Chapter 2
Electrochemical Biosensors and the Signaling

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Abstract An electrochemical biosensor is a type of sensor that uses biological materials as the sensitive component, electrodes as the conversion element, and current, potential or resistance as detectable characteristics of the signal. In recent decades, electrochemical biosensors have been developed for the detection of many biological elements. Biosensors designed to detect a range of targets have been extensively studied in terms of their transduction, biometric, and electrochemical components. This chapter presents a discussion of bio-receptors and electrochemical techniques that can be used to detect biological elements. Biological elements include tissue, living cells, an enzyme, antibody or antigen, and nuclei. Electrochemical methods include electric current, electric potential, impedance. This critical review will discuss the two components of electrochemical biosensors, biological elements and electrochemical techniques, in order to provide an easy to understand introduction to electrochemical biosensors.

Keywords Biosensors · Biorecognition elements · Electrochemical signaling · Electrochemical methods · Voltammetry · Electrochemical Impedance Spectroscopy

2.1 Introduction

Electrochemical biosensors combine the low detection limits of electrochemical transducers with the high specificity of biometrics [1]. Therefore, the electrochemical biosensor has the characteristics of high sensitivity and specificity. At the same time, the electrochemical biosensor also has the advantages of low cost, convenient use, portability, simple structure, and so on. Currently, the electrochemical biosensor also boasts the advantages of being cost-effective, user-friendly, portable, and having a simplified structure. Therefore, it provides precise quantitative or semi-quantitative

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analysis for various types of molecules, including small molecules, nucleic acids, proteins, cells, and more [2, 3].

Electrochemical biosensors employ recognition elements that have the ability to selectively react to targeted analytes, thereby reducing interference from other components present in the sample [3]. Biometric components may be antibodies or antigens, nucleic acids, enzymes, molecular imprinted polymers and peptide nucleic acids, etc. [3–5]. Sensor/transducer signals include current, potential, intensity, and phase of electromagnetic radiation, mass, conductivity, impedance, temperature, light, and viscosity [1, 3, 6, 7] (Fig. 2.1). The overall performance of electrochemical biosensors is determined by biometric components, sensors, and transduction methods [4].

Electrochemistry does not depend on the volume of the reaction, so rapid analysis of small samples can be achieved. (less than 1 μL) [8–10]. The electrochemical detection process requires no preparation or fewer experimental steps, specifically, electrochemical analysis of homogeneous samples, without the need to separate the antibody-antigen complex from the sample, is a step toward achieving electrochemical rapid detection. Because electrochemical biosensors are unaffected by chromophores, fluorophores, and particles in the sample composition, electrochemical sensors can detect colored or turbid samples, such as serum, plasma, or whole blood, without interference from lipoprotein, red blood cells, hemoglobin, and bilirubin [11, 12].

Electrochemical biosensors have made great progress in recent years in laboratory research, and translation from laboratory to clinic remains a challenge for many laboratories, including sensitivity, specificity, and reproducibility. The solution to these several key problems must also start from the basic origin of the electrochemical biosensors themselves, and promote the translation of electrochemical biosensors

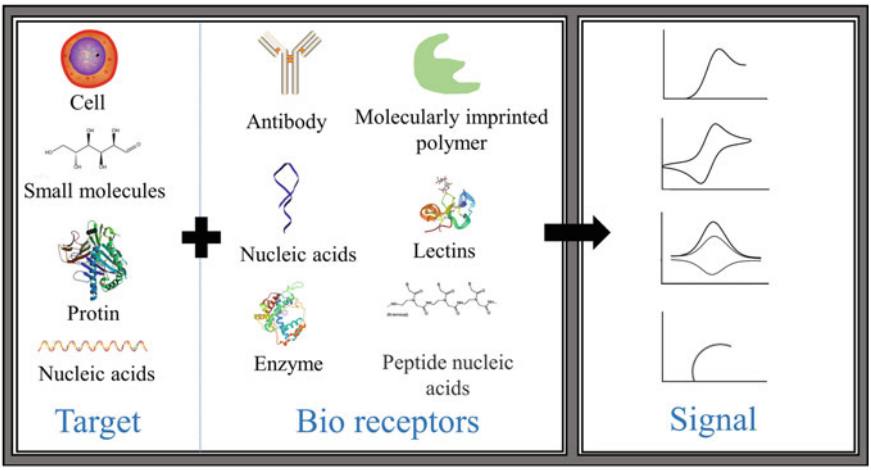

Fig. 2.1 Components of electrochemical biosensors

from laboratory to clinic by optimizing the recognition components, selecting the appropriate sensors and conduction methods, increasing the use of new materials and integrating interface technologies. In this section, we aim to provide a brief discussion of signaling and biorecognition elements.

