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REVIEW ARTICLE

Development of Electrochemical DNA Biosensors-A Review

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Abstract: Electrochemical DNA biosensors are used widely due to its sensitivity, accessibility and accuracy. This electrochemical era in DNA biosensors took almost three decades for its development and application. Purine bases in the DNA namely guanine and adenine oxidizes at the working electrode during application of a positive potential. This property of DNA has been utilized in electrochemical DNA biosensors in various fields from the clinical diagnostics, biomedical, forensic applications till environmental studies. With the use of nanotechnology, various nanomaterials have been utilized over the past years in order to increase the sensitivity of the biosensor. A clear understanding on the DNA immobilization strategy is required for sensor preparation for its various applications. A detailed account on the initial developments till current scenario in electrochemical DNA biosensors have been provided in order to understand the basics of electrochemical DNA biosensor and its application in developing a DNA biosensor.

Keywords: Electrochemical DNA biosensor, DNA damage, DNA hybridization, DNA immobilization and DNA interaction

Introduction

Electrochemical DNA biosensors have the potential to overcome the limits of other sensors due to rapid response, high sensitivity, selectivity and experimental convenience. They offer a greater advantage for the detection of toxins, anti-cancer elements, hybridization, biomolecules etc. Initially this was made possible with the availability of metal[1] and carbon based electrode[2] which enabled the immobilization of DNA molecules. Out of the four nuceobases found in DNA, only purine bases oxidation can be monitored electrochemically for the reason, they both were known to oxidize at a lesser potential, obtained during voltammetric measurements. Apart from voltammetric measurements, EIS and CV became promising electrochemical method[3] to study the film forming ability of DNA bases immobilized over the working electrodes using redox probe. Moreover, it was also proved that the changes in the oxidation of the purine at the electrode surface is directly associated with the film forming abilities and electron transfer characteristics of the redox probe[4]. The characteristics peak obtained from the electrochemical measurements were used to find the interaction of a molecule with the DNA strands. This article, review the initial development of DNA biosensors, methods adapted for its preparation for increased sensitivity and selectivity. We have also discussed the various strategies used for the immobilization of DNA for the construction of electrochemical DNA biosensor.

Initial developments

End of the twentieth century was the ear of the biosensors development and divergence. It was a period of very little research focusing the detection of DNA using a biosensor configuration[5]. In the initial stages, the most common application of biosensor involves the electrochemical detection of glucose using glucose oxidase. This enzyme has been used mainly with potentiometric and amperometric devices. Theoretically, DNA biosensor could offer an exceptionally sensitive and selective method for the detection of specific genes from within the genome of an organism. Practically one by one of the following techniques were completely established: identification of the presence of particular gene of interest using radioactive label[6], non-radioactive alternates including avidin/biotin and novel enzyme labeling systems. It should be noted that all these techniques required a specialized laboratories. In 1987, Mark E A Downs *et al.*[7] focused on the production of cheap and rapid detection of biological systems with high sensitivity, cost effective and quantitative features, resulting in the electrochemical detection of DNA using Stripping voltammetry.

Further developments happened when E. Palecek in 1988 demonstrated[8] the electrochemical behavior of nucleic acids. He demonstrated the nucleic acids to be electroactive species producing well developed voltammetric peaks on the mercury electrode. The nucleic modified electrode was simply prepared by immersing the mercury electrodes into the drop of DNA solution. This was the first nucleic acid modified electrodes prepared and this strongly decrease the quantities of DNA required for analysis[8]. A small damage in the double helical DNA was also sensitively identified[9]. Since then, several polarographic and voltammetric methods have been proposed for the direct quantification of nucleic acids, for the detection of hybridization and for the evaluation of DNA damage[9].

Chemistry of guanine and adenine oxidation at the working electrode

Out of the four nuceobases found in DNA, only purine bases oxidation can be monitored electrochemically for the reason, they both were known to oxidize at a lesser potential compared to pyramidine bases. Hydroxyl radicals add to purines giving raise to C4-OH-, C5-OH- and C8-OH- adduct radicals. C4-OH- and C5-OH- adduct radicals undergo dehydration and yield oxidizing purine (-H)* radicals, which reconstitute purines upon reduction. One-electro oxidation and one electroreduction of C8-OH- adduct radicals give rise to 8-hydroxypruines and formamidopyrimidines respectively. Analogous reactions of adenine yield 8-hydroxyadenine and 4-6-diamino-5-formamidopyrimidine. Both these products can be formed in the presence and absence of oxygen. However, formation of 8-hydroxypurines is preferred in the presence of oxygen. Hydroxyl radical generates multiple products in DNA of those discussed above[9,10].

