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Hybridization biosensor using 2-nitroacridone as electrochemical indicator for detection of short DNA species of Chronic Myelogenous Leukemia

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ABSTRACT

A new acridone derivative 2-nitroacridone (NAD) was synthesized in this paper, and it was found that NAD had excellent electrochemical activity on the glassy carbon electrode (GCE) with a couple reversible redox peaks at 0.051 V and 0.103 V, respectively. Voltammetry was used to investigate the electrochemical behavior of NAD and the interaction between NAD and salmon sperm DNA. In pH 4.0 phosphate buffer solution, the binding ratio between NAD and salmon sperm DNA was calculated to be 2:1 and the binding constant was 3.19×10^5 L/mol. A Chronic Myelogenous Leukemia (CML, Type b_3a_2) DNA biosensor was developed by immobilizing covalently single-stranded CML DNA fragments to a modified GCE. The surface hybridization of the immobilized single-stranded CML DNA fragment with its complementary DNA fragment was evidenced by electrochemical methods using NAD as a novel electrochemical indicator, with a detection limit of 6.7×10^{-9} M and a linear response range of 1.8×10^{-8} M to 9.1×10^{-8} M for CML DNA. Selective determination of complementary ssDNA was achieved using differential pulse voltammetry (DPV).

1. Introduction

Chronic Myelogenous Leukaemia (CML) is a clonal myeloproliferative disorder resulting from the neoplastic transformation of the primitive hemopoietic stem cell (Fialkow et al., 1967; Champlin and Golde, 1985; Kantarjian et al., 1993). Generally speaking, the CML patients does not appear any observable symptoms in their early stage, and the chronic course can last 3–5 years. These phenomena bring the difficulties to the diagnosis of CML. There has been much learned about the clinical disorder and how the chimeric oncogene BCR/ABL generated from the translocation of chromosome 9 to 22 (Philadelphia Ph. Chromosome) can lead to the pathogenesis of CML. BCR/ABL gene is the traditional gene for the disease, and it exists in almost all cases of CML patients (Butturini and Ralph, 1996; Jorge et al., 1996). There are many types of BCR/ABL genes, and Type b_3a_2 is one of the most common mutation types, which has been often studied. Thus, the detection of BCR/ABL gene will afford an early diagnosis and monitor of the disease, and further improve the facility of detecting minimal residual leukaemia cells in the CML patients, especially after the bone marrow transplantation (BMT).

In recent years, the monitoring methods for clinical diagnosis and prognosis about fusion gene of CML include chromosome analysis, Southern blot, polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH) and so on, but all of these methods have some limitations. Chromosome analysis is time-consuming and insensitive; the traditional Southern blot detection is hadrospecificity, while it is very insensitive to the detection of the low replication of the gene order, its detection method is complicated, and the cycle is too long, moreover it relies on hazardous radioactive labels, which limit its extensive application in clinic. PCR, an enzyme-based DNA amplification technology, is often employed toward these applications. Although PCR is extremely sensitive, it remains to be improved from the practical point of view. Its disadvantages include relatively long assay time, high assay cost, and error-prone nature that occasionally lead to "false-positive" signals. FISH technique requires complicated fluorescence coloration system and its sensitivity is still not high, so it cannot be widely utilized in clinic. Thus, it is very significant to develop and investigate CML gene detecting technique, which is convenient, fast, accurate, sensitive and economic for solving the actual problem of the early diagnosis and prognosis monitor of CML.

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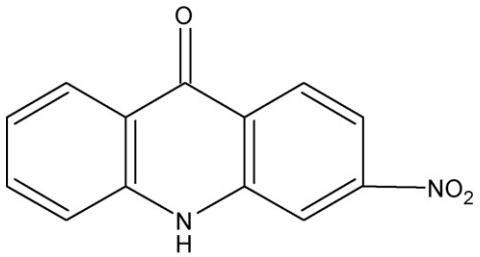
DNA biosensors based on nucleic acid hybridization, one of the most important biomolecular recognition processes, are currently undertaken wide investigation owing to their increasing importance in the diagnosis of disease with low-cost and low power requirements (Wang, 1999; Palecek and Fojta, 2001; Millan et al., 1992, 1994; Millan and Mikkelsen, 1993; Hashimoto et al., 1994a,b). Electrochemical techniques are very suitable for rapid and direct detection of specific DNA sequences with high sensitivity, small dimensions and good compatibility with transducer microfabrication technology (Wang, 2000; Mikkelsen, 1996; Yang et al., 2002; Hason et al., 2002; Wong and Gooding, 2003; Pang et al., 1996; Ozsoz et al., 2003). These sensors can be prepared by immobilizing single-stranded DNA probes on different electrodes and using electroactive indicators to measure the hybridization events between the DNA probes and their complementary DNA fragments. DNA hybridization detection deals with the use of electroactive indicators interacting directly and specifically with the DNA duplex, such as intercalators, DNA groove binders, metal complexes and threading agents, has been recently reviewed by Takenaka (2003). However, concerning DNA biosensor development, there are still some limitations with these indicators, for example, metal complexes such as $Ru(bpy)_3^{2+}$ (Armistead and Thorp, 2001), Ferrocene (Eunkyung et al., 2003), $[Co(bpy)_3]Cl_2$ (bpy = 2,2-bipyridine) (Millan et al., 1994), $[Co(phen)_3]Cl_2$ (phen) 1,10-phenanthro-line) (Wang et al., 1997), $[Os(bpy)_3]Cl_2$ (Mishima et al., 2000; Gore et al., 2003), $[Os(5,6\text{-dmphen})_3]Cl_2$ (5,6-dmphen) 5,6-dimethyl-1,10-phenanthroline) (Maruyama et al., 2001) and poly(4-vinylpyridine) derivative bearing $[Os(5,6\text{-dimethyl-1,10-phenanthroline})_2Cl]^{2+}$ (Liu and Anzai, 2004), have often been used for this purpose because of their high stability and reversibility in the redox reactions. In practice, DNA hybridization is detected by measuring the redox current for redox indicators adsorbed on the dsDNA chains on the electrode. In this protocol, it is a prerequisite for the redox indicators to be adsorbed more efficiently on dsDNA chains than on single-stranded DNA (ssDNA) for affecting sensitive determination of DNA hybridization. From this point of view, the binding efficiency of the metal complexes mentioned above should be improved because their binding affinity is still inadequate, which is due to the fact that the metal complexes bind to dsDNA chains through the electrostatic force between metal complexes and DNA backbone or through hydrophobic interactions. Therefore, metal complexes have been modified with intercalative ligands to improve the binding affinity to dsDNA (Wilhelmsson et al., 2002; Metcalfe et al., 2003; Li et al., 2007; Niu et al., 2006). However, there are still some limitations with these methods, including experimental complications, such as purification of the ligands and complexes. Until now, novel electroactive indicators still need to be developed for the sensitive and selective assay of the hybridization events between the DNA probes and their complementary DNA fragments.

