Detection of Morphine in ppb Range by Using SPR (Surface-Plasmon-Resonance) Immunosensor

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Highly sensitive detection of morphine (MO) in ppb range was realized by using an immunosensor based on surface-plasmon-resonance (SPR) phenomena. The addition of MO into the antibody solution was found to reduce the incident angle shift of the SPR sensor system sharply because of the inhibition effect of MO. Based on this inhibiting principle, the present sensor could detect MO very sensitively in the concentration range of 0.1 - 10 ng/cm^3 (ppb).

It is well known that morphine (abbreviated as MO) is one of useful drugs to relieve severe pains from patients, but its excessive or habitual use frequently causes toxic symptoms. In order to prevent such toxic symptoms from occurring, sensitive monitoring of MO in the patient's blood or urine is inevitable. Several methods, such as high-performance liquid chromatography (HPLC), radioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA) and so on, have been developed for this purpose. These methods, though highly sensitive, need time-consuming and/or tedious procedures so that simpler detection methods are highly desired. Quite recently, a compact electrochemical sensor for MO was proposed,[1] but its detection range 0.1 - 10 μg/cm^3 (ppm) is still insufficient to meet the high MO sensing required.

This situation motivated us to examine the possibility of sensitive MO monitoring by combining an immunoreaction and a surface-plasmon-resonance phenomenon (SPR). There have been several reports[2,3] on SPR-based immunosensors for detecting macromolecular proteins like IgE, but little work has been done for detecting small molecules like MO (molecular weight, MW: 285). As an example of detecting such a small compound, we have already reported that an inhibitive method using a small compound-protein conjugate is very useful for the case of detecting methamphetamine (MW: 149) based on a QCM technique.[4] In the present paper, we aimed at extending this method to an SPR-based immunosensor for MO.

Since MO has no immunogenicity because of its small molecular weight, it should be derived into a conjugate with protein. For this purpose, MO was demethylated to normorphine to prepare a normorphine-bovine serum albumin conjugate (abbreviated as NM-BSA, MW: ca. 69000). The anti-MO monoclonal antibody (MW: ca. 150000) was prepared by the method reported before.[5] Each of MO, NM-BSA, and antibody was dissolved in phosphate-buffered saline (PBS, pH=7.2). The sensor assembly used was composed of an SPR measuring system (SPR-20, Denki Kagaku Keiki Ltd.) attached with a gold-deposited slide glass (sensor chip), a flow-through cell and a micro-tube pump. Each solution (1 cm^3) was allowed to circulate through the flow-through cell at a rate of 0.26 cm^3/min. The NM-BSA was immobilized on the Au film of the sensor chip by a physical adsorption method,[4] before exposure to MO-containing solutions. As a sensing signal, a shift in incident angle of the SPR system was measured at room temperature.

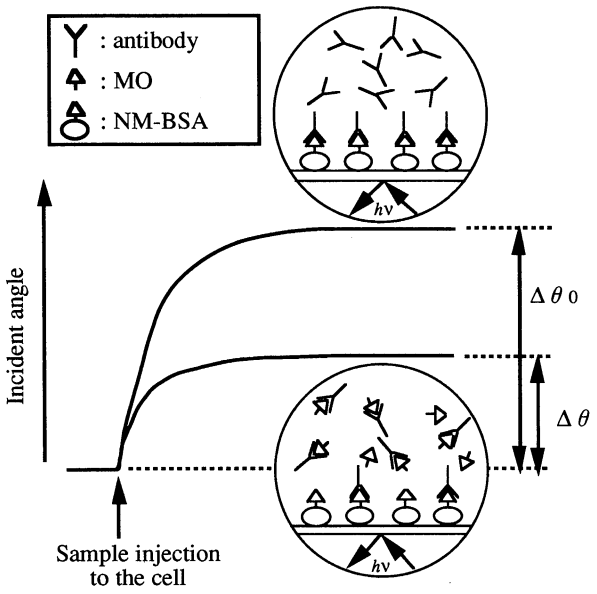

Figure 1. Principle of morphine (MO) detection by SPR immunosensor.