Current scenario

Several electrochemical biosensors with immobilized layer of DNA have been reported for the determination of electroactive and non-electroactive compounds interacting with DNA, detection of specific sequence of DNA and monitoring of DNA integrity[11]. These findings have directed the applications of DNA biosensors in clinical diagnostics, forensic and biomedical applications. In addition, electrochemical DNA biosensors represent a new alternative to study DNA interactions and DNA damage. DNA based diagnostic tests have received great development in many fields such as genetics, pathology, criminology, pharmacogenetics, food safety and forensics[12].

Pededo and Rivas[13,29] investigated the immobilization properties of DNA over the carbon electrode for the development of affinity based biosensors. It was found that electrochemical pretreatments, supporting electrolytes, halides, monovalent cations, length and composition of DNA, immobilization potential and time plays a major role in the electro chemical response of nucleic acids over carbon surface[13,29].

The application of single-stranded DNA in the literature is very limited. Unlike ds-DNA, ss-DNA from calf thymus have been immobilized over the electrode surface for the detection of different environmental contaminants such as PCB (Polychlorinated biphenyl) mixtures, atrazine, phthalate, hydrazine[14], PAH (Polycyclic Aromatic Hydrocarbons)[15]. In these studies it was reported that the ss-DNA immobilized electrode shows a higher oxidation signal as the bases in the ss-DNA are free to react with the neighbouring molecules. Inspite of this fact, ds-DNA is often used as most of the genome are double stranded and it make easier to understand the interaction of a compound which results can be used for further applications. However, ss-DNA is the one and only used in DNA hybridization studies.

DNA hybridization studies

The immobilization of nucleic acids on oxidized surfaces of glassy carbon electrodes using $co(phen)_3^{3+}$ as indicator was performed. It was found that DNA can strongly adsorbed on glassy carbon electrodes only if the solution containing DNA is evaporated to dryness on the electrode. Inercalators as redox labels for detecting DNA hybridization are particularly interesting because they interact with the "π-stack" formed by the DNA-base pairs in the DNA duplex. With some intercalators efficient electron transfer over long distance between the intercalator and the electrode can proceed via the DNA duplex but not with a single strand of DNA. The utilization of long-range electron transfer as the basis of a DNA hybridization biosensor was first introduced by Barton and co-workers[16], where electrochemical current was observed through DNA duplexes by using methylene blue as an intercalator. Similarly, planar aromatic molecules, daunomycin, ethidium bromide, acridine dyes, anthraquinone derivatives[16] have been used as intercalator to study DNA hybridization. Any disruptions in the DNA-base pairing affect the perfect π-stacking and causes attenuation in the electrochemical current which enables the detection of single-base mismatches without requiring stringency washes.

DNA damage studies

As discussed earlier, as, and when DNA come across a molecule which damages its structure, a change in oxidation signal of purine bases in the DNA was observed. This property of DNA was used for determination studies, provided the molecule damages DNA. DNA damaging properties of pollutants in the water have been explored for its application in the preparation of biosensor for analysis of waste water samples[17], environmental pollutants[18], aromatic amines[19], phenolic pollutants[20], *etc*. The DNA biosensor response indicated the binding of one or more molecules present in waste water sample with a promising correlation with the Toxalert response (an indispensable tool for high-throughput toxicity prediction)[17]. Yanyan Qui *et al*[21] developed the procedure for the electrochemical detection of bisphenol A radicals through electro-oxidation signals of guanine from DNA damaging property of BPA radicals.

DNA interaction studies

Application of electrochemical DNA biosensor for interaction studies developed very lately when compared to determination of compounds interacting with DNA and hybridization. Initially DNA interaction studied was performed by optical method and through various biological assays. Pandey & Weetall coupled a FIA (Flow Injection Analysis) system with

an evanescent wave biosensor for the detection of typical interactions[22]. The studies on the interaction of a ds-DNA with other molecules, especially with therapeutic drug will make an important significance in life sciences. These studies illustrate the mechanism of action of many drug compounds, designing of new DNA-drug biosensor and screening of drugs in vitro. This has become possible electrochemically through the electrochemical measurement of immobilized DNA after its interaction with analytes in the solution. Electrochemical signal after analyte-DNA interaction can provide evidence of interaction mechanism and the nature of complex formed, binding constant, size of binding site, and the role of free radicals generated during interaction in drug action[23].

There are number of modes by which different molecules interact with DNA. These include electrostatic interaction (generally non-specific) with the negatively charged nucleic acid sugar-phosphate structure, intercalation of planar aromatic ring systems between base pairs (planar organic molecules containing several aromatic condensed rings often bind to DNA in an intercalative mode; for example daunomycin, epirubicin and actinomycin D), and minor and major DNA grooves binding interaction. Minor groove binding makes intimate contacts with the walls of the groove and as a result of this interaction numerous hydrogen binding and electrostatic interactions occur between a drug and DNA (DNA bases and the phosphate backbone, example: mithramycin). Major groove bindings occur via the hydrogen bonding to the DNA and can form a DNA triple helix such as norfloxacin[24].

