DNA and RNA sensor

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Abstract This review summarizes recent advances in DNA sensor. Major areas of DNA sensor covered in this review include immobilization methods of DNA, general techniques of DNA detection and application of nanoparticles in DNA sensor.

Keywords: DNA detection, biosensor, immobilization of DNA, hybridization of DNA, nanoparticle.

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The development of DNA sensors attracts recent research attention directed at gene analysis, such as detection of genetic disorders, tissue matching and forensic applications[1,2].

In general, sensors based on the principle different from that of the traditional spectroscopic analysis and biochemical analysis. In sensor system recognition events (adsorption, combination, reaction etc.) is used to replace chemical separation process, and the combination of recognition with detection process eliminates the problems and errors associated with traditional chemical analysis[3].

The principle of biosensor is to fix one part of materials such as protein, nucleic acid, RNA, virus etc. onto a substrate to make a device that could recognize the protein, RNA, DNA. The recognition event could be expressed in a detectable signal. Sensing technology has great advantage over the traditional chemical analytical method in the aspect of simplicity and high detection speed.

DNA sensor is a kind of transducer based on the DNA hybridization principle, offers a kind of rapid and simple method for the detection of genetic variation and infections disease pathogens. Since Watson and Crick[3,4] discovered the double helix structure of deoxyribonucleic acid (DNA) in 1953 and established the molecular mechanism of genetics, the recognition of DNA and its sequence determination has attracted the attention of many scientists. There are two fundamental considerations for the DNA sensor construction. The first one is based on the Watson-Crick base match principle, i.e. G(guanine)-C(cytomine), A(adenine)-T(thymine) combination to form a base couple. The second one is based on the Hoogsteen three oligonucleotide hybridization principle, i.e. double helix A-T DNA interacts with T to form a T.A-T three base complex, or G-C DNA interacts with protonated C(C^+) to form a C.G-C complex. Most DNA sensors are based on the double helix hybridization principle. The whole process for DNA sensor formation is to immobilize a single strain oligonucleotide, generally containing 18—50 bases, on some solid supporting materials as a probe, which can recognize the complement DNA single strands in the analyte and express the extent of hybridization in a physical signal such as color, heat, conductivity, weight etc. Since RNA (Ribonucleic Acid) has similar structure with DNA, consisting of a long, usually single-stranded chain of alternating phosphate and ribose units with the bases adenine(A),

guanine(G), cytosine(C), and uracil(U) bonded to the ribose, the RNA sensor generally is named DNA sensor too.

Two key technologies in the sensor design are utmost important, i.e. the fixation technology and the signal transduction technology. In this paper we will discuss the limitation of current technology and the possible way people to overcome it. In addition, we will discuss the possibility for the future DNA sensor.

1 Immobilization technology of DNA

The fixation of DNA on the sensor substrate is the foundation of sensor construction, which consists of connecting the oligonucleotide probe to the surface of biosensor and keeping the probe high specific combination ability of the probe with the target DNA in analyte. A satisfactory fixation must meet the following requirements. (i) To modify the surface of sensor elements, such as metallic electrode, piezoelectric transducer, quartz fiber etc., for making necessary active surface groups (hydroxy-, amino- group etc.), which could connect to the DNA probe easily. (ii) To terminate or graft certain functional groups or segments on the target DNA so as to ease the combination between DNA probe and target DNA in analyte.

The fixing technology plays an important role in determining the sensitivity as well as the selectivity of DNA sensor. It could be catalogued as follows.

1.1 Immobilization by covalent force

In this method, the coupling agents or bi-functional compounds have been used to improve the connection strength and durability of the sensor by affecting the connection of DNA to the sensor surface. Since one end of DNA was fixed on the substrate surface, it has great freedom for the DNA conformation change and favors the DNA hybridization. There have been many relevant reports. For example, Yang et al.[5] treated the glass surface with ammonium propyl triethoxylsilicane (APTES), of which the hydrolyzed product reacted with the hydroxyl group on the glass surface and formed a very strong -O-Si-O- bond, making a NH_2-terminated surface. If the surface is treated by using bi-functional compounds such as glutaric aldehyde (GA), nitrophehemetyl chloride (NP) or maleic anhydride (MA), it could successfully fix a 5′-NH_2-DNA and 5′-SH-DNA on the substrate at room temperature, especially the 5'-SH-DNA one, which is obtained by using MA treatment, has better stability at high temperature. Other bi-functional agents such as di-bromoethylene, chloro-, bromo-, 3-methyl are also commonly used. They react to the substrate surface with one halide atom in the molecules and connect with the DNA probe at the 5′ ends with another halide atom. For example, Fang et al.[6] have terminated the graphite surface by amine group using APTES, and then added water-soluble carbon diimide hydrochloride (EOC) as a coupling reagent promoted to speed up the amide bonding formation between phosphate and amine.