2.2 Electrochemical Methods

Depending on the respective input and output signals, electrochemical biosensors can be classified as oltammetric/amperometric (typically generating a measurable current), impedimetric (monitoring resistance and reactance), potentiometric (the ratio of the potential energy of a unit charge in an electric field to the amount of charge it carries), conductometry (the velocity of charge flow in a substance), photoelectrochemical and electrochemiluminescent biosensors (measurable iuminescence signal), field effect transistor (FET, measurable ionic charge).

Voltammetric/amperometric is the most commonly used of these techniques, including cyclic voltammetry (CV), chronoamperometry (CA), differential pulse voltammetry (DPV), square wave voltammetry (SWV), linear sweep voltammetry (LSV) [13]. The classification of these techniques depends on how the question potential method is applied, which we will discuss in detail in the next section (Fig. 2.2). Among these techniques used for electrochemical biosensors, voltammetry/amperometry accounts for 80%, followed by EIS and potentiometry, etc. We describe the principles of all these techniques and their corresponding sensor applications.

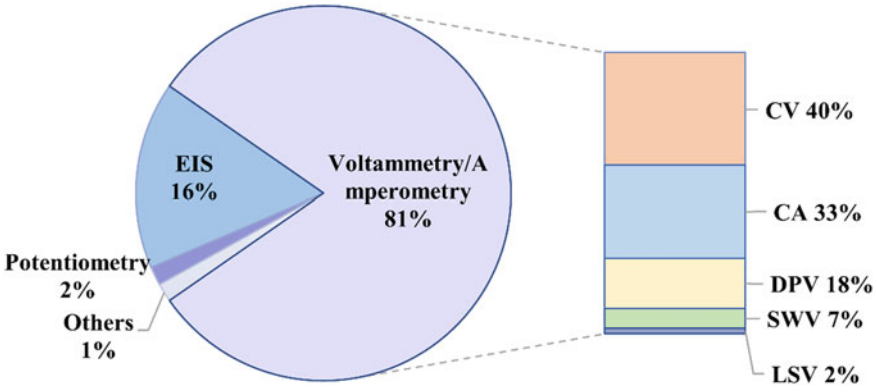

Fig. 2.2 Electrochemical techniques employed for biosensors. The different classifications of electrochemical techniques and their proportions, and the subclassifications of voltammetry/amperometry techniques and their proportions are shown in the figure. Both statistical analyses come from Web of Science, using the search terms "electrochemical biosensors" and "blood" from the year 2013 to the year 2023

2.2.1 *Voltammetry/Amperometry*

Voltammetry/amperage technology works by applying a potential to the working electrode and measuring the current resulting from the oxidation or reduction reaction of electrochemically active molecules at the working electrode. The redox-active molecules are either attached to the electrode surface or free in solution. The electron transport of attached redox molecules is limited by the mass transfer rate at the reaction molecule-electrode interface in terms of solution pH, ionic strength, temperature, solution viscosity, nano-limited domain space, etc. [13–18].

Voltammetric and amperometric biosensors can be classified according to the technique used to apply interrogation potentials, which is briefly discussed below:

(1) **Linear sweep voltammetry (LSV)**

In LSV, the potential is constant input, the current is output, and the potential is linear with respect to time. The peak current measured by the current–potential curve is linearly related to the concentration of the measured substance, which can be used for quantitative analysis and is more suitable for the determination of adsorbed substances (Fig. 2.3).

The number of electron transfers, the chemical reactivity of the redox molecule, and the scanning rate are all key factors affecting LSV. According to the Randles–Sevcik equation, the higher the number of electrons gained and lost in a reversible reaction, the higher the peak current. The larger the electrode area of the reaction, the higher the molarity of the reactant redox molecules and the corresponding increase in current.

In LSV, the current increases with the scan rate, while the potential varies with the scan rate. At a fixed scan rate, the potential shift depends on the type of redox reaction (reversible, quasi-reversible or irreversible). In a reaction process controlled by free diffusion, for a reversible reaction, the faster the scanning rate, the thinner the diffusion layer, the larger the potential gradient, and the increased current. The potential shift requires more response time than quasi-reversible or irreversible reactions or reactions with slower kinetic processes. During the control of the adsorption process, the relationship between peak current and sweep speed will not be that of the Randles–Sevcik equation but will be close to the one-square relationship.

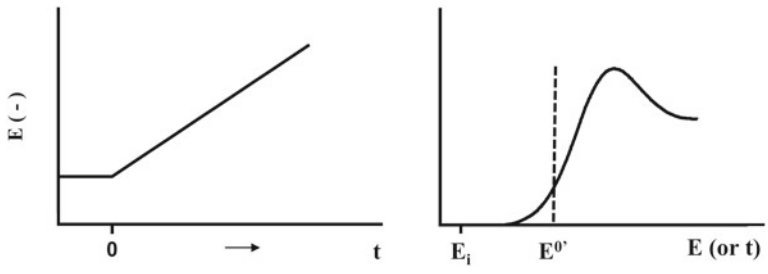

Fig. 2.3 The mechanism of LSV employed for electrochemical biosensors

LSV allows the study of materials in the general potential energy and time scale domains to gain insight into the thermodynamic and kinetic behavior of insertion reactions. In addition, LSV is highly sensitive to structural changes in the inserted compound. LSV is therefore a widely used method for the study of insertion compounds [19].

