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Research Article

ELECTROCHEMICAL LABELING AND DETECTION OF DNA: RECENT ADVANCES

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ABSTRACT

Labeled DNA probes have got enormous amounts of applications in various fields such as DNA sequencing and detection of mutations. Analysis of large number of samples in short period of time is always desirable. Methods of labeling of DNA are divided into three categories i.e. radiolabeling, fluorescence labeling and redox or electrochemical labeling. Though sensitive, use of radioactive labels is hazardous and associated with several regulatory issues. Fluorescent DNA probes have got drawback of expensive reagents and instrumentation. Electrochemical labeling is booming these days because of its simplicity, cost effectiveness and ease of miniaturation. This review article puts light on different techniques of DNA labeling and their detection, recent techniques of electrochemical labeling and its advantages.

Keywords: Electrochemical labeling, fluorescence labeling, DNA sequencing.

INTRODUCTION

Electrochemical properties of DNA were discovered about 45 years ago. At present there are quite a lot expectations regarding development of electrochemical transducer based devices for the detection of DNA sequences or mutations. Electrochemistry of nucleic acids is booming as it provides good alternative for well-developed fluorescent hybridization detection methods[1]. Fluorescent DNA probes come with drawback of expensive reagents and instrumentation. Over the last decade enormous advancement has been made in the field of biosensors. Gene chips featuring dense arrays of oligonucleotides have been successfully applied to problems in transcriptional profiling. However fluorescence based readout of these chips involves highly precise and sophisticated methods to analyze and interpret data. Recent development of DNA based electrochemical sensing offers lot of promises such as simple, accurate and inexpensive platform for patient diagnosis[2, 3].

Labeling of DNA/RNA

Labeled DNA probes have got enormous amount of applications. As science is progressing, genetic basis of more number of diseases is being known. These kinds of labeled probes are used for DNA sequencing as well as detection of mutations. In addition, most of the analytes of interest such as proteins, DNA, peptides are present in minute concentration, thus very sensitive and specific analytical techniques are required for their detection. Moreover, analysis of large number of samples in short period of time is desirable. Labeled DNA can fulfill all these requirements. Nucleotides can be labeled by isotopic and nonisotopic methods.

Isotopic labeling of nucleic acids and detection

Traditionally, labeling of nucleic acids has been conducted by incorporating nucleotides containing radioisotopes. Such radio labeled probes contain nucleotides with a radioisotope (often ^{32}P, ^{33}P, ^{35}S, ^{3}H and ^{14}C), which can be detected specifically in solution or, within a solid specimen by autoradiography. The intensity of an autoradiographic signal is dependent on the intensity of the radiation emitted by the radioisotope, and the time of exposure, which may often be long (one or more days, or even weeks in some applications). ^{32}P has been used widely in Southern blot hybridization, dot-blot hybridization, and colony and plaque hybridization because it emits high-energy β-particles, which afford a high degree of sensitivity of detection. It has the disadvantage of being relatively unstable[4].

Additionally, its high-energy β-particle emission can be a disadvantage under circumstances when fine physical resolution is required to interpret the resulting image unambiguously. Therefore, radionuclides which provide less energetic β-particle radiation have been preferred in certain procedures, for example ^{35}S-labeled and ^{33}P-labeled nucleotides for DNA sequencing and tissue *in situ* hybridization, and ^{3}H-labeled nucleotides for chromosome *in situ* hybridization. ^{35}S and ^{33}P have moderate half-lives while ^{3}H has a very long half-life. However, the latter isotope is disadvantaged by its comparatively low energy β-particle emission, which necessitates very long exposure times[4].

General method of radio labeling of DNA

In the initial step, free 3' hydroxyl group is created within the unlabelled DNA, by means of nuclease such as pancreatic deoxyribonuclease. At the same time, the 5' to 3' exonuclease activity of this enzyme will eliminate the nucleotide unit from 5' phosphoryl terminus of the nick. Finally, a new nucleotide with free 3'-OH group will be incorporated at the position where the original nucleotide was excised. One nucleotide unit in a 3' direction will shift along the nick. This 3' shift or translation of nick will result in sequential addition of new nucleotides to the DNA, while the pre-existing nucleotides will be removed. So, when radioactively labeled deoxyribonucleoside triphosphate is used as substrate, labeled one will replace the original unlabelled nucleotides in the DNA.

