

Biochip Technology for Immunosensors

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1. Introduction

Increase in sensitivity of an immunosensor requires immobilization of a large amount of antibodies. Good immobilization method and procedure must be developed for this antibody immobilization on the sensor chip. As large an amount of antibodies as possible must be affixed to the electrode surface without inactivation. One method is a physical process: antibody is adsorbed spontaneously to the surface [1]. This process is easy to manage, but surface-coating material must be chosen so that the largest possible amount of antibody is adsorbed. Another method of immobilization is to fix it chemically, but this process is thought somewhat troublesome. We develop the immobilization method by covalent bonding of antibody on QCM. The C-reactive protein (CRP) is well-known as a good marker protein to detect and diagnose pneumonia in human blood [2], and is chosen as target molecule for this sensor. Figure 1 shows the concept of immunosensor. Figure 2 indicates the experimental procedure of CRP detection by QCM

QCM-immunosensor techniques have been developed widely in the field of environmental monitoring, medical diagnosis, food industry, and so on. However, increased long-term stability is necessary to construct a novel QCM-immunosensor like the ELISA kit. To solve this problem, a short-cut method coats stabilizer reagents onto an antibody immobilized QCM. Therefore, we examined eight stabilizer reagents containing 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymers as superior stabilizers of immunologic activity of the antibody in ELISA under several storage conditions [3].

Surface plasmon resonance (SPR) technique has been used as a signal transduction method for real-time biochemical reaction monitoring for many years. As a result, commercial instruments have been developed to study a wide variety of biochemical binding events under controlled laboratory conditions [4]. Conventional monitoring of exhausted dioxins in the environment is a serious problem in Japan. It could be solved by developing a sensing system using SPR for dioxins. The

immunoreaction is most popular in its sensitivity and selectivity for environmental assays. Dioxins have highly toxic properties; thus we use 2,4-dinitrophenol (DNP) as a model compound for dioxins.

2. Experimental and Results

The QCM gold electrode was reacted with cyteamine and then activated by glutaraldehyde. Anti-CRP monoclonal antibody or its F(ab')₂ fragment of anti-CRP monoclonal antibody were immobilized via covalent bonding. Oscillation frequency of QCM immobilized anti-CRP antibody; and its F(ab')₂ fragment was measured at each reaction step after the dry process under nitrogen gas. The range of CRP concentration was settled from 1 ng dL⁻¹ to 100 µg dL⁻¹. Regression of CRP response denotes: $\Delta F_{\text{IgG}} = 29.42 \log [\text{CRP}] + 88.91$, ($r = 0.963$) and $\Delta F_{\text{F(ab)'2}} = 56.02 \log [\text{CRP}] + 211.45$, ($r = 0.987$), respectively. Frequency response of anti-CRP F(ab')₂ antibody immobilized QCM for CRP is two times larger than anti-CRP antibody immobilized QCM for CRP.

Various stabilizers (A: Block Ace[®], B: glycerin-PBS, C: Lipidure[®] Lipidure-B05/PBS, D: Lipidure[®]-Lipidure-J02/PBS, E: Lipidure[®]-Lipidure-H02/PBS, F: Lipidure[®]-H02 and casein hydrolysate mixture/PBS, G: PBS, H: StabilGuard[®]) were applied to QCM immobilized anti-2,4-dinitrophenol (DNP) antibody, and stored at 25°C and 55°C on 2 h for the acceleration test of long-term stability. To determine immunologic activity of anti-DNP antibody immobilized QCM, DNP-conjugated albumin was used for immunoreaction as an antigen [5].

Immunologic activities of the anti-DNP antibody were determined by relative frequency shift of QCMs compared with results without acceleration experiments. Results of blank experiments, without stabilizer, showed remarkably lower activities than the others. Anti-DNP activities of blank were 37% at 25°C and 8% at 55°C, respectively. Maximum immunologic activity with C stabilizer were obtained at about 80% at 25°C and 50% at 55°C, respectively. Stabilizers containing MPCs (C, D,

E, and F) show larger immunologic activity than conventional stabilizers (A and H). This implies that MPCs indicate superior stability of QCM-immunosensor for DNP detection.

