Electrochemical sensors for biomedical applications based on DNA hybridization detection using gold nanoparticle tags

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Abstract

Gold nanoparticles (AuNPs) stabilized by various functional groups have many applications, one use being as labels in biological systems. AuNPs have been used to signal hybridization in various DNA detection assays. The most important AuNPs based DNA detection strategies used so far will be revised. A magneto sensor based on the direct detection of AuNPs has been developed by our group and current results obtained will be described as well. Some problems related to the application of the developed AuNPs based genosensors in clinical analysis (i.e breast cancer or cystic fibrosis related DNA) and the future challenges will be discussed.

Keywords: Gold nanoparticles, DNA hybridization, genosensors, stripping voltammetry, biomedical applications.

1. Introduction

Detection of specific DNA sequences is of great importance in clinical diagnostics, gene therapy and a variety of biomedical studies for example for the detection of nucleic acids from pathogenic organisms. Electrochemical assays of nucleic acids have received considerable attention in connection with the detection of DNA hybridization. [1-4] DNA hybridization biosensors are a very attractive topic in the clinical diagnostics of inherited diseases and the rapid detection of infectious microorganisms.

Diverse techniques for detection of DNA hybridization have been developed. In most of them the hybridization event and electrochemical detection are carried out on the electrode surface. [5-9] In other cases, the electrode only acts as a detector of the hybridization event, [10-16] which occurs in a separate step, either because it takes place in a microwell [10] or because the hybridization event occurs on the surface of magnetic beads, which are separated from the hybridization solution and then redissolved [11, 12].

Electrochemical transduction of the hybridization event can be classified into two categories: label-based and label-free approaches. The label-based approach can be further subdivided into intercalator/groove binder, non–intercalating marker, and nanoparticle.

On the other hand, the label-free approach is based on the intrinsic electroactivity of the DNA purine bases or the change in interfacial properties (e.g. capacitance and electron transfer resistance) upon hybridization [17].

The sensitivities of label-based approach depend mainly on the specific activity of the labels linked to the oligonucleotide probe. Radioisotopic [16], fluorescent [18] and enzymatic [6,8,15] labels have been commonly used. The nanoparticles offer attractive properties to act as DNA tags [19].

Nowadays, the most widely used techniques in the detection of DNA hybridization are based on metal nanoparticles labeling [20].

The fact that nanoparticles present an excellent biocompatibility with biomolecules and display unique structural, electronic, magnetic, optical and catalytic properties have made them a very attractive material [21,22].

The gold nanoparticles (AuNPs) functionalized with oligonucleotides are extensively used as tags in many highly sensitive and selective DNA recognition schemes by means of electrochemical sensing.

The integration of nanoparticles DNA labelling technology in chips has been also studied in DNA analysis. [23]

Several groups, including ours, have developed AuNPs–based electrochemical DNA hybridization assays.

The aim of this review is firstly to give a historic prospective of AuNPs application to biology and medicine and on the other hand to try to overview the most recent developments in this field, principally in connection with its use as tags.
2. Synthesis of AuNPs

Different chemical, electrochemical and physical synthesis methods have been developed for the preparation of AuNPs of different sizes and shapes [21, 24-26].

The most common way of AuNPs preparation is the chemical reduction method of soluble metal salts such as potassium tetrachloraurate, by suitable reducing agents (e.g., hydrogen, boron hydride, methanol, citric acid, and others), and electrochemical reduction [24].

In order to stabilize the nanoparticles, to control their size and shape and to prevent them from aggregating, organic ligands need to be added which are typical colloid chemical stabilizers or electron-donor ligands, like phosphines, amines or thiols, which stabilize the particles electrostatically or sterically [27,28].

The classical chemical methods are based on the use of tetrachloroauric acid and other precursors, but there are other alternatives.

Li et al., [29] prepared AuNPs (13 nm) in an aqueous solution following the chemical reduction method [24] and Brust et al., [30] have developed a two-phase liquid system for preparing AuNPs in organic phase and posteriorly extended this synthesis to p-mercaptophenol-stabilized AuNPs in a single phase system [31] which opened a new way to the synthesis of AuNPs stabilized by a variety of functional thiol ligands.

