Surface Plasmon Resonance Based Immunosensor for Trace Level Analysis of TNT and Related Nitroaromatic Compounds Aiming for On-site Detection of Buried Landmines

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1. INTRODUCTION

The rapid increase in the number of landmines around the world due to the expanding incidents of war and terrorist attacks is a matter of great health and security concern for the international community[1)]. At the moment, trained dogs are the most sensitive and versatile sensors for field detection of landmines. But dogs tire quickly and cannot cover substantial land area. Thus, a great research activity is currently under progress for the development of field-ready landmine detection system (handheld and mobile devices), which mimic the chemical registry of the dog's nose without having their drawbacks. In the present study, we demonstrated a SPR immunosensor for the detection of TNT and related nitroaromatic compounds, based on the principle of indirect competitive immunoreaction[2,3)], using 2,4,6-trinitrophenol-bovine serum albumin (TNP-BSA) conjugate and anti-TNP antibody.

2. EXPERIMENTAL

All chemical used were of analytical grade. Phosphate buffered saline containing 1 vol.% ethanol (PBS, 0.1 M, pH 7.2) was used as a working solution. Pepsin solution was prepared using Glycine-HCl buffer (pH 2.0). All measurements were carried out in an air-conditioned room at 25±1°C. Standard TNT solutions in the concentration range from 0.001 ppb to 1000 ppb were prepared with PBS solution containing 20 ppm anti-TNP antibody and incubated for 10 min at room temperature.

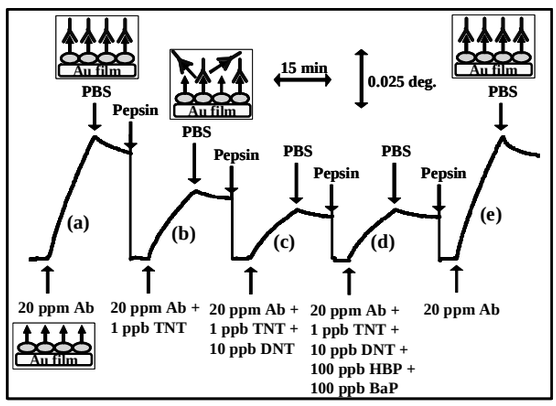
SPR instrument (SPR 670, Nippon Laser and Electronics, Japan) equipped with a flow injection system was used for SPR immunoanalysis. The SPR gold-chip was attached to the prism of a SPR instrument using a refractive-index matching liquid.

3. RESULTS AND DISCUSSION

TNP-BSA conjugate was attached to the SPR-gold sensing surface by physical immobilization. The remaining unoccupied sites at the TNP-BSA immobilized sensing surface were filled with BSA to avoid non-specific adsorption of protein. The immunoassay was performed by exposing the sensing surface to an anti-TNP antibody solution, which may or may not contain free TNT, and monitoring the surface binding by using SPR. The changes in the resonance angle shift observed as a result of inhibition of the anti-TNP antibody by different concentrations of TNT were recorded (Fig. 1). Three different anti-TNP antibodies, such as polyclonal antibody prepared in our laboratory, commercial polyclonal and monoclonal antibodies were used in the study for the immunoreaction with TNP-BSA conjugate. All the three immunosystems were utilized for the quantification of TNT and related nitroaromatic compounds. It was observed that each analyte has different inhibition, depending upon their affinity to the antibodies. The sensor surface was regenerated by a brief exposure to an aqueous pepsin solution, which liberates surface bound antibody and leaves the conjugate free for the next test.

Fig. 1 SPR response of a TNP-BSA immobilized gold-sensing surface to the flow of 20 ppm anti-TNP antibody (lab.) in the absence and in the presence of TNT and other analytes. Carrier solution: PBS, flow speed: 15 □l/min.

Dynamic ranges, defined by the analyte



concentrations that inhibited maximum signals by 15% and 85%, covers a wide concentration range between 60 ppt to 1000 ppb TNT with polyclonal laboratory antibody. Similar experiments with commercial anti-TNP antibody showed sensitivity to TNT in the range from 80 ppt to 1000 ppb and a sensitivity range from 10 ppb to 100 ppm was observed with monoclonal anti-TNP antibody. Experiments with endocrine-disrupting relevant chemicals such as, benzo[a]pyrene (BaP) and 2-hydroxybiphenyl (HBP) (Fig. 1, curve (d)) suggests the remarkable selectivity and negligible matrix effect of the immunoassay for favorable application to the analysis of practical samples.

4. CONCLUSIONS

We have demonstrated a novel immunoassay system for highly sensitive detection of TNT and related nitroaromatic signature compounds with desired analytical characteristics. The results are highly promising for development of field-portable sensor devices for on-site detection of landmines.

References

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