Review

Electrochemical Sensing of DNA Using Gold Nanoparticles

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Abstract
The electrochemical properties of gold nanoparticles (AuNPs) have led to their widespread use as DNA labels. This fact has improved the design strategies for the electrochemical detection of DNA through hybridization event monitoring. The reported DNA hybridization detection modes are based on either AuNP detection after dissolving or the direct detection of the AuNP/DNA conjugates anchored onto the genosensor surface. Various enhancement strategies have been reported so as to improve the detection limit. Most are based on catalytic deposition of silver onto AuNP. Other strategies based on the use of AuNPs as carrier/amplifier of other labels will be also revised. The developed techniques are characterized by sensitivities and specificities that enable further applications of the developed DNA sensors in several fields.

Keywords: Gold nanoparticles, DNA labeling, DNA sensing, DNA immobilization, DNA hybridization, stripping voltammetry

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1. Introduction

Nanotechnology refers to research and technology development at the atomic, molecular, and macromolecular scale, leading to the controlled manipulation and study of structures and devices with length scales in the 1 to 100 nm range. Objects at this scale, such as nanoparticles (NPs) take on novel properties and functions that differ markedly from those seen in the bulk scale [1].

NPs represent an excellent biocompatibility with biomolecules and display unique structural, electronic, magnetic, optical and catalytic properties which have made them a very attractive material [2] as labels in the detection of DNA hybridization [3] or various electrochemical techniques [5] between other applications.

Metal NPs have been known since antiquity and AuNPs, known for their use in staining glass, have been the subject of systematic study since 1857 with the pioneering work of Michael Faraday on the color of colloidal gold [6].

Gold nanoparticles (AuNPs) are the most frequently used among all the metal NPs. Colloidal gold or AuNPs is a suspension of submicrometer-sized particles of gold in a solvent usually water. The AuNPs suspension has usually either an intense red color (for particles less than 100 nm), or a dirty yellowish color (for larger particles) [7]. The NPs themselves can come in a variety of shapes: spheres, rods, cubes, triangles, ellipsoidal, are some of the more frequently observed ones. AuNPs of different shapes and sizes were reported by Dos Santos et al. [23].

Michael Faraday was the first scientific to attribute the red color of colloidal gold to its finely divided state and the modern scientific evaluation of colloidal gold did not begin until his work [6] and later by the other pioneering works of Turkevich [24] and Frens [8]. Synthesis of novel AuNPs with unique properties is subject of substantial research, with applications in a wide variety of areas, including medicine, electronics, nanotechnology [7]. Particularly important are optical, electrical, magnetic and catalytic properties. The intrinsic properties of a metal nanostructure can be tuned by controlling its size, shape, and crystallinity. [20, 25].

The size and properties of AuNPs are highly dependent on the preparation conditions [26]. The shown biocompatibility [27] makes the AuNPs as very interesting for several bioanalytical applications in general and for biosensor application particularly. They have become of great importance in different DNA detection methods such as optical, electronic [30]. Optical biosensors based on fluorescence are extraordinarily sensitive, and arrays containing thousands of unique probe sequences have been constructed [31].

Bla et al. [32] reported a novel optical readout scheme for AuNPs-based DNA microarrays on “Laser-Induced Scattering around a NanoAbsorber” which provides direct counting of individual NPs present on each array spot and stable signals, without any silver enhancement. Given the detection of nanometer-sized particles the linear dynamic
range of the method is particularly large and well suited for microarray detection.

Owing to their attractive properties AuNPs have been the most extensively NPs used so far in electrochemical biosensor applications in general and for DNA analysis particularly [33 – 35]. The aim of this review is to summarize the recent advances in AuNPs-based electrochemical DNA sensors emphasizing their use as DNA labels.

