Trends in Analytical Chemistry 79 (2016) 9–22

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Electrochemical coding strategies using metallic nanoprobes for biosensing applications

Enrique Valera [a,*], Alejandro Hernández-Albors [b,c], M.-Pilar Marco [b,c]

[a] Department of Chemistry, University of Illinois at Urbana – Champaign, 600 South Matthews Avenue, Urbana, IL 61801, USA
[b] Nanobiotechnology for Diagnostics (Nb4D) Group (IQAC-CSIC), Barcelona 08034, Spain
[c] CIBER de Bioengineria, Biomateriales y Nanomedicina (CIBER-BBN), Barcelona, Spain

ARTICLE INFO

Keywords:
Electrochemical biosensors
Electrochemical metallic nanoprobes
Multiplexing detection
Nanoparticles synthesis
Conjugation protocols
Clinical diagnosis
Food safety

ABSTRACT

As it is well known, electrochemical biosensors offer several very interesting advantages such as portability, low power consumption, low price, etc. However, in most of the cases these types of biosensors are weak in the simultaneous multiplexing detection. In general, these techniques are developed for single analyte detection, and when multiplexing is required, spatial separation is applied. As an alternative, the possibility of using electrochemical nanoprobes based biosensor in different configurations allows the implementation of multiplexed devices and opens the door to the point-of-care systems.

This review is focused in the use of electrochemical metallic-nanoprobes (electroactive metallic-nanoparticles chemically modified with a specific biological element) for electrochemical biosensing applications. First, the manuscript focus their attention in the electroactive nanoparticles synthesis, and then in the most common conjugation protocols. Finally, the work discuss about the usefulness of these nanoprobes for biosensing applications, especially in the fields of clinical diagnosis and food safety.

Contents

1. Introduction

As is well known, biosensors are analytical devices mainly consisting of a specific biological element and a transducer. While the biological element is responsible for the specific analyte recognition, the aim of the transducer is to convert the biorecognition process into a measurable signal. During the last 10 years few types of electrochemical biosensors have claimed the label-free detection/analysis of biomolecular interactions. Interested examples are the hybridization chain reaction (HCR) [1] and the impedimetric techniques [2]. While in the case of the HCR the binding of DNA to a substrate can accomplish the roles of recognition and signal amplification without any external inputs, in the case of the impedimetric biosensors the detection technique is based on the change in the electric impedance on the surface of the interdigitated electrodes. Although interested, these techniques are weak in the simultaneous multiplexing detection. In general, these techniques are developed for the detection of a

* Corresponding author. Tel.: +1 217 244 8129; Fax: +1 217 265 6290
E-mail address: evalerac@illinois.edu (E. Valera).

http://dx.doi.org/10.1016/j.trac.2015.12.014
0165-9936/

single analyte, and when multiplexing is required, spatial separation is applied [3].

When label-free methods are not applied, in order to have a measurable signal, scientists require the use of chemical labels. Concerning this, the use of enzymes (e.g. horseradish peroxidase) as electroactive tags is widespread [4–6]. However, their use requires the use of secondary mediators and substrates. Likewise, an additional limitation of their use as labels is also the difficult development of simultaneous multiplexing detection [7]. In the last years, many efforts have been conducted to develop alternatives to the use of enzymes as electroactive tags based on the use of biohybrid nanoparticles (nanoprobes).

In this review, electrochemical metallic nanoprobes are defined as electroactive metallic nanoparticles (e.g. AuNPs, CdS) chemically modified (bioconjugated) with a specific biological element (e.g. Ab, DNA). Although the use of, e.g. Ab in different detection techniques such as ELISA (Enzyme Linked ImmunoSorbent Assay) is well-established, the development of Ab-NPs nanoprobes has allowed an improvement in detectability and sensitivity in different techniques, leading the way toward the development of diagnostic tools much simple and sensitive. Likewise, electrochemical biosensors based on nanoprobes offer, in addition to an inherently amplified signal and a high selectivity, the possibility of simultaneous multiplexing as this was exceptionally reported by Prof. Wang [8–10]. In the technology developed, named Electrochemical Coding Technology, the simultaneous detection of multiple DNA [8], Ab [9], and aptamers [10] targets was described based on the use of three inorganic semiconductors nanocrystals (CdS, PbS and ZnS) as labels. As a different redox potential is associated to each metal ion, each nanoprobe offered a voltammetric signature with distinct electrical signals for the corresponding targets. To do that, a sandwich assay, involved a dual affinity event was performed. Thus, the corresponding amount of each target (three, one per type of particle) was captured by one of the three receptor-coated magnetic beads. Then, a solution of each nanoprobe (QD-biological element conjugate) was added and mixed. After the immunoassay, the resulting complex (magnetic particle / capture element / target / nanoprobe) was resuspended in a HNO_3 solution. Dissolution of the semiconductors nanocrystals thus proceeded. Following a magnetic separation, the acid solution (containing the dissolved metal ions) was transferred into an electrochemical cell. Finally, square-wave anodic stripping voltammetry (SWASV) measurements of the dissolved QDs were carried out. Schematic explanation of the Electrochemical Coding Technology is shown in Fig. 1.

