효소 LB막을 이용한 광섬유 유기인 바이오 센서의 특성 조사

정 정 원, 민 준 홍, 조 경 상, 최 정 우, 이 원 홍
서 강 대 학 교 화 학 공 학 과

Investigation of Characteristics of Fiber-Optic Organophosphorus Biosensor using Enzyme-containing Langmuir-Blodgett Film

Jung-won Jung, Junhong Min, Gyeong Sang Cho, Jeong-Woo Choi and Won Hong Lee
Department of Chemical Engineering, Sogang University

INTRODUCTION

Acetylcholinesterase(AChE)-containing LB film is constructed for the development of the fiber-optic biosensor to detect organophosphorus compounds in contaminated water. The organophosphorus compounds are widely used in industry, medicine and agriculture, and included some highly toxic pesticide[1]. The toxicity of this compounds can inhibit AChE, an essential enzyme of nerve tissue, founded in many other tissues and organs of human and animal origin[2]. The instrument for detection of organophosphorus, such as pesticide and toxic material, have been widely investigated[3,4,5].

The objectives of this study are to investigate the effect of immobilization of AChE and to develop a fiber-optic biosensor using LB technique for detection of organophosphorus compounds. The AChE-immobilized LB films can be formed by adsorption of enzyme to viologen monolayers due to the electrostatic forces between enzymes and monolayers. In this study, an analyte is PARAOXON(an organophosphorus plant protective) and the enzymes(AChE) are used as reaction catalysis for detecting organophosphorus. Based on the optimum pH for enzyme reaction and detectable wavelength of He-Ne laser, the pH sensitive dye, litmus, is chosen to express the inhibitory effect of an organophosphorus compound on a AChE. The optimum condition for the construction of enzyme-immobilized LB films and the characteristics of fiber-optic organophosphorus biosensor are investigated in this study.

EXPERIMENTAL

Materials

Acetylcholinesterase(EC 3.1.1.7: V-s, from electric eel) with a specific activity of 1000U/mg and acetylthiocholine iodide were obtained from Sigma chemical company (USA). The sensitive dye, litmus was supplied by BDH laboratory (England). Paraoxon(diethyl-p-nitrophenylphosphate, E600, liquid, 95% purity) was purchased from Sigma chemical company (USA). The Viologen was synthesized in our laboratory.

Immobilization of the enzyme

In this study, LB trough of Formherz type(Nima Tech., London) is used for immobilizing the enzyme on the substrate, which is treated to hydrophilic substrate. The viologen mixture in chloroform is spreaded on the subphase(pH 7.0) to form

monolayer at air/water interface, then left 30-40min in one compartment for evaporation of chloroform. After the evaporation, the viologen monolayer is compressed to the surface pressure of 37mN/m. The enzyme(AChE) solution is made by dissolving the solid enzyme to the same subphase and spreaded to another compartment. The viologen monolayer is moved to enzyme compartment with keeping the surface area constant. And the viologen monolayer is left for 1hr for the adsorption of enzyme molecules onto the viologen monolayer. After adsorption, the AChE-immobilized viologen monolayer is moved to the dipping compartment and transferred the enzyme-immobilized LB films to the hydrophilic substrate. That substrate was transferred with the viologen monolayer(1layer) before the enzyme-immobilized viologen monolayer is transferred to the substrate, because the transition of deposition type of enzyme-containing LB film is Z-type[6].

Experimental set-up

The experimental set-up is schematically shown in Fig.1. The reactor contains AChE-immobilized LB film. The three solutions (distilled water, substrate solution of litmus and acetylthiocholine iodide, inhibitor solution) is prepared. The solution of substrate and dye with potassium phosphate buffer (pH7.2) containing 180mM of NaCl and 48μM of $MgSO_4$ for enhancement of enzyme activity and distilled water are mixed via peristaltic pump (Marubish, Japan) for invarient concentration of substrate when the sample is introduced and flowed to enzyme reactor at first. After reaction period, the distilled water is exchanged with sample solution, which contained paraoxon of various concentration. Light emitting from the He-Ne laser(633nm) is guided through two optic fiber lines to input and output part of enzyme reactor. Transmitted light at two parts are guided through the opposite two fiber and then conveyed to two phototransistors, detectors. The detectors converted the optical signal into the electrical signal would be modulated to obtain a quantitative value related to analyte concentration. All experiments are carried out at room temperature (20 - 25℃).

RESULTS AND DISCUSSION

In this study, the optimum condition for the constrcution of AChE-containing LB films are investigated to be used for the fabrication of fiber-optic biosensor to detect the organophosphorus compound which owe their toxicity to an ability to inhibit AChE. Since AChE hydrolyzed acetylthiocholine to thiocholin and acetic acid, AChE activity could be measured by color change of the acid-base indicator. The blue color of litmus dye is changed to the red color by the pH decrease due to the formation of acetic acid. The change of color is measured by UV spectrophotometer(Shimadzu, Tokyo Japan).

To investigate the enzyme adsorption, π-A isotherm is obtained for the pure viologen monolayer and the AChE-immobilized viologen monolayer at air/water interface. π-A isotherm of viologen monolayer and AChE-immobilized viologen monolayer on subphase are shown in Fig.2. For the AChE-immobilized viologen monolayer, the distinction of the phase region is not clear and the limiting area increases. The value of the limiting area, the zero phase molecular area, of the viologen monolayer is less than that of the AChE-immobilized viologen monolayer. From these data, the adsorption of AChE on the viologen molecules makes the

increase of the area per molecular at zero pressure. This result indicates that enzyme(AChE) is adsorbed at head group of lipid monolayer by electrostatic force.