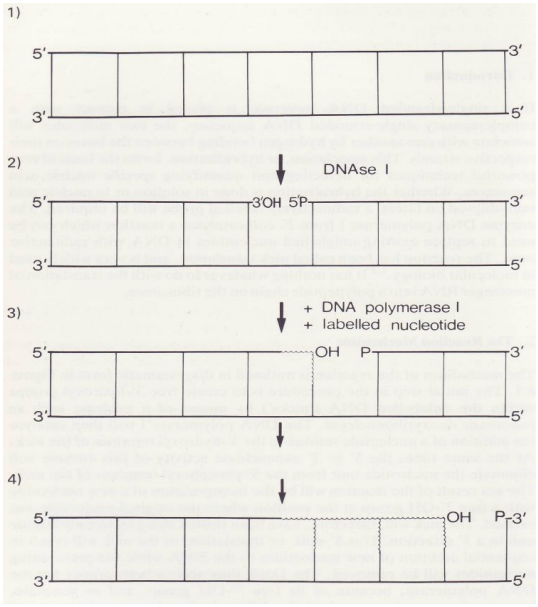

Fig.1: Generalised method of labelling DNA/RNA with radioactive probe. Step 2 shows

formation on nick. Labelled nucleotide is added after formation of nick, which later gets incorporated in the strand.[5]

Autoradiogrpahy

Autoradiography is a procedure for localizing and recording a radiolabelled compound within a solid sample, which involves the production of an image in a photographic emulsion. In molecular genetics applications, the solid sample mostly consists of size-fractionated DNA or protein samples that are embedded within a dried gel, fixed to the surface of a dried nylon membrane or nitrocellulose filter, or located within fixed chromatin or tissue samples mounted on a glass slide. The photographic emulsions consist of silver halide crystals in suspension in a clear gelatinous phase. Following passage through the emulsion of a β-particle or a γ-ray emitted by a radionuclide, the Ag^+ ions are converted to Ag atoms. The resulting latent image can then be converted to a visible image once the image is developed, an amplification process in which entire silver halide crystals are reduced to give metallic silver. The fixing process results in removal of any unexposed silver halide crystals, giving an autoradiographic image, which provides a two-dimensional representation of the distribution of the radiolabel in the original sample[4].

Direct autoradiography involves placing the sample in intimate contact with an X-ray film, a plastic sheet with a coating of photographic emulsion; the radioactive emissions from the sample produce dark areas on the developed film. This method is best suited to detection of weak to medium strength β-emitting radionuclides (e.g. ^{3}H, ^{35}S, etc.). However, it is not suited to high-energy β-particles (e.g. from ^{32}P) such emissions pass through the film, resulting in the wasting of the majority of the energy. Indirect autoradiography is a modification in which the emitted energy is converted to light by a suitable chemical (scintillator or fluorescence). One popular approach uses intensifying screens, sheets of a solid inorganic scintillator that are placed behind the film in the case of samples emitting high-energy radiation, such as ^{32}P. Those emissions, which pass through the photographic emulsion, are absorbed by the screen and converted to light. By effectively superimposing a photographic emission upon the direct autoradiographic emission, the image is intensified[6].

Radioactive compounds are useful and sensitive indicator probes, which provide easy and specific detection, localisation of nucleic acids. However, there are several limitations associated with it. Radioactive materials are hazardous as personnel handling it get exposed to high level of radiation, so safety precautions must be maintained while handling. Radioactive materials are extremely expensive to purchase, and there are various safety and regulatory issues associated with it regarding their disposal. Radioactive materials have limited shelf life, which further increases usage costs. These drawbacks lead an urge for development of non-radioactive labelling and detection techniques of nucleic acids[7].

NON-ISOTOPIC LABELLING OF NUCLEIC ACIDS

Direct labeling of nucleic acids

Fluorescence labeling of nucleic acids was developed in the 1980's and has proved valuable in many applications such as chromosome *in situ* hybridization, tissue *in situ* hybridization and automated DNA sequencing.

