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Threading intercalation to double-stranded DNA and the application to DNA sensing. Electrochemical array technique*

Makoto Takagi

Department of Applied Chemistry, Faculty of Engineering, Kyushu University, Higashi, Fukuoka, Japan

Abstract: Electrochemical labeling of double-stranded (ds) DNA is achieved by redox-active DNA intercalators. Threading intercalators derived from naphthalenediimide appended with ferrocene reporter group in the side arms are particularly useful for this purpose. This allows a sensitive electrochemical detection of DNA hybridization on an array, providing a great potential for low-cost, high-throughput, and quick DNA screening technique in post-genome study.

INTRODUCTION

The development of DNA sensing or gene detection is increasing its practical importance especially in conjunction with the development of micro fabrication technology toward chips and arrays. Nowadays, fluorometric detection is widely used, typically based on the DNA chips developed by Affymetrix Inc., while the potential of electrochemical method has long been suggested and studied [1]. DNA detection is basically a hybridization detection. A single-stranded DNA (ssDNA) is immobilized on a solid support as a probe, and a sample DNA mixture is allowed to react under hybridization conditions. The target DNA carrying the base sequence that matches that of the probe strand selectively forms a double-stranded DNA (dsDNA) and becomes bound to the solid support. After washing off the extraneous DNA in the sample, the dsDNA on the support is detected and quantitated by appropriate means (fluorometric, electrochemical, etc.).

Some of the keys in developing electrochemical DNA chips or arrays are (i) selective electrochemical labeling of dsDNA on the support, since otherwise the ssDNA probe strands that remain unhybridized can produce a serious background signal, and (ii) effective and reproducible immobilization of probe strand on the support (electrode) surface where the electric signal resulting from hybridization is to be collected. The author and coworkers have been involved in this line of study for the last 10 years.

As an electrochemical labeling agent (or redox-active DNA ligand), a combination of a DNA intercalator (acridine derivative) and groove binder (viologen derivative) was first studied [2,3]. Both the DNA binding modes, intercalation and groove-binding, are expected to be peculiar to dsDNA. This molecular design provided a strong binding affinity to dsDNA, but the standard redox potential of viologen falls in the negative side and is inconvenient in conventional electrochemical sensing purposes. In fact, as to the redox potential and electrochemical reversibility, there are not very many choices in organic materials for use in DNA labeling. Therefore, the use of ferrocene was considered, and we described the ferrocene labeling of oligonucleotide and its successful use in detecting targeted DNA fragments at fmol level on HPLC fitted with a conventional electrochemical detector [4]. Ferrocene

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derivatives are suitable for such labeling use because they are easy in synthetic handling and in the subsequent electrochemical measurements.

As to the immobilization of probe DNA to an electrode, traditional thiol/Au chemistry seems to be the best choice. In 1992, we first described DNA-immobilized gold electrode for sensory purpose [5], where DNA-binding drugs such as quinacrine were determined based on the concept of ion-channel mechanism [6].

Thus, all the basic elements in carrying out electrochemical DNA detection on a chip or an array seemed at hand, but the technique could not be practical until the kinetic and configurational aspects of DNA-intercalating ligands were clarified and taken advantage of as described below.

THREADING INTERCALATORS

Figure 1 shows some of the typical "threading" intercalators. Nogalamycin, one of the anthracycline antibiotics, is the most studied in detail as to its threading behavior [7,8]. The anthraquinone ring is the intercalating part, and in order for the ring to be fully intercalated one of the two bulky sugar moieties attached in the opposed sides of the ring has to penetrate the stack of base pairs. The situation is obviously different than the penetration by ordinary intercalators, since the molecular thickness of the sugar moiety is much greater and requires a base-pair dissociation (partial breakage of dsDNA structure) for its penetration. Such a process in effect results in kinetic inertness or stabilization of the complexes formed from threading intercalators and dsDNA as compared to those formed from classical intercala-

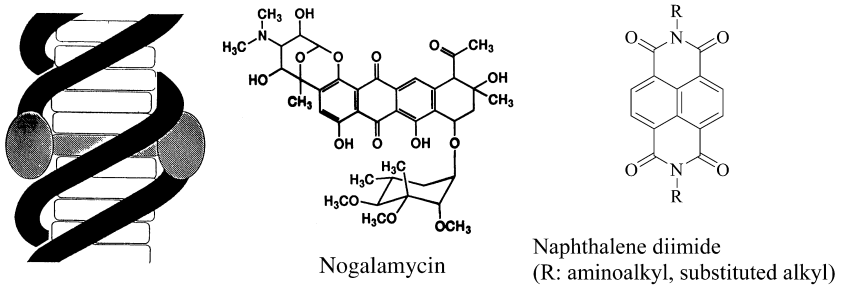

Fig. 1 Some typical natural and synthetic intercalators.

