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DNA-Based Label-Free Electrochemical Biosensors: From Concepts to Applications

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Abstract

Deoxyribonucleic acids (DNAs) have been used as excellent biomaterials to construct a range of biosensors via interactions between DNAs and biomolecules or chemical substances. DNA-based electrochemical biosensors with excellent sensitivity and selectivity have been widely employed in bio-chemical analysis due to their ease of operation, quick response, and inexpensive cost. However, most DNA-based electrochemical biosensors involve the tagging of electroactive chemicals or nanomaterials on DNAs as signal read-out elements, which unavoidably results in complicated operation and high costs. Label-free techniques are solutions for DNA-based electrochemical biosensors that do not require extra assay chemicals or time-consuming procedures. Because of its simplicity and low cost, DNA-based label-free electrochemical biosensors have piqued the interest of researchers as a potential analytical tool. The concepts and applications of DNA-based label-free electrochemical biosensing devices, encompassing heterogeneous and homogeneous modes with diverse amplification techniques, have been discussed and reviewed extensively in this study. Furthermore, the current limitations and future prospects of DNA-based label-free electrochemical biosensors are discussed.

Keywords: Label-free electrochemical biosensors; DNA; Biochemical analysis; Clinical diagnosis

Introduction

Electrochemical biosensors have been created by combining electrical transduction components with biological recognition elements. On the one hand, the electronic components provide high-performance electrochemical biosensing platforms for detecting target molecules with high sensitivity and quick response. The biorecognition components, on the other hand, contribute to the great selectivity of biosensors through the particular recognition of biomolecules. These advantages make electrochemical biosensors extensively employed in a variety of disciplines, including biochemical analysis, environmental monitoring, food safety control, and so on. DNAs are the bearers of genetic information and serve critical roles in directing numerous biological processes as the foundation materials of biological heredity. Furthermore, DNA has evolved into an effective building block for the development of novel devices in biosensor technology, with the development of DNA-based electrochemical biosensors accelerating in the last 13 years. Most DNA biosensors are based on the hybridization of a certain DNA-base sequence with its complementary strand. Furthermore, functional DNAs such as DNA aptamers and DNAzymes may be used to build highly selective DNA biosensors. DNA aptamers are single-stranded nucleic acids or peptide molecules that are chosen by in vitro systematic evolution of ligands. Combining the sensitivity of electroanalytical methods with the inherent bioselectivity of DNAs' biological components, DNA-based electrochemical biosensors have the advantages of simplicity, fast response, low cost, high sensitivity, miniaturisation, and low power and volume requirements, and have been developed for detecting various biological

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molecules, including nucleic acid (e.g., DNA and RNA) targets and non-nucleic acid targets. The detection of nucleic acid targets is mostly dependent on the development of double-helical structures based on Watson-Crick base pairing, whereas matching aptamers are often used for high affinity and specificity identification of non-DNA targets. Traditional DNA-based electrochemical biosensors, on the other hand, sometimes need the tagging of electroactive molecules or nanomaterials as signal read-out components, which increases complexity and potentially impairs biomolecule function. Furthermore, labelling requires time and effort. The cost of synthesising DNA with electrochemical signal molecular markers is too high. Furthermore, one redox label (e.g., ferrocene and methylene blue) is frequently tagged to a single biomolecule, resulting in a weak electrochemical signal and decreased bioaffinity. The increased need for efficient and Portable Point-of-Care (POC) testing has created new obstacles for classic DNA-based electrochemical biosensors. As a result, the label-free technique offers appealing alternatives in the fabrication of DNA-based electrochemical biosensors for biomolecule analysis.

