per two PET units on surface. PET-NH_3^+ substrate at certain charge density was immersed into pepsin solution, where pepsin was induced to produce negative charges. Driven by electrostatic attraction, the pepsin molecules moved towards the film and deposited on it finally.

The pepsin/ PET-NH_3^+ assembled film was studied by XPS spectra (Fig. 2). The result showed that there was about 6.2% nitrogen in the pepsin/ PET-NH_3^+, which was larger than that in the PET-NH_3^+ film. Comparison of Fig. 1 and Fig. 2 revealed that the ratio of -CONH- was significantly increased (from 30% to 80%). Because the nitrogen composition in pepsin was about 11.2%, and the -CONH- ratio was high in pepsin, it was concluded that pepsin molecules had effectively deposited on the PET-NH_3^+ film by electrostatic attraction.

The AFM image of PET surface modified with PAH is shown in Fig. 3. The root-mean-square (RMS) roughness of this film was 0.25 nm. The AFM image of the pepsin/PET-NH_3^+ assembled film is shown in Fig. 4. The PET-

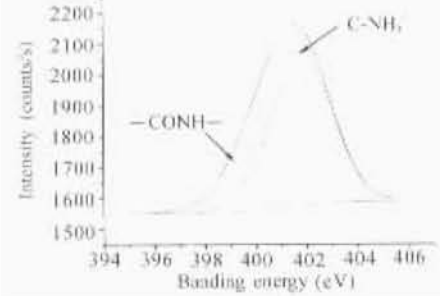

Fig. 1 N_{1s} region XPS spectra of PET-NH_3^+ film

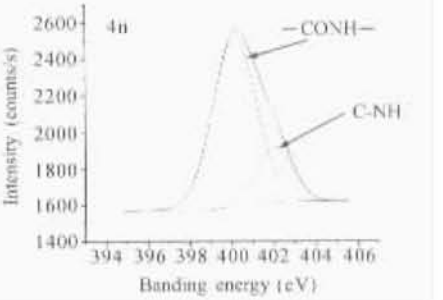

Fig. 2 N_{1s} region XPS spectra of pepsin film on PET-NH_3^+

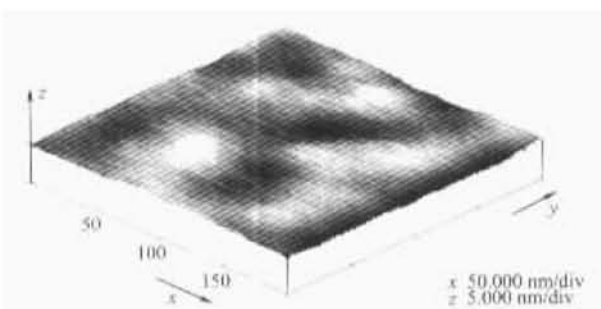

Fig. 3 AFM image of PET surface modified with PAH.

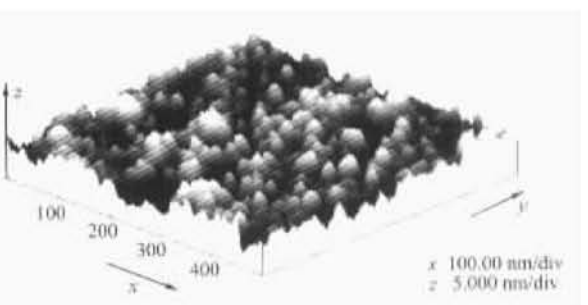

Fig. 4 AFM image of pepsin film on PET-NH_3^+ (pH = 5.0)

NH_3^+ substrate surface had RMS roughness of 1.1 nm and was well covered with pepsin molecules. The vertical height of pepsin in Fig. 4 is ca. 1.3 nm; this value agrees well with the radius of pepsin molecules ($3 \times 3 \times 3$ nm), which indicated the pepsin molecules arranged on the surface closely. But most of the pepsin molecules on the surface had ca. 18 nm diameter, which was larger than the actual diameter. This was probably due to tip convolution effect in AFM, which increases the x-and y-dimensions of imaged molecules.

The AFM image of pepsin/ PET-NH_3^+ film assembled in pH=2.0 solution is shown in Fig. 5. The RMS roughness was 0.61 nm. Comparison of the two images of pepsin/ PET-NH_3^+ assembled film on the same substrate in Fig. 4 and Fig. 5 shows that there are some larger structures (about 40 nm) in Fig. 5. The pI value of pepsin is 1.0. Molecules aggregates are formed easily during assembling, because the pepsin molecules carry few charges in pH=2.0 solution and there is smaller repulsion between molecules.

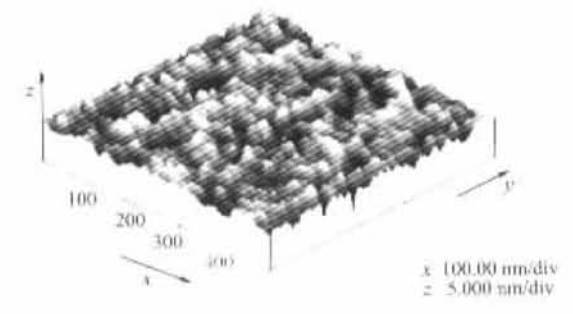

Fig. 5 AFM image of pepsin/ PET-NH_3^+ assembled film in pH=2.0 solution

The biocatalytic activity of self-assembled pepsin was determined by hydrolysis of hemoglobin to amine acid in pH=2.0 buffer at 30°C for 30 min. Forlin reagent was used as indicator to react with amine acid and the reaction was followed by monitoring the absorbance at 540 nm. Table 1 shows the results of the activity measurement.

As shown in Table 1, the pepsin self-assembled in pH=2.0 solution is more active than that self-assembled in pH=5.0 solution. Free pepsin has highest activity in pH=1.52.0 solution; in other words, the pepsin molecule has more favorable conformation in pH=2.0 solution than in pH=5.0 solution. This indicates that the conformation of pepsin molecules is kept well during the self-assembling process by molecular-deposition; and that the conformation of self-assembled pepsin molecules is consistent with that of the self-assembling solution.

Table 1 Activity of pepsin self-assembled under different condition

No.	Activity of pepsin (PE)	
	Self-assembled in pH=2.0 solution	Self-assembled in pH=5.0 solution
1	27.1	19.5
2	31.6	22.5

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