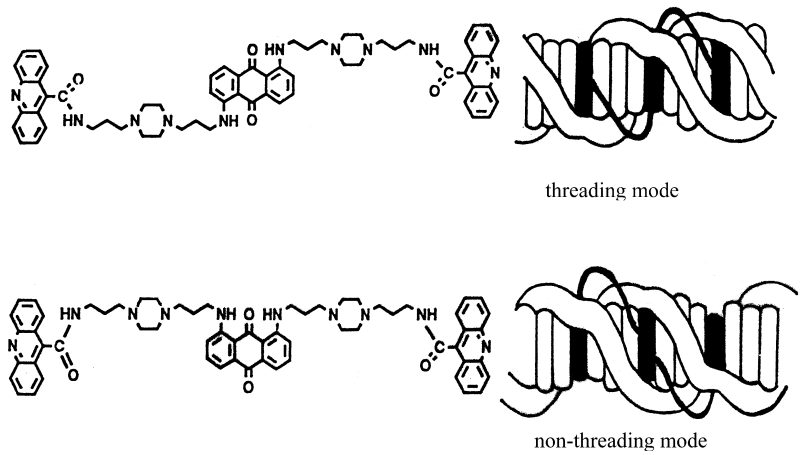

Fig. 2 Threading and non-threading intercalation by 1,5- and 1,8-disubstituted anthraquinone derivatives (trisintercalators).

tors. The situation is similar for synthetic threading intercalators shown in Fig. 1, where bulky substituents are introduced at two nitrogens in the opposite positions in naphthalene bis(dicarboximide). To further help envisage the topology difference between the threading and non-threading intercalation, the DNA-binding of isomeric synthetic trisintercalators are schematically illustrated in Fig. 2 [9]. A review was made on threading intercalators emphasizing its aspect as a DNA structural probe [10].

DNA-PROBE MODIFIED ELECTRODE AND ELECTROCHEMICAL DNA SENSING

The principle of gene detection using hybridization indicator is illustrated in Fig. 3 along with typical electrochemically active DNA ligands so far studied [11,12]. Methylene blue is a typical intercalator, while the other ligands are groove binders. On the other hand, we have developed a ferrocene-appended naphthalene diimide derivative (FND, Fig. 4) specifically designed for the present purpose [13,14].

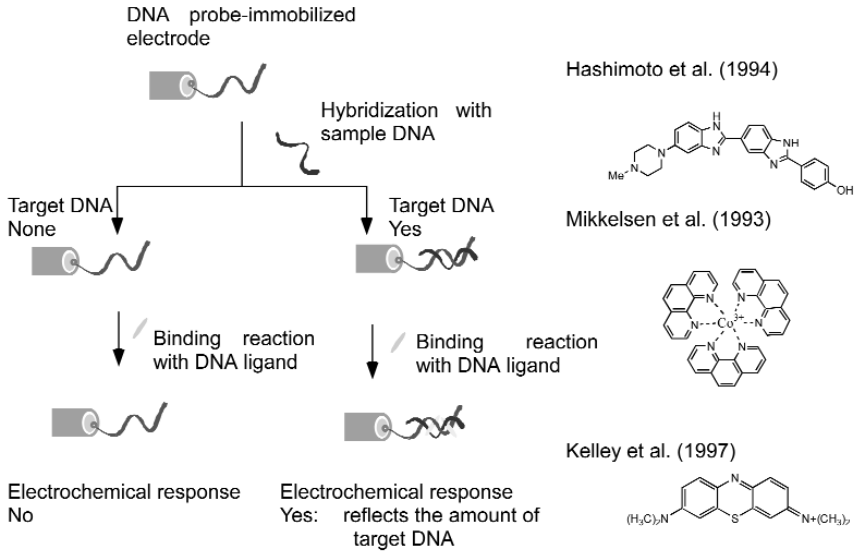

Fig. 3 Principle of gene detection based on hybridization indicators. Electrochemically active DNA ligands used.

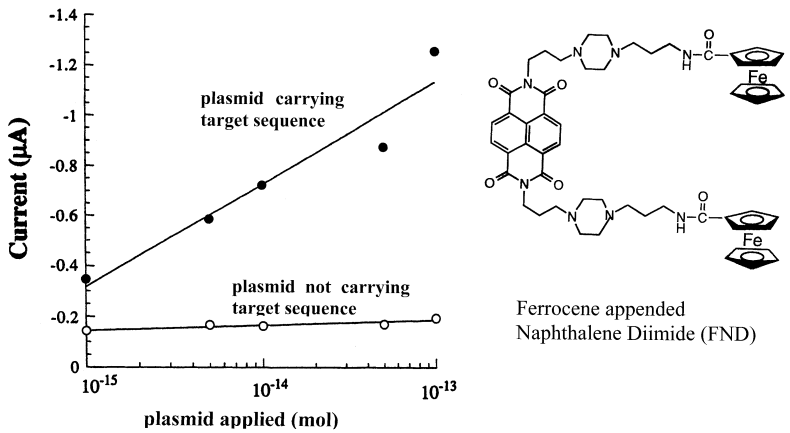

Fig. 4 DPV detection of plasmid DNA carrying yeast choline transport gene using ferrocene-appended naphthalene diimide (FND) as hybridization indicator.

