Electrochemical Genosensor Design Devoted To Clinical Analysis

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DNA arrays and gene chip technology have emerged among the most promising methods for the detection of viral and bacterial pathogens, hereditary diseases, and genetic abnormalities. The basis of these techniques is the specific recognition of target gene sequences with complementary oligonucleotide probes attached to a solid phase.

Recent advances in biosensors based on nucleic acid hybridization recognition have led to the development of genosensor technology for DNA sequence analysis. Specifically, electrochemical hybridization biosensors demonstrate great promise for pathogen identification, mutation detection, and genomic sequencing. These novel, sequence-specific hybridization processes either involve monitoring the oxidation signal of the electroactive bases of DNA or employing an electroactive hybridization indicator that emits different signals to discriminate between single-stranded and double-stranded DNA. Successful attempts to exploit the electrochemical detection of hybridization events and base pair mismatches using sample amplicons in order to obtain reliable, clinically-relevant measurements have already been reported.

We present a robust and simple method for the direct detection of multiple point mutations in the Mycobacterium tuberculosis rpoB gene during the development of rifampin (RIF) resistance using an electrochemical genosensor. The field effect sensor is designed to detect multiple point mutations simultaneously and does not require additional lengthy and expensive genotyping methods based on polymerase chain reaction (PCR) amplification. The device contains five different capture probes, each covalently attached to a different graphite electrode, which are designed to hybridize with several sequence segments within the bacterial rpoB gene hotspot region. Unmodified PCR amplicons are captured at the genosensor interface via hybridization with guanine-free oligonucleotide probes. Point mutations are detected by monitoring guanine oxidation with differential pulse voltammetry (DPV). Changes in the peak potential corresponding to guanine oxidation provide an electrochemical signal for hybridization that can be used to determine the presence of point mutations conferring RIF resistance. Numerous factors affecting the hybridization of target sequences are optimized to improve the sensitivity. Sequences unrelated to the immobilized capture probes are used to confirm the genoassay’s selectivity.

References