Towards Genoelectronics: Electrochemical Biosensing of DNA Hybridization

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Abstract: Deoxyribonucleic acid (DNA) offers the chemist a very powerful recognition tool. The use of DNA as a selective recognition element in biosensors represents an exciting new area of chemistry. Electrochemical sensing devices offer innovative routes for interfacing the nucleic acid recognition system with the signal-generating element at the molecular level. Such devices are also ideally suited for shrinking DNA diagnostics and meeting future requirements of large-scale genetic testing. This article reviews new concepts for electrochemical biosensing of DNA sequences, and for using electrical fields to regulate DNA interactions in connection with the development of genoelectronic chips. Particular attention is given to new label-free gene-sensing systems based on monitoring changes in electronic or interfacial properties accompanying DNA hybridization.

Keywords: biosensors · conducting polymers · DNA recognition · genoelectronics · nucleic acid recognition

Introduction

Nucleic acid recognition processes offer unique possibilities for DNA diagnostics. In particular, the use of Watson–Crick base-pair recognition is extremely important for the diagnosis of genetic or infectious diseases, environmental testing for bacterial contamination, rapid detection of biological warfare agents, and forensic investigations. Wide-scale DNA testing requires the development of fast, easy-to-use, inexpensive, tiny biosensing devices. DNA biosensors are small analytical devices consisting of a nucleic acid recognition layer immobilized on a physical transducer (Figure 1). The goal is to convert the DNA hybridization event into a useful analytical signal. DNA biosensors, based on optical, electrochemical, or piezoelectric transducers, are presently under intense investigation.

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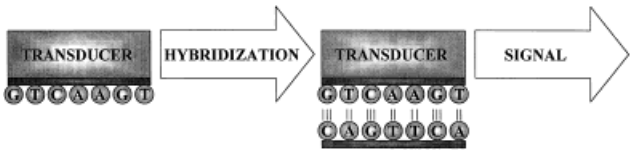

Figure 1. Processes involved in the operation of a DNA hybridization biosensor: base-pair recognition, signal transduction, and readout.

Electrochemical devices offer a special promise for rapid, direct detection of specific DNA sequences.[1, 2] The high sensitivity of electrochemical transducers, coupled to their compatibility with modern microfabrication/miniaturization technologies, low cost, minimal power requirements, and independence of sample turbidity, make them particularly attractive for shrinking DNA diagnostics, and for reaching mass markets. In addition, electrochemistry offers a unique route for electrical control of DNA hybridization and denaturation processes, and for the use of specific DNA interactions to induce electrical signals.

The new term genoelectronics will be used below to describe the interface of nucleic acid recognition and electronic systems. Genoelectronics is a new subclass of bioelectronics, which generally deals with the coupling of biological functions to electronics. Such molecular interfacing technology has been used in the past primarily for enhancing the electron transfer between redox enzymes and electrode surfaces.[3] We, and several other groups, are trying to extend this molecular interfacing approach into regulating DNA interactions, and into exploiting DNA recognition events for inducing direct electrical signals. Such innovative strategies for designing electrochemical DNA biosensors are the focus of this Concepts article.

Discussion

First-generation electrochemical DNA biosensors: Early work in the mid-1990s focused on indicator-based DNA hybridization biosensors.[1] This approach relies on measuring changes in the peak current of a redox-active marker that preferentially binds to the target:probe duplex. For this purpose, the duplex formed (in the hybridization event) is exposed to a solution of the indicator, and the increased electrochemical response—due to the change in the surface

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concentration of the indicator—serves as the hybridization signal.

The properties of the redox indicator are very important for the performance of these first-generation DNA biosensors. The desired properties of such markers include:

a) High discrimination between single- and double-stranded DNA; substantially different interactions with the probe and the probe:target duplex.
b) Reversible electron transfer with a low redox potential.
c) Stability of both the reduced and oxidized forms.
d) Low toxicity and cost.