(2) **Cyclic voltammetry (CV)**

CV is a common method used in electrochemical research. CV controls the change in electrode potential, with the voltage fluctuating between the two values at a fixed rate, but returning to the minimum/maximum when the voltage reaches a maximum/minimum voltage reversal. The potential range includes both reduction and oxidation reactions (depending on the REDOX molecule used), and current–potential curves are recorded (Fig. 2.4). Therefore, CV is often used to study the properties, mechanism, and kinetic parameters of electrode reactions. It can also be used for the quantitative determination of reactant concentration, adsorption coverage on electrode surface, electrode active area, electrode reaction rate constant, exchange current density, reaction transfer coefficient, and other kinetic parameters.

For CV technology, scan rate and potential are key factors. The scan time must provide sufficient time for the complete redox reaction, and the potential must contain the complete redox reaction [20]. Therefore, the scanning time must be sufficient to allow meaningful chemical reactions to occur. And the scan range for CV measurements is highly dependent on the specific redox-active molecules.

(3) **Differential pulse voltammetry (DPV)**

DPV uses successive rising potential pulses in step wave mode, with a series of forward and reverse pulses as excitation signals. The electrolytic current $\triangle i$ within the cycle is obtained by subtracting the positive and negative pulse current within the cycle. With the increase of potential, the electrolytic current $\triangle i$ for several cycles is measured continuously and $\triangle i \sim E$ is plotted as pulse difference curve (Fig. 2.5). DPV can increase the sensitivity of electrochemical detection, and the application of step pulse potential can improve the effect of charge current generated by CV when the linear potential changes and the influence of non-Faraday current caused by the diffusion layer.

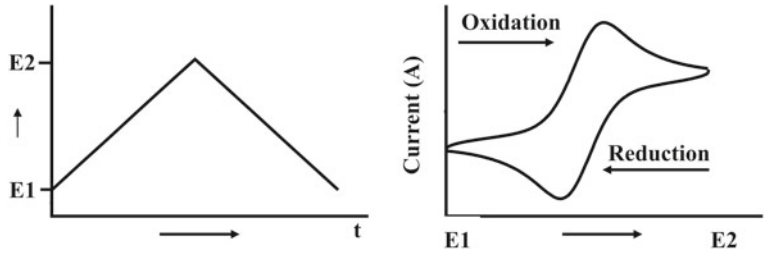

Fig. 2.4 The mechanism of CV employed for electrochemical biosensors

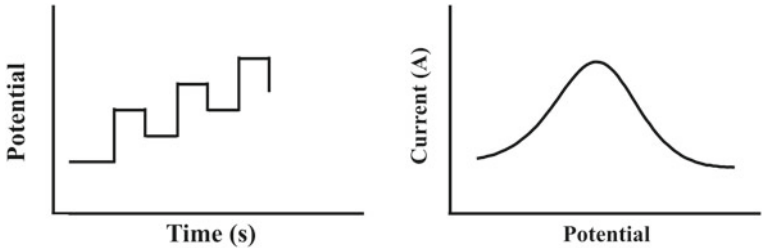

Fig. 2.5 The mechanism of DPV employed for electrochemical biosensors

(4) **Square wave voltammetry (SWV)**

SWV is a technique in which potentiostat superimposes potential in the form of symmetric square wave (forward and reverse) on the basis of step linear scanning. Forward and reverse potentials have the same duration and are applied at the same frequency. The forward and reverse pulses of current subtracted from each other will result in a differential current and potential curve (Fig. 2.6). The forward and reverse components of SWV are curves measured experimentally and are directly related to the oxidation and reduction reactions of redox molecules [21].

SWV can be used not only for quantitative analysis, but also for the study of chemical reaction mechanisms, kinetics and thermodynamics. SWV is characterized by its ability to distinguish between background (capacitive) current and Faraday current, as well as its rapid analysis rate. Compared to DPV, the consumption of electroactive components is lower, and in SWV scanning, the low concentration of dissolved oxygen in the solution has no time to diffuse to the electrode surface for reaction, so no nitrogen deoxygenation is required. And the SWV current is 3–4 times higher than the corresponding DPV response.

