Analytical techniques for dna extraction

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Development of DNA sensors for highly sensitive detection of sequence specific DNA has become crucial due to their extensive applications in clinical diagnosis, pathogen detection, gene expression studies, and environmental monitoring. ref Along with complementary base-pair hybridization between long oligonucleotide for DNA detection, several DNA sensors employ short oligonucleotide (â‰¤10 base pair) to this goal. Ref Easley and co-workers constructed the electrochemical proximity assay (ECPA) for highly sensitive and highly selective quantitative detection of protein, where target-induced DNA hybridization between 5, 7, or 10 complementary base system brings redox tag close to the sensor surface resulting direct electrochemical readout.

To date, numerous analytical techniques have been established for DNA detection, such as electrochemistry, fluorescence, surface plasmon resonance, chemiluminiscence, quartz crystal microbalance and so on. Ref Among these methods, electrochemical DNA (E-DNA) sensors have attracted much attention owing to their reliability, simplicity, rapid response, low cost and portability, low sample consumption, ability to work in complex-multicomponent samples and remarkably high sensitivity and selectivity. ref The basic principle of E-DNA sensor is based on immobilization of single stranded DNA probe, a selective biological recognition element, on a sensor surface followed by incubation with sample containing the target biomolecules. When a target-induced molecular recognition event (hybridization) takes place the sensor translates that to a measurable electrochemical signal which is directly correlated to the target concentration. In recent years, numerous research groups have studied the

performance of these sensors by investigating the effect of immobilized probe structure and probe surface density, nature of the redox reporter used, target length, ionic strength of buffer and modifying the frequency of the square-wave voltammetry employed. ref Nevertheless, distance dependence of the redox tag relative to the electrode surface to achieve maximum signal has never been explored. As solid-phase hybridization is very distinct from that in solution-phase in terms of kinetics and thermodynamics, ref sensor performance may be sensitive to the location of the redox reporter because surface charge would likely alter the hybridization rate of negatively charged DNA which, in turn, alters the signaling properties of E-DNA sensors. Especially for short oligonucleotide (â‰¤10 base pair) hybridization near surface the effect may lead to very … due to their low binding energy which is not sufficient to overcome…. Here, we describe a detailed study of the extent to which the location of the redox reporter can be varied to achieve maximum signal within shorter response time in effort to design efficient E-DNA sensors with improved sensitivity.

Prior to this work, these electrochemical DNA (E-DNA) and electrochemical, aptamer based (E-AB) sensors have been reported against specific DNA and RNA sequences, 2 proteins, 3, 4 small molecules, 5-7 and inorganic ions. 8, 9 Because all of the sensing components in the E-DNA/EAB platform are covalently attached to the interrogating electrode, the approach requires neither exogenous reagents nor labeling of the target. Likewise, because their signaling is linked to specific, binding-induced changes in the dynamics of the probe DNA (rather than changes in adsorbed mass, charge, etc.), these sensors function well when challenged with complex, contaminant-

ridden samples such as blood serum, soil extracts, and foodstuffs. 5, 7, 9, 10 These attributes render the E-DNA/E-AB platform an appealing approach for the specific detection of oligonucleotides and other targets that bind DNA or RNA. 11-13

In the above methods, electrochemical biosensors are much popular because of their simple instrumentation setup, low sample and reagent consumption as well as high sensitivity and selectivity (Wenetal., 2012; Lu etal., 2012; Wenetal., 2011; Farjamietal., 2011; Xia etal., 2010; Xiang andLu, 2012; Pei etal., 2011; Farjamietal., 2013; Liu etal., 2013b).

Electrochemical methods, 1, 11 being simple, portable and low-cost, are particularly attractive for DNA detection. 12âˆ'16

Electrochemical methods have been used extensively in DNA detection assays, as summarized in recent review articles. 15, 16

Among these protocols, the electrochemical biosensors have attracted particular attention in different fields owing to its small dimensions, easy operation, rapid response, low cost, high sensitivity and selectivity [10, 11].

Among these techniques, the electrochemical techniques have received great interests owing to its superior characteristics of rapid response, low-cost, small-size, simple operation, and good selectivity [13-16].