The DNA 5-phosphate end could fix to the electrode surface via covalent bond with the amine group. The other kinds of siliconiting reagent like glycopropelene trimethy silicane (GOPS) have also been used. Because of the long chain of glycol oxide these kinds of probes have more mobility. The covalent method could provide a stable modified surface and can easily to realize the hybridization. However, to get a controlled density and orientation nuclear acid probe is still a hot topic in the DNA sensor study.

1.2 Immobilization by biotin-avidin interaction

Both Biotin and streptavidin are large proteins, a tetramer of 4 methylene groups and have the molecular weight of 68 KD and 60 KD respectively. Evert methylene group has one combinative site for biotin with a combination constant K_d of 10^{15} mol • L^{-1}. The avidin could be adsorbed on the substrate surface at first and then the oligonucleotide-biotin complex will combine with the avidin by the biotin-avidin interaction. Sui et al.[7] reported the fixation of DNA modified by biotin using this method. They transferred the dipalmitoyl phosphatidylethanoamide (DPPE) and biotinated DPPE to the gold surface and combined then with avidin. Experimental results showed that avidin combined with the biotin on the surface via its two combining sites for fixing and the other 2 sites reacting with the bionated DNA so as to make a DNA

probe. Mediating the amount of DPPE, biotinated DPPE and avidin, Sui et al. could decrease the unspecific adsorption to a minimum extent. This system has the advantage of easy operation, but the existence of a large amount of proteins will decrease the sensitivity and selectivity of sensors.

1.3 Physical adsorption method

The physical adsorption method for fixing the DNA probe to the solid substrate is based on the noncovalent bonding. Mascini et al.[8] fixed the oligonucleotide probe to the graphite electrode only by applying a certain electric potential to the electrode. This method is relatively simple and without any reagent or specific nuclear acid modification. The main disadvantage is that nonspecific adsorption exists and the fixed nucleotide is prone to being desorbed from the substrate. More over, the hybridization efficiency will be decreased due to more groups will lose their reactivity by multiple sites adsorption. To overcome this disadvantage, Moser et al.[9] changed the component of the modifier layer and introduced the electrocharged group.

1.4 Surface modification by synthetic method

This method based on photochemical and electrochemical synthetic reaction. A representative example of combining the photoresist with a photosynthetic process *in situ* has been developed by Affymetrix Company[10]. This method utilizes the photoresist technique to synthesize and fix the required oligonucleotide probe on the substrate, and could get higher fixation efficiency and simplify the process from synthesis to fixation. However, the higher fixation efficiency will cause the lower detection sensitivity to the target DNA and the purification of the synthetic probe is difficult. The electrochemical method is based on the electro-polymerization reaction. For example, Livache et al.[11] used a Pt electrode as a working electrode putting in an electrolyte containing pyrrole and a pyrrolyl-oligoneucleoted compound and cyclo-scanned in the range between –0.3 and 0.85 V. As a result, the oligonucleotide probe was fixed on the Pt electrode through the polypyrrole binding. Both methods have been used to develop the DNA chip.

1.5 Self-assembly technology

The driving force of self-assembly technology is the interaction between molecules, which could induce a highly organized molecular membrane formation. For example, SH-DNA could easily attach to the gold electrode through the SH-bonding[12], and an organized molecular membrane is formed. The surface structure obtained by this method has high orientation and good stability that makes hybridization easier. However, this method requires a high purity SH-bonding which needs a complicated purify procedure. Owing to the higher hydrophilicity of numerous base groups it is difficult to get a compact packing structure. Bard et al.[13] modified the Au surface by a layer of alkyl-biphosphate aluminum and fixed the DNA to the electrode surface by the interaction of Al^{3+} and the PO^{4-}-DNA.

