A Novel Receptor-based Surface-plasmon-resonance Affinity Biosensor for Highly Sensitive and Selective Detection of Dopamine

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We have established a new surface-plasmon-resonance (SPR) based affinity biosensor for highly sensitive and selective detection of dopamine (DA) employing a D_3 dopamine receptor (D-RC) and a home-made DA-bovine serum albumin (DA-BSA) conjugate. The proposed biosensor is simple, reproducible and exhibit excellent sensitivity for DA (detection limit = 85 pg/mL (ppt)) without any significant interference from ascorbic acid (AA), uric acid (UA), and other DA analogues.

Dopamine is one of the significant neurotransmitters in the mammalian central nervous system (CNS) and plays a prime role in Parkinson's disease, cardiovascular, renal, and hormonal systems.[1,2] Owing to the significant importance in the human physiological system, there is a challenging interest in the development of novel analytical methods for highly sensitive and selective detection of DA in its active biochemical constitution for improvement of quality and longevity of human life.

Consequently, various analytical methods including HPLC, capillary electrophoresis, fluorescence, and amperometry have been demonstrated for detection of DA.[3–7] Reviewing the wide number of reports, it can be realized that the selective detection of DA remains a challenge because of the presence of large excess of AA, UA, and other analogous endogenous compounds with DA in biological samples, which limits their application to in situ analysis. Various strategies have been employed in electrochemical-based sensors for alleviation of this selectivity problem such as, coupling with chromatographic separation,[3,4] surface pretreatment,[8] or using perm-selective membranes.[7] However, the desired selectivity remains unreachable. Moreover, they often involve time consuming and complicated procedures.

In this context, we aimed to establish a simple and reliable method for highly sensitive and selective detection of DA with a possible scope for in situ detection without prior separation in medical diagnostics. For this purpose, we have developed a new optical affinity biosensor using a DA receptor (D-RC) as a biorecognition element. As a matter of fact, receptors are attractive choice for use as a biorecognition element in sensor technology due to their excellent affinity and specificity towards the target analyte.[9] The sensitive and selective interaction of D-RC with DA was monitored using SPR technique. SPR is an attractive optical tool for rapid and real-time analysis of biomolecular interaction.[10–12]

In order to functionalize the sensor surface with DA (molecular weight = 153), we synthesized a protein conjugate of DA using BSA protein. Here, a residue containing equimolar concentrations of DA, *N*-hydroxysuccinamide and *N*,*N*′-dicyclocarbodiimide was mixed with a solution of BSA in phosphate buffered saline (PBS) and the resulting conjugate was dialyzed (in PBS at ca. 5 °C), lyophilized and stored at −20 °C. The DA-BSA conjugate was immobilized onto an SPR gold-chip surface by simple physical adsorption.[13] The gold chip consists of a ca. 5 nm layer of chromium and ca. 50 nm layer of gold coated over a BK7 type microscopic glass slide ($20 \times 13 \times 0.7\,mm^3$) by high vacuum sputtering method and was attached to the prism of the SPR instrument (SPR 670, Nippon Laser and Electronics, Japan) by using a matching liquid.[11] PBS (0.1 M, pH 7.2) was used to prepare all the solutions and also as a carrier buffer.

Figure 1 shows the SPR response observed for the preparation of sensor surface. The DA-BSA conjugate was allowed to flow over the gold surface at a rate of 20 μL/min. Increase in the resonance angle (ca. 0.175°) indicates the immobilization of the DA-BSA conjugate on SPR-gold surface (curve a). After completion of any injection, the flow was switched back to PBS automatically. As can be seen, the response angle remained stable after an initial desorption of the loosely bound conjugate, which indicates good and stable immobilization of the conjugate. In order to avoid non-specific adsorption, 1000 μg/mL of BSA was introduced over the conjugate surface (curve b). A small shift in the resonance angle (ca. 0.015°) clearly indicates that the gold surface was fully covered by the DA-BSA conjugate. Moreover, BSA covered any of the remaining gold surfaces left unoccupied by the DA-BSA conjugate.

Detection of DA was carried out using the principle of indi-

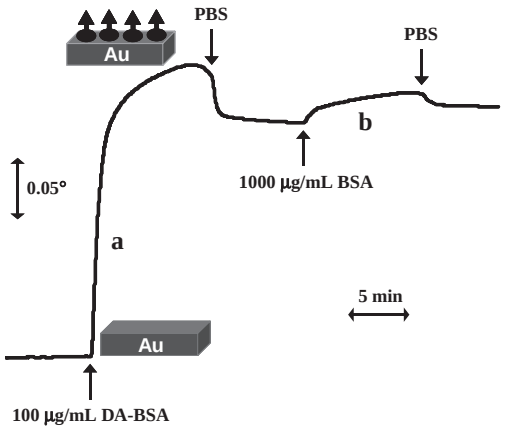

Figure 1. Sensorgram for physical immobilization of DA-BSA conjugate and BSA onto an SPR-gold surface. Carrier solution: PBS, flow speed 20 μL/min, flow duration: 10 min.