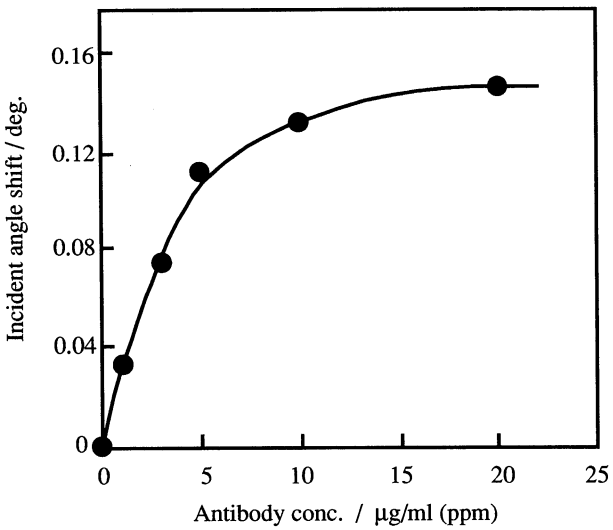

Figure 2. Dependence of the incident angle shift of NM-BSA immobilized SPR sensor on the antibody concentration.

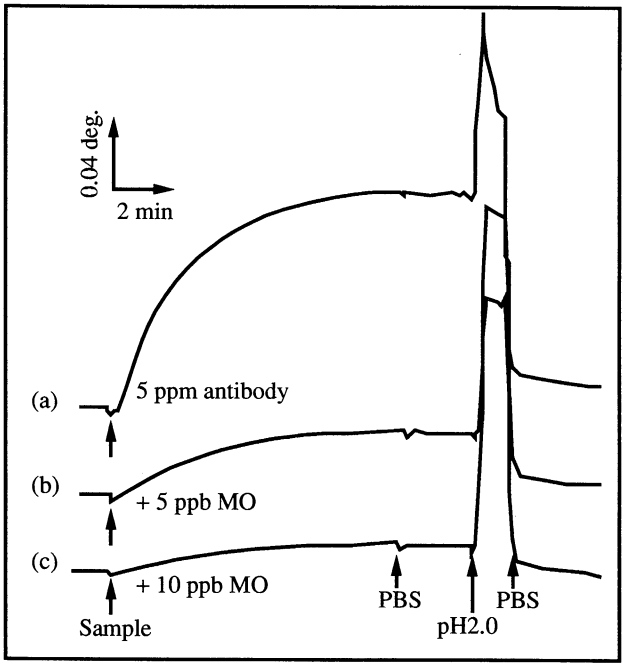

Figure 3. Response transients of NM-BSA immobilized SPR sensor to 5 ppm antibody solution without MO (a), with 5 ppb MO (b) and 10 ppb MO (c).

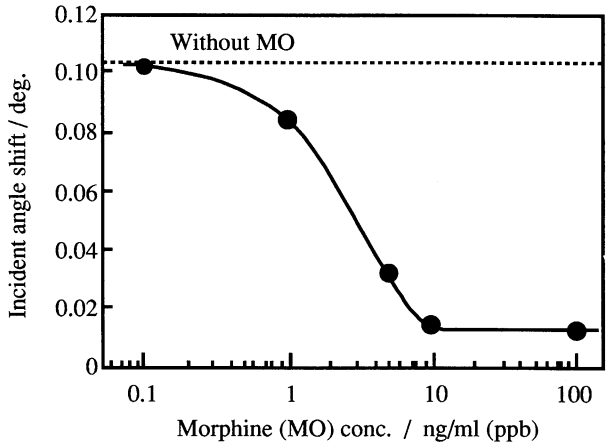

Figure 4. Incident angle shift of NM-BSA immobilized SPR sensor as correlated with the concentration of MO under the fixed concentration (5 ppm) of antibody.