The fluorescent labels are used in direct labeling of nucleic acids by incorporating a modified nucleotide (often 2'deoxyuridine 5' triphosphate) containing an appropriate fluorophore, a chemical group that fluoresces when exposed to a specific wavelength of light. Commonly used fluorophores in direct labeling are fluorescein, a pale green fluorescent dye, amino methyl coumarin, a blue fluorescent dye and rhobdamine, a red fluorescent dye.[8]

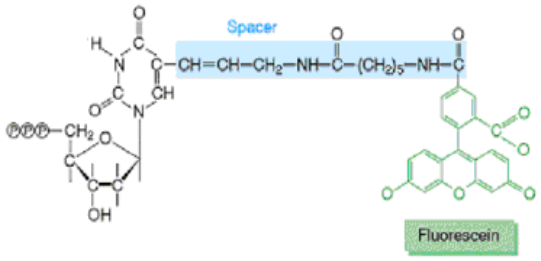

Fig. 2: Fluorescein dye attached to oligonucleotide with spacer. Method of direct labelling of nucleic acids. [5]

Fluorescent labeling of nucleic acids is usually carried out by enzymatic reactions. Organic fluorophores are chemically introduced into primer or nucleoside triphosphates and are then incorporated either using PCR amplification or using DNA or RNA polymerases. Introduction of active amino or thiol groups' synthesized oligonucleotides is preferred, as it provides acceptors for subsequent chemical labeling. In one of the method developed by Prodnikov and co workers, aldehyde groups were introduced into DNA by partial depurination and into RNA by oxidation of its 3'-terminal ribonucleoside with sodium periodate. It was then followed by direct or indirect attachment of one flurophores molecule to aldehyde group of fragment[9].

One more commonly used method is coupling of succinimidyl ester of flurophores and primary alkylamine modified oligonucleotides. Coupling of phosphoramidite derivatives of flurophores to oligonucleotides during solid phase oligonucleotides synthesis is also used. Wang and co workers have reported site specific fluorescent labelling of DNA using Staudinger ligation (Staudinger ligation is a reaction in which combination of an azide with a phosphine produces iminophosphorane intermediate. Later, hydrolysis produces phosphine oxide and amine) with high efficiency. An oligonucleotides was modified at its 5' end by an azido group, was selectively reacted with 5-[N-(3'-diphenylphosphinyl-4'-methoxy carbonyl) phenyl carbonyl) aminoacetamido] fluorescein (Fam) to produce Fam labeled nucleoside.[10]

A significant disadvantage of single labeled probe is that quantification and /or visualization of the target is made difficult by strong fluorescence background because of unbound probes, which requires an extra step for removal from medium of probes that are not hybridized to target. To overcome this problem concept of Molecular Beacons (MB) was introduced by Tyagi and Kramer in 1996. A MB is an oligonucleotide (ON) that contains fluorophores and a quencher at different positions of a strand. This ON strand is composed of a probe region (loop) complementary to target sequence and a region of self-complementary nucleotides. In the absence of target, the complementary parts of the probe hybridize together forming a hairpin structure. Because of close proximity enforced by the stem, the quencher deactivates the excited state of fluorophore, resulting in strong quenching of fluorescence. However, in presence of target, the probe region of MB hybridizes to it, promoting the opening of hairpin conformation and separating fluorophore and quencher from each other. Because of enforced separation after hybridization with target, fluorophore fluorescence is not quenched [11,12].

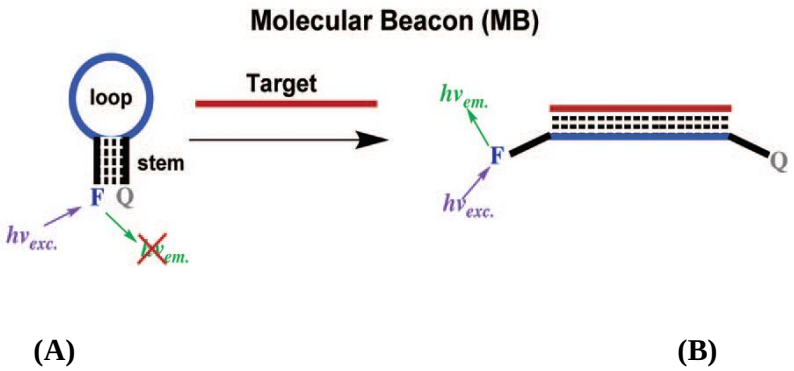

Fig. 3: (A) is MB showing loop of self complementary nucleotides, with a fluorophore and quencher. (B) MB attached to target.[11]

Indirect labeling of nucleic acids

It usually features the chemical coupling of a modified reporter molecule to nucleotide precursor. After incorporation into DNA, an affinity molecule, a protein or other ligand, which has a very high affinity for the reporter group, can specifically bind the reporter groups. A marker molecule or group is attached to later, which can be detected by suitable assay. The reporter molecules on modified nucleotides should be placed sufficiently far from nucleic acid backbone to facilitate their detection by affinity molecule and a carbon spacer is required to separate nucleotide reporter group[5].