DNP-conjugated bovine serum albumin (DNP-BSA) induced immunoreaction with an anti-DNP monoclonal antibody immobilized SPR chip. We report DNP detection using a competitive reaction between DNP and DNP-BSA for anti-DNP monoclonal antibody immobilized SPR chip. The experimental procedure for DNP detection by SPR follows in ref. [6].

The competitive reaction between DNP and DNP-BSA for the anti-DNP antibody immobilized SPR chip was measured by reflectance change of SPR. Regression analysis of response of the competitive reaction between DNP and DNP-BSA denotes: $\Delta\text{Reflectance} = -0.0005 \log [\text{DNP}] + 0.0005$, ($r = 0.990$). A linear relationship is obtained in the range from 1 ng mL⁻¹ to 10 pg mL⁻¹ of DNP. We examine increasing SPR response using a sandwich reaction of a second anti-DNP antibody for bounded DNP-BSA on a chip. Regression of the sandwich reaction by anti-DNP antibody response denotes: $\Delta\text{Reflectance} = -0.0014 \log [\text{DNP}] + 0.0016$, ($r = 0.969$). Response of the sandwich reaction is three times greater than the competitive reaction response.

Performance of the Dioxin immunosensor for actual environmental sample using QCM with immunized anti-dioxin antibodies will be presented at SSDM 2002.

Acknowledgments

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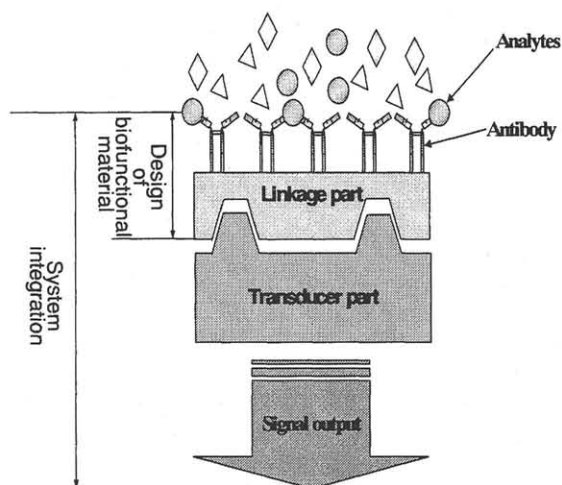


Fig. 1 Concept of immunosensor

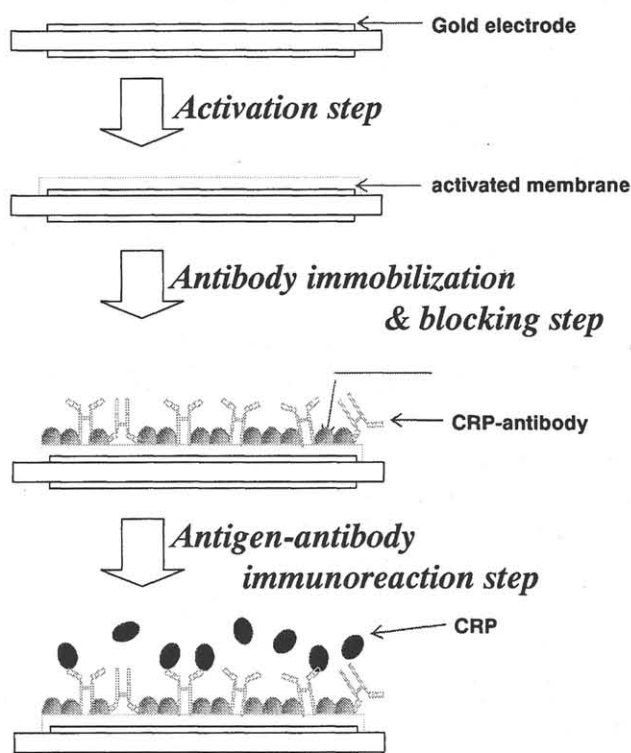


Fig. 2 Experimental procedure of CRP detection by QCM