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New approaches in the development of DNA sensors: hybridization and electrochemical detection of DNA and RNA at two different surfaces

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Abstract

Up to now, the development of the electrochemical DNA hybridization sensors relied on solid electrodes, on which both the hybridization and detection steps have been performed. Here we propose a new method in which the DNA hybridization is performed at commercially available magnetic beads and electrochemical detection on detection electrodes (DE). Due to minimum nonspecific DNA adsorption at the magnetic beads, very high specificity of the DNA hybridization is achieved. Optimum DE can be chosen only with respect to the given electrode process. It is shown that high sensitivity and specificity in the detection of relatively long target DNAs can be obtained (a) by using cathodic stripping voltammetry at mercury or solid mercury amalgam DEs for the determination of purine bases, released from DNA by acid treatment, and (b) by enzyme-linked immunoassay of target DNA modified by osmium tetroxide,2,2′-bipyridine (Os,bipy) at carbon DEs. Direct determination of Os,bipy at mercury and carbon electrodes is also possible. © 2002 Published by Elsevier Science B.V.

Keywords: DNA sensors; Hybridization; Electrochemical detection; Magnetic beads

1. Introduction

Determination of the DNA sequence of individual eukaryotic genomes is still too difficult to any current technologies. New approaches are therefore sought and, among them, sequencing by DNA hybridization is gaining importance. This kind of DNA (and RNA) sequencing can be performed by means of the DNA hybridization sensors with fluorescence detection. Recently, it has been shown that this detection can be replaced by electrochemical one, which should be less expensive and simpler. Such sensors are well suited for decentralized diagnostics as well as for determination of bacteria and viruses (including biological warfare) in the field.

Currently developed DNA hybridization sensors are using single-stranded (ss) short (15–25 nucleotides) oligodeoxynucleotide (ODN, probe DNA) immobilized on an electrode (reviewed in Refs. [1–3]). The ODN-modified electrode is immersed in target DNA solution to test its nucleotide sequence. When the sequence of target DNA matches that of the probe (based on the complementary Watson–Crick pairing), a probe–target (hybrid) duplex DNA is formed at the electrode surface. The hybridization event (DNA duplex formation) is detected electrochemically in various ways [4].

This system works quite well with synthetic ODNs when probe and target DNAs are of about the same lengths. In a real DNA sequence analysis with longer PCR products, viral or chromosomal DNAs, the target DNAs are substantially longer than the probe. With longer target DNAs, difficulties connected with the nonspecific DNA adsorptions frequently arise, resulting in a loss of specificity and decreased sensitivity. Elimination of the nonspecific DNA adsorption at the electrodes (such as carbon or gold ones) usually applied for the DNA hybridization is very difficult.

At this meeting, we propose for the first time a new method based on separation of DNA hybridization from the electrochemical detection. We show that using this method, it is possible to analyze long target DNAs in the presence of a large excess of nonspecific DNA.

2. Experimental

2.1. Chemicals

Oligodeoxynucleotides (ODNs) were synthesized by VBC-Genomics (Vienna, Austria). Dynabeads Oligo$(dT)_{25}$

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and magnetic particle concentrator MPC-S were products of Dynal (Oslo, Norway). The nucleic acid concentration was determined spectrophotometrically using a HP 8452 spectrophotometer. OsO_4 was supplied by JMC (Great Britain); other chemical reagents were products of Sigma (St. Louis, MO).

2.2. Hybridization at the Dynabeads

Aliquots (usually 20 μl) of Dynabeads Oligo$(dT)_{25}$ (DBT) were washed twice in 1:1 volume of 0.2 M NaCl and 50 mM phosphate buffer, pH 7.0. Then 20 μl of DNA solution in the same buffer was added to the DBT. The samples were shaken for 30 min at 26 °C to allow hybridization between the DNA in solution and the oligo(dT) chains on the bead surface. After hybridization, the DBT was washed five times with 100 μl of 0.3 M $NaClO_4$. DNA was released from DBT by heating (85 °C, 2 min) into 20 μl of triple-distilled water.