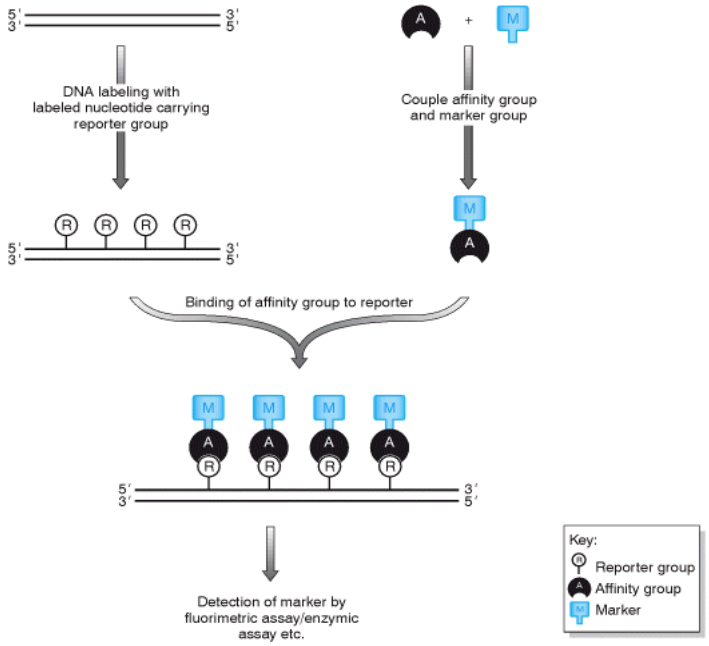

Fig.4: Generalized diagram of indirect labeling of DNA [5]

One major non-isotopic labeling method is biotin-avidin system. Biotin (vitamin H) has many features that makes it suitable candidate for probe. The interaction between biotin and avidin (a 68000 dalton glycoprotein from egg white) has one of the highest binding constants (K_d= 10^{-15}) known. When avidin is coupled with an appropriate indicator molecules (fluorescent dyes, electrodense proteins, enzymes or antibodies), minute quantities of biotin can be detected. This specific binding property of biotin-avidin complex has been used to develop methods for the visual localization of specific proteins, lipids and carbohydrates. Biotin directly attached to nucleotide those functions, as an efficient polymerase substrate is a versatile tool, in experimental protocols and in the detection methods that could be used[13].

Langer and co-workers have reported one method of synthesizing biotin labeled nucleotides. In this method, first 5-C mercurated derivatives of UTP and dUTP were prepared by addition of mercuric acetate. Then this mercurated derivative was attached with allylamine linker by a reaction catalysed by palladium. AA-dUTP was then added to mixture of Biotinyl-N-hydroxysuccinimide ester and dimethyl formamide (DMF). Biotin labeled nucleotide was then separated by ion exchange chromatography[14].

Chollet and Kawashima have reported one method of synthesizing 5' biotin labeled nucleotides. In this method oligodeoxynucleotides were synthesized using solid phase phosphoramidite method on an automated machine. The oligodeoxynucleotide (1) was first converted to its 5'-amino-alkylphosphoramidate (3) derivative. Biotin was introduced by mixing 5'-amino-alkylphosphoramidate with biotin-N-hydroxysuccnimidyl ester in DMF[15].

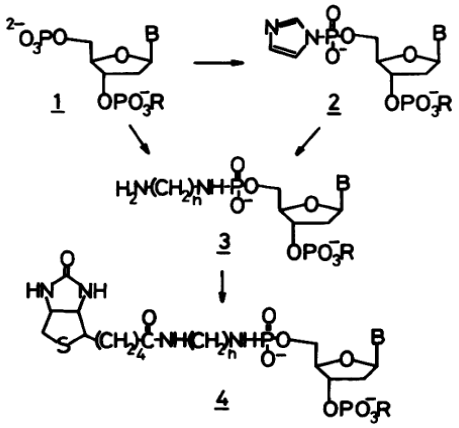

Fig. 5: Chemical method of labelling of DNA with biotin at 5' position. R= 5'-oligodeoxynucleotide , B= A,C,G,T; n=6 [15]

