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Ultrasensitive and Highly Selective Detection of Testosterone Using a Surface Plasmon Resonance Sensor Combined with Molecularly Imprinted Films

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An ultrasensitive and highly selective testosterone detection method by surface plasmon resonance (SPR) combined with molecularly imprinted films (MIFs) was developed. The coupling angle shifts were highly selective to testosterone and showed a linear relationship to the logarithmic concentrations (from 10^{-15} to 10^{-10} mol L^{-1}) of the testosterone in the acetonitrile. The selectivity of the thin testosterone-imprinted films was examined by 10^{-4} mol L^{-1} progesterone acetonitrile solution, no observable binding was detected.

Testosterone (MW: 288.42) is an important androgenic steroid that acts as the principal male sex hormone. An accurate measurement of testosterone is required for correct diagnosis and appropriate treatments. Testosterone concentrations are commonly measured by immunoassays;[1] however, existing testosterone measurements based on immunoassays suffer from a number of serious problems including insufficient sensitivity, cross-reactivity, inaccuracy, limited linear range, poor inter-method agreement, and imprecision. Various chromatographic methods have also been reported for the determination of testosterone in biological samples;[2] however, these methods involve complicated instrumentation and time-consuming extraction steps.

Surface plasmon resonance (SPR) is an optical phenomenon that has been used for highly sensitive detections of adsorption on gold surfaces.[3] Recently, label-free detections based on thin imprinted films on SPR sensor substrates have also been reported.[4] The detection system without using labeling agents is expected to play an important role as a simple, fast, and ultrasensitive method. SPR biosensors based on an immunobiosensor system for testosterone, demonstrated limits of detection of 3.7 pg mL^{-1} with standard in running buffer and 15.4 pg mL^{-1} in a stripped human saliva matrix.[5] Although this technique provides poor sensitivity for testosterone detection, it requires an expensive special antibody, a qualified operator, and multiple steps. By combining a microfluidic system integrated molecular imprinted polymer film in a SPR sensor, testosterone molecules can be detected with a concentration ranging from 0.1 to 500 μM.[6] However, this technique provides poor limits of detection for routine analysis. In order to improve the limit of detection, we employed a SPR sensor system combined with molecularly imprinted films (MIFs) for highly sensitive detection of testosterone. Testosterone possess a rigid and stereochemically complex hydrocarbon skeleton with a variety of substituents at the extremities, thus it is ideally suitable for MIP studies.[7]

MIFs were prepared by copolymerizing methacrylic acid and ethylene glycol dimethacrylate in the presence of testosterone on the SPR substrates. The SPR substrate consists of a glass slide (LaSFN9, 2.5 cm × 3.0 cm) coated with a gold layer (50 nm in thickness). The SPR instrument used in this study is home-built, and SPR substrates were prepared in the laboratory. All the above information is supplied in the Supporting Information.[8]

The SPR sensor chip coated with the imprinted polymer films was loaded to the sensor. A flow cell with the volume 0.78 mL was pressed against the sensor chip surface to flow liquid samples over the sensor surface at a flow rate of 0.5 mL min^{-1}. The imprinted testosterone templates were removed from the imprinted polymer films by injecting 5 mL solution of acetonitrile and acetic acid (v:v = 8:2). The SPR angular reflectivity spectra were measured independently before and after rinsing. Typical SPR curves obtained in acetonitrile were shown in Figure 1. Because the release of testosterone templates in the MIFs should generate a decrease of refractive index, the shift of the angle of coupling angle from 68.10 to 67.30° suggested that removal of testosterone molecules from MIFs was successful.

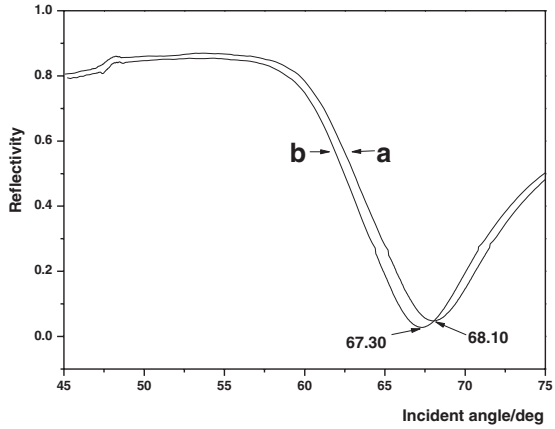

Figure 1. SPR angular reflectivity spectra independently measured before and after MIFs coated on the surface of SPR substrate. a: before rinsing; b: after rinsing.

The rebinding of testosterone molecules was observed upon a sequential injection of a series of testosterone acetonitrile solutions at different concentrations from 10^{-15} to 10^{-10} mol L^{-1}. Each sample of 3 mL was injected and circulated through the flow cell for 15 min, followed by a 2 min rinsing with acetonitrile, as seen in Figure 2a, the capture of testosterone molecules during a sample flow was manifested as a gradual increase in the reflectivity signal due to the capture in the cavities in the MIFs with the increase of the refractive index. Some decreases of reflectivity signals were observed especially under higher concentrations possibly due to the desorption of testosterone molecules that physically adsorbed on the surface of MIFs by injecting pure acetonitrile solvent. The higher concentrations often correspond to larger decreases of reflectivity signals. Figure 2b shows the SPR angular reflectivity spectra after rebinding testosterone molecules under various concentrations by MIFs coated on the SPR substrate surface, the inset graph is the enlarged range around coupling angles. Obviously, coupling angle shifts toward higher angles during adsorption at higher concentrations. A plot of the coupling angles shift $\Delta\theta$ versus logarithmic concentrations of the testosterone (from 10^{-15} to 10^{-10} mol L^{-1}) showed good linearity (R^2 = 99.48%), as can be seen from Figure 3. The coupling angles

