Site-Selective Deposition of Enzyme/Polyelectrolyte Multilayer Films on ITO Electrodes Controlled Electric Fields

Lixin Shi, Junqi Sun, Junqiu Liu, Jiacong Shen,* and Mingyuan Gao*†
Key Lab for Supramolecular Structure and Materials of Ministry of Education, JiLin University, ChangChun 130023, P. R. China
†*Institute of Chemistry, The Chinese Academy of Sciences, Beijing 100080, P. R. China*

(Received August 26, 2002; CL-020727)

Electric field directed layer-by-layer assembly (EFDLA) approach was for the first time employed to realize site-selective deposition of enzyme/polyelectrolyte films on two independent indium-tin-oxide electrodes. Enzyme activities and thickness of the resulted films were investigated and compared with those of normal layer-by-layer self-assembled films of enzyme. All results prove that the site-selective deposition was successfully realized at applied voltage of 1.2 V.

The fabrication of ultrathin molecular films of enzymes has attracted great attention worldwide due to the potential application in biological sensors.[1–4] Layer-by-layer (LbL) self-assembly method has been proven to be a powerful technique to immobilize enzymes in ultrathin molecular films.[5] Utilizing chain reactions described in many literatures, maltose sensor has been produced by constructing a heterostructure of glucose oxidase and glucoamylase using the LbL self-assembly method.[6] A recent development in fabrication of lateral patterning structures using Electric Field Directed Layer-by-layer Assembly method (EFDLA) enables the preparation of a multi-component lateral structure consisting of two types of independent patterns of different materials on a common substrate.[7–9] Based on the fact that charged species will move either along or against the direction of electric fields, by defining the polarities on electrodes that are placed on a common substrate, a site-selective deposition of multilayer films can be realized. In this method, conducting electrodes not only supply electric fields but also serve as supporting substrates for resulted films. In current study, this novel approach is for the first time used to fabricate lateral structure of enzyme/polyelectrolyte ultrathin films. Glucose isomerase (GI) was adopted as a model enzyme. Site-selective deposition of GI and poly(diallyldimethyl-ammonium chloride) (PDDA) alternating films on structured indium-tin-oxide (ITO) glass was carried out in the presence of static electric fields. In brief, two independent electrodes on one rectangular piece of glass were fabricated. On one of the electrodes negatively charged glucose isomerase and positively charged PDDA alternating layers were selectively deposited in the presence of electric fields that were established between the two electrodes. Since depositions of negatively charged GI on positive electrode would be accelerated, whereas depositions of positively charged PDDA would be decelerated, by switching the polarities on two electrodes each time a new layer was deposited, a series of favorable depositions of GI and PDDA were enabled on a pre-selected electrode, which resulted in a site-selective deposition.

The isoelectric point of GI is 4.7. GI solution with an activity of 714.4 U/ml at pH 7.0 in phosphate buffer solution (0.1 M) was used in the investigation. PDDA solution of 2 mg/mL was prepared. Two independent ITO stripes of 2 mm in width separated by a 2 mm non-conducting gap on glass were produced by chemically etching one rectangular piece of ITO glass through a mask in the mixture of Zn powder and diluted HCl. The structured ITO glass was cleaned and then immersed in hydrogen peroxide at 80 °C for several minutes to make the ITO surface hydrophilic. The film deposition started with PDDA. After deposition of the first PDDA layer, the structured ITO glass was cleaned with Milli-Q water to remove the excess PDDA. Then the polarities on the two ITO electrodes were switched to deposit the first layer of GI on the top of PDDA layer. After this dipping cycle was completed, the second layer of PDDA was deposited under a reversed polarities on the growth electrode. By repeating the above procedures, a multilayer film deposited on the pre-defined electrode was obtained. Typically the deposition time in PDDA and GI was 4 min, respectively. Following the cysteine-carbazole method, the amount of fructose converted from glucose was used for determination of the enzyme activity[10,11] using 2 M glucose as substrate. In this work, one unit of GI activity was defined as the amount of enzyme required to produced 1 μmol fructose in 1 min in 0.1 M phosphate buffer (pH 7.0) at 60 °C.