2. Synthesis and Characterization

2.1. Synthesis

After the first reported synthesis by Faraday [6], standard protocols for the preparation of AuNPs in aqueous solution were established by Turkevich [24, 36] and refined later by G. Frens [8]. Generally monodisperse spherical AuNPs, suspended in water with a diameter of around 10 – 20 nm, have been used to be produced. HAuCl₄, dissolved in deionized water and heated then until boiling is used and then sodium citrate solution is added. The color of the solution will gradually change from faint yellowish to wine-red. The sodium citrate first acts as a reducing agent, and later the negative citrate ions around the AuNPs surface introducing the charge that repels the particles and prevents them from aggregating. The formation of AuNPs can be observed by a change in color since small NPs of gold are red. Subsequently several other methods have been developed to prepare AuNPs with different sizes and shapes [37 – 40]. Several reducing agents/modes such as citric acid, [8, 24, 41] sodium borohydride (NaBH₄), [38, 42, 43] sodium ascorbate, [44] amines, [41, 45] and sonochemical [46] or electrochemical [38, 47] reduction have been reported. The stability of the colloidal suspension is the most important prerequisite in utilizing AuNPs. In order to stabilize the NPs, to control their size and shape and to prevent them from aggregating organic ligands as typical colloid chemical stabilizers or electron-donor ligands, like phosphines, amines or thiols, which stabilize the particles electrostatically or sterically [48] have been also used.

Alkylthiol passivated AuNPs of around 5 – 6 nm have been reported by Brust et al. [42] by using NaBH₄ as reducing agent. Ultrasound has become an important tool for the synthesis of metal NPs [49]. Based on this method [50] AuNPs have been produced by using hydroxyl and sugar pyrolysis radicals as reducing agents.

Colloidal solutions containing AuNPs of various sizes (5 to 80 nm) were prepared by a new method introduced by Slouf et al. [51]. They used a combination of the techniques already described [24] based on several-step reduction of HAuCl₄ water solution by combination of Na(BH₄)₂ and NH₂OH solutions. Recently, Newman and Blanchard [41] 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 Au⁺, with the subsequent formation of AuNPs.

Kimling et al. [52] have examined in detail the growth of AuNPs by reduction by citrate and ascorbic acid to explore the parameter space of reaction conditions. It is found that AuNPs can be produced in a wide range of sizes, from 9 to 120 nm, with defined size distribution, following the earlier work of Turkevich [24] and Frens [8].

The synthesized AuNPs have been characterized by means of various optical (spectroscopic, microscopic etc.) or electrochemical techniques so as to obtain information about structure, morphology, size and composition including their electrochemical behavior.

2.2. Optical Characterization

Transmission electron microscopy (TEM) has been used extensively as a way of AuNPs characterization. Nevertheless other techniques such as scanning electronic microscopy (SEM), scanning tunneling microscopy (STM), atomic force microscopy (AFM) and X-ray powder diffractometry (XRD) have been also used.

In a study reported by Dos Santos et al. [23] AuNPs of different shapes and sizes produced through the reaction of fulvic acid (FA) and gold tetrachloride were characterized by high-resolution transmission electron microscopy (HRTEM) and their optical field enhancing properties tested in surface-enhanced Raman scattering (SERS). Nuclear magnetic resonance (NMR) spectroscopy and TEM were also used to characterize AuNPs stabilized by 2,2’ : 6,2’-terpyridinylolanehtiol [53].

Chirea et al. [54] described the construction and characterization of structural and charge transport properties of electrostatically layer-by-layer self-assembled polyelectrolyte/AuNPs films composed of cationic poly(1-lysine) (pLys) and mercaptosuccinic acid stabilized AuNPs with an average diameter of 2.5 nm. The assemblies were characterized using UV-vis absorption spectroscopy, AFM as well as electrochemical methods.

Frenkel et al. [55] characterized their thiol-protected AuNPs by using TEM to measure their size distribution and extended X-ray absorption fine-structure (EXAFS) to measure their coordination numbers and nearest-neighbor distances. The authors presented a self-consistent analysis of the EXAFS spectroscopy data of ligand-stabilized metal nanoclusters. Their method employs the measurement of the coordination numbers and metal-metal bond-length decrease that can be correlated with the average diameter and structure of the NPs in the framework of the surface tension model and different structural motifs. To test the method, they synthesized and analyzed a series of dodecanethiol-stabilized AuNPs where the only control parameter was the gold/thiol ratio, varied between 6:1 and 1:6.

Recently, Scaffardi et al. [56] sized AuNPs by optical extinction spectroscopy. The measurement of optical extinction is used to determine the size of nearly spherical AuNPs suspended in solution, produced by a ‘reverse micelles’ process. For the small particles used in their work there had a very good agreement between the determina-
tion of the radius by optical methods and TEM techniques. Extinction measurements with a commercial spectrophotometer can be an economical and simple alternative when special electronic microscopy (TEM or SEM) is not available.