(5) **Chronoamperometry (CA)**

Chronoamperometry, applies a stabilizing potential to a working electrode and measures steady-state current as a function of time [22]. The CA regulation mandates an initial, as well as a high and low potential. After the initial potential, from the initial value to either a higher or lower potential. After a duration of τ (pulse width),

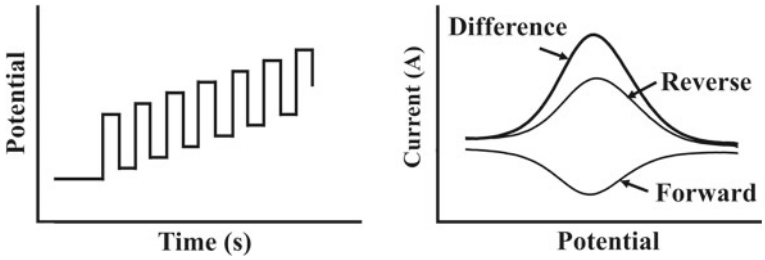

Fig. 2.6 The mechanism of SWV employed for electrochemical biosensors

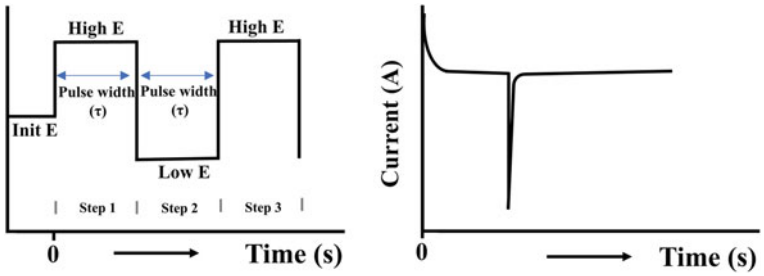

Fig. 2.7 The mechanism of CA employed for electrochemical biosensors

the potential will shift in the opposite direction (either from high to low potential or from low to high potential) and persist for τ time. The current response is contingent on initial and final potential values (Fig. 2.7).

The change in current is caused by an increase or decrease in the thickness of the diffusion layer on the electrode [23–26]. Notably, CA is a sensitive technique that does not require redox molecules. Compared to other detection methods, timing amperometry is usually used to quantify known analytes due to its better signal-to-noise ratio [27], and CA can be applied alone or in combination with other electrochemical techniques.

(6) **Chronocoulometry (CC)**

The CC method is a determination of the change of charge over time, it may be performed during a potential scan or a fixed potential step [28]. The CC method requires an initial potential (Initial E) and a final potential (Final E) and is a change of potential from an initial potential to a final potential with charge as a function of time, which will change to a third value (usually the initial potential value) after holding the second potential for a period of time τ. Thus, the potential step experiment of the CC method can be a single or double step (Fig. 2.8).

If the Faraday current resulting from electrolysis of molecular substances in solution does not occur at any of the potentials, the response is the current due to electrode charging, and the response decreases exponentially with the peak current. Usually,

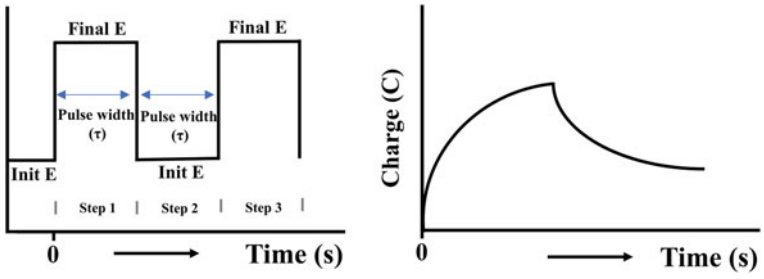

Fig. 2.8 The mechanism of CC employed for electrochemical biosensors

the Faraday response does not occur at the initial potential, but arises at the final potential. During the CC test, redox active molecules are electrolyzed as soon as they reach the surface of the working electrode; therefore, the magnitude of the CC current depends on the rate of transfer of electrochemically active material from the solution to the surface of the working electrode, i.e., the rate of diffusion of redox molecules.

CC measurements are not used for absolute concentration measurements, and are usually used to measure electrode area and diffusion coefficient.

The advantage of CC is that the signal increases with time, the response occurs in the second half of the potential, and the signal has a good signal-to-noise ratio. In addition, CC retains information about the initial response by accumulating charge during the test and can be used to detect adsorbed substances on the surface of the working electrode [29].

2.2.2 Electrochemical Impedance Spectroscopy (EIS)

EIS uses a small sine wave potential or current as a disturbance signal to generate an approximately linear response of the electrode system, thus measuring the impedance spectrum of the electrode system over a wide frequency range [30]. EIS can investigate intrinsic material characteristics or specific mechanisms that affect the conductivity, resistivity, or capacitance of an electrochemical system. EIS can measure the conductivity of a medium and indicate the interaction between the electrode surface and the target.

