

IMMUNOSENSOR FOR HERBICIDE 2,4-DICHLOROPHENOXYACETIC ACID

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Direct monitoring of affinity interactions in real time can be performed using advanced optical transducers based on surface plasmon resonance and resonant mirror techniques. On the other hand, a more straightforward technique is now available – the measurement of mass changes resulting from the formation of affinity complexes at the sensing surface [1]. The relevant physical transducer - piezoelectric quartz crystal - functions as a chemical or electrochemical nanobalance (EQCN) and is now widely used. Piezoelectric detectors address such different areas as monitoring of plating, affinity biosensing and electrochemical measurements [2].

For biosensing applications, the simple direct counting was the approach successfully adopted [1]. However, the frequency resolution became the limiting factor in further progress. To improve this situation, we have combined the well-characterized pair of herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and the corresponding anti 2,4-D monoclonal antibody (mAb) with the highly sensitive EQCN equipment available from ELCHEMA. The performance of the obtained immunosensors in kinetic studies of the competitive and direct immunoassays of the model hapten, is characterized in this contribution.

The improved highly sensitive piezoelectric immunosensor has been developed and evaluated using a model interaction of antibody with the model hapten - herbicide 2,4-D. For immobilization of 2,4-D, the self-assembled layers of cystamine, 4-aminothiophenol or 3,3'-dithio-bis(propionic acid N-hydroxysuccinimide ester) were formed on smooth and rough crystals coated with gold or silver electrodes. The immunochemical interactions performed well in all cases, the aminothiophenol on gold was chosen as the optimum with regard to regeneration of immunosensing surfaces. The kinetics of interaction of surface-bound 2,4-D with free antibody provided significantly higher kinetic

parameters (kinetic association rate constant) when using optically smooth crystals compared to common rough crystal. Therefore, the smooth crystal should be preferred for future kinetic studies. The competitive assay of the herbicide 2,4-D achieved the limit of detection 10 ng/l using the monoclonal anti-2,4-D antibody F6C10. Finally, a direct assay format has been evaluated using a thicker layer of glutaraldehyde-crosslinked antibody on the sensing surface. The direct binding of a small herbicide molecule was followed in real time. The detected concentration of 2,4-D (5 µg/l) was low enough for future direct monitoring of this herbicide in water.

References

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