2.3. DNA depurination

Hydrolysis of ODN was performed by adding 20 μl of 1 M $HClO_4$ to sample of the same volume of 2.0-μM ODN (related to the monomer) and heating for 30 min at 65 °C. The sample was cooled down, neutralized with NaOH and 20-μl aliquot was added to 1 ml of the background electrolyte and used for voltammetry measurements.

2.4. Modification of DNA with Os,bipy

DNA samples (50 μg ml^{-1}) were incubated with 2 mM OsO_4 and 2 mM 2,2′-bipyridine (Os,bipy) in 0.1 M Tris–MHCl (pH 7.4) at 37 °C for 3 h. To remove unreacted Os,bipy, the samples were dialyzed against 0.1 M Tris–HCl (pH 7.4) using Slide-A-Lyzer MINI Dialysis Units at 5 °C overnight.

2.5. Voltammetry

Electrochemical measurements were performed with an Autolab analyzer (Eco Chemie, The Netherlands) in connection with a VA-Stand 663 (Metrohm, Zurich, Switzerland) using a three-electrode system consisting of Ag/AgCl/3M KCl electrode as a reference and platinum wire as an auxiliary electrode. The working electrodes were either hanging mercury drop electrode (HMDE) or pyrolytic graphite electrode (PGE).

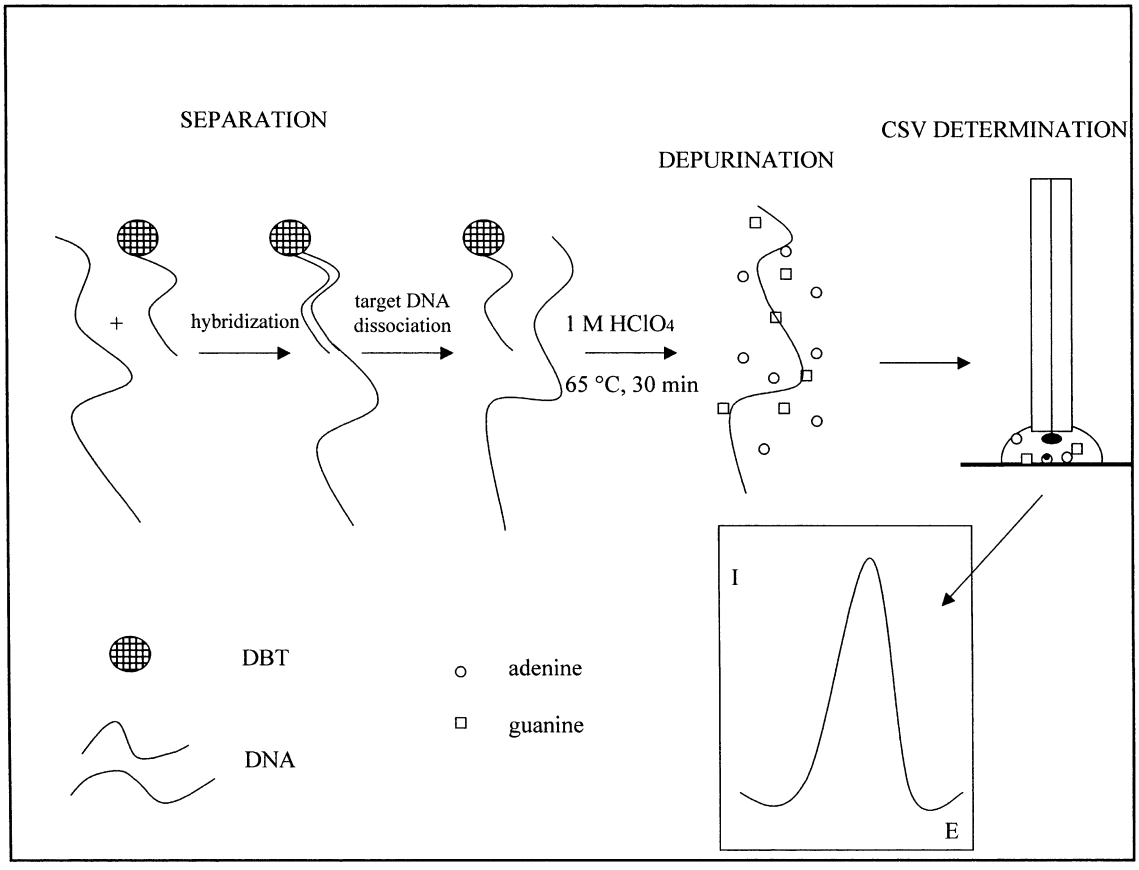

Fig. 1. Scheme of DNA (or RNA) hybridization in which Dynabeads oligo$(dT)_{25}$ (DBT) are used as surface H and a hanging mercury drop electrode (HMDE) as DE. The detection is based on the cathodic stripping voltammetry (CSV) of adenine released from DNA by acid treatment. Adenine, producing a sparingly soluble compound with the electrode mercury, can be determined by CSV at nanomolar concentrations [6].

2.6. Cathodic striping voltammetry (CSV) at HMDE

CSV measurements were performed using differential pulse technique at HMDE with a drop area of 0.41 mm^2. All CSV experiments were carried out with the following parameters: E_i=0.18 V, t_A=120 s, pulse amplitude=50 mV, step potential=5 mV. Borax buffer (0.05 M, deaerated prior to each measurement) was used as a background electrolyte.

2.7. Pyrolytic graphite electrode