In fact, the prerequisite to realize the enzyme lateral structure using the EFDLA method is to ensure that enzyme isn't denatured in the presence of electric fields. High voltages can induce electrolysis of water, which will decrease enzyme activity. However, low voltages will not provide sufficient driving force to effectively control the deposition process. Therefore, voltage-dependent site-selective deposition was investigated and characterized by enzyme activities of 10-bilayer films obtained at DC voltages of 0.6, 0.8, 1.0, 1.2 and 1.4 V, respectively. The results are given in Figure 1. Typically, we name the electrode on which favorable depositions occur as growth electrode and the electrode on which unfavorable depositions take place as counter electrode. Under 0.6 V, GI deposition takes place on both electrodes. There is no big difference between two electrodes in enzyme activity. As the voltage increases, the enzyme activities of films on the growth electrode increase, whereas the enzyme activities of films on the counter electrode decrease. This leads to variation of deposition selectivity S which is characterized by enzyme activities ($S = (A_{gr} - A_c)/(A_g + A_c)$, A_{gr}, enzyme activity of the film on growth electrode; A_c, enzyme activity of the film on counter electrode). The voltage-dependent S is summarized in Table 1. The selectivity S reaches its maximum 95.2% at around 1.2 V and then slightly decreases. The following decrease of S is caused by a slight increase in enzyme activity of the counter electrode. At this moment, we don't know the reason for this extraordinary phenomenon. However, the enzyme activity measured from the growth electrode increase all the time

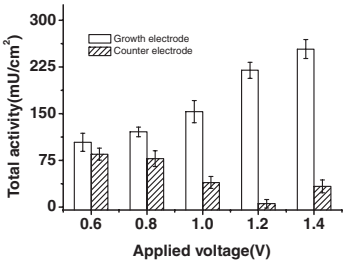

Figure 1. Enzyme activities of the films on both growth electrode and counter electrode against the applied voltage.

Table 1. Deposition selectivity *S* between the counter electrode and growth electrode characterized by enzyme activities

Votage/V	0.6	0.8	1.0	1.2	1.4
Selectivity	10.2	21.6	59.0	95.2	76.9

with the increase of voltage, which implies that within the investigated voltage range, the GI enzyme preserves its biological function. Figure 2 presents FTIR-RAS spectra of 10-bilayer films produced at 1.2 V and 0 V, respectively. The latter one corresponds to normal LbL self-assembled film. Almost no distinct peak shift is observed. It can therefore be concluded that the assembled GI enzyme in the presence of electric fields have at least the same properties as that in the normal LbL self-assembled films.[12]

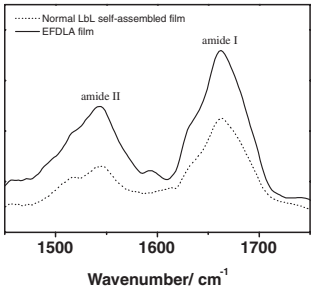

Figure 2. FTIR-RAS spectra of 10-bilayer GI/PDDA films fabricated by normal LbL self-assembly method and EFDLA method, respectively.

Using 1.2 V as optimized voltage, site-selective deposition of layer-by-layer assembled films with different number of bilayers were fabricated. Ellipsometric measurements were performed to characterize the thickness of films on both counter electrode and growth electrode.[13] The results were compared with those of normal self-assembled films as shown in Figure 3. The thickness of the film on growth electrode increases linearly against the number of bilayers.[14] This suggests that almost equal amount of enzyme can be deposited during each dipping cycle. In contrast, the thickness of the films on counter electrode remains almost unchanged as the number of bilayers increases. The different deposition behaviors occurring to the growth and counter electrodes lead to effective site-selective deposition. In comparison with normal LbL self-assembled films, the films on counter electrode have higher film thickness. The increasing factor is about 1.62 on average. If the increased film thickness on growth electrode is partly contributed by more effective adsorption of GI driven by electric fields, the increased film thickness should give rise to higher enzyme activities.

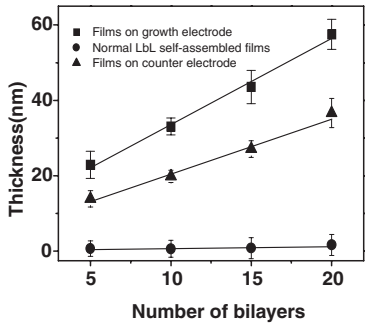

Figure 3. Thickness of GI/PDDA films against the number of bilayers.