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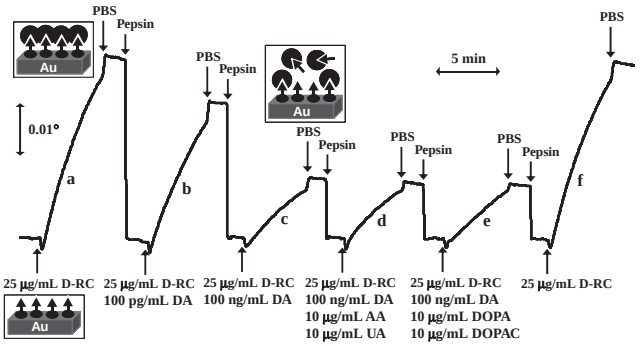

Figure 2. SPR response for the affinity reaction of DA-BSA conjugate with D-RC in the absence and in the presence of DA and its analogues. Carrier solution: PBS, flow speed: 40 μL/min, flow duration: 5 min.

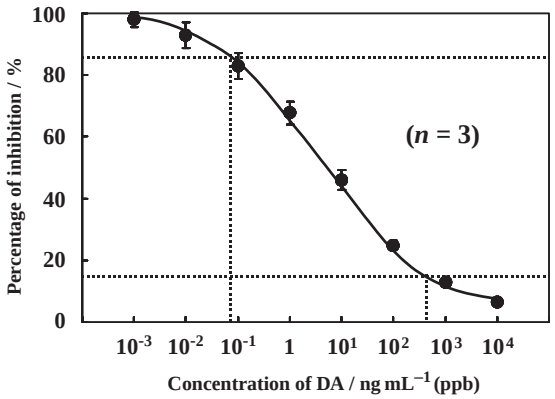

Figure 3. Dependence of the percentage of inhibition on the concentration of DA (detection range: 15 to 85% of inhibition).

rect competitive inhibition, which is a promising tool for highly sensitive detection of small molecules.[13] Here, equal volumes of D-RC (RBI Co., U.S.A.) were incubated with different concentrations of DA for 10 min and introduced over the conjugate surface. It is expected that the interaction of D-RC with DA-BSA conjugate would be inhibited in the presence of free DA in solution. Figure 2 shows the SPR response observed for the affinity reaction between DA-BSA conjugate and D-RC in the absence and in the presence of DA and its analogues. The concentration of the D-RC was 25 μg/mL (ppm) and the duration of the injection was 5 min. The D-RC showed good interaction with DA-BSA conjugate, as can be evidenced from a resonance angle shift by 0.035° (curve a). The conjugate immobilized assay format used in this study has a major advantage that it could be reused by simple regeneration of the conjugate surface by injecting a suitable eluent enabling multiple analyses.[11,13] Here, the conjugate surface was regenerated to its original state by injecting 5 μg/mL of pepsin solution (prepared in glycine–HCl buffer, pH 2.0) for 30–60 s. As a matter of fact, the conjugate surface remained stable and the affinity reaction with D-RC did not vary significantly for nearly 20 cycles of measurement.

For detection of DA, D-RCs incubated with different concentrations (from 0.001 ng/mL (ppb) to 10 μg/mL (ppm)) of DA were injected over the DA-BSA conjugate. As expected, decreases in the resonance angle shift were observed in proportion to the concentration of DA (Figure 2, curves b and c). All concentrations were measured at least in triplicate and a calibration curve was plotted between the percentage of inhibition and the logarithm of DA concentration (Figure 3). Based on the calibration curve, DA could be detected in the concentration range from 85 pg/mL (ppt) to 700 ng/mL (ppb). This sensitivity is significantly higher than most of the sensors reported for detection of DA.[3–8]

In order to evaluate the specificity of the present biosensor, interference from the possible coexisting compounds for DA detection were examined. The curve d in Figure 2 corresponds to the addition of 10 μg/mL (ppm) each (100 fold excess) of AA and UA to the solution of DA and D-RC mixture. The response curve revealed that they had no significant influence on the resonance angle shift. We have also studied the interference from other DA analogues such as 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-(3,4-dihydroxyphenyl)alanine (DOPA) for the measurement of DA (Figure 2, curve e), which also showed negligible influence on the resonance angle shift for DA. The results clearly indicate that the D-RC has high selectivity towards DA, which is highly appreciable for analysis of DA in its complex biological format with enhanced reliability. The present affinity biosensor is reliable and reproducible as can be seen from the curve f of Figure 2, which showed almost similar resonance angle shift (original response) for the interaction of D-RC with DA-BSA conjugate after multiple analysis cycles.

In conclusion, the new methodology presented here using receptor as a recognition element for DA detection based on SPR technique proved to be a potential tool for highly sensitive and selective detection of dopamine with good reliability. The sensing idea is simple and the high affinity molecular recognition of the D-RC provided remarkable specificity for DA against AA, UA, and other DA analogues. The proposed method provides a new and promising route for reliable and economic biochemical diagnosis of risk of DA related disorders. Application of the proposed biosensor for real-time analysis of DA is currently under progress in our laboratory.

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