Before each measurement, the PGE was electrochemically pretreated at −1.7 V for 60 s in the background electrolyte followed by renewing the surface using sticky tape. DNA was adsorbed at the electrode surface from 7-μl aliquots, containing 0.2 M NaCl, 10 mM Tris–HCl, pH 7.4, for 1 min followed by rinsing the electrode by distilled water and transfer of the electrode to the voltammetric cell. Signals of the DNA-Os,bipy adducts as well as the intrinsic DNA oxidation signals were measured by square-wave voltammetry at following settings: initial potential −1.0 V, final potential +1.6 V, amplitude 25 mV, frequency 200 Hz. As a background electrolyte, 0.2 M acetate buffer (pH 5.0) was used. All measurements were done at room temperature.

3. Results and discussion

We hybridize DNA at one surface with the immobilized probe DNA (surface H) and perform the electrochemical detection at another surface (detection electrode, DE). Finding optimum conditions in this system is much easier than in the usual one-surface systems (with DNA probe attached directly to the electrode) because optimum conditions for hybridization are not always the same as those of the electrochemical detection and vice versa. For example, (a) strong adsorption of DNA at DE can be exploited to accumulate DNA at DE, on the other hand, strong adsorption of DNA at surface H can decrease or even eliminate the specificity of the DNA hybridization; (b) the DE should be small to be able to detect small volumes of DNA hybrids while the hybridization surface H should be sufficiently large to efficiently capture target DNA; (c) surface H should be suitable for the probe immobilization while DE should be best suited for the given electrode process (probe immobilization is not necessary); (d) surface H does not have to be conductive but the electrode has to be conductive, etc. In our work, we used commercially available superparamagnetic Dynabeads oligo(dT), with the covalently attached DNA probe $(dT)_{25}$, as surface H and the mercury, solid amalgam or carbon DEs for the DNA and RNA hybridization Fig. 1.