Some drawbacks of biotin labeled nucleic acid probes are also been reported. These probes often give rise to unspecific side reactions, when used for in situ hybridization, as biotin itself occurs in almost all natural materials. Sometimes streptavidin/avidin used for detection of the hybrids tend to bind unspecifically to tissues and membranes (especially highly charged nylon membranes) resulting in increased unspecific background signals. In order to avoid these drawbacks an alternative method is developed, for labeling nucleic acid probes with cardenolide digoxigenin, which occurs in Digitalis plant. For detection of digoxigenin labeled hybrids, Fab fragments of a highly specific polyclonal sheep antibody coupled to alkaline phosphatase are applied. Kessler and co-workers have reported one method of labeling nucleic acids with digoxigenin. In this method digoxigenin modified nucleoside was prepared by reaction of 3-O-methylcarbonyl-ε-

aminocaproic acid-N-hydroxysuccinimide ester with 5-aminoallyl substituted UTP[16].
Detection techniques of all these assays mainly include chemiluminescent, colorimetric and fluorescent techniques.

Chemiluminescent detection of DNA

The method employs a biotin streptavidin system which binds an enzyme specifically to target DNA and upon exposure to substrate, the enzyme catalyses a chemiluminescent reaction. A Polaroid or X-ray film captures the image within seconds. In this method, a biotinylated oligonucleotides was used in a primer extension for DNA sequence or as probe for hybridization to a target DNA. Biotinylated alkaline phosphatase is bound to biotinylated 5' end of oligonucleotides via streptavidin bridge and catalyses light reaction by cleaving off a phosphate group from an added chemiluminescent substrate[17].

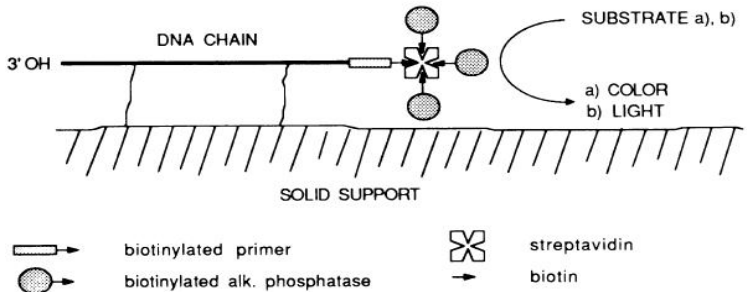

Fig. 6: Chemiluminescent detection of DNA. Biotin streptavidin complex is specifically bound to UV- immobilised target- DNA, through which alkaline phosphates catalyses a colour or light reaction depending on substrate added. In case of chemiluminescent substrate cleavge of phosphate group destabilises the 1, 2 dioxetane ring structure and molecule decomposes with emmision of visible, yellow, green light. [17]

Fluorescent detection of DNA

When indirect labeling method is used, hybridization event can be detected by using fluorescent detector. Fluorescent detector is a technique, which is used to study specimens, which are made to fluoresce.

ELECTROCHEMISTRY OF DNA

In 1957, it was first found that, DNA and RNA gave reduction and oxidation signals upon interactions with electrodes when analyzed using polarography. Later, it was shown that all NA bases produce anodic signals due to formation of sparingly soluble compound with electrode mercury. Ability of NA bases to associate with electrodes was shown in 1960 by V. Vetterl. In the middle of 1980's, more sensitive, voltametric analysis of NA was introduced. It was shown that DNA and RNA could be easily immobilised on the surface of mercury or carbon electrode, simply by immersing electrode in small volume of (3-10 μl) of nucleotide solution. Due this strong adsorption samples it was then easy to analyte even in small volume. First electro active marker was synthesized using osmium tetroxide complexes with nitrogen ligands (Os, L) forming stable DNA- Os, L adducts, which produced redox couples and yielded catalytic signals at mercury electrode[18].

Millan and Millenseck first introduced the concept of electrochemical DNA biosensor in 1993. They demonstrated its utility for detecting the cystic fibrosis ΔF508 detection sequence associated with 70% of cystic fibrosis. DNA biosensors convert hybridisation event into an analytical signal. A basic DNA biosensor is designed by immobilisation of DNA strand on a transducer surface and its recognition of target sequence by hybridisation. DNA duplex or triplex formed on transducer is known as hybrid. This event is then converted to an analytical signal by transducer, which can be electrochemical, optical or gravimetric[19].