Although different techniques have been used to study drug-DNA interactions, there is not a single technique that can be employed to resolve drug-DNA interactions. Hence, there is a high demand for developing new and improved techniques to determine the drug-DNA interactions. Electrochemical studies of drug-DNA interaction have attracted considerable attention and show great promise for elucidating the mechanisms of drug-DNA interaction. Different types of electrode materials have been used for the investigation of drug-DNA interaction such as carbon paste electrode, gold electrode, pencil graphite electrode, glassy carbon electrode and screen printed electrodes. Drug-DNA interactions have been investigated with electrochemical techniques such as cyclic voltammetry, square wave voltammetry, differential pulse voltammetry and chronopotentiometry[25].
The mechanism of interaction can be investigated in three different ways:

1. DNA modified electrode
2. Drug-modified electrode
3. Interaction in solution

In all the three approaches, pre- and post- electrochemical signals of either the drug or DNA can be monitored to elucidate the mechanism of the drug-DNA interaction. Electrochemical DNA biosensor consists of a nucleic acid recognition layer that is immobilized over an electrochemical transducer. Electrochemical DNA biosensors can be used to investigate the interactions of DNA with drugs over the wide potential range, at any ionic strength and over a wide pH range. In the last decade, the binding of small organic molecules to DNA and its alterations has been described on the basis of the variation of the electrochemical signal of guanine and adenine[24].

The interaction of daunomycin[26] and ciprofloxacin[27] with DNA was studied by using DNA modified carbon based electrode. The decrease in guanine signal was used as an indicator of the interaction mechanisms. The ionic strength of the solution was found to be strongly related to the binding of the molecules. It was proved that ciproflaxin might bind to the DNA in two different modes; electrostatic or intercalative. For drugs, electrostatic binding of the drugs was found significant as compared with binding in the intercalative

mode. The interaction of rifampicin with double and single stranded DNA was found to be intercalative[28].

The interaction of sildenafil citrate over carbon based electrode with ds-DNA was investigated under different ionic strength conditions. The attenuation in the guanine oxidation signal was noticed due to conformational changes resulting from the electrostatic interaction between sildenafil citrate and DNA. However, electrostatic contribution posed by the cationic species was not affected by increasing the ionic strength of the solution. It was proposed that the electrostatic contribution from the cationic form is not significant and major contribution of the interaction of sildenafil citrate with the DNA is through intercalation with only a minor electrostatic contribution[24].

Strategies used for the immobilization of DNA over working/Modified electrode

The most simplest method of immobilization of DNA involves the electroadsorption of short DNA templates on working electrode to develop a stable biorecognition layers for further application. One such report using this technique showed detection limit[29] of 25 µg/L. In the recent years, development of highly sensitive and selective DNA biosensor is the main focus of research with its application in various fields discussed so far. In order to achieve DNA with high sensitivity, nanostructures with different morphology have been used for the modification of substrate aiming at increasing the immobilization concentration of probe DNA and improving the electrochemical signal of the immobilized DNA. This high sensitivity and have been reported based on nanomaterials based electrochemical methods. The nanomaterials used for electrode surface modification includes metal nanoparticles, semiconductor nanoparticles, nanowires, carbon nanotube, nanopores[30]. The basic scheme of DNA biosensor preparation is displayed in Figure 1 with which any individual with strong base in chemical bonding can propose a scheme for DNA biosensor preparation.

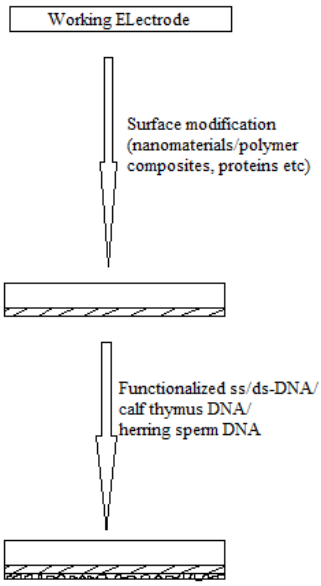

Figure 1. Basic scheme of DNA biosensor preparation

Kelley research group fabricated controlled nanowire and Pd nanostructures modified electrodes and achieved sensitive DNA detection through controlling the orientation of probe