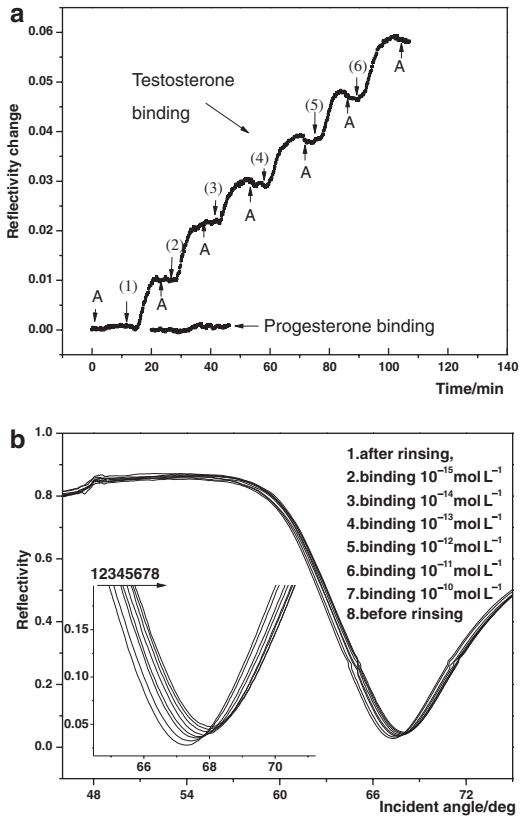

Figure 2. a. The kinetics of the SPR reflectivity changes due to the rebinding of template testosterone molecules dissolved at the concentrations of (1) 10^{-15}, (2) 10^{-14}, (3) 10^{-13}, (4) 10^{-12}, (5) 10^{-11}, and (6) 10^{-10} mol L^{-1}, and the binding of progesterone analog molecules dissolved in acetonitrile at the concentration of 10^{-4} mol L^{-1}. Between injections of samples, the SPR substrates were rinsed with pure acetonitrile (A). b. SPR angular reflectivity spectra measured after rebinding with various concentrations of testosterone by MIFs coated on the SPR substrate, inset graph is the enlarged spectra around the coupling angles.

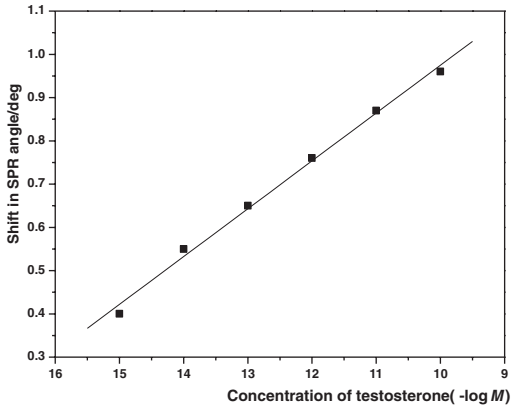

Figure 3. Calibration curve for the detection of testosterone by SPR sensor coated with testosterone-imprinted polymer films, fitted with linear function (linear equation being $y = 0.11x + 2.08$ and correlation coefficient $R^2 = 99.48\%$ ($n = 6$)).

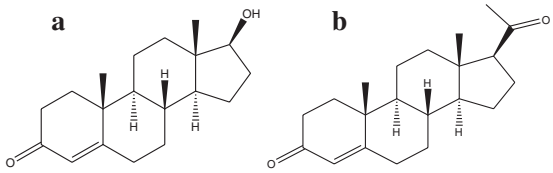

Figure 4. Chemical structure of template and template analog. a. Testosterone. b. Progesterone. Progesterone differs in the chemistry of carbonyl group located at the C17 location.

shifted to 68.06° after binding with 10^{-10} mol L^{-1} solution of testosterone. Compared with the angle 68.10° before rinsing, it demonstrated that the cavities formed by departure of template testosterone in the MIFs were almost all taken by testosterone analyte molecules.

The selectivity of the SPR sensor was evaluated by injection of progesterone sample dissolved in acetonitrile at concentration of 10^{-4} mol L^{-1}. The kinetic measurements of the SPR reflectivity changes were shown in the Figure 2a. There was no obvious change of the reflectivity signal when the progesterone sample was flowed over the SPR substrate. This demonstrated that the testosterone-imprinted films showed very good selectivity to the testosterone analyte. Progesterone was chosen because of its similar structure to that of testosterone, where progesterone differs only by the carbonyl group located at C17 (Figure 4). That such minor differences have profound effects on the rebinding, is testament to the ability of MIFs to accurately recognize the template molecule of testosterone. The present work is a preliminary result, and further studies of the reasons for the ultrahigh sensitivity are under investigation.

In conclusion, the present study demonstrated the testosterone-imprinted synthetic polymer-coated SPR substrates showed ultrahigh sensitivity to detect testosterone molecules. The proposed method reported in this work is measurable at ultralow concentration of testosterone, and this approach may lend itself to the development of field-based SPR sensor combined molecularly imprinted polymer for the high-sensitivity detection of other steroids.

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8 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