Later, this approach was interestingly applied to the development of a barcode system [11]. Thus, the position and amplitude of the current peaks (displayed at the redox potentials of the Zn, Cd and Pb) were used as linear analog coding signals, and then digitally processed in order to create barcodes.

The advantages, above commented, of the electrochemical nanoprobes based biosensors open the door to these devices of interesting applications such as clinical diagnosis, detection of antibiotics and pesticides in food safety, pathogenic detection and environmental monitoring.

This review aims to illustrate with recent examples all the achievements and new strategies of the use of electrochemical metallic nanoprobes for biosensing applications. To this goal, first we will focus our attention in the synthesis of the electroactive metallic nanoparticles, describing the most used chemistry techniques. Then, we will center our interest in the most common conjugation protocols, basically Ab-NPs and DNA-NPs nanoprobes, emphasizing how the conjugation affects the detectability of the biological elements. Finally, we will discuss about the usefulness of these nanoprobes for biosensing applications, especially in the fields of clinical diagnosis and food safety.

2. Synthesis of the metallic nanoparticles

As can be concluded from Section 1, current approaches in nanotechnology are closely related with electrochemistry and electroanalysis, between other fields. As consequence, certain nanomaterials, such as nanocrystals or QDs, are attractive probe candidates because their small size and high surface-to-volume ratio, chemically tailored properties and robustness. Functionalization of different nanomaterials provide almost unlimited combinations of various compositions, dimensions and shapes of materials, that could be conjugated to different biomolecules such as DNA, proteins and peptides among others, developing nanoprobes for different applications, i.e. nanodiagnostics. In this Section, we broach different synthesis strategies for most nanomaterials used in biosensing, mainly AuNPs, QD and metallic nanocrystals such as CdS, Pbs and ZnS. All strategies described in this section are summarized in Table 1.

2.1. Synthesis of gold nanoparticles (AuNP)

Gold nanoparticles have been widely studied for their unique optical properties because of their high absorption coefficients, allowing high sensitivity in optical detection [12], but also for their catalytic activity in development of highly sensitive detection methods in the field of electrochemistry [13] and/or with metal deposition for signal [14]. There are two mainly reasons that could explain the usefulness of these nanoparticles, first the characteristic surface plasmon band giving the nanoparticles an intense color, allowing a control in the synthesis procedure and in the characterization of final products; and second, the easiness of the AuNPs to be functionalized by direct incubation with thiol and carboxylic compounds, allowing their fast and simple attachment to different biomolecules. The morphology and surface chemistry of nanoparticles could be usually controlled by synthetic strategy chosen. AuNPs are synthesized by chemical or electrochemical reduction of a Au(III) in the presence of capping agent, blocking the nanoparticle size in nanometer range and stabilizing the colloidal structure in the solvent used. A common approach is to use capping agents with high affinity for gold, such as thiolated agents. Most common used methodology to prepare AuNPs is the citrate reduction method, described by Turkevich et al. [15]. The use of citrate as a capping agent allows the replacement by other capping agents in an easily way, such as thiol agents, bringing further attachment of biological probes, such as DNA, peptides or proteins for biosensors development [16,17]. Other methodologies for AuNPs synthesis based in reduction methods, instead of citrate reduction, are described in literature. These methods allow the control of the nanoparticle size by slow addition of the reactants involved [18,19]. More information regarding synthesis of gold nanoparticles methodologies could be found in the cited literature [20].

2.2. Synthesis of quantum dots (QDs)

As is well known, quantum dots are nanocrystal made of semiconductor materials (III-V or II-VI), such as ZnS, ZnSe, CdS, CdSe, CdTe. These crystals, which are small enough to exhibit quantum mechanical properties, confine their exactions in all three spatial directions. Many methodologies for the synthesis of QDs are reported in literature. *"top-down"* strategies, such as lithography has allowed the synthesis of QDs soaked in semiconductor thin layer [21]. However, the most common used protocol for the synthesis of QDs are based in *"bottom-up"* strategies, due to the stability of the colloidal suspension of the obtained QDs. "Bottom-up" strategies could be divided in *wet-chemical* and *vapor-phase* methods. Wet-chemical involves conventional precipitation methods such as microemulsions, sol-gel [22,23], hot-solution decomposition [24], and sonic waves or microwaves [25] among other. On the other hand,