2.3. Electrochemical Characterization
Quinn et al. [57] reported the preparation of hexanethiol-capped Au (C6S-Au) particles to obtain thiol protected AuNPs (0.81 nm, Au147) so-called monolayer protected clusters (MPCs) with improved monodispersity. In order to investigate MPCs redox properties electroanalytical techniques: Cyclic voltammetry (CV), differential pulse voltammetry (DPV), and chronoamperometry at a Pt microelectrode were used. A DPV response for the as-prepared Au147 MPCs showing 15 evenly spaced peaks characteristic of charge injection to the metal core was obtained. The authors presented the first report of 15 quantized double layer charging peaks at room temperature which is a clear confirmation that MPCs are indeed multivalent redox species.

The reaction of the phosphine-protected AuNPs Au55 (PPh3)12Cl6 ("Au55") with hexanethiol (C6H13SH) and other thiols with the aim to obtain relatively monodisperse NPs was described by Balasubramanian et al. [58]. The voltammetry of the reaction product with C6H13SH displays a well-defined pattern of peaks qualitatively reminiscent of Au38 NPs, but with quite different spacing (0.74 ± 0.01 V) between the potentials of initial oxidation and reduction steps (electrochemical gap). Correction of this "molecule-like" gap for charging energy indicates a HOMO-LUMO gap energy of about 0.47 V. CV and Osteryoung square-wave voltammetry (OSWV) were carried out in a single compartment cell containing 1.4 mm Pt disk working, Pt wire counter, and Ag wire quasireference electrodes (QRE), under argon.

AuNPs electrodeposited onto glassy carbon-electrodes (AuNPs/GC) in the presence of two different additives: cysteine and iodide ions were studied by Deab et al. [59]. The electrochemical characterization of the AuNPs/GC was performed via the measurements of the reductive desorption patterns of a thiol (e.g., cysteine) self-assembled monolayer as well as the CV response toward the oxygen reduction reaction in alkaline medium.

The redox behavior of a ruthenium-terpyridine complex of AuNPs (Ru-Tpy–Au, core size 5.5 nm) was studied also by CV using platinum wire as counter and glassy carbon or gold as working electrodes [53].

3. DNA Immobilization
Owing their large specific surface area and high surface free energy, AuNPs can strongly adsorb DNA. The negative charges as a result of the adsorption of citrate (used in most of the fabrication processes) enhance the electrostatic adsorption between AuNPs and DNA strands. DNA can also be immobilized onto AuNPs through special functional groups such as thiols and others, which can interact strongly with AuNPs [60–62].

DNA oligonucleotides that contain several adenosyl phosphothiolate residues at their ends have been used to interact directly with the metal surface of NPs [63]. A limited number of linkers to immobilize DNA oligonucleotides onto AuNPs has been used [64, 65]. Figure 1 is a schematic of the methods used for conjugating oligonucleotides to AuNPs. Monomaleimido gold clusters have been coupled with thiolated DNA oligomers to synthesize probes for homogeneous nucleic acid analyses and ensure a 1:1 DNA/AuNP connection with interest for sensitivity improvements [66, 67] (See Fig. 2).

The synthesis of a novel trithiol-capped oligodeoxyribonucleotide and AuNPs conjugates prepared from it, was reported by Li et al. [61] This novel trithiol DNA oligonu-
cleotide can be used to stabilize particles >30 nm in diameter which is essential for many diagnostic applications [61].

Cai et al. [68] immobilized the oligonucleotide with a mercaptohexyl group at the 5'-phosphate end onto the 16 nm diameter AuNPs, which were self-assembled on a cysteamine-modified gold electrode and discovered that the saturated immobilization quantities of single strand DNA on the modified electrode were about 10 times larger than that on a bare gold electrode.

4. Applications in DNA Analysis

The analysis of specific gene sequences in the diagnostic laboratory is usually based on DNA hybridization in which the target gene sequence is identified by a DNA probe able to form a double stranded hybrid with its complementary nucleic acid with high efficiency and specificity [69]. NPs in general and AuNPs particularly offer attractive properties to act as DNA hybridization tags [70] with interest in developing sensitive electrochemical genosensors.