Sensors based on various indicators (both intercalators and groove binders) have been developed. These include the use of cationic metal complexes such as tris(2,2′-bipyridine)ruthenium(III) ($[Ru(bpy)_3]^{3+}$)[4] or tris(1,10-phenanthroline)cobalt(III) ($[Co(phen)_3]^{3+}$),[5] or planar aromatic organic compounds, such as the dye Hoechst 33258[6] or daunomycin[7] (e.g., Figure 2A and B). Most of these markers do not fully discriminate between single- and double-stranded DNA, and hence duplex formation is detected from the change (increase) in the indicator signal. Duplex-specific indicators, with negligible affinity (and hence response) to the single-stranded probe, are highly desired. One such attractive indicator, developed recently by Takenaka's group,[8] couples the threading intercalation properties of naphthalene diimide with the redox activity of ferrocene moieties (Figure 2C). Because of the large difference in the rate of its dissociation from single- and double-stranded DNA, this threading intercalator offers remarkable discrimination between the probe and the duplex.

Besides the use of external electroactive indicators, it is possible to use electrochemically active (ferrocene-bound) oligonucleotides in connection with a sandwich-type hybridization assay (Figure 3).[9] The enhanced oxidation current due to the increased surface concentration of the ferrocenyl units thus reflects the concentration of the target. Covalently bound ferrocene markers, coupled to phenylacetylene molecular wires, are being used in the DNA chips being developed by Clinical Micro Sensors (CMS) Inc.[2] The CMS system also includes a self-assembled monolayer of a hydroxy-terminated alkanethiol, aimed at minimizing nonspecific adsorption and electroactive interferences. Instead of redox tags, enzyme labels can be used to accomplish the electrochemical biosensing of DNA sequences (in a manner analogous to electrochemical immunosensors). Heller's group[10] demonstrated that the base-pair recognition of model oligonucleotides can be monitored directly as an electrical current in connection with the use of a target labeled with horseradish peroxidase (HRP). In this system, the hybridization event resulted in the wiring of the enzyme to the transducer (via an electron-conducting redox hydrogel), hence leading in a continuous hydrogen peroxide electroreduction current. Dramatic enhancement of the sensitivity of enzyme DNA biosensors may be achieved in connection to bienzyme recycling amplification schemes.

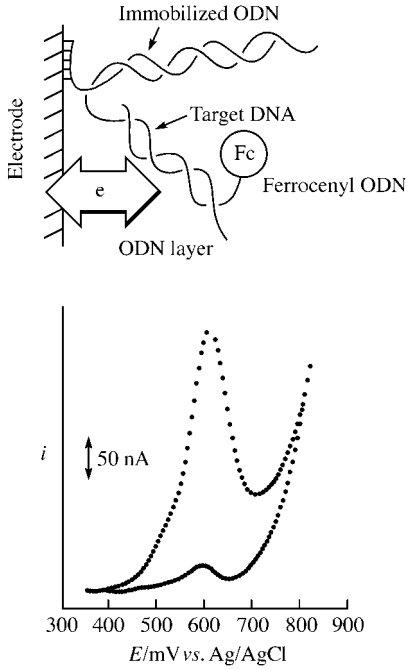

Figure 3. An electrochemical gene-sensing system based on the use of a ferrocenyl oligonucleotide (Fc-ODN), sandwich-type hybridization, and pulse-voltammetric detection of the preconcentrated ferrocenyl unit. (Reproduced with permission.[9])

Towards genoelectronics: second-generation electrochemical DNA biosensors: Direct (indicator-free) electrochemical detection of hybridization events represents a very attractive

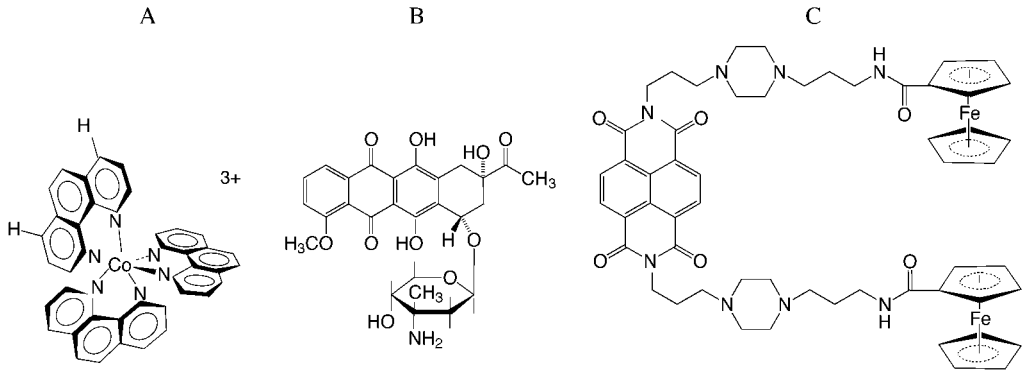

Figure 2. Examples of redox-active markers used in DNA hybridization biosensors: A) tris(1,10-phenanthroline)cobalt(III); B) daunomycin; C) ferrocenyl naphthalene diimide.