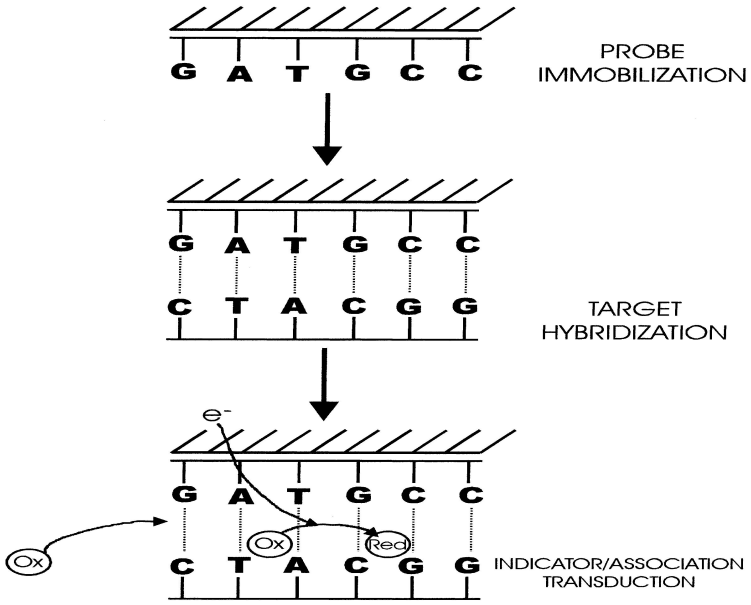

Fig. 7: Major processes involved in electrochemical biosensor based on use of redox indicators[20]

Electrochemical detection

Electrochemical methods are attractive option for DNA diagnostics, as electrochemical reactions give an electronic signal directly; there is no need of expensive instrumentation. DNA microarrays consist of arrayed spots of immobilised single stranded nucleic acid capture (test sites) to detect fragments with specific base pair sequence in complex DNA or RNA sample solutions. The DNA microarray technology is currently used as highly paralleled analytical tool for gene expression profiling mainly in drug discovery related research. The main challenges for successful development of DNA microarrays are immobilisation of capture probes, identification of test sites and simple, sensitive and specific detection technique. Laser scanning fluorescence microscopy is highly developed and standard for detection of hybridization. However, size, price, complexity and low stringent selectivity are major drawbacks of fluorescence based detection techniques. Electrochemical DNA chips can be much simpler in instrumentation and are easier to miniaturize since electrochemical based DNA microarrays are compatible with microfabrication.[21] Detection of hybridization event involves monitoring increased current signal of a redox indicator (which recognizes DNA duplex) or from other hybridization-induced changes in electrochemical parameters such as capacitance or conductivity. Immobilized probe sequences can be accomplished with an inexpensive electrochemical analyzer. Electrochemical signaling strategies are based on direct or catalyzed oxidation of DNA bases as well as redox reactions of reporter molecules or enzymes recruited to the electrode surface by specific DNA probe-target interactions. Electrochemical detection is conducted by three techniques, namely, direct electrochemistry of DNA, Indirect electrochemistry of DNA and by using DNA specific redox indicator [22].

Direct electrochemistry of DNA

The earliest electrochemical sensing strategy was based on reduction and oxidation of DNA at a mercury electrode, simply the amount of DNA reduced or oxidized would be equivalent to amount of DNA captured. Palecek and co workers showed various methods to discriminate single and double stranded DNA through direct DNA reduction around 40 years ago. Later DNA oxidation was carried out through adsorption stripping voltametry (ASV). This technique is sensitive as electrostatic build-up of analyte at electrode surface is induced before detection step. Thought sensitive this method is associated

with certain drawbacks like significant background currents at relatively high potentials required for direct DNA oxidation[23, 24].

Indirect electrochemistry of DNA

This methodology uses an electrochemical mediator for detection. In this method an electrode is held at a potential that oxidizes the reduced metal complexes, which then come into contact with DNA. Guanine residues in DNA can reduce the metal complex, regenerating reduced mediator. The enhanced signal reflects amount of guanine available for oxidation. E.g. use of polypyridyl complexes of Ru (II) and Os (II) to mediate electrochemical oxidation of guanine[23].

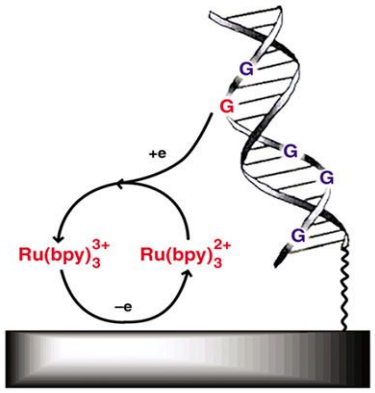

Fig. 8: Indirect electrochemistry of DNA.