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**Fig. 2.** Schematics of A) Formation of particle-linked DNA network structure due to the interconnection between magnetic beads (MB) in the case where AuNPs modified with more than one DNA strands are used; B) The previous network is not created by using the 1:1 Au-DNA connection (C). The reaction of maleimido-Au67 with thiol-oligonucleotide that make possible the 1:1 Au-DNA connection. Adapted from *Langmuir*, 2005, 21, 9625.

**Fig. 3.** Schematic (not in scale) of the different strategies used for the integration of gold nanoparticles (AuNPs) into DNA sensing systems: A) Previous dissolving of AuNP by using HBr/Br₂ mixture followed by Au(III) ions detection; B) direct detection of AuNPs anchored onto the surface of the genosensor; C) conductometric detection, D) enhancement with silver or gold followed by detection; E) AuNPs as carriers of other AuNPs; F) AuNPs as carriers of other electroactive labels.
Figure 3 is a schematic of the most important strategies used to integrate AuNPs in DNA detection systems. These strategies consist of: (A) The electrochemical detection of AuNP label by detecting the gold ions released after acidic dissolving; (B) Direct detection of AuNPs anchored onto the surface of a conventional genosensor (based on stripping voltammetry); (C) Silver enhancement using conductimetric technique; (D) Enhancement of AuNPs anchored to conventional genosensor surface by using silver or gold; (E) Using of AuNPs as carriers for other electroactive labels.

Details of the above strategies will be discussed in the following sections.

4.1. Detection Based on AuNPs Dissolving

AuNPs bound to a DNA can be detected indirectly, by oxidatively dissolving the AuNPs into aqueous metal ions and then electrochemically sensing the ions. The great majority of the AuNPs-based assays have been based on chemical dissolution of the AuNPs tags in a hydrobromic acid/bromine (HBr/Br2) solution followed by accumulation and stripping analysis of the resulting Au(III) solution.

Authier et al. [71] developed an electrochemical DNA detection method for the sensitive quantification of an amplified 406-base pair human cytomegalovirus DNA sequence (HCMV DNA). The HCMV DNA was extracted from cell culture, amplified by polymerase chain reaction (PCR), and then quantified by agarose gel electrophoresis. The HCMV DNA was immobilized on a microwell surface and hybridized with the complementary oligonucleotide-modified AuNPs, followed by the release of Au by treatment with a HBr/Br2 solution, and the indirect determination of the solubilized Au(III) ions by ASV at a sandwich-type screen-printed microband electrode (SPMBE). AuNPs of 20-nm were used. The combination of the sensitive Au(III) determination at a SPMBE with the large number of Au(III) released from each gold nanoparticle probe allows detection of as low as 5 pM amplified HCMV DNA fragment. Wang et al. [72] developed an AuNPs based protocol for the detection of DNA segments related to the breast cancer BRCA1 gene. This bioassay consisted in the hybridization of a biotinylated target DNA to streptavidin coated magnetic bead-binding biotinylated probe and followed by binding of streptavidin-coated AuNPs (5 nm) to the target DNA, dissolution of the AuNPs and electrochemical detection using potentiometric stripping analysis (PSA) of the dissolved gold tag at single use thick film carbon electrodes, obtaining a detection limit of $4 \times 10^{-9}$ M.

The sensitivity of the detection is usually improved by the silver enhancement method. A better detection limit was reported when a silver enhancement method was employed, based on the precipitation of silver on AuNPs tags and its dissolution (in HNO3) and subsequent electrochemical potentiometric stripping detection. The new silver-enhanced colloidal gold stripping detection strategy represented an attractive alternative to indirect optical affinity assays of nucleic acids and other biomolecules. The high sensitivity and selectivity of the new protocol was illustrated for the detection of DNA segments related to the BRCA1 breast-cancer gene. A detection limit of around 150 pg mL$^{-1}$ (i.e., 1.2 fmol) was obtained [73, 74].

The same group reported a new strategy for amplifying particle-based electrical DNA detection based on oligonucleotides functionalized with polymeric beads carrying numerous AuNPs tags which an ultrasensitive electrochemical stripping detection of the dissolved gold tags was carried out [75].