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approach for DNA biosensors. Such a route greatly simplifies the sensing protocol (since it obviates the need for the indicator addition/association/detection steps) and offers an instantaneous detection of duplex formation. Such an effective avenue for detecting DNA sequences can greatly benefit from elegant genoelectronics schemes based on the judicious interface of nucleic acid systems and electrode transducers.

Two major routes can be exploited for this indicator-free hybridization detection. The first involves monitoring changes in electronic or interfacial properties accompanying DNA hybridization, while the second relies on the intrinsic DNA signal associated with the electroactivity of nucleic acids.

Conducting polymer molecular interfaces: A key factor concerning the development of genoelectronic devices is the achievement of an efficient interface between the nucleic acid system and the electronic transducer. Conducting polymer molecular interfaces are particularly suitable for modulating DNA interactions, for inducing electrical signals accrued from such interactions, and for localizing DNA probes onto extremely small surfaces. Of particular interest and importance is the use of base-pair recognition for switching the electronic properties of conducting polymers. Such an innovative approach for detecting DNA hybridization is being explored by several groups. For example, Garnier's team has demonstrated that a 13-mer oligonucleotide-substituted polypyrrole film (Figure 4) displays a decreased current response during the duplex formation.[11] Such a change in the electronic properties of polypyrrole has been attributed to bulky conformational changes along the polymer backbone due to its higher rigidity following the hybridization. Similarly, Bäuerle and Emge[12] have demonstrated that the base-pair recognition can induce changes in the electronic properties of nucleobase-functionalized polythiophene films. Rather than covalently attaching the oligonucleotide probe to the monomer, we are currently examining its physical entrapment and/or actual doping within electropolymerized films, and developing transistor-like electronic devices based on the electrodeposition of oligonucleotide-containing conducting polymers across the gap between closely spaced microband electrodes. Ultimately, it may be possible to eliminate the need for conducting-polymer molecular interfaces and to exploit perturbations in the charge transfer through the DNA itself for detecting mutations, to rely on potentially different rates of electron transfer through ss- and ds-DNA for probing the hybridization, and to use nucleic acid wires in the design of electronic devices. Recent activity in this direction is encouraging.[13, 14]

Direct detection of hybridization based on changes in interfacial properties: Other physical parameters (besides the conductivity of electropolymerized films) can be exploited for direct label-free electrochemical detection of specific DNA sequences. In particular, the hybridization event can lead to changes in various interfacial properties that may be exploited for rapid detection of the duplex formation. For example, Souteyrand et al.[15] used a probe-coated field-effect silicon device for in situ impedance measurements of DNA sequences. The device displayed well-defined shifts of the impedance curves, corresponding to changes in the surface charge induced by the base-pair recognition. Similarly, Johansson's group has demonstrated recently that changes in the capacitance of a thiolated oligoncleotide modified gold electrode, provoked by hybridization to the complementary strand (and the corresponding displacement of solvent molecules from the surface), can be used for rapid and sensitive detection of DNA sequences.[16] Nikolelis and co-workers[17] described the use of self-assembled bilayer lipid membranes (BLMs) for the direct monitoring of DNA hybridization. A decrease in the ion conductivity across the lipid membrane surface (containing the single-stranded probe) was observed during the formation of the duplex. This was attributed to alterations in the ion permeation associated with structural changes in the BLM