Among these approaches, electrochemical methods have been shown to be superior over the other existing measurement systems, 11 because electrochemical transduction possesses a potential allowing the development of rapid, simple, low-cost, and portable devices. 12-14

As an alternative to conventional techniques, electrochemical DNA biosensors have attracted considerable interest owing to their intrinsic advantages, including good portability, fast response, and remarkably high sensitivity (Sun etal., 2010). More importantly, a number of DNA biosensors have been developed and extensively applied for the determination of biomarkers (Huang etal., 2014).

Microfabrication technology has enabled the development of electrochemical DNA biosensors with the capacity for sensitive and sequence-specific detection of nucleic acids. 1-5 The ability of electrochemical sensors to directly identify nucleic acids in complex mixtures is a significant advantage over approaches such as polymerase chain reaction (PCR) that require target purification and amplification.

Electrochemical DNA sensors are reliable, fast, simple, and cost- effective devices that convert the hybridization occurring on an electrode surface into an electrical signal by means of direct or indirect methods.

the electrochemical DNA (E-DNA) sensor is one of them. This sensor platform, the electrochemical equivalent of optical molecular beacons, exhibits notable sensitivity, specificity and operational convenience whilst also being fully electronic, reusable and able to work in complex, contaminant-rich samples [4-6].

Compared with other transducers, electrochemical ones received particular interest due to a rapid detection and great sensitivity. Combining the characteristics of DNA probes with the capacity of direct and label-free electrochemical detection represents an attractive solution in many different

fields of application, such as rapid monitoring of pollutant agents or metals in the environment, investigation and evaluation of DNA-drug interaction mechanisms, detection of DNA base damage in clinical diagnosis, or detection of specific DNA sequences in human, viral, and bacterial nucleic acids [2-8].

The determination using electrochemical biosensor methods has attracted much interest because of their simple instrumentation, high specificity, sensitivity, rapid, and is inexpensive with potential for applications in molecular sensing devices.

Amongst the electrochemical transducers, carbon electrodes such as glassy carbon, carbon fibre, graphite, or carbon black exhibit several unique properties.

Recent engineering advances have enabled the development of electrochemical DNA biosensors with molecular diagnostic capabilities (2, 8, 18, 33, 47). Electrochemical DNA biosensors offer several advantages compared to alternative molecular detection approaches, including the ability to analyze complex body fluids, high sensitivity, compatibility with microfabrication technology, a low power requirement, and compact instrumentation compatible with portable devices (18, 48). Electrochemical DNA sensors consist of a recognition layer containing oligonucleotide probes and an electrochemical signal transducer. A well-established electrochemical DNA sensor strategy involves “ sandwich” hybridization of target nucleic acids by capture and detector probes (5, 7, 46, 50).

First reported in 2003, electrochemical DNA (E-DNA) biosensors are reagentless, single-step sensors comprised of a redox-reporter-modified nucleic acid " probe" attached to an interrogating electrode. 1 Originally used for the detection of DNA2âˆ'9 and RNA10 targets, the platform has since been expanded to the detection of a wide range of small molecules, 11, 12 inorganic ions, 13, 14 and proteins, 12, 15âˆ'17 including antibodies, 18, 19 via the introduction of aptamers and nucleic-acid-small molecule and nucleic-acid-peptide conjugates as recognition elements (reviewed in refs 20 and 21).

Irrespective of their specific target, all of these sensors are predicated on a common mechanism: binding alters the efficiency with which the attached redox reporter approaches the electrode due to either the steric bulk of the target or the changes in the conformation of the probe. 1, 12, 18 Given this mechanism, these sensors are quantitative, single-step (washfree), and selective enough to perform well even in complex clinical samples. 12, 15 They are likewise supported on micrometer- scale electrodes22 and require only inexpensive, handheld driving electronics (analogous to the home glucose meter23), suggesting they are well suited to applications at the point-of-care.

Among these, the electrochemical detection of DNA hybridization appears promising due to its rapid response time, low cost, and suitability for mass production. 11, 12 The E-DNA sensor, 13-16 which is the electrochemical equivalent of an optical molecular beacon, 17-20 appears to be a particularly promising approach to oligonucleotide detection because it is rapid, reagentless, and operationally convenient. 21, 22 The E-DNA sensor is

comprised of a redox-modified " stemloop" probe that is immobilized on the surface of a gold electrode via self-assembled monolayer chemistry. In the absence of a target, the stem-loop holds the redox moiety in proximity to the electrode, producing a large Faradic current. Upon target hybridization, the stem is broken and the redox moiety moves away from the electrode surface. This produces a readily measurable reduction in current that can be related to the presence and concentration of the target sequence. Both E-DNA sensors13-16 and related sensors based on the binding-induced folding of DNA aptamers23-28 have been extensively studied in recent years. Nevertheless, key issues in their fabrication and use have not yet been explored in detail.