Electrochemical impedance sensing technology can be used to monitor changes in the electrical properties of the electrode surface caused by material modification or molecular identification. For example, changes in electrode conductance can be measured using EIS due to antigen/antibody binding and antibody-antigen reactions on the electrode surface (Fig. 2.9) [31].

2.2.3 Photoelectrochemical and Electrochemluminescent (ECL) Biosensors

The principle of photoelectrochemical (PEC) sensors relies on the process by which light irradiation is absorbed by the electrode material, generating a measurable current, also known as photocurrent. The photocurrent information is proportional to the concentration of the analyte, thus facilitating its application in analytical chemistry. Moreover, the correlation between photocurrent, wavelength, electrode potential, and solution composition can reveal valuable details about the properties, energetics, and kinetics of the optical processes involved.

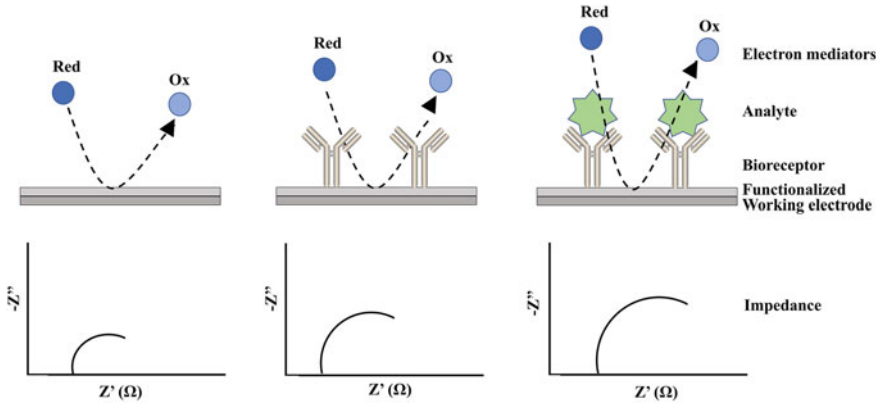

Fig. 2.9 The mechanism of EIS employed for electrochemical biosensors [32]. Copyright 2020 Springer Nature

The design principle of PEC is to enhance light absorption and optimize load separation efficiency. In the photoelectric conversion process, chemical information or analyte concentration is initially converted into a specific physical and chemical parameter. Subsequently, the heterogenous electron transfer resulting from the change in the dynamics of the interfacial charge transfer is detected as an electrical signal, to achieve the detection target.

PEC analysis can be classified into two types based on the electronic transmission method: photoanode and photogalvanic analysis. Based on the N-type semiconductor, the optical anode mode is used as a light-sensitive material and interacts with electron donors in electrolytes. In contrast, the optoelectronic cathode mode depends on the P-shaped semiconductor material that acts as an electron receptor in PEC.

ECL biosensors use the conversion of electrochemical energy into light at the electrode interface [33]. In an electrochemical reaction, luminescence is observed when the molecule is in an excited state. To initiate this phenomenon, a potential is applied to the electrode. The intensity of the emitted light is measured using a photodetector, typically a photomultiplier tube [34].

High responses are often achieved by the conversion of tags by repeater molecules, such as ruthenium complexes such as $Ru(bpy)_3^{2+}$ [35, 36]. The application of ECL to a variety of NPs is a recent development. This includes popular forms such as binary, core–shell, and doped QDs, as well as single-element NPs such as Si, Ge, Ag, Au. Metal oxide semiconductors, up-converting NPs, molecular nanoaggregates, and more complex hierarchical assemblies are also explored for their diverse ECL mechanisms and reaction pathways [37]. Compared with other luminescence methods, electrochemical luminescence instruments have been widely used due to their simplicity and low background signal.

Among all the methods described above, due to its flexibility in signal interpretation and ease of operation, voltammetry including CV, EIS, SWV, DPV, etc., is the most applicable technique that has been widely used in biosensors.

2.3 Biorecognition Elements for Electrochemical Biosensors

As the "core" component of electrochemical biosensors, Biorecognition elements have the essential function to promise the sensitivity and specificity of electrochemical biosensors. The unique structure of biorecognition elements influences the performance characteristics of electrochemical biosensors. Therefore, before conducting an in-depth analysis of electrochemical biosensors, we should first have a basic understanding of each biometric element. Loads of biorecognition elements exist, from natural existence to artificial design and synthesis. Here, we focus on several of the most commonly-used biometric elements.