Recently, Newman and Blanchard [32] reported the controlled formation of AuNPs using amine reducing. The reduction of HAuCl₄ occurs due to transfer of electrons from the amine to the metal ion, resulting in the formation of Au⁰, with the subsequent formation of AuNPs.

3. AuNPs based DNA detection strategies

3.1 Detection via dissolving into gold ions

AuNPs bound to a DNA can be detected indirectly, by oxidatively dissolving the AuNPs into aqueous metal ions and then electrochemically sensing the ions.

A variety of stripping detection of hybridization events based on gold-DNA probes via dissolving into gold ions have been reported [10,33,34]. The great majority of the AuNPs-based assays have been based on chemical dissolution of the AuNPs tag (in a hydrobromic acid/bromine mixture) followed by accumulation and stripping analysis of the resulting Au³⁺ solution.

The HBr/Br₂ solution is highly toxic and therefore methods based on direct electrochemical detection of AuNPs tags, which would replace the chemical oxidation agent, are urgently need [35].

3.2 Direct detection of the AuNPs onto the sensing electrode without the need for dissolving

Direct detection of AuNPs but not in connection with the detection of DNA hybridization was reported earlier by our group (see Figure 1), and Costa-García’s group [36,37]. In our protocol a graphite-epoxy composite electrode (GECE) is used, in which surface the AuNPs are adsorbed.

![Figure 1 Schematic diagram of the electroanalytical protocol for direct voltammetric determination of AuNPs using a graphite-epoxy composite electrode GECE. Obtained with permission from [36]](image)

Ozsoz et al., [38] described an electrochemical genosensor based on colloidal AuNPs for detection of Factor Leiden Mutation for which the oxidation signal of colloidal gold was measured following the direct detection by using differential pulse voltammetry. Direct electrochemical oxidation of the AuNPs was observed at a stripping potential of approximately +1.2 V.

Recently our group reported a novel nanoparticle-based detection of DNA hybridization based on magnetically induced direct electrochemical detection of the 1.4 nm Au₆₇ quantum dot tag linked to the target DNA. The Au₆₇ nanoparticle tag was directly detected after the DNA hybridization event, without need of acidic (i.e., HBr/Br₂) dissolution [35].

Figure 2A-E represents the main steps involved in the assay. The binding of the probe DNA2 to the paramagnetic beads (A) is achieved via the streptavidin-biotin interaction. The resulting DNA2 modified paramagnetic beads (B) are hybridized then with the target DNA1, marked with Au₆₇ nanoparticle in the ratio 1:1. The resulting Au₆₇-DNA1/DNA2 paramagnetic bead conjugate (C) is collected magnetically on the surface of a transducer with built-in magnet (Figure 2D), which is triggering the direct electrochemical detection (E). The formed Au₆₇-DNA1/DNA2-paramagnetic
bead conjugates with a single DNA duplex link between the nanoparticle and the paramagnetic bead ensure their individual handling and consequently the sensitivity of the assay is not hindered by sharing one gold tag by several DNA strands as it was in previous assays [35].

The use of AuNPs also for amplification and augmentation of electrochemical detection in DNA sensors using a variety of detection schemes has been recently reviewed [40,41].

Kawde and Wang, [41], reported a triple-amplification bioassay that involves a hybridization event in which the DNA target is labeled with the gold-loaded carrier sphere. This assay combines the use of carrier-bead amplification platforms with an ultrasensitive electrochemical stripping detection of multiple gold nanoparticle tracers. Also a sensitivity enhancement was achieved.

4. Biomedical applications

Several protocols with potential biomedical application have been developed. Table 1 summarizes information of some typical electrochemical genosensors based on DNA hybridization detection using gold nanoparticle tags.

