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The lipase monolayer film self-assembly on the negatively charged poly(ethylene terephthalate) substrate

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Abstract: The PET-CO_2^- film was prepared and the lipase was assembled on the surface of the PET-CO_2^- substrate. The structure at the surface and activity of lipase/PET monolayer were studied by ATR-FTIR and AFM, and other methods.

Keyword: Self-assembled, monolayer, lipase, PET-CO_2^- substrate.

The artificial assembly of enzymes is of considerable interest in basic research for development of enzyme engineering as well as for technological applications.

Since 1991, the molecular deposition developed by Decher and others has been a versatile method for the protein and enzyme molecules self-assembly as a novel technique of immobilized enzyme. The glucose isomerase and the bienzymes of glucose oxidase and glucoamylase were assembled using molecular deposition on the surface of the cationized quartz slide or silicon crystal by J. Shen[1,2] . More than ten kinds enzyme, such as lysozyme and glucose oxidase were assembled by T. Kunitake[3]. In this paper, the lipase was assembled on the surface of the PET-CO_2^- substrate. The structure at the surface and activity of lipase/PET monolayer were studied by attenuated total reflection infrared (ATR-FTIR) and atomic force microscopy (AFM), and other methods.

Experimental

Substrate preparation.

PET films (Mylar,5 mil) were rinsed with distilled water and methanol, extracted in refluxing hexane for 2 h, and dried at 20℃ for 12 h and under reduced pressure (2mmHg) for 8 h. PET-CO_2^- film was prepared by introducing clean film to 1.5 mol/L NaOH solution for 12 min at 60 ℃. The film was subsequently rinsed with 0.1 mol/L HCl, distilled water, methanol, hexane, and dried at the same condition.

The lipase molecular-deposition assembling.

The pH 2 buffer solution was made up with 0.2 mol/L hydrochloric acid and 0.2 mol/L patassium chloride at 35℃. The lipase solution was prepared with pH 2 buffer solution. Thus lipase molecules were induced to have positive charges. The lipase monolayer is assembled at 35℃. After layer deposition, film samples were rinsed with water and dried

at reduced pressure.

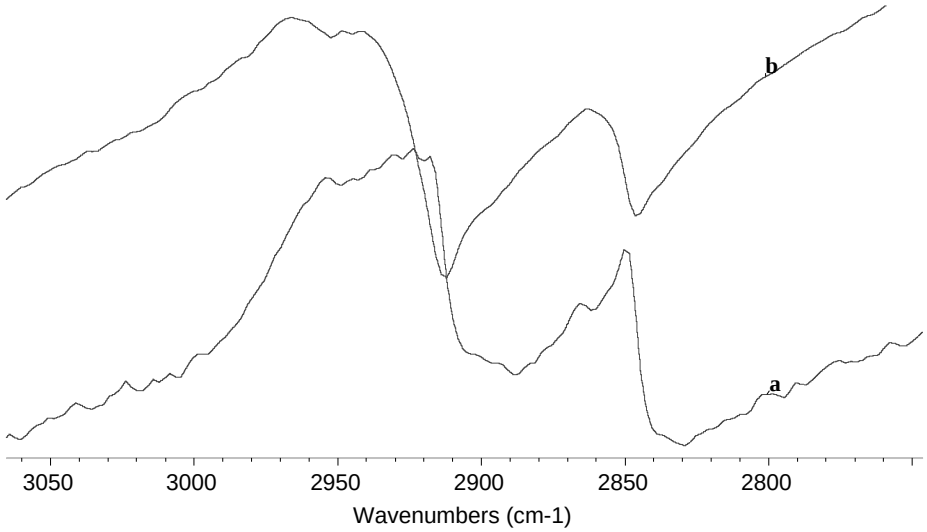
FTIR spectroscopy.
A Nicolet Magna IR560 spectrometer, equipped with attenuated total reflection accessory was used. The spectra were recorded with resolution of 4 cm^{-1}. The internal reflection element ZnSe was employed, at an angle of incidence of 45° and 32 scans. The penetration depth was approximately 0.88 um at 1000 cm^{-1} [4].

Atomic Force Microscopy.
AFM images were recorded with a Nanoscope ⅢA system (Digited Instrument Inc., Santa Barbara, California, USA) in the resonance mode and measured at room temperature under air. Lipase activity was measured in BP63 method by reference[5].

Results and Discussion

The lipase monolayer film assembly
The lipase molecules are induced to be positively charged when the pH value 2.0 of solution is used. The negatively charged substrate PET- CO_2^- was immersed in a lipase solution for 1h. In this way, the PET- CO_2^- substrate was covered with a lipase monolayer by electrostatic interaction and its surface charge was reversed. The lipase/PET monolayer film was characterised by means of ATR-FTIR and AFM shown in **Figure 1** and **Figure 2**, respectively.