The principle of the MO detection method used here is shown schematically in Figure 1. The NM-BSA-immobilized chip increases its incident angle by $\Delta\theta_0$ on contact with an antibody solution of a fixed concentration due to the immunoreaction. When the antibody solution is mixed with a certain amount of MO, a part of antibody will react with MO to become inactive. On exposure of the mixed solution to the sensor chip, the immunoreaction takes place to a reduced extent, causing the shift in incident angle to be attenuated from $\Delta\theta_0$ to $\Delta\theta$. If one chooses properly the amount of immobilized NM-BSA and concentration of antibody, the incident angle shift ($\Delta\theta$) would be a sensitive function of the concentration of MO injected to the antibody solution.

First, NM-BSA was immobilized on the sensor chip by a physical adsorption method. On exposure to an NM-BSA solution (100 ppm), the incident angle of the sensor increased rapidly and reached a steady state in about 15 min. The total increment of incident angle due to the adsorption of NM-BSA was as large as ca. 0.85 degree. No incident angle shift was observed on subsequent exposure to the flow of PBS solution, confirming that the NM-BSA-immobilization is stable enough. Finally the chip was exposed to a BSA solution (1000 ppm) to block the non-specific adsorption sites of the sensor chip. This treatment resulted in an increase in incident angle by ca. 0.12 degree. After this treatment, the incident angle no longer changed by the flow of 20 ppm BSA solution.

The NM-BSA immobilized chip thus prepared was exposed to various concentrations of antibody solution. These measurements could be repeated, because the antibody adsorbed on the chip could be cleaned off by exposure to glycine/HCl buffered solution (pH 2.0). With increasing concentration of the antibody, the incident angle shift ($\Delta\theta_0$) increased conspicuously up to ca. 5 $\mu g/cm^3$ (ppm) and then gradually approached to an saturation value (ca. 0.15 degree), as shown in Figure 2. From this behavior, the use of 5 ppm antibody solution was judged to be best for the MO sensing experiments based on the inhibitor method.

Then, the monoclonal antibody solution (5 ppm) was mixed with MO to various concentrations, prior to flowing over the sensor chip. Figure 3 exhibits a series of response transients obtained. With no MO added, the incident angle shifted upward by about 0.1 degree. With an increase in MO concentration, the shift decreased remarkably due to the inhibition effect of MO. These measurements could be repeated about 10 times on the same sensor chip, by washing off the adsorbed antibody with glycine/HCl buffered solution (pH 2.0). The incident angle shifts were reproducible to about $\pm 5\%$ on the repeated runs.

As shown in Figure 4, $\Delta\theta$ was very sensitive to a change in MO concentration in the range 0.1 - 10 ng/cm^3 (ppb). Above this range, the shift became almost null because whole the antibody (5 ppm) had been inactivated with excessive amounts of MO. It is quite notable that the present assay system can detect MO even at concentrations less than 1 ng/cm^3 (ppb). Such sensitive detection of small molecules like MO by using SPR technique has not been reported so far.

References

1 D. Kriz and K. Mosbach, *Anal. Chim. Acta*, **300**, 71 (1995).
2 S. Löfas, M. Malmqvist, I. Rönnberg, E. Stenberg, B. Liedberg, and I. Lundström, *Sensors and Actuators B*, **5**, 79 (1991).
3 U. Kunz, A. Katerkamp, R. Renneberg, F. Spener, and K. Cammann, *Sensors and Actuators B*, **32**, 149 (1996).
4 N. Miura, H. Higobashi, G. Sakai, A. Takeyasu, T. Uda, and N. Yamazoe, *Sensors and Actuators B*, **13/14**, 188 (1993).
5 T. Usagawa, Y. Itoh, E. Hifumi, A. Takeyasu, Y. Nakahara, and T. Uda, *J. Immunol. Methods*, **157**, 143 (1993).