Acridine and acridone derivatives are well-known anti-tumor drugs and their planar structure confers on these molecules the ability to bind DNA by intercalation. The acridone is highly fluorescent and stable (Legg and Hercules, 1969; Siegmund and Bendig, 1980; Siegmund et al., 1985) against photo-degradation, oxidation, and heat. Further, acridone is a rather small molecule can be easily synthesized with high yield using inexpensive materials. Several types of acridone derivatives have been prepared (Faller et al., 1997; Reymond et al., 1996; Bahr et al., 1997), used as a fluorescent label for peptides and amino acids. One recent focus in our laboratory has been the development of some new probes of acridone for the direct monitoring of DNA hybridization events. Acridone is an azine dye, which is similar to the other planar dyes in the chemical structure belonging to the acridine, thiazine and xanthene groups. We have demonstrated the interaction of acridones with DNA spectrophotometrically (Chen et al., 2005a,b, 2006). However, based on our knowledge, acridone is not electroactive, so it cannot be used as electrochemical probe, and no electrochemical report on the interaction mechanism of acridone–DNA has been appeared in the literature. Therefore, an acridone derivative 2-nitroacridone (NAD) (see Scheme 1) has been designed and synthesized as the electrochemical label for DNA hybridization.



Scheme 1. Molecular structure of NAD.

NAD had excellent electrochemical activity on the glassy carbon electrode (GCE) with a couple reversible redox peaks. In this paper, the interaction between NAD and salmon sperm DNA was investigated in detail using CV and spectroscopic techniques. Moreover, the hybridization of the immobilized 18-base singlestranded CML DNA fragment with its complementary DNA fragment was confirmed by electrochemical methods using NAD as a novel electrochemical indicator. Based on which an electrochemical DNA biosensor was developed for detection of CML DNA with a detection limit of 6.7×10^{-9} M, which could be used to perform the clinic diagnosis and might has potential application in designing of novel anti-CML drugs.

2. Experimental

2.1. Apparatus

PerkinElmer Spectrum 2000 FT-IR spectrophotometer (USA). Elementarvario EL III elemental analyzer, Varian UNITY-500 nuclear magnetic resonance analyser. UV/vis absorbance spectra were measured with a UV-2501PC spectrophotometer (SHIMADZU, Japan) using a quartz cell with 1.0 cm optical pathway. All electrochemical measurements were carried out using CHI 660B Electrochemical Workstation (Shanghai CH Instruments, China). A three-electrode system was employed with Pt wire as auxiliary electrode, Ag/AgCl/KCl (salt) as reference electrode, and glass carbon electrode (GCE) as working electrode.

2.2. Reagents

Acridone (AR) was purchased from The Third Agents Factory of Shanghai (China). Salmon sperm DNA (1.0 mg/mL) was purchased from Aldrich. The concentrations of DNA per nucleotide phosphate ([DNA]) were calculated according to the absorbance at 260 nm by using $\varepsilon_{DNA} = 6600\,M^{-1}\,cm^{-1}$. Denatured ssDNA was produced by heating a dsDNA solution in a water bath at 100 °C for 5 min, immediately followed by rapid cooling in an ice bath. Phosphate buffer solution (PBS) with different pH were prepared by mixing the stock solutions of 0.02 M NaCl and 0.05 M NaH_2PO_4–Na_2HPO_4, and then adjusting the pH with 0.05 M H_3PO_4 or 0.05 M NaOH. The pH value of each solution was checked with pH meter prior to measurements. The 18-base synthetic oligonucleotides were purchased from Shanghai Sangon Biological Engineering Technology