Electrochemical biosensors, combining the sensitivity of electroanalytical methods with the inherent bio-selectivity of the biological component, have found extensive application in diverse fields because of their high sensitivity with relatively simple and low-cost measurement systems. 1 For example, by assembling artful target-responsive DNA architectures on the electrode surface, a series of electrochemical bioanalysis methods have been proposed for the sensing of specific biomarkers, such as DNA and proteins. 2-5 The typical sensing schemes of these designs involve the immobilization of an efficient probe on the electrode surface, incubation with target biomolecules, and measurement of the output electrochemical signal. 6, 7

A wide variety of nanomaterials including metal nanoparticles, oxide nanoparticles, quantum dots, carbon nanotubes, graphene and even hybrid nanomaterials have found attractive application in electrochemical

biosensing, such as detection of DNA, proteins and pathogens and the design of biological nanodevices (bacteria/cells). 14, 15

Electrochemical transducers offer broad opportunities in DNA sensor design due to simple experiment protocols, inexpensive and mostly commercially available equipment.

Among various detection methods, the electrochemical approach attracted much attention due to its rapidness, low cost, high sensitivity and compatibility with portability [10, 11]. The E-DNA sensor [12, 13], an electrochemical method derived from the optical molecular beacon[14, 15], is particularly promising because it is reagentlessness andoperation convenience. In brief, the E-DNA sensor is composed of a redox-modified hairpin-like stem-loop DNA probe that is immobilized on the electrode surface. Without a target, the stem-loop structure holds the redox probe close to the electrode surface, pro-ducing a large current. Upon hybridization with a target, the stem is opened and the redox label moves away from the electrode surface and the current is decreased. This current change is directly related to the target DNA concentration.

Many different versions of the E-DNA sensor have been reported to date [7-9]. A popular construct of this type of sensors is a folding-based E-DNA sensor comprised of a redox-labeled DNA stem-loop probe covalently attached to a gold disk electrode. In the absence of a target, the stem-loop conformation holds the redox label in close proximity to the electrode, facilitating electron transfer. In the presence of and binding to a complementary DNA target, hybridization forces the redox tag farther from

the electrode, impeding electron transfer and producing an observable reduction in redox current [4-6].

In this approach, a single-stranded DNA (ssDNA) probe is immobilized on a surface and exposed to a sample containing the specific complementary target sequence, which is captured by forming a double-stranded DNA(dsDNA) molecule. This recognition event (hybridization) is then transduced into a readable signal.

In this strategy, the target is anchored to the sensor surface by the capture probe and detected by hybridization with a detector probe linked to a reporter function. Detector probes coupled to oxidoreductase reporter enzymes allow amperometric detection of redox signals by the sensor electrodes (28, 34). When a fixed potential is applied between the working and reference electrodes, enzyme-catalyzed redox activity is detected as a measurable electrical current (11, 16, 27). The current amplitude is a direct reflection of the number of target-probe-reporter enzyme complexes anchored to the sensor surface. Because the initial step in the electrochemical detection strategy is nucleic acid hybridization rather than enzyme-based target amplification, electrochemical sensors are able to directly detect target nucleic acids in clinical specimens, an advantage over nucleic acid amplification techniques, such as PCR.

Electrochemical methods are typically inexpensive and rapid methods that allow distinct analytes to be detected in a highly sensitive and selective manner [22-25]. Although electrochemical DNA sensors exploit a range of distinct chemistries, they all take advantage of the nanoscale interactions

among the target present in solution, the recognition layer, and the solid electrode surface. This has led to the development of simple signal transducers for the electrochemical detection of DNA hybridization by using an inexpensive analyzer. DNA hybridization can be detected electrochemically by using various strategies that exploit the electrochemistry of the redox reaction of reporters [26] and enzymes immobilized onto an electrode surface [27], direct or catalytic oxidation of DNA bases [28-31], electrochemistry of nanoparticles [32-35], conducting polymers (CPs) [35-37], and quantum dots [38].