3.1. Mercury and solid amalgam electrodes

In this paper, we wish to show the usefulness of the mercury electrodes enabling highly sensitive label-free selection of DNA and RNA. Mercury electrodes have not been so far applied in the DNA hybridization sensors probably due to the strong hydrophobic interactions of ss nucleic acids with the mercury surface [5], interfering with the specific probe–target interaction at these electrodes. If DNA hybridization is performed at surface H, mercury electrodes prove to be excellent DE, which can be applied for different purposes, including:

(a) Highly sensitive label-free detection of purine bases (forming sparingly soluble compounds with the electrode mercury [6]) released from DNA by acid treatment [4]. Nanomolar and subnanomolar concentration of DNA (related to the monomer content) can be determined by

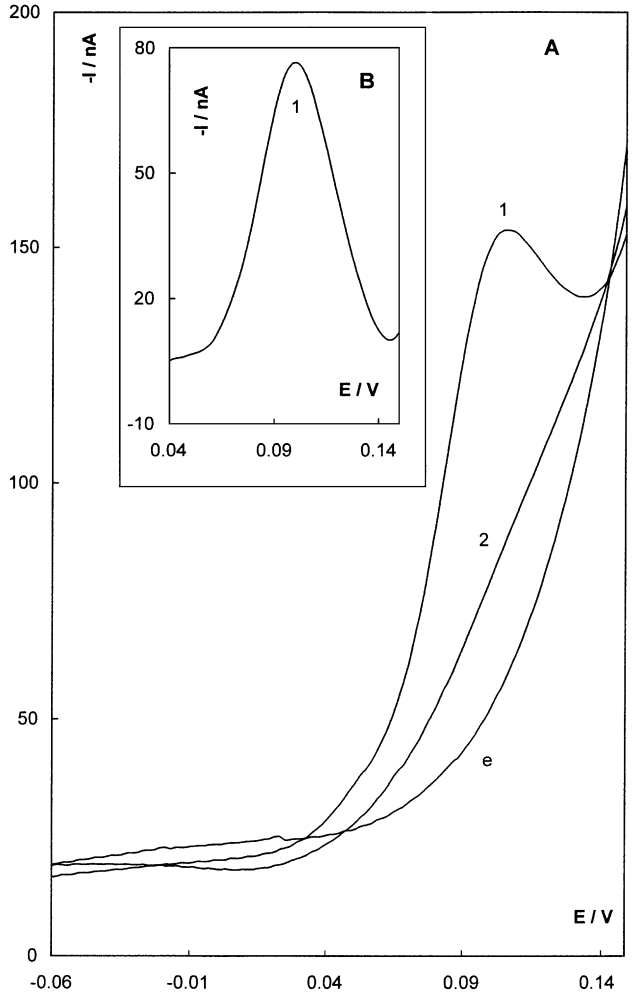

Fig. 2. Differential pulse-stripping voltammograms obtained (A1) after specific hybridization of $(A)_{25}(GAA)_4$, and (A2) after nonspecific interaction of $(GAA)_4$ at DBT; e: background electrolyte (without baseline correction); (B1) is baseline corrected (moving average) curve A1. ODNs (2 μM) were incubated with DBT, washed, released from DBT and acid-treated as described in Experimental. An aliquot of ODN, 20-μl, was added to 1 ml of background electrolyte. HMDE, E_i=0.18 V, t_A=120 s, pulse amplitude=50 mV, potential step=5 mV. Background electrolyte: 0.05 M borax.

the cathodic stripping voltammetry (CSV) in the presence or absence of copper ions [4,7,8]. Fig. 2 shows a well-developed peak of 37-mer ODN $(A)_{25}(GAA)_4$, specifically captured by DBT, contrasting with almost flat curve of the noncomplementary $(GAA)_4$ [or $(CTT)_4$, not shown] differing little from the background electrolyte. Similar results were obtained with longer target DNAs, including a DNA PCR product, and in the presence of large excess of noncomplementary DNA [4].

(b) Determination of chemically modified DNA such as:

(i) DNA prelabeled or postlabeled with osmium tetroxide,2,2′-bipyridine (Os,bipy); with this DNA voltammetric signals due to the catalytic hydrogen evolution can be obtained at very low DNA concentrations [9], similar to CSV mentioned under (a);
(ii) cystein-labeled nucleic acids producing signals due to the catalytic hydrogen evolution in cobalt-containing background electrolytes [10], similar to signals of proteins and peptides.