By cysteine-carbazole method, the enzyme activities of films were measured and shown in Figure 4. Quite similar results were obtained with respect to enzyme activities. On growth electrode, the enzyme activity increases linearly with the increase of the number of bilayers. In addition, the enzyme activity of the films on growth electrode is increased by a factor of 1.57 comparing with that of normal self-assembled films. This is in very good agreement with the increasing factor for film thickness and proves that the electric field can effectively enhance the adsorption of GI without destroying its catalytic properties. Thus just by increasing the number of bilayers or film thickness, a certain catalytic ability can be reached on growth electrode. However, on counter electrode enzyme activities are independent of the number of bilayers. Therefore, site-selective deposition as well as site-dependent function is successfully realized. This is a very important step towards the fabrication of multi-enzyme chips based on lateral structures of different enzymes. Such multi-enzyme chips will enhance detecting ability for analyzing a complicated biological system by excluding the interference from different analytes.[15] Since the biochips are more tolerant to defects than electronic devices, the success of site-selective deposition achieved by the EFDLA method will open up a promising way to biochip fabrications.

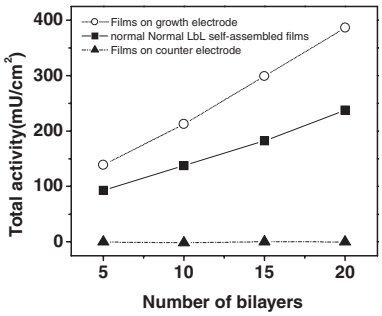

Figure 4. Enzyme activities of GI/PDDA films vs. the number of bilayers.

In summary, films on counter electrode and growth electrode prepared by EFDLA method were investigated by enzyme activity measurements, ellipsometric measurements and FTIR-RAS spectroscopy. The results prove that the site-selective deposition of GI/PDDA multilayer films can successfully be realized at 1.2 V.

This research was supported by the Major State Basic Research Development Program (G2000078102), the National Natural Science Foundation of China (Grant No. 20174013).

References

1 G. Decher and J.-D. Hong, *Makromol. Chem. Macromol. Symp.*, **46**, 321 (1991).
2 G. Decher, J.-D. Hong, and J. Schmitt, *Thin Solid Film.*, **831**, 210 (1992).
3 W. Kong, X. Zhang, M. L. Gao, H. Zhou, W. Li, and J. C. Shen, *J. Macromol. Rapod Commun.*, **15**, 405 (1994).
4 G. Decher, *Science*, **227**, 1,232 (1997).
5 J. I. Anzai, H. Takeshita, Y. Kobayashi, T. Osa, and T. Hoshi, *Anal. Chem.*, **70**, 811 (1998).
6 a) Y. P. Sun, X. Zhang, C. Q. Sun, B. Yang, and J. C. Shen, *J. Macromol. Chem. Phys.*, **197**, 147 (1996). b) W. Muller, H. Ringsdorf, E. Rump, G. Wildburg, X. Zhang, L. Angermaier, W. Knoll, M. Liley, and J. Spinke, *Science*, **262**, 1706 (1993).
7 F. Palmisano, D. Centonze, and P. G. Zambonin, *Biosens. Bioelectron.*, **8**, 471 (1993).
8 K. Sieradzki, S. R. Brankovic, and N. Dimitrov, *Science*, **2**, 138 (1999).
9 J. Q. Sun, M. Y. Gao, and J. Feldmann, *J. Nanoscien. Nanotech.*, **1**, 133 (2001).
10 D. Y. Ryu, S. H. Chung, and K. Katoh, *Biotechnol. Bioeng.*, **19**, 159 (1977).
11 S. Z. Zhang, *Science Press.*, **2**, 587 (1984).
12 E. A. Ponomarenko, A. J. Waddon, K. N. Bakeev, D. A. Trrell, and W. J. Macknight, *Macromolecules*, **29**, 4340 (1996).
13 a) M. Lestlius, B. Liedberg, and P. Tengvall, *Langmuir*, **13**, 5900 (1997). b) H. Tokuhisa, M. Zhao, L. A. Baker, V. T. Phan, D. L. Dermody, M. E. Garcia, R. M. Crooks, and T. M. Mayer, *J. Am. Chem. Soc.*, **20**, 4492 (1998).
14 M. Y. Gao, J. Q. Sun, E. Dulkeith, N. Gaponik, U. Lemmer, and J. Feldmann, *Langmuir*, **18**, 4098 (2002).
15 M. C. Shin, H. C. Yoon, and H. S. Kim, *Anal. Chim. Acta*, **329**, 223 (1996).