E-DNA sensors, the electrochemical analog of optical molecular beacons [e. g., 1-4], are based on the hybridization-induced folding of an electrode-bound, redox-tagged DNA probe. In their original implementation, the concentration of a target oligonucleotide is recorded when it hybridizes to a stem-loop DNA probe, leading to the formation of a rigid, double stranded duplex that sequesters the redox tag from the interrogating electrode [1]. Follow-on E-DNA architectures have dispensed with the stem-loop probe in favor of linear probes, leading to improved binding thermodynamics and, thus, improved gain [5], as well as strand-invasion, hairpin and pseudoknot probes producing signal-on sensors [6-8]. Because E-DNA sensors are reagentless, electronic (electrochemical) and highly selective (they perform well even when challenged directly in complex, multicomponent samples such as blood serum or soil) [e. g., 9], E-DNA sensors appear to be a promising and appealing approach for the sequence-specific detection of DNA and RNA [see, e. g., 10, 11].

E-DNA signaling arises due to hybridization-linked changes in the rate, and thus efficiency, with which the redox moiety collides with the electrode and transfers electrons.

To design efficient DNA-electrochemical biosensors, it is essential to know the structure and to understand the electrochemical characteristics of DNA molecules.

Motivated by the potential advantages of the E-DNA sensing platform, numerous research groups have explored their fabrication and optimization over the past decade. Specifically, efforts have been made to improve the platform’s signal gain (change in signal upon the addition of saturating target) by optimizing the frequency of the square-wave potential rampemployed, 11 the density with which the target-recognizing probes packed onto the electrode, 11, 24 probe structure, 25 the redox reporter employed, 26 and the nature of the monolayer coating the electrode. 25

Contributing to these studies, we describe here a more comprehensive study of the extent to which the square-wave voltammetric approach itself can be optimized to achieve maximum signal gain. Specifically, we have investigated the effect of varying the square-wave frequency, amplitude, and “ potential step-size” on the gain of E-DNA sensors, evaluating each parameter as a function of the others as well as of the structure of the E-DNA probe, its packing density, the nature of its redox-reporter, and the monolayer chemistry used to coat the sensing electrode.

E-DNA sensors are a reagentless, electrochemical oligonucleotide sensing platform based on a redox-tag modified, electrode-bound probe DNA.

Because E-DNA signaling is linked to hybridization-linked changes in the dynamics of this probe, sensor performance is likely dependent on the nature of the self-assembled monolayer coating the electrode. We have investigated this question by characterizing the gain, specificity, response time and shelf-life of E-DNA sensors fabricated using a range of co-adsorbates, including both charged and neutral alkane thiols.

The signaling mechanism of E-DNA sensors is linked to a bindingspecific change in the flexibility of the redox-tagged probe; upon hybridization, the relatively rigid target/probe duplex hampers the collision of the electrochemical tag thus decreasing the observable amperometric signal [5, 12]. This, in turn, suggests that E-DNA signaling may be sensitive to changes in surface chemistry which, due to surface charge and steric bulk effects, would likely alter the dynamics of a negatively charged DNA probe. However, despite rapid growth in the E-DNA literature [reviewed in 13] the extent to which surface chemistry affects E-DNA signaling has not been established; all previous E-DNA sensors were fabricated using hydroxyl-terminated alkane thiol self-assembled monolayers (SAMs) [e. g., 1, 3, 5, 7, 9]. Here we address this question and describe a study of E-DNA sensors fabricated using co-adsorbates of various lengths and charges in an effort to further optimize E-DNA performance.

For example, while it is likely that the signaling properties of these sensors depend sensitively on the density of immobilized probe DNA molecules on the sensor surface (measured in molecules of probe per square centimeter) [see, e. g., refs 5 and 29-36], no systematic study of this effect has been reported.

Similarly, while it appears that the size of the target and the location of the recognition element within the target sequence affect signal suppression, 24 this effect, too, has seen relatively little study. Here we detail the effects of probe surface density, target length, and other aspects of molecular crowding on the signaling properties, specificity, and response time of the E-DNA sensor.

However, the sensitivity is one of the most important limiting factors for the development of electrochemical DNA biosensors.