We[14] have used a simple self-assembly method to prepare the DNA probe. In this method, the Pt electrode surface was modified with polypyrrole obtained by electropolymerization, and a high stability and high conductivity hydrophobic surface was formed, and then the octadecyl amine was coated by adsorption to make the surface charged positively, and the single strain DNA with PO^{4-} end was fixed on the surface by electrostatic force.

Both Bard's and our methods have the advantage of avoiding the HS attachment procedure and the DNA probe has a higher activity.

1.6 Langmir-Blogett technology

Langmiur-Blogett technology could form a monolayer of biological substance, and can accurately mediate the monolayer accumulation. Because of the water-soluble property of DNA that makes the monolayer spread difficult, some special spreading methods have to be adopted. Nicolini et al.[15] have spread the octadecyl amine on the solution surface to form a monolayer and to make a DNA $C_{18}NH_2$ complex by adsorption of the DNA from the solution, and then transfer the monolayer to the solid substrate by horizontal lift transfer method to make multiplayer devices. The single strand DNA is situated in the mid-

dle of multiplayer LB film. We[16] have found that during such adsorption process, the single strand nucleotide could be adsorb to the octadecylamine by electrostatic attraction at pH = 7. The molecular area in π-A curve of Langmuir film of $C_{18}NH_2$ has been increased by adsorption of the DNA from the soultion, indicating the penetration of single nucleotide into the monolayer. It would possibly produce a nonsoluble DNA-lipid complex at first and then spread it on the solution surface.

2 DNA detection technology

Another important element for biosensor manufacture is the transducers. They play a role of detecting the minor change of the hybridization between DNA probe and the target DNA and transferring it to a recording signal. According to whether they use label indicators, DNA sensor could be divided as:

(i) Indicator type. For example, the fluorescent or electroactive indicator, including DNA probe attached type or cDNA attached type. In these kinds of biosensor, the hybridization extent could be detected by measuring the signal of indicators. The key problem is to choose the suitable hybridization indicator.

(ii) Without indicator type. Quartz crystal microbalance and surface plasma resonance (SPR), utilizing the change of mass or light reflection, are the examples of such transducers.

These kinds of methods are easy to establish and mediate on line. According to the transfer signal, they could be divided as electrochemical, photochemical, surface wave and mechanical method.

2.1 Electrochemical detection

The principle of electrochemical DNA sensor is based on the interaction between the electroactive indicators and the DNA single strand or double strand. It can quickly detect the sequence and genetic disorder of DNA, having the advantage of low cost, easy to internalization and automatization. According to the detecting ways, they could be divided into voltammetry method, chronopotentiometry method and electrochemiluminescence method.

DNA sensor of the voltammetry method is based on the fact that the added oxidation active reagent reacts with the hybridized double helix DNA molecules will cause a cathodic or anodic electric current of potential change. The active reagent could be organometallic compounds, antibiotics acridine dyes, and benzoamide dyes etc. that could interact with DNA. Bard et al.[17] investigated the interaction between metallic covalent complexes, such as $M(bpy)_3^{m+}$, $M(phen)_3^{m+}$, (bpy = 2, 2 bipyridil, phen = 1, 10, phenantroline, M= Co, Os, Ru) and double strand DNA, indicating the reaction between DNA and metallic complex mainly through (1) hydrophobic force, which drives the metallic complex to penetrate into the double strain DNA, and induce the interaction between the DNA bases and metallic complexes; (2) electrostatic force, which makes the attraction between the opposite charge species, the negative charged sugar/phosphate structure in DNA and metallic complexes. Mikkelsen et al.[18] have used $Co(bpy)_3^{3+}$ and $Co(phen)_3^{3+}$ as the hybridization indicator. Because these metallic complexes will be enrichmented in the double helix DNA formed after hybridization, the measured electric current is much higher than the single strain DNA. Mikkelsen et al. have used an octadecylamine or stearic acid modified carbon paste electrode to detect the cystic fibrosis ΔF 508 sequence deficiency with an 18-base oligodeoxynucleotide. Mascini et al.[19] have found that when some redox reagent such as acridine orange, benzoamide dyes Hoescht 33258 and daunomycin were used as additive, the detected electric peak was shifted towards positive side and the shift extent was related to the target DNA concentration, whereas for the mismatched DNA sequence, the peak shift was very slight. Hashimoto et al.[20] have used HS-DNA to modify the Au electrode and used Hoescht 33258 as indicator, the detection limitation of target DNA could reach 10^{-7}—10^{-13} g/mL.