During the adsorption of enzyme molecules onto the viologen monolayer for 1hr, the pressure increase for various spreading enzyme unit is shown in Fig.3. As the spreading enzyme amount increase, the pressure increase upto 300unit. The change of pressure is not good from 300unit to 500unit, so optimum spreading amount of enzyme to form the AChE-immobilized LB films is selected as 300unit.

The effect of the difference of signal on the surface pressure when the enzymes are adsorbed onto the viologen monolayer with equal number of layer is shown in Fig.4. As the surface pressure increases, the signal increases upto 37mN/m. This represents that the optimum surface pressure for the adsorption of enzyme is 37mN/m. When this result is compared with that of Fig.1, 37mN/m surface pressure is optimum for enzyme adsorption.

The sensor signal with multi-layer of enzyme LB film is detected according to the various organophosphorus compounds concentration is shown in Fig.5. It represents that signal is proportional to organophosphorus compounds concentration when detection time is 5min. The linear range of 0 ~ 2.0 ppm of organophosphorus compounds concentration and sensor signal is obtained. The linear range of organophosphorus compounds concentration vs. sensor signal is necessary because the primary standard and secondary standard of Korean effluent water is 0.5ppm and 1ppm, respectively.

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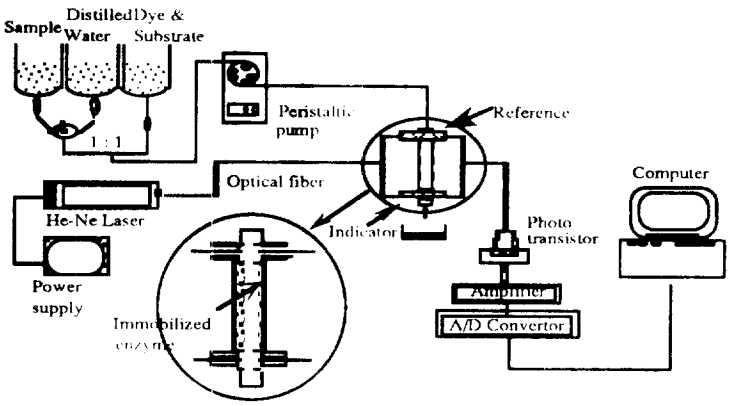

Fig.1 Schematic diagram of detection system

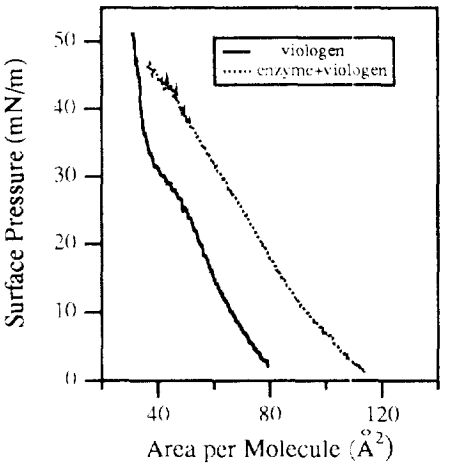

Fig.2 π-A Isotherm of viologen monolayers before and after adsorbing enzyme

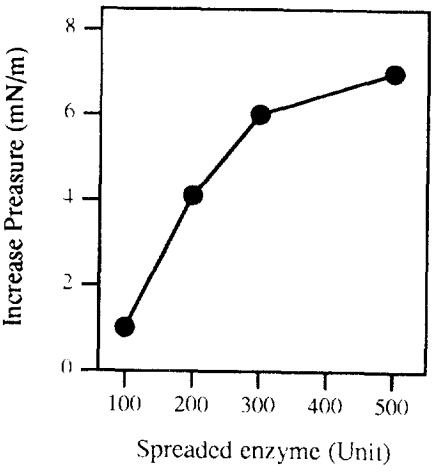

Fig.3 Increase pressure for adsorption enzyme of different unit

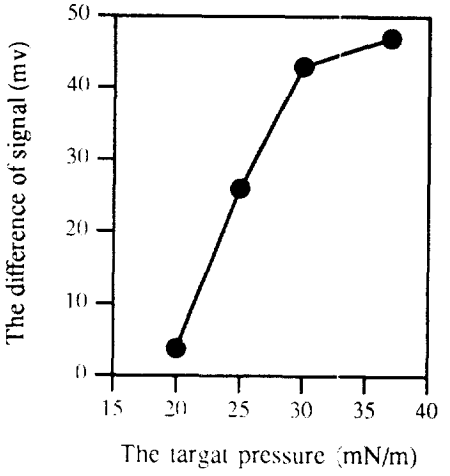

Fig.4 The difference of the sensor signal for targat pressure

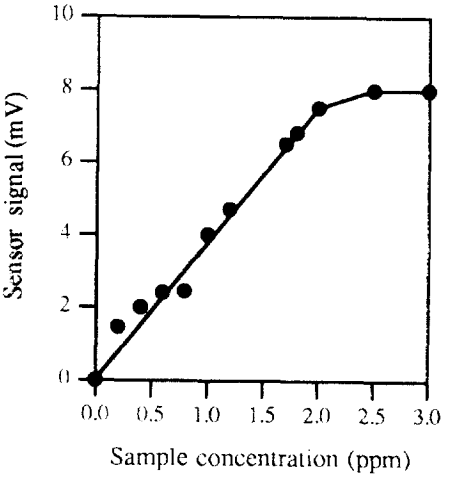

Fig5 The sensor signal on the paraoxon concentration