4.2. Direct Detection

The HBr/Br2 solution is highly toxic and therefore methods based on direct electrochemical detection of AuNPs tags anchored onto the surface of the DNA genosensor, which would replace the chemical oxidation agent, are urgently needed [67].

Direct detection of AuNPs but not in connection with the detection of DNA hybridization was reported earlier by our group and Costa-García’s group [76, 77].

The application of AuNPs as oligonucleotide labels in DNA hybridization detection assays using a magnetic graphite-epoxy composite electrode (M-GECE) has been reported by Pumera et al. [67]. The novel gold nanoparticle-based protocol for detection of DNA hybridization was based on a magnetically trigged direct electrochemical detection of gold quantum dot tracers. It relies on binding target DNA (DNA1) with Au$\text{Cl}_2$ quantum dot in a ratio 1:1, followed by a genomagnetic hybridization assay between Au$\text{Cl}_2$-DNA1 and complementary probe DNA (DNA2) marked paramagnetic beads. DPV was used for a direct voltammetric detection of resulting Au$\text{Cl}_2$ quantum dot-DNA1/DNA2-paramagnetic bead conjugate on M-GECE. The electrochemical oxidation of Au$\text{Cl}_2$ quantum dots to AuCl$^+$ was performed at +1.25 V (vs Ag/AgCl) for 120 s in the nonstirred solution. Immediately after the electrochemical oxidation step, DPV was performed. During this step the potential was scanned from $+1.25$ V to 0 V, resulting in an analytical signal due to the reduction of AuCl$^+$ at potential $+0.4$ V. The DPV peak height at a potential of $+0.4$ V was used as the analytical signal in all of the measurements. The background subtraction protocol involving saving the response for the blank solution and subtracting it from the analytical signal was used.

Castañeda et al. [78] reported two Au-NPs based genosensors designs for detection of DNA hybridization. Both assay formats were also based on a magnetically induced direct electrochemical detection of the Au-NPs tags on M-GECE. The first assay is based on the hybridization between 2 single strands biotin modified DNA probes: a capture DNA probe and a target DNA related to the BRCA1 breast cancer gene, which is coupled with streptavidin-AuNPs (10 nm). The second assay is based on hybridization between 3 DNA strands: a biotin modified capture DNA probe, a target DNA, related to cystic fibrosis gene, and DNA signaling probe modified with AuNPs via biotin–
streptavidin complexation reactions. In this assay the target is ‘sandwiched’ between the others two probes. The Au-NPs tags were directly detected after the DNA hybridization event without the need of acidic dissolution. The electrochemical detection of AuNPs by DPV was performed in both cases with the same conditions described previously [67] (See Fig. 4).

An electrochemical genosensor based on AuNPs for detection of Factor V Leiden mutation from PCR amplicons which were obtained from real samples was described by Ozsoz et al. [79] The authors covalently bound amplicons to a pencil graphite electrode (PGE) and hybridized oligonucleotide-AuNPs conjugate a these electrode-bound targets. The oxidation signal of AuNPs was measured directly by using DPV at PGE. Direct electrochemical oxidation of the AuNPs was observed at a stripping potential of approximately +1.2 V. The response is greatly enhanced due to the large electrode surface area and the availability of many oxidizable gold atoms in each nanoparticle label. The detection limit for PCR amplicons was as low as 0.78 fmol.

4.3. Enhancement Methods

Enhancements by precipitation of silver onto the AuNPs labels have been reported so as to achieve amplified signals and lower detection limits [73, 80]. The use of other particles as AuNPs labels carriers are also used.

4.3.1. Enhancement with Silver

Mirkin’s group [30] has developed an electronic DNA detection approach with high sensitivity and selectivity. In their approach, a small array of microelectrodes with 20 μm gaps between the electrodes leads is constructed, and probe sequences are immobilized on the substrate between the gaps. Using a three-component sandwich approach, hybridized target DNA is used to recruit AuNPs-tagged reporter strands between the electrodes leads. The NPs labels are then developed in the silver enhancer solution, leading to the precipitation of silver metal onto the AuNPs. The deposition of silver closes the electrical connection between the two flanking microelectrodes, and target captured is signaled by a sharp drop in the resistance of the circuit. The binding events localize AuNPs in an electrode gap; silver deposition facilitated by these NPs bridges the gap and leads to readily measurable conductivity changes. With this method, they demonstrated a sensitivity of 500 fM with a point mutation selectivity factor of 100000:1 in target DNA.