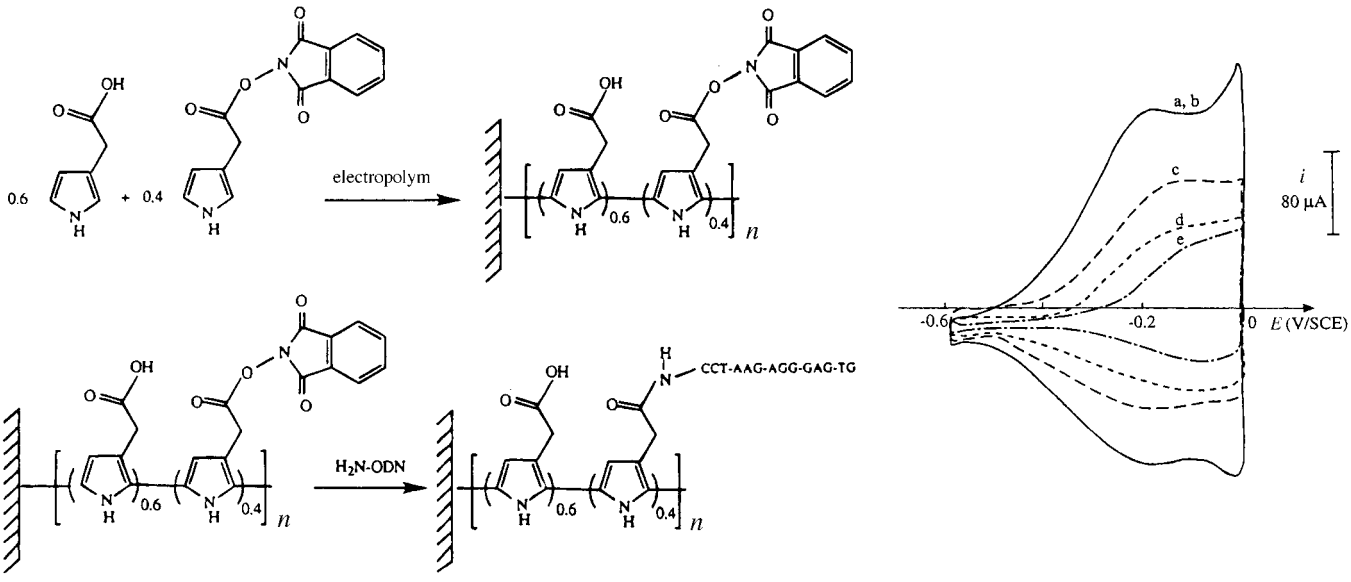

Figure 4. Preparation of the electropolymerizable oligonucleotide (ODN)-substituted polypyrrole, along with its voltammetric hybridization response to increasing levels of the DNA target: 0 (a,b), 66 (c), 165 (d), and 500 (e) nmol. (Reproduced with permission.[11])

accrued by the desorption of the ds-DNA. New avenues for generating the hybridization signal are currently being explored in several laboratories.

Intrinsic electroactivity of DNA for detecting hybridization: The intrinsic electroactivity of DNA[18] opens up unique opportunities for direct, indicator-free detection of DNA sequences. Of the four nucleobases, only guanine and adenine are readily oxidized at solid carbon electrodes.[19] Previously, we exploited the sensitivity of the guanine oxidation signal to the DNA structure for detecting the formation of the surface duplex.[20] The decreased guanine response of the immobilized probe was used for detecting the DNA hybridization. A more attractive approach is the use of an inosine-substituted (guanine-free) probe to detect the hybridization through the appearance of the target guanine signal (Figure 5).[21] The

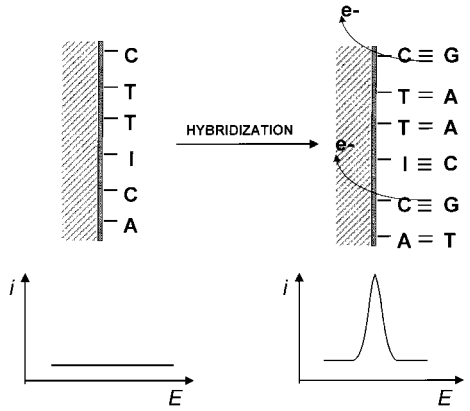

Figure 5. Direct, label-free, electrochemical detection of DNA hybridization based on the use of inosine-substituted probes and anodic monitoring of the target guanine oxidation signal.

electrocatalytic oxidation of guanine by a $[Ru(bpy)_3]^{3+}$ redox mediator has also been exploited for electrochemical sensing of DNA hybridization.[22] In this case, the formation of the double helix precludes the direct collision of the mediator with the guanine residue, hence attenuating the rate of electron transfer. New schemes for detecting DNA hybridization based on the electrochemical oxidation of the target sugar backbone at copper electrodes are currently under development in Kuhr's laboratory.[23]

