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Preparation of a Polyclonal Antibody and a Bioassay for Nitroaromatic Compounds by an Enzyme-Linked Immunosorbent Assay Technique and a Surface Plasmon Resonance Biosensor

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Sensitive and selective detection of 2, 4, 6-trinitrophenyl derivatives (TNP derivatives) based on surface plasmon resonance (SPR) was performed using polyclonal anti-TNP antibody and *N*-(TNP)-ovalbumin (TNP-OVA) conjugate. TNP-bovine serum albumin (TNP-BSA) conjugate was injected into the mice, and polyclonal anti-TNP antibody was gained after purification of the sera using protein G. TNP-OVA was immobilized on the Au film of the SPR sensor chip by physical adsorption. The incidence angle shift of the TNP-OVA immobilized sensor increased steeply with increasing concentration of anti-TNP antibody up to 20 μg/ml and increased only slightly above this concentration. The additions of TNP derivatives into the anti-TNP antibody solution were found to decrease the incidence angle shift because of the inhibition effects of TNP derivatives. The lowest detection limit for trinitrotoluene (TNT) by SPR was 1×10^{-7} g/ml, whereas for TNP-6-aminohexanoic acid (TNP-aha) it was 3×10^{-9} g/ml. Evaluations of affinity constants of anti-TNP antibody were performed. Analyses of SPR data were carried out by assumptions of the Langmuir isotherm and equilibrium state of immunoreaction. The value of association constant between anti-TNP antibody and immobilized TNP-OVA (K_1) was 6.4×10^6 M^{-1}. The values of association constants between antibody and TNP-aha (K_2) were 2.7 and 8.5×10^6 M^{-1} when 20 and 10 μg/ml antibody were used, respectively.

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