Silver enhanced technology, using voltammetry techniques, has been used also by Cai et al. [81] The DNA target was immobilized onto a chitosan-modified glassy carbon electrode (GCE) and hybridized with gold nanoparticle-modified DNA probes. This electrode was subjected to silver enhancer solution for 8 min to coat the gold particle with a thick shell of metallic silver. The voltammetric signal was increased by 88 times after silver enhancement strategy.
The same group reported [82] an electrochemical methodology that enables the rapid identification of different DNA sequences on microfabricated electrodes. Their approach starts with an electropolymerization process on a patterned indium tin oxide (ITO)-coated glass electrode, followed by a selective immobilization of biotin-tagged probes on individually addressable spots via the biotin–streptavidin linkage. An exemplary target mixture containing *E. coli* and *Stachybotrys chartarum*, an airborne pathogen, is then introduced. Recognition of the DNA hybridization event of the immobilized probes with the target pathogen PCR products or synthetic oligonucleotides is achieved by chronopotentiometric stripping utilizing the catalytic silver electrodeposition process on the DNA-linked nanogold shells. The ability to selectively immobilize different oligonucleotide probes together with a sensitive electrochemistry-based detection for multiple species, as demonstrated in this study, is an important step forward for the realization of a portable bioanalytical microdevice for the rapid detection of pathogens.

Lee et al. [83] reported an improved electrochemistry-based sequence-specific detection technique by modifying the electrode surface using polyelectrolytes or utilizing the electrode whose surface exhibits the lowest background signal. The electrochemical DNA-hybridization detection utilizing AuNPs labels in combination with silver enhancement was successfully demonstrated on the gold and indium tin oxide (ITO) electrodes. For the gold electrode, a significant reduction in the background silver staining was achieved by modifying the electrode surface with polyelectrolyte multilayer films of poly-allylamine hydrochloride (PAH) and poly-styrenesulfonate (PSS). The DNA probe was immobilized onto the (PAH/PSS)3-modified gold electrode via an avidin–biotin interaction for the sequence-specific detection of target sequences. For the ITO electrode, its inherent low silver-deposition property was exploited to develop a sensitive DNA-detection platform. The electrode was modified with a SAM of MPA, to which a thiol-modified probe was attached through a disulfide linkage [83].

Later the same group [84] reported a simple, rapid and sensitive method for the electrochemical AuNPs-based DNA detection with an electrocatalytic silver deposition process. The catalytic silver electrodeposition on AuNPs surfaces using an ITO as the electrode material instead of carbon paste, at certain potentials, without any chemical pretreatments of the electrode was performed. The ITO electrode surface was first coated with an electroconductive polymer, poly(2-aminobenzoic acid), to enable the chemical attachment of avidin molecules for the subsequent probe immobilization. The AuNPs labels were bound to the formed hybrids, via streptavidin–biotin interaction. Finally, silver was electrodeposited on the AuNPs surface and quantified directly by scanning the electrode to obtain the silver oxidation signal (See Figure 5).

### 4.3.2. Enhancement with Gold

Dequaire et al. [85] developed a new efficient protocol for the sensitive quantification of a 35 base-pair human DNA...
cytomegalovirus nucleic acid target (tDNA). In this assay, the hybridization of the target adsorbed on the bottom of microwells with an oligonucleotide-modified AuNPs detection probe (pDNA-Au) was monitored by the anodic stripping detection of the chemically oxidized gold label at a SPMBE. Thanks to the combination of the sensitive Au(III) determination at a SPMBE with the large amount of Au(III) released from each pDNA-Au, pM detection limits of tDNA can be achieve. Further enhancement of the hybridization signal based on the autocatalytic reductive deposition of ionic gold (Au(III)) on the surface of the AuNPs labels anchored on the hybrids was first envisaged by incubating the commonly used mixture of Au(III) and hydroxylamine (NH2OH). However, due to a considerable nonspecific current response and of poor reproducibility it was not possible to significantly improve the analytical performances of the method under these conditions. This strategy, which led to an efficient increase of the hybridization response, allowed detection of tDNA concentrations as low as 600 aM and thus offers great promise for ultrasensitive detection of other hybridization events (See Fig. 6).