DNA. The detection limit towards target DNA for this nanostructured electrode was estimated to be approximately 1.0 fM[31]. Dendritic gold nanostructure modified DNA biosensor was developed by Feng *et al*[32] with the same detection limit. Detection limit lower than 1.0fM has been reported by Chang and his co-workers[33] by fabricating an electrochemical DNA biosensor based on conducting polyaniline nanotube array. Further the detection limit has been decreased (till 28aM) by using an electrochemical sensitive DNA biosensor with the combination of signal amplification technology of DNA-Au bio bar code[34]. 10 pM detection limit towards the target DNA was achieved by preparing nanogold aggregates modified electrode with the use of methylene blue as an electrochemical indicator[35]. Electrochemical DNA biosensor based on Carbon nanotubes doped with palladium nanoparticles showed a detection limit of 0.12 pM towards the detection of target DNA[36]. These reports proves that the introduction of nanomaterials during surface modification efficiently increase the electrode surface area and enhance the DNA immobilization character. Few of the most significant strategies used for DNA immobilization are listed below:

- For the DNA biosensor which uses MWCNT for electrochemical property enhancement, chitosan is the common binder used. Chitosan acts as scaffold for dispersing MWCNT and incorporating firmly MWCNT and biomolecules at different electrodes[37]. In addition, the chemical treatment of MWCNT-CHIT immobilized at carbon electrode surface has been proven to be strongly influencing the adsorption and electrooxidation of DNA, once cross-linked with glutaraldehyde[38].
- Acetic acid-plasma treatment on gold-supported aligned carbon nanotubes, generated from pyrolysis of iron (II) phthalocyanine, followed by grafting single-strand DNA chains with an amino group at the 5' phosphate end [AmC65'→3') onto the plasma-induced –COOH group through the amide formation, in presence of EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride] coupling agent was developed by Pingang and Liming[39] in order to increase the sensitivity and selectivity.
- Mercapto-saline coating of a platinum electrode were prepared by addition of Mercapto-saline to a mixture of acetate buffer and acetone which were later post baked, incubated with iodoacetic acid and activated with water-soluble carbodiimidies for the immobilization of DNA[40].
- The clean gold electrode soaked in cysteamine (monolayer of cysteamine/gold electrode), later dipped in gold colloid solution was able to immobilize ss-DNA on the colloidal Au-modified electrode forming self assembled (-*S*-$(CH_2)_2$-NH-Au-ss-DNA) pattern[41]. Hollow gold nanospheres have also been implemented for the modification of electrode surface via a 1,6- hexane dithiol liking agent to fabricate electrochemical DNA biosensor with the hybridization detection limit up to 1pM[42]. Gold electrodes modified with multi-wall carbon nanotubes (MWCNT) produced by chemical vapor deposition technique with Ni as catalyst were also employed. The carbon nanotubes were activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and DNA oligonucleotides with amino terminal groups were covalently immobilized for 12 h[43]. MB was also used by Fang's group[44] for detecting the hybridization event at the surface of zirconium oxide modified gold electrode. All these strategies required chemically modified DNA sequences, *e.g.*, thiol or amine modifications, highly specialized equipment and in general high adsorption times. MB was also employed as a DNA indicator in a biosensor based on graphite electrode modified with CHI doped with CNT fabricated to detect salmon sperm DNA. In this case the film was stabilized immersing the electrode in 0.1 M NaOH for 30 min[45]. High adsorption times of DNA and MB were employed and no direct DNA bases oxidation signal was analyzed.

- Glassy carbon electrode alternatively dipped in the aqueous solution of poly(diallyldimethyl ammonium chloride) (PDDA) containing NaCl and DNA-SWNT hybrid suspension, respectively formed multi-layer films of DNA-SWNT over the glassy carbon. This set up was used for the detection of arsenic (III) with the detection limit of 0.05 µg/L at pH 7[46].
- Modification of electrode using electropolymerization technique was performed for the detection of target DNA with the detection limit[47] of $3.5x10^{-13}$ M. Aminobenzoic acid (ABA) was first electropolymerized on the surface of the electrode modified with MWCNT with carboxyl groups by cyclic voltammetry. Gold nanoparticles were subsequently introduced introduced to the surface of polyABA-MWCNT composite film by electrochemical deposition method onto which functionalized ss-DNA was immobilized.

Conclusion

It can be seen that carbon nanotubes represent an increasingly important group of nanomaterials with unique geometrical, mechanical, electronic and chemical properties. Such properties of CNT make them also extremely attractive for the task of electrochemical detection. The adsorption properties of CNT, reflecting their huge surface area and graphene sheet structure have been exploited for extending the scope of adsorptive stripping voltammetry towards important compounds that do not exhibit surface-active properties at conventional electrodes. Such unique application of CNT-modified electrodes has been used for the ultra trace measurements of the common nitroaromatic explosive 2, 4, 6- trinitrotoluene [48]. The modification of the working electrodes using nanostructures can be constructed by either one of the following strategy separately or in combination: Direct electrostatic assembly, covalent linking, polymer entrapment, co-mixing, sol-gel and electro deposition[49]**.** With all these developments, DNA electrochemical biosensors appears as interesting analytical tools for the presence of carcinogens, drugs, mutagen, pollutants with binding affinities for DNA.

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