ELECTROCHEMICAL DNA BIOSENSORS

Electrochemical Genosensing Based on Rigid Carbon Composites. A Review

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Abstract: The development of novel and sensitive assays for DNA hybridization and detection has become an increasingly important research field. The design and fabrication of DNA-modified surfaces and materials that are reproducible, stable, and selective to complementary DNA sequences are the main goal in the development of emerging analytical tools such as DNA chips or user-friendly diagnostic devices for detecting a few DNA sequences such as electrochemical genosensors. Carbon-based materials are widely used for this task due to their electrochemical, physical, and mechanical properties; their commercial availability; and their compatibility with modern microchip fabrication technology. Various approaches for electrochemical DNA determination are reviewed, in which the common element is the use of rigid carbon composite material as the electrochemical transducer. A stable DNA layer can be easily obtained by physical adsorption of DNA on graphite-epoxy composite (GEC). Moreover, a universal affinity platform for electrochemical genosensing can be easily achieved by modifying the graphite-epoxy composite with avidin to obtain an avidin biocomposite (Av-GEB) whereon biotinylated DNA can be rapidly single-point attached. Additionally, DNA-modified magnetic beads are easily attached to magneto-graphite-epoxy composite (m-GEC). The main strategies for electrochemical

Received 4 May 2005; accepted 28 July 2005

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Financial support from Ministry of Education and Science (MEC), Madrid (Project BIO2004-02776), is acknowledged. M. I. P. acknowledges the financial support from the Juan de la Cierva Program from MEC (Madrid) and the Universidad Nacional del Litoral (Argentina).

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genosensing when using rigid carbon composites such as electrochemical transducer are discussed. Parameters such as ease of preparation, robustness, sensitivity, surface regeneration, costs, and transfer to mass production of these different DNA detection strategies are also considered.

Keywords: Graphite-epoxy composite, avidin biocomposite, DNA, hybridization genosensor, enzyme-label electrochemical genosensor, label-free electrochemical genosensor, amperometric genosensors, DNA biosensor, magnetic beads

INTRODUCTION

The use of nucleic acids recognition layers represents a new and exciting area in analytical chemistry that requires an extensive study. The growing demand for genetic information in an increasingly broad range of disciplines has instigated research into the development of new techniques for genetic analysis. The Human Genome Project (HGP) (Baltimore 2001) has stimulated the development of analytical methods that yield genetic information quickly and reliably. Examples of this development are the DNA chips (Bowtell 1999; Collins 1999; Lander 1999) and lab-on-a-chips based on micro fluidic techniques (Sanders and Manz 2000). Additionally, the knowledge obtained from the HGP has expanded the market that requires genetic devices, hence, generating new applications. However, this expanding market is not contradictory to simple, cheap, and user-friendly analytical devices—especially for industrial applications.

Therefore, the development of new methodologies possessing the convenience of solid-phase reaction, along with the advantages of rapid response, sensitivity, and ease of multiplexing, is now a challenge in the development of new biochemical diagnostic tools. Electrochemical DNA biosensors can meet these demands, offering considerable promise for obtaining sequence-specific information in a faster, simpler, and cheaper manner compared to traditional hybridization assays. Such devices possess great potential for numerous applications, ranging from decentralized clinical testing, to environmental monitoring, food safety, and forensic investigations.

In order to prepare analytical devices for the electrochemical detection of DNA, the immobilization of the biological species must be carefully considered. The most successful immobilization techniques for DNA appear to be those involving multisite attachment (either electrochemical or physical adsorption) or single-point attachment (mainly covalent immobilization or strept(avidin)/biotin linkage) (Pividori et al. 2000). Single-point attachment is beneficial to hybridization kinetics, especially if a spacer arm is used. However, among different DNA immobilization methodologies reported, multisite adsorption is the simplest and most easily automated procedure, avoiding the use of pretreatment procedures based on previous activation/ modification of the surface transducer and subsequent DNA immobilization. Such pretreatment steps are known to be tedious, expensive, and time-consuming. Furthermore, the adsorption