DNA-based label-free electrochemical biosensors are built around either the intrinsic electroactivity of target nucleic acid molecules or the detection of electrochemical signal changes associated with the hybridization reaction without the use of electroactive molecules as labels on DNA probes or target analytes. Label-free techniques were mostly depended on in the early development of DNA-based electrochemical biosensors, particularly employing the guanine electrochemical oxidation signal. Purines guanine (G) and adenine (A) have been shown to oxidize at significantly lower positive potentials than pyrimidines thymine (T) and cytosine (C). Paleek, for example, achieved the earliest DNA detection by directly oxidizing guanine on a mercury drop electrode. Following that, the same group conducted a series of research projects that laid the groundwork for the creation of label-free DNA electrochemical biosensors. Furthermore, Wang et al. devised numerous label-free electrochemical techniques for detecting DNA hybridization based on the inherent redox activity of the target guanine. The use of electrochemically active compounds in the fabrication of DNA-based label-free biosensors has grown in popularity in recent years. In fact, in conventional label-free biosensors, electrochemically active compounds display three states: direct addition to the solution, alteration on the electrode surface, and binding with DNA probes via electrostatic contact or intercalation. A number of experiments have been conducted to date in order to create modified electrodes for label-free electrochemical detection. Gooding et al. announced the invention of Self-Assembled Monolayer (SAM) technology for electrode modification, allowing for molecular control of the interfaces. The unwanted adsorption of nonspecific DNA or indicator molecules on gold electrodes can be reduced using the thiol-linked DNA probe SAM. Direct charge transfer through Double-stranded DNA (dsDNA) has been achieved for the detection of base pair mismatches by combining DNA redox intercalators with DNA SAM on a gold electrode. Dharuman and Hahn discovered that short chain alkane diluents at the HS-ssDNA/diluent binary and ternary mixed monolayer could control non-specific DNA-Au interactions as well as the DNA hybridization discrimination efficiency of label-free electrochemical biosensors in the presence of dsDNA specific intercalators. Furthermore, signal amplification technologies such as tool enzyme-based isothermal amplification, DNA strand displacement reaction-based enzyme-free amplification, nanomaterial-based signal amplification, and others have been used to increase the sensitivity of label-free electrochemical biosensing. Notably, in addition to single-mode signal amplification, multiple or even triple signal amplification schemes have been developed to boost sensitivity.

Polymerase Chain Reaction (PCR) is well known as the conventional and fundamental method for sensitive detection and quantification of nucleic acids, which is critical for molecular diagnostics. PCR technology has progressed from end-point analysis using gel electrophoresis to real-time fluorescent quantitative PCR (qPCR) and digital PCR (dPCR), with findings extending from qualitative analysis to relative and absolute quantitation, respectively. Next-generation sequencing (NGS) technologies have evolved as a result of the advancement of genetic engineering, and they may give reliable and accurate genomic sequence information for a variety of life sciences, including DNA/RNA sequencing, epigenetics, metagenomics, and so on. Many NGS equipment, such as the Ion Torrent Personal Genome Machine, are now in use (PGM).via exponential enrichment (SELEX). Overall, the DNA-based label-free electrochemical biosensors not only eliminate the time-consuming labelling processes and decrease the experimental time, but they also have no influence on the physical and chemical characteristics of molecules, preserving intermolecular affinity and molecular activity. Meanwhile, the label-free approach can prevent interference generated by the second detection of labelling chemicals and increase electrochemical analytical sensitivity. Furthermore, the mobility and cost of electrochemical instruments based on label-free detection enable biomolecules to be examined in a decentralized context, such as at the point of care. As a result, DNA-based label-free electrochemical biosensors have gotten a lot of interest in biological analysis, point-of-care diagnostics, and biomedicine. Some reviews have been published on DNA-based electrochemical biosensors, with the majority of them focusing on labelling techniques for signal read-out. Although label-free solutions for DNA-based electrochemical biosensors have been referenced in certain publications, no review has been published that provides a clear and complete introduction as well as a critical assessment of DNA-based label-free electrochemical biosensors. We introduce and outline the

concepts and uses of DNA-based label-free electrochemical biosensors in heterogeneous and homogeneous modes in this study. We also discussed the current limitations and future prospects of DNA-based label-free electrochemical biosensors.

Label-free electrochemical biosensors based on heterogeneous DNA

The heterogeneous (solid-phase) DNA-based label-free electrochemical biosensors are typically performed on electrode surfaces immobilized with DNA probes as biological recognition elements, in which analytes can be detected by translating changes in DNA structures and electrochemical properties into an electrical signal when analytes interact with the immobilized DNA probes on the heterogeneous interface. The immobilized DNA of heterogeneous label-free electrochemical biosensors offers several advantages, including reusability, low sample consumption, and excellent sensitivity..