Chrono-potentiometric sensor is a highly sensitive device, which offers high sensitivity and effective background compensation. Wang et al.[21,22] have reported a sensor for HIV1 detection. In this experiment a single-stranded DNA similar to 21-mer oligonucleotide, or 36-mer oligonucleotide, was immobilized on

the carbon paste electrode and $Co(phen)_3^{3+}$ was used as indicator. The detection limitation was 4×10^{-9} mol • L^{-1}. When 21-mer nuclear acids hybridize, the electric current peak value of fully matched target DNA was 5.2 times that of 3 bases mismatched DNA. They have also used hypoxanthine to replace the Guanine (G) in the probe and detected the change of G cathodic peak after hybridization without using indicator. The detection limitation was about 120 ng/mL.

Electrochemical luminescence (ECL) sensor is another kind of electrochemical sensor. The principle of this method is to apply a certain electric potential to an electrode, which is coated with some electro-chemical luminescence substances, or to an electrode that is situated in a solution containing some such electro-chemical luminescence substances. This method combines the high sensitivity of chemical luminescence and the high selectivity of electrochemical method. A device has been developed in the market that can quantitatively determine the PCR product of DNA to be detected (QPCR5000 System, PE Company). It is easy to immobilize the DNA probe with $Ru(bpy)_3^{2+}$ via avidin-biotin bonding, and in the presence of Tripropyl amine (TPA) a detection limitation of 10^{-15} mol • L^{-1}. DNA could be obtained. The detection speed is about 50 samples per hour. Bard et al.[23] fixed the DNA probe by Al^{3+} and PO_2^- attraction in stead of avidin-biotin interaction and then introduced $Ru(bpy)_3^{2+}$ and TPA as mentioned above to detect the DNA by the optical signal caused by the photoelectric oxidation.

Lee[24] has inserted conducting ions in-between the base pairs of a DNA molecule to form an electrically conductive DNA (M-DNA). Their M-DNA is capable of carrying on electric current in the same way that a wire is capable of supplying electricity.

Lee[25] also discovered that M-DNA has the ability to selectively combine other biological molecules such as environment toxic, drugs or proteins, which could repel the metallic ions, decreasing the conductivity of M-DNA and could be used as a DNA sensor.

2.2 Optical biosensor —— fiber optical biosensor

This kind of sensor is based on the lightwave guide and wave attenuation principle. When the light total internal reflection takes place at the bottom of the mirror, the thickness of solution medium could reach 300 nm that could make the maximum light adsorption in detection, enhancing the analytical sensitivity. They could mainly be constructed into fluorescence type, surface plasma type and resonance mirror type.

The principle of preparation of a fluorescence type DNA sensor is to label the fluorescence indicator to the DNA probe or target DNA, or to the hybridized double helix DNA. To measure the fluorescence signal caused by the implantation of fluorescence indicator in double helix DNA is the principle of this method. For example, Krull et al.[26] used a sensor where DNA probe was fixed on the quartz fiber by covalent bond. The DNA on the fiber hybridized with the target DNA in the solution. After 45 min, the fiber with hybridized DNA was immersed into a solution containing fluorescence dye EB (ethidium bromide) and its fluorescence intensity was measured after reaction. The intensity of fluorescence was proportional to the degree of hybridization and could be used as a measure of the amount of detected DNA and the detection limitation is 86 μg/L by warm buffer DNA. It was reported that after washing out the EB and the target by warm buffer solution the DNA sensor could be used repeatedly and kept a sustained performance after one year storage time. Graham et al.[27] immobilized the 16 mer and 20 mer oligonucleotide as probe on the surface of optical fiber. It has been found that the concentration of oligonucleotide labeled by fluorescence was proportional to the detected florescence intensity, and thus the DNA sequence could be detected at a nanogram level. The surface of sensor could be regenerated repeatedly many times. A DNA sensor was reported by Bier et al.[28] where cyanine dye YoYo and Picogreen was inserted as a fluorescence indicator into a double strain DNA. This kind of sensor can discriminate DNA strains with 2 mismatched bases and the detection limitation will reach 300 pg/mL and can keep their sensitivity after 60 times utilization.