We tested several classical intercalators and groove binders for electrochemical hybridization indicators on our ssDNA-probe modified gold electrodes, but these classical ligands proved unsatisfactory in achieving high detection sensitivity and critical discrimination among base mutation sequences of practical importance.

FND and the related family of derivatives (naphthalene diimides with different ferrocene and amine moieties), on the other hand, behaved well. The difference from the classical DNA ligands is presumably connected to the kinetic inertness of the threading ligands (slow rates of both complex formation and dissociation), though its verification is a matter of further research. In gene detection with FND, an anodic current at 520 mV (against Ag/AgCl reference electrode) due to ferrocene was measured by differential pulse voltammetry (DPV). Sometimes, the current before hybridization (i_0) and that after hybridization (i) were compared, and the % increase [$\Delta i = 100(i - i_0)/ i_0$] due to dsDNA formation on the electrode was taken as the measure of the presence (and the amount) of the targeted gene or DNA fragments in the sample DNA.

Figure 4 shows the calibration line for the determination of recombinant plasmid carrying the yeast choline transport gene. The gold electrode of a surface area of 2 mm^2 was modified with d(CCGCTTATCTTCAGTTTTCG) at a density of 19 pmol/mm^2. The electrode was allowed to undergo hybridization conditions in the presence of a varying amount of plasmid DNA samples, and finally DPV measurements were made in the presence of FND. It is feasible to determine plasmid DNA down to fmol levels. By reducing the modification density of probe DNA on the electrode, the detection of zmol level DNA is possible under favorable conditions [13]. The electrochemical detection of base pair mutation is also practical by using FND [14,15].

DNA MICROARRAY (ELECTROCHEMICAL ARRAY: ECA)

DNA hybridization detection by electrochemical means seems to be promising so far as one practices on a single electrode. Meanwhile, it is desirable in practical applications that the performances obtained are extended to an integrated multi-electrode array system. Preliminary study was made by using a 25-electrode "electrochemical chip" (ECA chip) [15]; gold galvanized spots of 1.0 mm diameter were placed in five rows (A–E) and five lines (1–5) each being apart by 4.5 mm (Fig. 5). Considerable variations were observed among 25 electrodes with respect to the surface area and the probe modification density. However, by taking advantage of Δi values, meaningful statistical information on the nature of DNA sample could be derived.

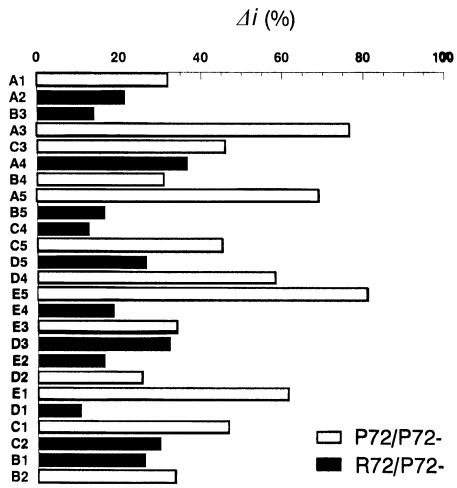

Fig. 5 One base mismatch detection on ECA.

Figure 5 shows the results of one-base mismatch detection in p53 gene, a gene coding a cancer repression protein. Two types of probe oligonucleotides, P72 and R72, were immobilized on the electrode on the chip in an alternate way; the electrodes' corresponding open bars in Fig. 5 were loaded with P72, and those corresponding filled bars were loaded with R72 probe. The whole ECA was then subjected to hybridization procedure with P72- oligonucleotide, the complementary strand to P72. A subsequent DPV measurement with added FND gave a current increase Δi as summarized in Fig. 5. The P72/P72- pair constitutes a fully matched base-pairing, while the R72/P72- pair addresses a G-G mismatched duplex on the electrode. Obviously, a P72/P72- pair is featured with a greater Δi value as compared with an R72/P72- pair.

TUM-gene, Inc. (Chiba, Japan; http://www.tum-gene.com/) is now developing ECA chips and the associated measuring assembly for commercialization based on the present technique.

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