Reaction of ruthenium taking place with guanine.

$$Ru(bpy)_3^{2+} \longrightarrow Ru(bpy)_3^{3+} + e^-$$

$$Ru(bpy)_3^{3+} + G \longrightarrow Ru(bpy)_3^{2+} + G^+ \quad [17]$$

Detection using DNA specific redox indicator

By analogy to fluorescence-based methods, several techniques have been put forward in whom target DNA sequences are labeled with redox active reporter molecules.

Several approaches have been developed for detection using DNA specific redox indicator, which involves conjugation of oligonucleotides with electroactive reporters, use of soluble electroactive intercalators, redox enzyme mediation and measurement of direct label free electrochemical process. All of these methods rely critically upon hybridization of complementary nucleic acid sequence for specific recognition while some use charge transport through π stack of duplex DNA.[25]

As a reagent for non-destructive labeling of DNA for electrochemical detection, Ihara and coworkers report a DNA ligand, in which viologen unit connected two aminoacridine parts. But it had certain drawbacks like its non-specificity in DNA binding and cathodic potential applied for probe detection have been obstacles for its analytical applications. For convenient analysis, ferrocene modified oligonucleotides were synthesized[25].

Ihara and coworkers reported one method. In this method, aminohexyl linked oligonucleotides were synthesized on a DNA synthesizer, based on cyanoethylphosphoramidite chemistry. Aminohexyl linker was used to facilitate labelling of ferrocene. At the final step of synthesis, coupling reaction of terminal amine of the oligonucleotide and activated ester of ferrocene carboxylate was carried out in aqueous solution containing DMSO. In can be explained with the help of figure depicted below[26].

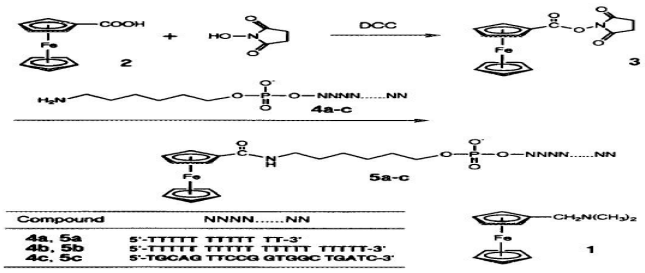

Fig. 8: Synthesis of ferrocene labeled nucleotide by using phosphoramidite chemistry[19]

One more method is reported by Wlassoff and King. In this method mercuration of deoxyuridine triphosphate was carried out. Then an allyamine linker was attached at C-5 position in presence of palladium catalyst. Addition of ferrocene carboxylic acid was then carried with HBTU as a condensing agent.[27].

Staudinger ligation is used in a wide range of applications as a general tool for bioconjugation, including specific labelling of nucleic acids. Kosiova and co-workers have reported one method for labelling nucleosides with ferrocene by using Staudinger ligation method. In this reaction 2'-Azido-2'-deoxyuridine was chosen as a substrate. The ferrocene derivative (4-ferrocenyl-4-oxabutanoic acid) was used as an electrochemical label. Reaction of 4-ferrocenyl-4-oxabutanoic acid reactive ester with iminophosphorane derivative gave 2' ferrocene labelled uridine[28].

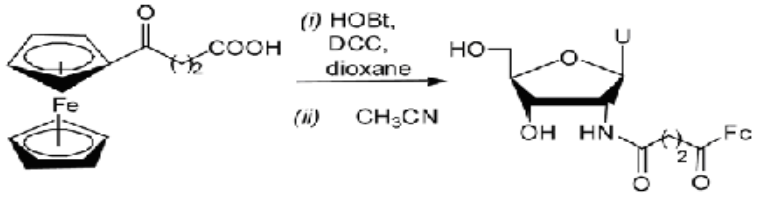

Fig. 9: Synthesis of ferrocene labelled uridine by using Staudinger ligation[28]

In one of the approaches, three-component sandwich assay is performed. In this method the redox label has been attached to synthetic sequence specifically designed to bind an overhang portion of the probe-target complex. It can be explained with the help of figure (10).