Surface plasma resonance (SPR) sensor is based on the change of refractive index of target substance on the metallic membrane surface. Generally, it is built

up from a gold or silver membrane coated on a prism, and to contact with a medium of another refractive index. If the light source is polarized light, its reflection at the prism-metal will become a minimum when the reflecting angle reaches a certain value (resonance angle). The resonance angle is very sensitive to the medium, which contacts with metal membrane. When single strand DNA fixed on the metallic surface hybridized with the matched target DNA strand in the solution, the reflective index will change too. There are two detection ways. The one way is SPR scan, where people measure the change of resonance angle, i. e. measure the relationship between the intensity of reflection light and the incident angle. Bier et al.[29] have found that in the case of DNA binding by avidin-biotin interaction the more bases of DNA probe were matched, the higher the resonance signal and the smaller the reflection light. The other way is SPR microscopy. In this method, the incident angle is fixed near the resonance angle. The reflection intensity is measured by charge couple device (CCD). The investigation by Corn et al.[30] has shown that this method can discriminate single, double strand DNA, and even some DNA mutants.

Resonance mirror is another kind of sensor, a measurement similar to the SPR. The only difference is to replace the metallic membrane with TiO_2 or PbO membrane that has high reflectivity and insulation property. This method measures the change of resonance angle for the specific bonding of the sensitive surfaces. Watts et al.[31] used this method to detect the hybridization of DNA and got the detection limitation of 263 pg/mm^2.

2.3 Surface acoustic wave detection

The transducer for these kinds of sensor is piezoelectric crystal. Nowadays, most of them are the quartz crystal microbalance. This kind of biosensor is based on the principle of mass changing. At the beginning, the crystal has a definite frequency, which will change after a tiny substance appeared on its surface. In 1992 Okahata et al.[32] fixed a 10-mer oligonucleotide on a gold electrode in a 9 MHz QCM to detect the DNA in aqueous solution. Afterward, they[33] used a 27 MHz QCM to enhance the sensitivity of QCM by 10 times, and the recognition ability of mismatched sequence DNA has also been enhanced. Using multilayer avidin to increase the recognition capacity of DNA probes one can increase the sensitivity by 4—5 times[34]. Wang et al.[35] fixed the HS-DNA probe in QCM to detect the G and A mutation at 540 th site in P 53 gene. It was found that even if the complement bases have the amount less than 20% of the non-complement bases, the detection limitation of about 1 μg/mL could be reached in spite of the influence of non complement bases. However, the frequency measurement is often influenced by many factors such as liquid viscosity, visco-elastivity of membrane as well as surface roughness that causes a certain disputes in application. According to the change of viscosity, Yamaguchi et al.[36] used impedance analysis to investigate the adsorption, fixation and hybridization of DNA and have got very nice results. Thompon et al.[37] investigated the hybridization kinetics taking place at the TSM (thickness shear mode) acoustic wave devices on the solid liquid surface by using acoustic network analysis. They[38] also used on-line acoustic wave detector to study the interactions of an 86-amino acid protein (Tat) with a viral messenger RNA transcript (TAR), showing the important significance of acoustic method in HIV study.

Over the years, a number of investigators have suggested various inhibitors, such as aminoglycoside abtibiotics[39] and other chemicals[40,41], Tat peptide analogs[42,43], TAR RNA decoys[44,45] and TAR ribozyme[46]. These agents affect the interaction between Tat and TAR, thereby preventing transcriptional activation of HIV-1 genome either by steric hindrance, sheer displacement mechanism or by deprivation of the functional molecules. Among them, inhibitors against the HIV transcriptional transactivator protein, Tat, are highly important and might even be critical in the fight against AIDS due to the fact that the transcription of viral RNA increases by hundreds-fold following binding of Tat to TAR[47].