3.2. Carbon electrodes

Adenine (A) and guanine (G) residues in DNA and RNA are oxidizable at carbon electrodes [11–13]. Carbon electrodes can be applied as DE in DNA hybridization either for label-free detection of G and A as well as for the detection of Os,bipy-modified target DNA [9,14] when working with model ODN systems and possibly also for target DNA amplified by PCR. The oxidation of A and G is irreversible while peaks of DNA-Os,bipy adducts show a certain degree of reversibility [14]. In adsorptive transfer-stripping (AdTS) experiments, previous separation of the DNA adduct by dialysis or other methods is not necessary, if a proper washing procedure is used. As expected, the sensitivity of these techniques depends very much on the base content of the analyzed DNA (Fig. 3). Os,bipy reacts preferentially with thymines (T) and DNAs with a high content of this base can be detected at low concentrations as shown in Fig. 3A. On the other hand, DNAs with high contents of G and/or A (and low content of T) can be better determined by means of oxidation signals (Fig. 3).

DNA-Os,bipy adducts are immunogenic [15] and can be determined at high sensitivities by electrochemical enzyme-linked immunoassay [16] at carbon and other electrodes. Using alkaline phosphatase and electroinactive 1-naphthyl phosphate as a substrate, we measured the electroactive product (1-naphthol) at carbon electrodes. The DNA hybridization was performed at DBT and long target deoxyoligonucleotides (such as 67- and 97-mers) and a DNA PCR product (226 base pairs) were detected at high sensitivity and specificity. Large excess of noncomplementary DNA did not prevent the determination [16]. Several possibilities exist to improve performance of this method, including the application of better substrate/product systems, other Os,L