2.3.1 Aptamers

Aptamers are short (usually 10–80 bases in length) single-stranded DNA or RNA oligonucleotides, with their 3D folded structures, which can identify target analytes through hydrogen bonding, electrostatic interaction, or base-stacking and other interactions, and combine with target analytes to form a stable complex [38]. The aptamer sequence of specific analytes can be manually isolated from oligonucleotide libraries through in vitro screening procedures, e.g., systematic evolution of ligands by exponential enrichment (SELEX) [39]. The main steps of the SELEX method include incubation, separation, amplification, and purification. Aptamers produced by this method can combine with a wide range of target analytes, including small molecules, ions, proteins, cells, viruses, etc. The recognition property of aptamers is similar to that of antibodies, but aptamers have some obvious advantages:

(1) Changes in temperature or the addition of exogenous reagents cause aptamers to undergo reversible conformational changes without losing their binding capacity [40, 41].
(2) Aptamers are produced by chemical synthesis, are easy to modify, and cost-effective.
(3) Since the production process of aptamers does not involve any biological system, batch-to-batch variations are passively minimized in comparison to antibody production.

With these advantages, biosensors based on electrochemical capacitors have been extensively studied. According to different design strategies, electrochemical aptamer sensors can be divided into three categories: (1) "aptamer-target" direct detection mode; (2) "target induced strand-displacement" competitive detection mode; (3) sandwich detection mode.

In direct detection mode, the aptamer can bind directly to the target and induce a conformational change in the probe, thus changing the electron transfer dynamics between the redox reporter molecule and the electrode, resulting in measurable

changes in electrical signals. Some aptamers do not undergo conformational changes after binding directly to the target, but binding to the target will also change the electron transfer dynamics between the redox reporter molecule and the electrode in the solution, resulting in measurable changes in electrical signals [42].

The competitive detection mode depends on the competition between the complementary aptamer sequence and the target [43]. Specifically, the complementary strand of the aptamer with redox reporter-labeled is fixed to the electrode and forms a dsDNA conformation with the aptamer strand. When the target analyte exists, the aptamer binds to the target is present, the complex falls off, and the electrochemical signal changes.

Sandwich electrochemical aptamer sensors typically have higher selectivity than single aptamer engagement, but require at least two aptamer recognition sites for the target analyte, and the two aptamers need to have different recognition sites. In sandwich electrochemical sensors, one aptamer modifies the signal molecule as the electrical signal element and the other aptamer with the target as the capture element [44].

2.3.2 DNAzymes

DNAzymes are oligonucleotide molecules that can bind target analytes with high specificity and enzymatic properties, which can be obtained through in vitro selection. Target-responsive DNAzymes can serve as excellent biological recognition elements because they can be rationally designed to utilize DNA complementarity for controlled capture and release. In biological systems, DNA enzymes may carry the risk of nuclease degradation. The current solution to this problem is to directly select DNAzymes from biological samples containing nucleases or to make chemical modifications to prevent exonuclease digestion. To date, DNAzyme biosensors have been developed using various amplification strategies such as hybridization chain reaction (HCR) and catalytic hairpin assembly (CHA) as promising recognition elements [45].

2.3.3 Peptides

Peptides are short chains of amino acids linked by peptide bonds and consist of 20 amino acids based on different sequence information [46]. Their building blocks are the same as proteins, so the peptide with a specific sequence can display the target recognition ability. According to different sources, peptides can be divided into natural peptides and artificial peptides. The standard synthetic protocol for artificial peptides is called Fmoc (Fluorenylmethyloxycarbonyl) and t-Boc (tert-butoxycarbonyl) solid-phase peptide synthesis (SPPS) [47, 48]. Because of its better

chemistry, conformational stability, and high specificity, easy modification, versatility, and chemical versatility. Peptide-based biosensors have been developed for electrochemical detection of various targets such as cells, proteases, nucleic acids, metal ions, and more. Generally, the combination of peptides and the target analyte will not produce measurable signals. Therefore, Peptide-based electrochemical biosensors modify redox reporter molecules on peptides to convert target information into measurable electrochemical signals.

2.3.4 Peptide Nucleic Acids (PNAs)

Peptide nucleic acids are a unique DNA analogue [49]. The backbone used is N-(2-aminoethyl) glycine, with pyrimidines and purines bound to it via methylene carbonyl groups. Similar to DNA, PNA also binds to specific RNA and DNA sequences based on Watson Crick's complementary pairing principle. Compared to hybridization between nucleic acids, PNA has the following advantages:

(1) Because PNA molecules have no electrical charge, there is no charge repulsion between RNA or DNA strands and the electrically neutral PNA strand. And the PNA/DNA(RNA) duplex has extraordinary thermal stability and is less affected by medium ion strength [50, 51].
(2) DNA/PNA hybrid complexes exhibit high sensitivity to the presence of a single mismatched base pair, allowing accurate discrimination of single base mismatches. Therefore, peptide nucleic acid biosensors are mainly used to analyze single nucleotide polymorphisms (SNP) [49].
(3) PNA is a synthetic molecule with an amide bond backbone, which distinguishes it from phosphodiester bonds present in DNA. This chemical composition makes it highly resistant to hydrolysis by protease or nuclease enzymes, providing superior stability [52].