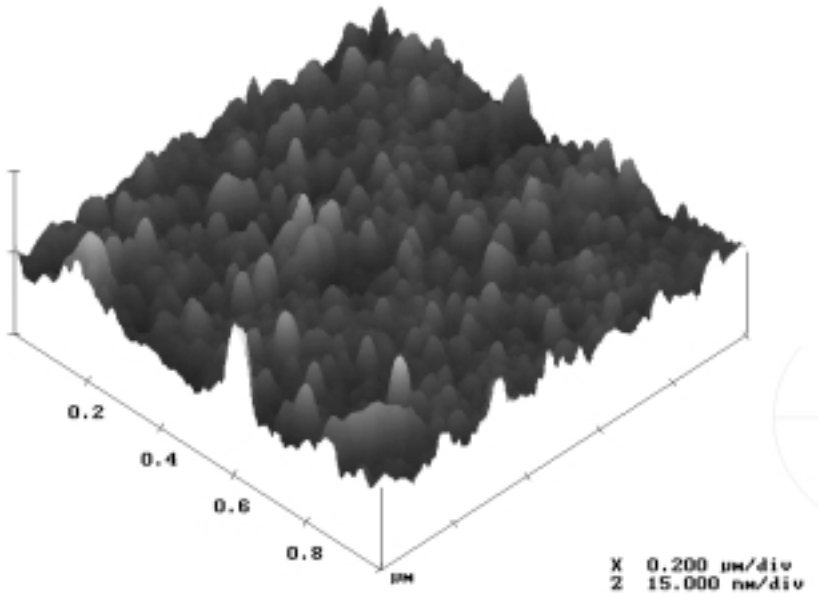
Figure 1. ATR-FTIR spectra for the lipase/PET and ionized PET film at surface
a. ionized PET film at surface b. lipase/PET monolayer film



As shown in **Figure 1**, The ATR-FTIR spectra of lipase/PET monolayer is different from that of PET- CO_2^- substrate. There are two bands of 2850 cm^{-1} and 2920 cm^{-1} for the

-CH_2 group of PET molecular chain in PET- CO_2^- substrate shown in **Figure1a** and two bands of 2860 cm^{-1} and 2970 cm^{-1} for $-CH_3$ group of lipace in lipase/PET monolayer shown in **Figure1b**. That is, the surface composition of film about 0.3 um deep is changed. It confirms the presence of lipase in the monolayer.

Figure 2. AFM image in scale of 1.0×1.0 μ m of the lipase/PET monolayer film



The lipase/PET monolayer self-assembly film was investigated with atomic force microscopy (AFM) shown in **Figure 2**. AFM images of lipase/PET monolayer indicated that the surface fluctuation is in the range of nanometer scale and the depth of monolayer is less than 15 nanometers.

The interface of lipase/PET monolayer self-assembly film

In surface of PET- CO_2^- substrate, there are two kinds of carboxyl groups as –COOR and $-COO^-$. The feature of 1700 band in FTIR spectra shown in **Figure 3a** is for the –COOR carboxyl group and the 1600-1655 bands for $-COO^-$ carboxylate group. After the lipase molecules are assembled, the bands of more stretching mode of carboxylate in the PET-CO_2^- substrate disappear, as shown in **Figure 3b**. The cationic and anionic interaction is the power to make the stretching mode of carboxylate to be the same. On the other hand, the volume of the enzyme molecule is so large that the streching of carboxylates in the PET- CO_2^- substrate has been limited, as in **Figure 4**.

Enzyme activity of lipase/PET monolayer self-assembly film

The performance activity of enzyme is an important parameter for the immobilization of self-assembly. It is the activity ratio per gram of self-assembly enzyme and solution enzyme. The results are shown in **Table 1**.

Figure 3. ATR-FTIR spectra for the lipase/PET and ionized PET film at surface
a) PET- CO_2^- film at surface;
b) lipase/PET self-assembly film

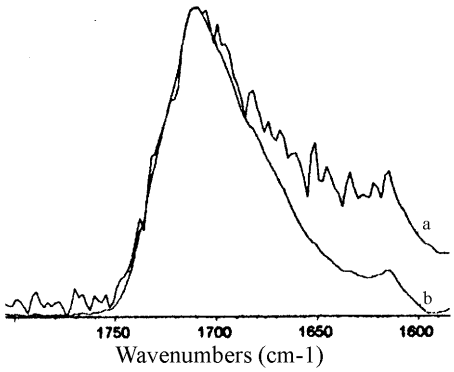


Figure 4 Model for electrostatic interaction of lipase/PET monolayer self-assembly film at interface

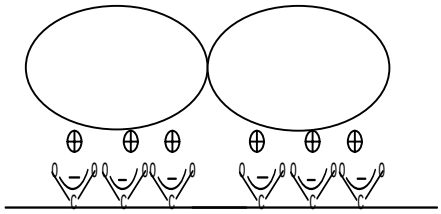


Table 1 Performance activity of lipase/PET monolayer self-assembly film

	activity of enzyme (U/g)		performance activity of enzyme
	solution enzyme	lipase/PET monolayer	
1	28264.95	53487.12	1.89
2	24725.60	59905.58	2.42
3	25423.50	49208.15	1.94

As shown in **Table 1**, the performance activity of lipase/PET monolayer self-assembly film is about 2.0. The result indicated the larger activity lipase by self-assembly on the PET- CO_2^- substrate can be obtained, and the similar result of glucose isomerase self-assembly film on quartz slide[1]. The advantage of self-assembly in comparison with the common enzyme immobilization method is that high activity can be obtained by the formation of an enzyme monolayer.

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