4.4. AuNPs as Amplification Units

AuNPs can be used as carrier for other AuNPs or other electroactive labels enhancing by this way the DNA detection compared to the use of single labels (single AuNP or a single electroactive molecule).

4.4.1. Carriers of AuNP Labels

A new strategy for amplifying particle-based electrical DNA detection based on oligonucleotides functionalized with polymeric beads carrying numerous AuNPs tags was described by Kawde and Wang [75]. The gold-tagged beads were prepared by binding biotinylated metal NPs to streptavidin-coated polystyrene spheres. Such use of carrier-sphere amplification platforms was combined with catalytic enlargement of the multiple gold tags and an ultrasensitive electrochemical stripping detection of the dissolved gold tags. This amplified electrical transduction allows detection of DNA targets down to the 300 amol level, and offers great promise for ultrasensitive detection of other biorecognition events.

4.4.2. AuNPs as Carriers for other Electroactive Labels

Another signal amplification strategy is to attach electroactive 6-ferrocenylhexanethiol molecules onto the AuNPs labels [86, 87]. AuNPs/streptavidin conjugates covered with 6-ferrocenylhexanethiol were attached onto a biotinylated DNA detection probe of a sandwich DNA complex. Due to the elasticity of the DNA strands, the ferrocene caps on gold nanoparticle/streptavidin conjugates are positioned in close proximity to the underlying electrode modified with a mixed DNA capture probe/hexanethiol self-assembled monolayer.
and can undergo reversible electron-transfer reactions. A detection level, down to 2.0 pM (10 amol for the 5 μL of sample needed) for oligodeoxynucleotide samples was obtained. The amplification of the voltammetric signals was attributed to the attachment of a large number of redox (ferrocene) markers per DNA duplex formed [86] (See Fig. 7).

5. Conclusions

Various electrochemical strategies to detect DNA hybridization by employing gold nanoparticles (AuNPs) as labels have already emerged. The majority of AuNP based DNA assays have been based on chemical dissolution of gold nanoparticle tag in a HBr/Br₂ solution followed by accumulation and stripping analysis of the resulting gold ions solution. The HBr/Br₂ solution is highly toxic and therefore methods based on direct electrochemical detection of AuNP tags, which replace the chemical oxidation agent, have been also reported.

Silver or even gold precipitation onto AuNP-DNA conjugates have been reported so as to improve the detection limit. Table 1 summarizes some of the results obtained by using different strategies. Although clear improvements have been demonstrated by the same authors upon comparing their results (with and without enhancement) it is not so clear the improvement when comparing different laboratories. The improvements by using enhancement strategies seem to be a compromise between signal augmentation and the reproducibility. The enhancements strategies by precipitation of gold or silver onto AuNPs or the use of AuNPs as carriers of other AuNPs or electroactive labels require a careful attention so as to avoid the irreproducibility problems.

Most of the electrochemical strategies reported up to date suffer from the fact that the hybridization event is still separated from the detection. Only in few cases these two processes are already integrated being the whole electrochemical assay compacted in a classical sensor model. The electrochemical detection of AuNPs using stripping methods can further be improved. The use of microelectrode including arrays may probably improve the detection limits allowing their application in the study of other biomolecules interactions. The potential for detecting single molecule interactions by detecting individual gold colloid label opens the way toward new applications.

The electrochemical properties of AuNPs make them extremely easy to detect using simple instrumentation. In addition, these electrochemical properties may allow designing simple and inexpensive electrochemical systems for detection of ultrasensitive, multiplexed assays. Clearly, AuNPs have a promising future in designing DNA sensors. Their utilization will be driven by the need for smaller detection platforms with lower limits of detection. Further efforts should be directed to enhancement strategies so as to avoid efficiently take their advantages.

Obviously the DNA electrochemical detection by using AuNPs will have an important impact on the development of specific and sensitive assays for clinical diagnosis, detection of pathogenic microorganisms in foods and the environment as well as for other applications including proteomics.

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7. References


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Table 1. Electrochemical genosensors using AuNPs as label. GE: gold electrode; 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, GCE: classy carbon electrode.

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