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LB FILM CONTAINING ACETYLCHOLINESTERASE FOR FIBER-OPTIC ORGANOPHOSPHORUS SENSOR

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Abstract LB film containing Acetylcholinesterase(AChE) was fabricated for the development of the fiber-optic biosensor to detect organophosphorus compounds in the contaminated water. The AChE-immobilized LB film was formed by adsorbing the enzyme molecules onto viologen monolayer using the electrostatic force. The optimal amount of the dissolved enzyme for the LB film formation were determined based on the enzyme adsorption isotherm and relative adsorption amount. The surface characteristics of AChE-immobilized LB film was analyzed by the atomic force microscope(AFM). The surface topography of the AChE-immobilized viologen LB films were obtained for the various conditions of the surface pressure. The response time to steady signal and the detection range of the sensor were 5 min and 0~2 ppm, respectively.

INTRODUCTION

The detection of the ground water contamination due to the wide use of pesticides has been needed for the quality control of water supplied. It is known that acetylcholinesterase (AChE), the essential enzyme in the nerve tissue, is inhibited by organophosphorus compounds widely used as pesticides and nerve toxin for military purposes.[1,2,3]

Langmuir-Blodgett(LB) film technic has been used to the formation of organized molecular film for the sensor device.[4,5] The advantages of this technique are the ultrathin film deposition, highly ordered molecular array, easiness of packing and stacking molecules, low temperature and biomimetic membrane fabrication.[6,7] It is necessary to investigate the microscopic morphology of the LB films containing enzyme to observe the image of the LB films using atomic force microscopy(AFM).[5]

The objectives of this study are to develop a fiber-optic biosensor using enzyme-viologen LB film for the detection of organophosphorus compounds. To optimize the enzyme adsorption condition of the AChE contained LB film for the increment of the sensor signal output, the activity measurement by enzyme reaction, analysis of the relative amount of amino groups of the adsorbed enzyme and topology observation of the LB film by AFM were done.

EXPERIMENTAL DETAILS

Acetylcholinesterase (AChE, EC 3.1.1.7: V-s, from electric eel) with a specific activity of 1000u/mg and acetylthiocholin iodide (ATCh) were obtained form Sigma Chemical Company (St. Louis). The multicompartment trough made by Nima Tech. (Coventry, England) was used to form the hetero LB film. The topology of AChE contained LB film was observed by AFM (autoprobe CP, Park Scientific Instruments, USA). The chloroform was used as the spreading solvents for the lipid and viologen

The experimental set-up was schematically shown in Fig.1. and the details were described previously.[3]

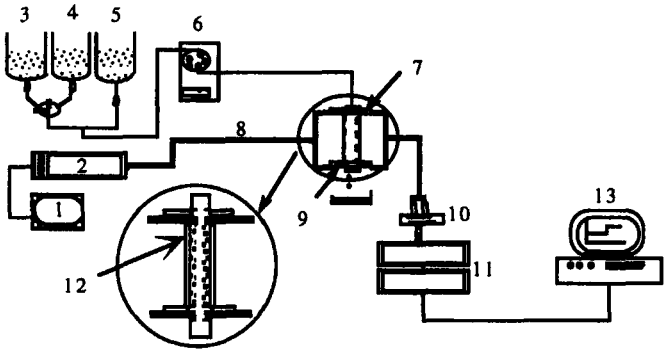


FIGURE 1. Schematic diagram of detecting system

For measurement of adsorbed enzyme amount in the LB films, an *O*-phthaldialdehyde and 2-mercaptoethanol were used. The measurement of fluorescence intensity for a complex of *O*-phthaldialdehyde and amino group of AChE, the fluorescence material, at 455nm was done to estimate the adsorbed AChE amount.[6]

RESULTS AND DISCUSSION

π-A isotherms for the lipid monolayer, viologen monolayer, AChE-immobilized lipid and AChE-immobilized viologen monolayer as shown in Fig. 2. In the AChE-immobilized lipid monolayer and AChE immobilized viologen monolayer, the phase regions were not clearly distinguished from one another. The values of area per molecule for the lipid and viologen monolayer were less than those for the AChE-immobilized lipid and viologen monolayer. It can be considered that these results were due to the adsorption and permeation of enzyme molecules into the pure lipid and viologen monolayer. From π-A isotherm, viologen was chosen as the more suitable material for adsorption of AChE than the lipid due to the large increment of molecular area.