Our laboratory[48—50] has used a quartz crystal microbalance (QCM) to study the interactions of poly

(allylamine hydrochloride), polyacrylate acid (PAA) and polyamidoamine (PAMAM) dendrimers with TAR RNA. The results showed that they bind strongly with TAR RNA resulting in blocking the interaction of Tat with TAR. According to an equation similar to Langmuir Adsorption Isotherm suggested by our laboratory, the surface combination coefficient (K_D^{-1}) of Tat-TAR is 1.1×10^5 (mol •L^{-1})$^{-1}$, which is consistent with what was reported in literature[51], whereas the combination coefficient of PAMAM-TAR is 1.8×10^7 (mol • L^{-1})$^{-1}$, 100 times higher than that of Tat-TAR, suggesting PAMAM dendrimer has much stronger affinity with TAR than Tat does.

2.4 Chemical force microscope (CFM) detection

Atomic force microscope can be used to study the interaction between the molecules on the surface whereas chemical force microscope (CFM) is a deviation of AFM, where the AFM probes were modified by biomolecules, donors or acceptors. By measuring the interaction force between modified probe and the target molecules on the substrate, we could discriminate or recognize the target molecules. Frank et al.[52] used polyepoxy, a glue emulsion particle, coated with single strain DNA to the AFM probe and scanned the target c-DNA on the substrate. Since the complement DNA has more sites for interaction, the friction force was 1.5 times larger than the non-complement one, and thus could distinguish the complement and non-complement DNA sequence.

In 1999 in the 7th international conference on nanotechnology, Lieber from Harvard University reported the result of high sensitivity detection of oligonucleotide DNA sequence with one mismatched base. They used synthesis 14-mer oligonucleotide acid to modify the pinpoint of CFM and found that the binding force will decrease by 30% when there was only one mismatched base in the target DNA sequence. Combining the data from binding force and elastic deformation, one could discriminate the type of DNA mismatch.

The sensitivity of this method is affected by the surface morphology and the time of binding, whereas the selectivity is affected by the condition of measurement, signal/noise ratio, etc. The key point in this recognition method is to make a high biological affinity pinpoint.

3 Application of nanoparticles in DNA sensor

Mirkin laboratory[53] invented a new kind of DNA sensor based on the color change of nanoparticles. They modified the Au nanoparticle surface with single strand DNA. If these particles meet another gold particles whose surface is modified with complement DNA single strand, the nanogold particle color will change due to the hybridization that makes the nanoparticle aggregated. The aggregation of nanogold particle makes the wavelength of absorbent peak shift to a long wavelength and the gold sol color changes from red to blue. After transferring this system to solid substrate, Mirkin et al.[54] detected the oligonucleotide to a concentration of 10^{-10} mol • L^{-1}.

Willner et al.[55] and Zhou et al.[56] reported a new way to improve the sensitivity of QCM sensor by introducing nanogold particle as amplifier to improve the detection limitation down to less than 10^{-12} mol • L^{-1}. In their works, they have described a novel microgravimetric technique for gene detection where a sandwich-type ternary complex consisting of an oligodeoxynucleotide immobilized on a QCM electrode, a target DNA and a gold nanoparticle modified by oligonucleotide is formed to give an amplified frequency signal.

Our lab[14,57] has found that when QCM was modified by nanogold particle the immobilization ability and sensitivity of QCM sensor will be improved greatly. Combing the surface modification and amplifier by nanogold technology, a DNA detection limitation down to 10^{-16} mol • L^{-1} was possible. In our previous study[58] on the particle size effect on the bare QCM gold surface, the amplification effect increased with the particle size of the amplifier rising in the beginning, but subsequently dropped down when the particles reached a certain size. On the contrary, in this system the amplification effect continually grows along with the particle size, since the amount and

solidity of the immobilized HS-DNA are evidently improved by the nanogold modification of the QCM surface.

4 Conclusion and prospect

The important elements of a biosensor are the fixation of active components and the way to transfer the biological signal to be a readable and measurable signal such as light, weight, electricity etc. Organized molecular assemblies have the advantage of controlling the DNA density on the surface and the compact package on the surface can speed up the responsibility. Generally speaking, DNA sensor without labeling indicator has great advantages in avoiding the contamination of indicators, on the other hand, if the indicator has some specific recognition ability and could enhance the determination limitation to a great extent, it is worth adding photo or electric indicators.

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