Authier et al., [10] developed an electrochemical DNA detection method for the quantitative detection of amplified human cytomegalovirus DNA sequence (HCMV DNA). The HCMV DNA was immobilized on a microwell surface and hybridized with the corresponding complementary oligonucleotide-modified Au-NPs. The resulting conjugate was treated with HBr/Br₂, resulting in the oxidative dissolution of the AuNPs. The solubilized Au³⁺ ions were then electrochemically reduced and accumulated onto the electrode and subsequently determined by anodic stripping voltammetry using a sandwich-type screen-printed microband electrode.

Table 1. Electrochemical genosensors using gold nanoparticle tags, with potential biomedical applications reported.

<table>
<thead>
<tr>
<th>Target DNA</th>
<th>Sample</th>
<th>Potential biomedical application</th>
<th>Detection technique</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of the target DNA= 406 bases</td>
<td>Cell culture</td>
<td>Human cytomegalovirus</td>
<td>ASV at SPMBE</td>
<td>[10]</td>
</tr>
<tr>
<td>Biotin-5’GAA CAA AAG GAA GAA AAT C</td>
<td>Synthetic</td>
<td>Breast cancer</td>
<td>PSA at SPEs</td>
<td>[33]</td>
</tr>
<tr>
<td>5’TCT CAA CTC GTA-phosphate(CH2)3-CONH-CH(CH2SH)-CONH-(CH2)6-OH</td>
<td>Synthetic</td>
<td>_</td>
<td>DPV at M-GECE</td>
<td>[35]</td>
</tr>
<tr>
<td>Factor V-Wild-Type Target: 5’-GAC AGG CGA GGA ATA CAG GTA TT-3’</td>
<td>Real PCR amplicons</td>
<td>Detection of Factor V Leiden Mutation</td>
<td>DPV at pencil-graphite electrode</td>
<td>[38]</td>
</tr>
<tr>
<td>Biotin-5’GAA CAA AAG GAA GAA AAT C’</td>
<td>Synthetic</td>
<td>Breast cancer</td>
<td>DPV at M-GECE</td>
<td>[42]</td>
</tr>
<tr>
<td>5’ATA TAT ATA GCA GCA GCA GCA GCA GCA GAC GAC GAC GAC GAC TCT C3’</td>
<td>Synthetic</td>
<td>Cystic Fibrosis</td>
<td>DPV at M-GECE</td>
<td>[42]</td>
</tr>
</tbody>
</table>

DPV=Differential pulse voltammetry; ASV=Anodic Stripping voltammetry; SPMBE=Sandwich-type screen-printed microband electrode; PSA=Potentiometric stripping analysis; SPEs=Screen-printed electrodes; M-GECE=magnetic graphite-epoxy composite electrode.

An electrochemical genosensor based on colloidal AuNPs for detection of Factor Leiden mutation was reported by Ozsoz et al., [38]. The work has a
realistic potential application, since experiments were carried out using real PCR-amplicons. Wang et al., developed a protocol for the detection of nucleic acid segments related to the breast cancer BRCA1 gene in a metallogenomagnetic assay [33].

Recently we have worked on the application of AuNPs as oligonucleotide labels in DNA hybridization detection assays using a magnetic graphite-epoxy composite electrode (M-GECE) developed previously by our group [37].

Different AuNPs-DNA conjugates such as those obtained via biotin-streptavidin [42] or via thiol linkage [35] can be observed in our protocols (see Figures 2 and 3). Both have proved to be highly sensitive in detecting nucleic acid probes in hybridization studies.

The potential use of the M-GECE in biomolecular detection and medical diagnostics seems with special interest.

The M-GECE genosensor have shown great promise for rapid, sensitive, reliable, and low cost DNA hybridization detection in clinical diagnostics.

The possible nonspecific adsorption is one of the limitations which must be overcome before the implementation of the M-GECE in biological fluids.

New challenges and requirements for the design of an ideal electrochemical genosensor suitable for nucleic acid analysis in biomedical applications include high sensitivity and high specificity protocol that can be carried out in a relatively short time offering at the same time low detection limit. On the other hand, systems that can be miniaturized and automated present a great advantage over conventional technology, especially if detection is needed in the field.

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