The surface pressure was increased in proportion to the amount of dissolved enzyme upto 300 unit as shown in Fig. 3. It can be considered that the increase of surface pressure was caused by the enzyme adsorption onto viologen monolayer. Based on the surface pressure increase by AChE adsorption and the relative amount of amino groups of the adsorbed enzyme as shown in Fig. 4, the optimal amount of dissolved AChE on subphase was determined as 300 unit. The determined optimal amount is verified by observation of topology of enzyme-viologen monolayer by AFM. (data are not shown)

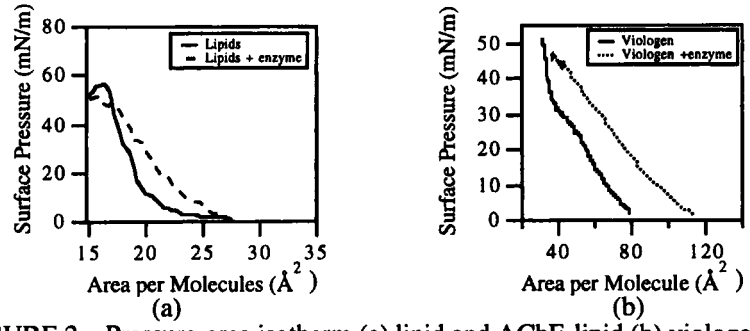


FIGURE 2. Pressure-area isotherm (a) lipid and AChE-lipid (b) viologen and AChE-viologen

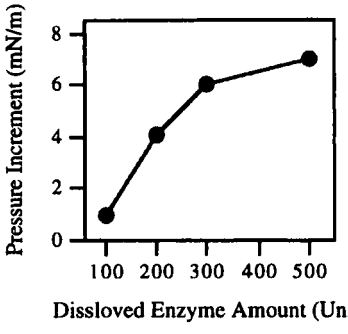


FIGURE 3. Pressure increment to dissolved enzyme amount

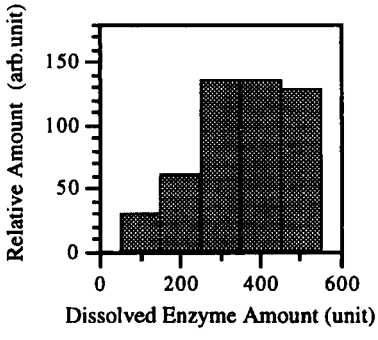
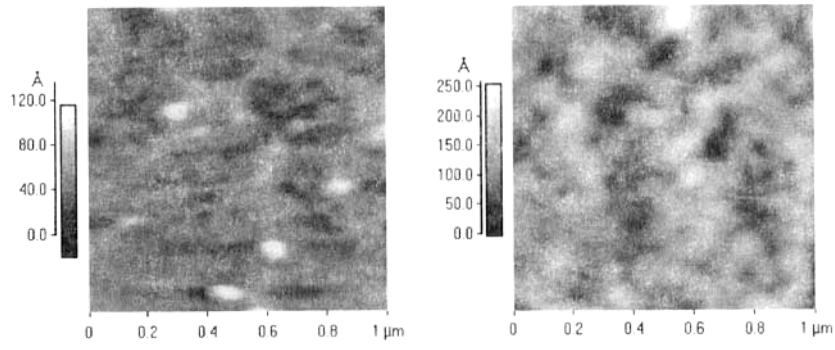


FIGURE 4. Relative amount to dissolved enzyme amount

The surface topographies of AChE-immobilized LB film with 1μm of scan area was shown in Fig. 5. In the topographies, the higher parts of AChE-immobilized LB film correspond to AChE molecules. The amount of AChE adsorbed onto the substrate was increased as the surface pressure of viologen was increased in Fig. 5. Same result were obtained by the activity measurement by enzyme reaction and the analysis of relative amount of amino groups of the adsorbed enzyme.(data are not shown). Based on these results, the optimum dipping surface pressure was determined as 35mN/m

The sensor signal output of the sensor composed of AChE-viologen hetero LB film was obtained with the various organophosphorus compound concentration as shown in Fig. 6. The signal was proportional to the organophosphorus compound concentration and the detection time was 5min. The detection range of the sensor with linear relationship between the signal output and analyte concentration was 0~2.0 ppm.



(a) 25 mN/m (b) 35mN/m

FIGURE 5. Topographic images of AChE-viologen hetero LB films

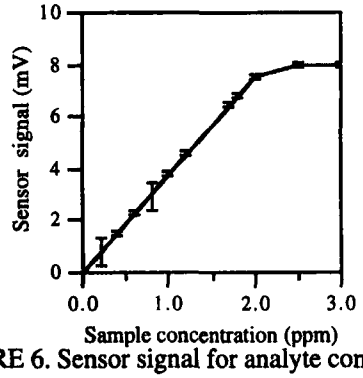


FIGURE 6. Sensor signal for analyte concentration

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