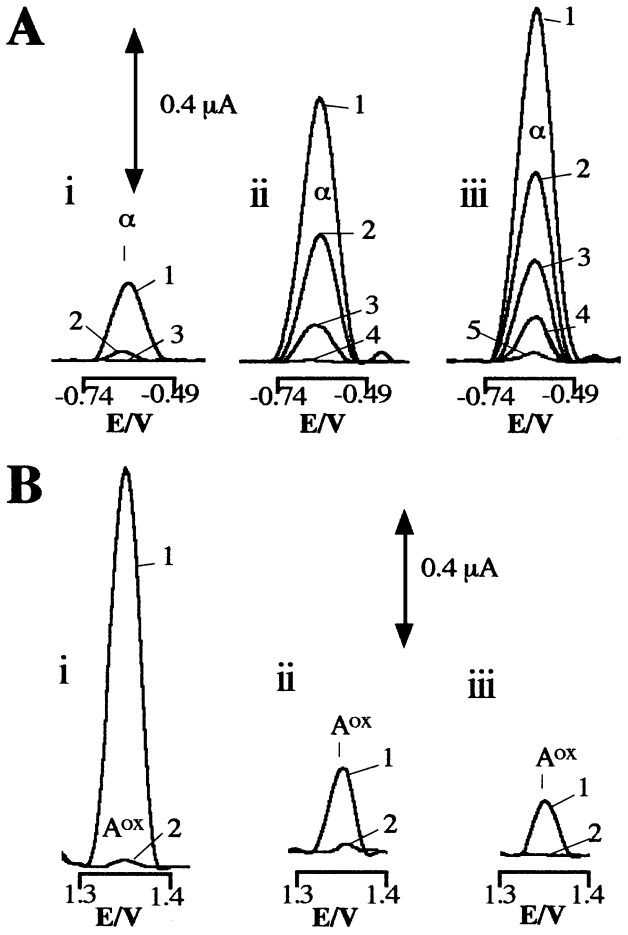

Fig. 3. Square-wave voltammograms of covalently Os,bipy-labeled DNA (A) and of underivatized DNA oligonucleotides (B) at the PGE. (i) 26-mer ODN $(A)_{14}(G)_7(T)_5$; (ii) 51-mer ODN $(A)_{14}(G)_7(T)_{20}$; (iii) 71-mer ODN $(A)_{14}(G)_7(T)_{50}$. A—Peak due to the DNA-Os,bipy adduct: (1) 200, (2) 100, (3) 50, (4) 25, (5) 13 ng ml^{-1}. The ODNs (50 μg ml^{-1}) were treated with 2 mM Os,bipy in 100 mM Tris–HCl, pH 7.6 at 37 °C followed by dialysis. B—Intrinsic adenine oxidation signal: (1) 250, (2) 125 ng ml^{-1} of unmodified ODNs. DNA was adsorbed at the electrode surface from 7-μl aliquots for 1 min followed by rinsing the electrode by distilled water and square-wave voltammetric measurement in 0.2 M sodium acetate, pH 5 (initial potential −1.0 V, final potential +1.6 V, amplitude 25 mV, frequency 200 Hz).

complexes for DNA modification (in combination with relevant antibodies), etc.

4. Conclusions

Electroactivity of nucleic acids (NAs) was discovered more than 40 years ago [17,18] by means of mercury electrodes. Almost 20 years later, carbon electrodes were introduced in the NA research [11] and both types of electrodes have been applied in biochemical analysis of [13,19–21] (reviewed in Ref. [22]). Mercury electrodes have shown a remarkable sensitivity for small changes in the DNA structure and produced an early evidence of DNA premelting and polymorphy of the DNA double helix

(reviewed in Ref. [23]). DNA-modified electrodes were introduced about 15 years ago [24] and in the last decade, they have been applied in the development of sensors for the DNA hybridization (reviewed in Refs. [1–3] and damage (reviewed in Ref. [3]). At this meeting, we show for the first time a new type of the DNA hybridization system, which differs from the previous ones (reviewed in Refs. [1–3] by separation of DNA hybridization from the electrochemical detection. In this new system, the hybridization is performed at surface H, optimized for the given purpose and the detection at the optimal DE. We also show that advantages of mercury and solid amalgam electrodes can be utilized in DNA hybridization sensors. The magnetic beads used in this paper as surface H are well suited for incorporation in a microfluidic device [25], which can easily handle all the necessary steps, including washing and the enzymatic reaction, using pumping and magnetic field systems [25,26]. Application of microfluidic systems enables miniaturization and automation, resulting in less reagent and sample consumption, more efficient and faster hybridization, etc.

The usefulness of the magnetic beads in the development of the DNA hybridization sensors is underlined by the contribution of Professor Joseph Wang presented at this meeting, reporting the use of another type of magnetic beads (covered with streptavidin to which biotinylated ODN probe is bound). Biotin-labeled 19-mer was used as a target DNA and streptavidin-labeled alkaline phosphatase was bound to the biotinylated target DNA and the product of the enzymatic reaction was determined at disposable screen-printed carbon electrodes [26]. He also reported an alternative detection of the DNA hybridization by means of gold nanoparticle tags on biotin-labeled ODN 19-mer target followed by precipitation of silver on the nanoparticles.

Recently, Azek at al. [27] immobilized a PCR-amplified DNA fragment on carbon electrode and detected the presence of the specific sequence of the human cytomegalovirus using a biotinylated probe. Streptavidin conjugated to horseradish peroxidase was used to quantify the extent of the DNA hybridization. This technique may be quite efficient and useful when relatively short DNA PCR products are used. It may however be expected that its efficiency will decrease with the length of the PCR products and particularly in unamplified DNA samples, where the length of the specific nucleotide sequence to be detected will represent too small fraction of DNA adsorbed at the electrode surface.

Our results show that application of the commercially available beads, developed for separation of mRNA by hybridization [28], is well suited for DNA hybridization with minimum nonspecific DNA adsorption at the surface. We believe that these beads in combination with a suitable DE, enabling measurement of high electron yield (catalytic) signals, represent a significant step toward an automatic DNA hybridization sensor capable to analyze long PCR products and possibly also natural, unamplified DNA samples. Present state of the development of the sensors for DNA hybridization and damage is reflected in a special issue of Talanta [29] devoted to this subject.

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