Towards genoelectronic chips: Electrical regulation of DNA interactions: The use of electrical fields for modulating DNA interactions offers unique opportunities for electrochemical devices. Recent studies at Nanogen demonstrated that a fine control of the electrical field can be used for facilitating the sample preparation (e.g., extracting the DNA using a series of high-voltage pulses),[24] accelerating the hybridization event by enhanced transport of the negatively charged target under positive fields, and promoting denaturation of the duplex (i.e., regeneration of the probe). The last possibility was also exploited for discriminating against one-point mutations by applying current pulses for dissociating complexes containing mutations.[25] A fine control of the hybridization and denaturation steps may also be achieved through thermal modulation of the transducing electrode (i.e., in connection with heat-pulsing sequences). Electrical fields can also be used for moving samples around and to drive the separation of nucleic acids. Such an ability to modulate the transport and interactions of nucleic acids holds great promise for on-chip assays, integrating the sample handling with the DNA detection. Advanced microfabrication and micromachining technologies[26] provide a unique means for producing high-density arrays of individually addressable oligonucleotide-coated microelectrodes, and for providing the microfluidic network essential for integrating the sample transport, reactions, and DNA amplification, separation, or detection in a chip format (Figure 6). Unlike bulky optical readout systems, the electrochemical control can be readily integrated on the chip. By performing all the steps of the biological assay on a single chip we expect significant advantages in terms of cost, speed, simplicity, and automation.

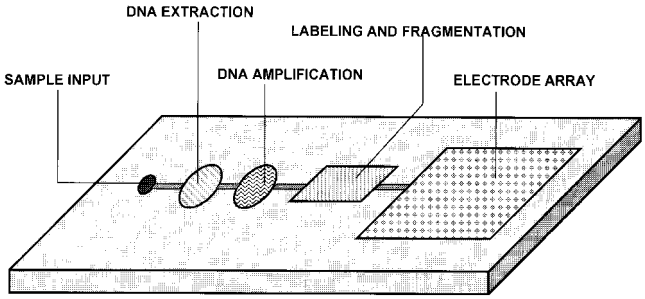

Figure 6. Layout of a microfabricated DNA chip, integrating sample pretreatment, amplification, and array detection (along with the necessary microfluidic network).

Challenges and Prospects

Few scientific areas have witnessed dramatic changes of the magnitude observed recently in DNA analysis. To continue this advance and to address market needs in the 21st century, future devices must link high-quality performance with speed, simplicity, and low cost. Electrochemical devices are not only uniquely qualified for meeting the size, cost, and power requirements of future DNA testing, but offer innovative routes for creating an interface at the molecular level between the DNA-recognition and signal-transduction elements. Yet the two important requirements of high selectivity and sensitivity remain to be demonstrated. Most devices have been shown to distinguish between relatively simple sequences, but have not been applied to the identification of point mutations. Dramatic improvements in specificity, including discrimination against single-base mismatches, have been demonstrated in peptide nucleic acid (PNA) probes.[27] The high specificity of PNA probes is attributed to their neutral pseudopeptide backbone, which results in stronger binding to complementary DNA sequences. While the application of PNA probes successfully addresses the selectivity problem, further improvements in the sensitivity will be necessary before the promise of commercial devices can be realized. The detection limits of the devices discussed above are currently approaching the picomolar (10^{-12} M) range. Various research

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groups are introducing new signal-amplification concepts that they hope will push the detectability to the femtomolar (10^{-15} M) domain. Advances in probe technology, such as the design of highly-branched (tree-like) DNA dendrimers, have also been shown to dramatically enhance the sensitivity in connection to a greatly increased hybridization capacity.[28] Dendritic PNA probes, coupling the high specificity of PNA with the amplification feature of nucleic acid dendrimers, can also be envisioned.

Over the past five years we have witnessed tremendous progress towards the development of electrochemical DNA biosensors. It is hoped that the continuous attention to fundamental issues, such as nucleic acid recognition, charge transfer through DNA, molecular (tailored) interfaces, and surface characterization, or direct signal transduction, coupled with major technological advances in the fields of microfabrication and micromachining will lead to powerful, miniaturized, easy-to-use instruments for DNA diagnostics that will accelerate the realization of large-scale genetic testing.

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