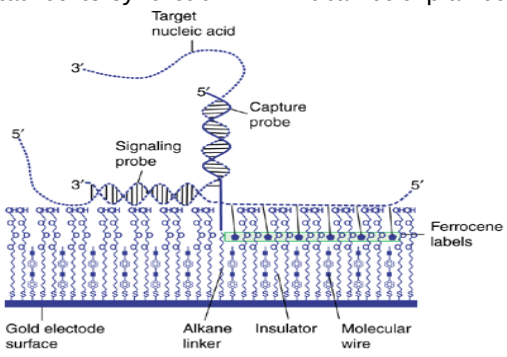

Fig. 10: The gold electrode is functionalised with DNA probe sequence, the oligophenylmethyl molecular wires, and polyethylene glycol insulator molecules. The target is captured on electrode and it hybridises to second reporter sequence,

labelled with ferrocene. Thus, the target is not labelled instead 'sandwiched' by the capture probe and signal probe. The molecular wires provide a pathway for electron transfer between the ferrocene label and electrode[29].

Colloidal gold nanoparticles have also been employed to signal hybridization in a sandwich-based assay. In one of the study performed, labelled target was captured by probe strands immobilized on a pencil graphite electrode and hybridization is detected electrochemically with appearance of characteristic gold oxidation signal. Wang et.al. have developed a technique in which these nanoparticles encode DNA sequences. Target DNA is hybridized with probe modified magnetic beads, and separated magnetically from pool of analytes and hybridized again with nanoparticles-labeled reporter strands. The products are isolated and nanoparticles are dissolved and analyzed by ASV. In one study conducted by Vernon and co workers, for detection of human papillomavirus, two chips were spotted with capture probes consisting of DNA oligonucleotides sequence specific for HPV types. Electrically conductive signal probes were synthesized to be complementary to a distinct region of the amplified HPV target DNA. The electrochemical signal was recorded when the HPV amplicon hybridizes to the capture probe and ferrocene-labeled signal probe, thereby bringing the reporter molecule, ferrocene, into contact with the self-assembled monolayer on the gold electrode. An alternating current voltammogram is obtained when HPV is detected in a sample but no electronic signal is registered when HPV DNA is absent from the sample[30, 31, 32].

Inouye and co-workers developed a method based on charge transport through π stack of duplexes. In this technique, π conjugated ferrocene modified analogue was connected at 5' end of single stranded oligonucleotides, so it behaved like 'wire-like' electrochemical probe. When the hybridized complex was attached onto gold electrode at 3' end of probe DNA, π way was fully opened from ferrocene tag of nucleoside residue to gold electrode, by means of conjugated acetylene synthetic base-stacked base pairs bridges. Transport from electrodes was insulated if a mismatched pair is present[33].

Other than ferrocene there are several other examples of compounds, which are used electrochemical markers. One example is of ruthenium, which is discussed above. One more example includes methylene blue (MB). Kelly and co workers have reported one such method. In the electrocatalytic process, electrons from electrode surface flow to intercalated MB. MB is then reduced to leucomethylene blue (LB). LB goes on to reduce ferricyanide in solution, thereby regenerating MB^+ catalytically, leading to amplification of the hybridization signal[26].

Second-generation electrochemical DNA biosensors

Direct (indicator free) electrochemical detection of hybridization events represents a very attractive approach for DNA biosensors. Such a route greatly simplifies the sensing protocol, since it offers an instantaneous detection of duplex formation. Two major routes are exploited for this indicator free hybridization detection. The first involves monitoring changes in electronic or interfacial properties accompanying DNA hybridization, while second relies on the intrinsic DNA signal associated with electroactivity of nucleic acids[27].

CONCLUSION

Over the past decade, there has been a tremendous progress towards the development of electrochemical nucleic acid biosensors. Such devices are of considerable interest due to their tremendous promise for obtaining sequence-specific information in a faster,

simpler, and cheaper manner compared to traditional nucleic acid assays. In addition to excellent economic prospects, such devices offer innovative routes for interfacing (at the molecular level) the DNA-recognition and signal-transduction elements, i.e. an exciting opportunity for fundamental research. The realization of instant decentralized (medical, environmental, or forensic) DNA testing would require additional developmental

work. The future research in this field will lead to new electrical detection strategies, that coupled with major technological advances, will result in powerful, miniaturized, easy-to-use instruments for DNA diagnostics. Such instruments will undoubtedly accelerate the realization of large-scale genetic testing.

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