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Bioelectrochemistry 56 (2002) 157–158

Bioelectrochemistry

www.elsevier.com/locate/bioelechem

Mixed urease/amphiphile LB films and their application for biosensor development

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Received 1 June 2001; received in revised form 27 November 2001; accepted 30 November 2001

Abstract

The optimal conditions for the formation of a stable mixed urease/octadecylamine (ODA) film at the air–water interface are determined. This film is efficiently transferred onto the hydrophobized surface of a pH-ISFET, and the features of the urea enzymatic field-effect transistor (ENFET) are determined: detection limit 0.2 mM, response time 15 s, and dynamic range 0–20 mM.

Keywords: Mixed LB films; Urea ENFET; Urease; Octadecylamine; Behenic acid

1. Introduction

Langmuir–Blodgett technology has been considered as a convenient tool for designing artificial systems (LB films) with biological functions such as biosensors [1]. The ultrathin, structure-ordered films with the incorporation of biomolecules much resemble the biological membrane environment [2] and, thus, the preservation of a high enzymatic activity is expected. Until now, many kinds of biosensors have been designed with the LB films as the biosensitive part; however, few papers are concerned with urease mixed LB films [3]. The aim of this work is focused on the formation of stable mixed urease/amphiphile Langmuir monolayers at the air–water interface, the monolayer transfer and the biosensor characteristics of the enzymatic field-effect transistor (ENFET) obtained by the deposition of the LB film on a pH-ISFET.

2. Experimental

Urease (EC 3.5.1.5) from Jack Beans with a specific activity of 800 U mg^{-1} was used. Langmuir experiments were performed on a Langmuir trough from NIMA (model 611). The chloroform solution of octadecylamine (ODA) or behenic acid (BA) with a concentration of 1 mg/ml was spread (22 μl used for ODA and 25 μl for BA each time) onto the subphase.

Infrared spectroscopy was performed on Perkin-Elmer 2000 (GB) with a standard ATR accessory. The response of the ISFET modified by the mixed LB film to the addition of urea solutions was conducted with a laboratory ISFET meter (IMT, Neuchatel, Switzerland) in a phosphate buffer solution with a pH of 5.5.

3. Results and discussions

The adsorption of urease in the subphase onto the ODA or BA monolayer can reach equilibrium after about 2–3 h, and the associating process is much similar to that of the reported BuChE/ODA monolayer [4]. The adsorption process is driven by the electrostatic interaction between urease and ODA or BA. In the case of ODA, at a pH value of the subphase of 5.5, the positively charged ODA head can interact with the negatively charged portion of the urease surface and induce the formation of a stable mixed Langmuir monolayer at the air–water interface. A similar adsorption process can occur, but with a reverse electrostatic

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PII: S1567-5394(02)00045-2

interaction, for urease and BA. According to the comparison of the $\Pi - T$ curves of ODA/urease and BA/urease, the slope of the pressure growth of ODA/urease is higher than that of BA/urease. This means that the ODA/urease complex is more stable than the BA/urease one. The adsorption process for ODA and urease is nearly complete after 2 h, whereas for BA, the process is complete after at least 3 h.

After the adsorption equilibrium is reached, the formed mixed urease/ODA monolayers are compressed to different target pressures. As shown in Fig. 1, for the mixed monolayer of ODA/urease with urease concentrations of 4 and 12 mg/l in the subphase, there are obvious phase transition points, which take place at a surface pressure of 32 mN m^{-1}, and different molecular areas with different urease concentrations. This can be attributed to the extrusion process of urease molecules from the entrapped state to the adsorption state on the positively charged head of the ODA monolayer. For the highest urease concentration (16 mg/l), the curve does not exhibit obvious phase transition points at a definite surface pressure. This may be due to the fact that entrapped urease molecules in the monolayer cannot be easily expelled.

At a surface pressure between 35 and 40 mN m^{-1} and at a trough temperature of 5 °C, the formed mixed ODA/urease monolayer shows high stability, which has been demonstrated by the molecular area ratio A/A_0–time evolution isotherms. This kind of mixed monolayer can be effectively transferred onto the surfaces of SiO_2/Si substrates or of ISFET that were previously hydrophobically treated by the grafting of octadecyltrichlorosilane (OTS), with a transfer ratio between 80% and 110% by the vertical dipping method. The FTIR-ATR spectra display the existence of the absorption bands centered at 1657 and 1543 cm^{-1} due to the protein peptidic bond, and the peak intensities increased with the increase of the number of the deposited ODA/urease monolayers.