Due to its distinctive characteristics, PNA has recently become a popular choice for building electrochemical biosensors. According to the different signal generation mechanisms, PNA-based electrochemical biosensors are mainly divided into two types: direct signal detection mode and indirect signal detection mode. The electrical signal of the direct detection model sensor is derived from changes in guanine oxidation signals before and after PNA and DNA hybridization [53]. Indirect detection uses PNA as a capture probe, introducing an electroactive label during sensor construction, and sensor signal changes are provided by the label's direct redox signal.

2.3.5 Proteins

Proteins have also been integrated into electrochemical sensors as biological recognition elements, with the gradual development of phage, bacterial, and ribosome-related technologies. For example, metal ion detection uses the interaction between metals and proteins to cause changes in their function and/or conformation. Specifically, the recognition proteins are attached to an electrode surface. Upon binding of metal ions, it changes to a more compact configuration, which opens the electrode surface for metal ions to enter and thus increases the capacitance of the electrode surface. However, this strategy requires high protein specificity [54–56]. Recently, a protein-based electrochemical biosensor has been developed for the detection of proteins and peptides. This type of sensor is based on the basic principle of conformational changes caused by proteins binding to target analytes and applied redox reporter to modify proteins to indicate the occurrence of binding events [57]. The signal transduction of this sensor is closely related to the placement of redox reporters on proteins.

2.3.6 Antibodies

Antibodies, also known as immunoglobulins, are highly soluble glycoproteins involved in the immune system's defense mechanisms. Antibodies have a Y-shaped structure and are composed of two parts, each consisting of a light and a heavy chain. The larger molecular weights are called heavy chains, the smaller molecular weights are called light chains, and the light and heavy chains are joined together by disulfide bonds [58, 59]. The light (V_L) and heavy (V_H) chain region of the antibody with a large sequence variation of about 110 amino acids near the N-terminus is called the variable region, which accounts for 1/4 and 1/2 of the heavy and light chains, respectively. The variable region determines the specificity of the antibody and is the site where the antibody recognizes and binds the antigen [60].

Various types of antibody-based biological recognition components, including monoclonal antibodies and derivatives thereof, such as antigen-binding fragments, single-chain variable fragments, or single-chain antibodies, may serve as capture or signal probes in the development of immunosensor applications [61]. Antibody-based sandwich electrochemical sensors typically employ a surface-bound antibody as the capture probe. Once attached to the target antigen, a reporter molecule, labeled as a secondary antibody, is introduced into the detection system. It binds to the same antigen, creating an electrochemical signal that depends primarily on the identification of distinct epitopes by the two antigens [62].

2.3.7 Enzymes

Enzymes are a type of protein or RNA created by living cells that are highly specialized and highly effective catalysts. The catalytic efficiency of enzymes depends on the integrity of both the primary and spatial structure of enzyme molecules. Enzyme activity may be lost due to the molecular denaturation of enzymes or depolymerization of subunits. Enzymes are classified as biological macromolecules with molecular weights ranging from 10,000 to millions of macromolecules. Enzymatic biosensors, as they are called, employ enzymes as recognition elements, which are highly specific to their substrates. Enzyme electrochemical biosensor uses enzymes as biometric molecules to capture signals generated by the reaction between the target and the enzyme through various chemical signal transducers. Enzyme electrochemical biosensors can be divided into catalytic systems or enzyme affinity systems according to the principle of biological selectivity. Usually, the signals are proportional to the concentration of the target, so as to achieve quantitative analysis of the target.

Enzymatic sensors have wide applications in detecting different substances in biological specimens, including glucose, cholesterol, or lactic acid, and in analyzing toxicity levels in environmental monitoring, food inspection, quality assurance, and biomedical and pharmaceutical sensing [63–68].

2.3.8 Lectins

Lectin is a glycoprotein or glycobinding protein that can be isolated from a variety of sources, including plants, invertebrates, and higher animals. Because of its ability to agglutinate red blood cells, it is referred to as lectin, commonly used as plant lectin [69, 70]. Lectins' ability to recognize complex carbohydrate structures found in glycoproteins and glycolipids, mainly located in cell membranes, specifically glycogroups present on the surface. Lectins have the unique ability to bind exclusively to a specific type of glycogen group [71–74]. This forms the basis for the development of lectin sensors that can directly examine intact cell surfaces, as well as glycolipids, glycoproteins, and sugars present in membranes [75–77]. There are some disadvantages, such as the need to label lectins, which can reduce the performance of the biosensor.

2.3.9 Epitopes

Epitopes are short continuous peptide chains [78–81] that specifically recognize the corresponding antibodies and have the advantage of being easy to obtain, synthesize and modify, which provides a convenient option for antigen–antibody interactions.