Using mimetic enzymes

Natural protein-assisted enzyme amplification is often employed to increase detection sensitivity, but their applications are limited due to problematic storage and a laborious labelling process. Mimetic enzymes have distinct benefits over standard natural enzymes, such as a simple structure, stable characteristics, and ease of production. So far, different mimetic enzymes, such as porphyrin-based enzymes (intercalated into grooves of negatively charged dsDNA) and DNAzymes (made by G-quadruplex), have been reported to be used to build DNA-based label-free electrochemical DNA biosensors with good stability and high sensitivity.

Using nanomaterials

Nanomaterials have distinct advantages, such as a high surface-to-volume ratio and outstanding electrical characteristics, and may be used as electrode modified materials as well as carriers of signal components. More intriguingly, due to their redox characteristics, mimic enzyme activity, and particular electrical properties, some materials, such as noble metallic nanoparticles, can be used directly as signal molecules. As a result, nanomaterials have emerged as promising options for the development of label-free electrochemical biosensors capable of detecting a wide range of biomolecules.

Electrochemical biosensors based on homogeneous DNA that are label-free

The label-free electrochemical biosensors based on DNA have been developed in a homogenous way. The immobilization-free technique encourages the creation of simple and efficient homogeneous DNA-based label-free electrochemical biosensors with greater binding efficiency between targets and recognition probes and a quicker response rate in solution than in solid phase. Similarly to heterogeneous assays, the signal in homogeneous electrochemical assays is generated by linking DNA with electroactive molecules and allowing electroactive probes to diffuse from solution to electrode surface. Furthermore, homogenous reactions reduce separation and washing stages, resulting in low cost and great amplification efficiency. As a result, various immobilization-free electrochemical biosensing approaches for DNA detection emerged. So far, an increasing number of researchers have dedicated themselves to developing homogeneous DNA-based label-free electrochemical biosensors for bioanalysis that are more appropriate for POC applications.

DNA-based label-free electrochemical biosensors' dependability

In the realm of biological investigation, DNA-based label-free electrochemical biosensors have proven to be extremely reliable. First, enhanced detection accuracy and sensitivity were obtained by using signal amplification techniques (e.g. exonuclease-assisted autocatalytic target DNA recycling amplification, RCA, HRCA, HCR, CHA, EXPAR in the manufacturing of DNA-based label-free biosensors). Furthermore, the DNA-based label-free electrochemical biosensors exhibit remarkable selectivity for detecting a variety of analytes. The detection of nucleic acids is mostly dependent on the creation of a double-helical structure based on Watson-Crick base pairing, which allows for high selectivity. More importantly, as compared to immobilization-based electrochemical biosensors, immobilization-free techniques eliminate complex probe immobilization processes, making them cost-effective and simple to apply. However, because the signal produced in homogeneous label-free electrochemical biosensing platforms is mostly dependent on the diffusion of electroactive probes from solution to electrode surface, they frequently have lower sensitivity and specificity when compared to heterogeneous platforms.

Conclusions and perspectives

We outlined the concepts and progress of DNA-based label-free electrochemical biosensors in this study by emphasizing various instances from both the heterogeneous and homogeneous sides. DNA-based label-free electrochemical biosensors have shown to be potent approaches in the domains of bioanalysis due to their benefits of ease of operation, quick response, low cost without extra assay reagents, and lack of time-consuming processes. Despite these tremendous achievements, there are still several obstacles to

overcome in producing label-free electrochemical biosensors, ranging from analytical performance to applications. First, in label-free electrochemical biosensing platforms, particularly in homogeneous mode, signal generation frequently depends on the diffusion of electroactive probes from solution to electrode surface, resulting in very poor sensitivity. As a result, the investigation of functional nanomaterials-modified electrodes and signal amplification techniques should increase detection sensitivity. Second, most DNA-based label-free electrochemical biosensors are currently used in vitro, with in vivo and in situ experiments being rare. Aptamers, as a potential molecular probe, may be easily integrated with label-free techniques, broadening the scope and value of applications. Finally, the development of point-of-care (POC) devices for diagnostics and sample analysis is gaining popularity. As a result, label-free solutions that integrate with portable equipment to accomplish speedy and sensitive detection are in high demand.