Fig. 2 shows the response to urea of ENFET obtained by the deposition of six mixed ODA/urease films on a pH-ISFET after stabilization of the mixed LB film by reticulation in a glutaraldehyde vapour. The steady-state response of the biosensor is achieved within 15 s, the detection limit for the urea determination is 0.2 mM, and the dynamic range is from 0 to 20 mM of urea concentration in the logarithmic scale.

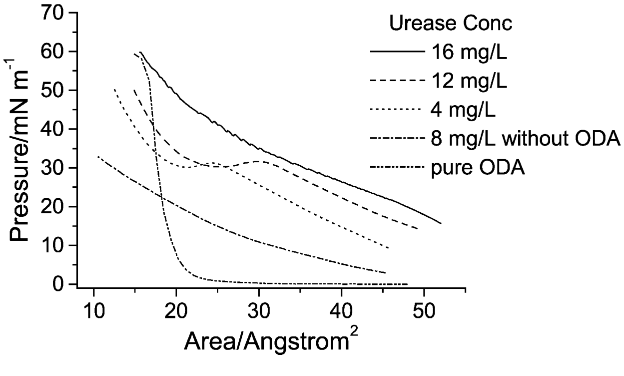

Fig. 1. Surface pressure–molecular area isotherms for pure and mixed ODA/urease monolayers after adsorption equilibrium (pH 5.5).

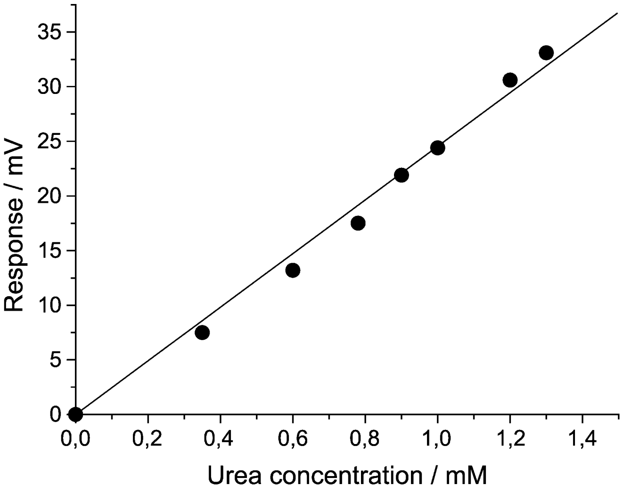

Fig. 2. Response of the mixed ODA/urease LB film-modified ISFET as a function of urea concentration in the phosphate buffer solution, pH 5.5.

4. Conclusion

The stable mixed amphiphile/urease monolayer can be obtained at the air–water interface by carefully modulating the amphiphilic type (ODA and BA in this case), the urease concentration in the subphase, associating time, surface pressure and the temperature of the subphase. The formed urease-associated amphiphile monolayer can be efficiently transferred onto the hydrophobic surface of SiO_2/Si substrates and ISFET with a high transfer ratio. Thus, good biosensor characteristics of ENFET obtained by deposition and stabilization of a mixed amphiphile/urease LB film on a pH-ISFET are obtained.

Acknowledgements

This work was supported by the AFCRST.

References

[1] V. Rosilio, M.M. Boissonnade, J. Zhang, L. Jiang, A. Baszkin, Penetration of glucose oxidase into organized phospholipid monolayers spread at the solution/air interface, Langmuir 13 (1997) 4669–4675.

[2] A.P. Girard-Egrod, R.M. Morélis, P.R. Coulet, Bioactive nanostructure with glutamate dehydrogenase associated with LB films: protecting role of the enzyme molecules on the structural lipidic organization, Thin Solid Films 292 (1997) 282–289.

[3] S. Arisawa, R. Yamamoto, Quantitative characterization of enzymes adsorbed on to Langmuir–Blodgett films and the application to a urea sensor, Thin Solid Films 210/211 (1992) 443–445.

[4] J.M. Chovelon, K. Wan, N. Jaffrezic-Renault, Influence of the surface pressure on the organization of mixed Langmuir–Blodgett films of octadecylamine and butyrylcholinesterase: 1. Film preparation at the air–water interface, Langmuir 16 (15) (2000) 6223–6227.