Epitopes can specifically recognize the corresponding antibody, and because epitopes are easy to obtain, synthesize and modify, it provides a scheme for the detection of antibodies in the blood. It is worth noting that the rapid response time of epitopes to targeted antibodies is up to minute, so epitope sensors are a powerful means of antibody analysis [82–85].

2.3.10 Molecularly Imprinted Polymers (MIPs)

MIPs are widely used as recognition elements for biosensors due to their ease of synthesis, cost-effectiveness, flexibility in the design of recognition elements for target analytes, and the ability to mimic natural enzyme analogs [86]. MIPs are synthetic matrices used as detection materials in biosensor fabrication due to their exceptional recyclability, thermal stability, and specificity in comparison to biorecognition components. Based on the function, charge, and morphology of the target, imprinted polymer receptors exhibit selective binding, causing changes in their structural characteristics and signals, enabling effective detection [87–89]. However, several challenges remain for MIP technologies, including low affinity, incomplete removal of template molecules, low conductivity and electrocatalytic activity, and inefficient mass transfer.

2.4 Conclusion and Outlook

Electrochemical biosensors with high specificity and sensitivity for target detection are of great importance for clinical prevention, diagnosis, and prognosis of related diseases. The application of electrochemical biosensors focuses on the selection of appropriate test methods and the identification of the original components, which can effectively improve the detection performance of the sensor. Common electrochemical biosensors can be classified into voltammetric/ampere, impedance, potential, conductivity, photoelectrochemical and electrochemiluminescent, field effect transistors, etc. Biorecognition elements may be tissue, living cells, an enzyme, antibody or antigen, and nuclei. Due to the different detection methods and characteristics of biometric originals, the corresponding sensors have different research priorities. Although electrochemical sensor detection has made great progress in recent years, there are still some challenges in translation from laboratory to clinic. For example, most sensors can only detect a single target, instability, and poor immunity to interference.

The simultaneous detection of multiple markers helps In the accurate diagnosis of diseases, while ability of electrochemical biosensors to identify multiple analytes makes them very popular in practical applications. The senso's the ability to achieve peak separation and improved sensitivity to specific analytes is critical to the success of this application. Peak separation is closely related to the detection method,

nanomaterials, and the signal molecule used. The use of nanomaterials and signal molecules facilitated the precise segregation of various analytes, allowing accurate quantitative measurements. CV, SWV, and DPV can be altered by nanomaterials or by the use of multiple signal molecules, allowing complete differentiation of multiple peak currents, and enabling simultaneous detection of multiple targets [90, 91].

The simultaneous use of multiple different potential signal molecules is a prerequisite for the simultaneous use of multiple targets, and the signal molecules commonly used in electrochemical sequencing sensors currently including methylene blue, ferrocene, anthraquinone, thionine, nile blue, woster's blue, multicolor electrochemiluminescence, luminol, isoluminol, and their derivatives, acridinium ester derivatives, ALP, and HRP has been also explored for electrochemical biosensors. Methylene blue is the most widely used signaling molecule in electrochemical biosensors. Most current electrochemical studies use a single redox molecule, i.e., MB or Fc [92, 93]. In addition, the position of the redox marker affects DNA interfacial electrochemistry. Specifically, limitations in ion accessibility affect the current of the redox marker located at the bottom of the DNA monolayer, while the redox marker at the top is unaffected [94]. It is important to note that there are generally three requirements for the simultaneous use of multiple reporter molecules. First, their potential does not overlap, allowing both to be monitored simultaneously. Second, both are fairly stable. Finally, their physical properties are very similar, so they respond consistently to environmental changes that cause drift. Further improvements and development of nanomaterials and signal molecules will provide sensitive and selective substrates by which multianalyte detection can be performed.

The design of novel nucleic acid probes and the development of related interfacial sensing principles are important factors driving the development of sensing technologies. In the field of electrochemical biosensing, a lot of related research work has been done by many excellent groups at home and abroad. Self-assembled single-molecule layers on the sensor surface, which act as molecular closure layers, can eliminate or reduce the non-specific adsorption of DNA probes on the electrode surface to improve the sensitivity, specificity, and lifetime of electrochemical sensors. Researchers have greatly enhanced antifouling properties by using alkanethiol molecules with different hydrophilic portions [95], alkanethiol molecules with different hydrophobic portions [96], and mimetic phosphatidylcholine [93] to improve detection performance (e.g., stability, sensitivity, and specificity).

The combination of multifunctional materials, recognition elements, and electrochemical methods enhances selectivity and stability. To achieve this, researchers are exploring innovative materials and diverse signal molecule potentials, redox peak potential/current, amplitude, scan rate, bionic film, etc. These techniques have been used to improve one or both of these deficiencies. Although electrochemical biosensors face some challenges, they have a significant clinical impact in diagnosing and treating diseases. In addition, these sensors play a crucial role in collecting data for biomedical research.

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