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Potentiometric sensing of nuclease activities and oxidative damage of single-stranded DNA using a polycation-sensitive membrane electrode

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ABSTRACT

A simple, general and label-free potentiometric method to measure nuclease activities and oxidative DNA damage in a homogeneous solution using a polycation-sensitive membrane electrode is reported. Protamine, a linear polyionic species, is used as an indicator to report the cleavage of DNA by nucleases such as restriction and nonspecific nucleases, and the damage of DNA induced by hydroxyl radicals. Measurements can be done with a titration mode or a direct detection mode. For the potentiometric titration mode, the enzymatic cleavage dramatically affects the electrostatical interaction between DNA and protamine and thus shifts the response curve for the potentiometric titration of the DNA with protamine. Under the optimized conditions, the enzyme activities can be sensed potentiometrically with detection limits of 2.7×10^{-4} U/μL for S1 nuclease, and of 3.9×10^{-4} U/μL for DNase I. For the direct detection mode, a biocomplex between protamine and DNA is used as a substrate. The nuclease of interest cleaves the DNA from the protamine/DNA complex into smaller fragments, so that free protamine is generated and can be detected potentiometrically via the polycation-sensitive membrane electrode. Using a direct measurement, the nuclease activities could be rapidly detected with detection limits of 3.2×10^{-4} U/μL for S1 nuclease, and of 4.5×10^{-4} U/μL for DNase I. Moreover, the proposed potentiometric assays demonstrate the potential applications in the detection of hydroxyl radicals. It is anticipated that the present potentiometric strategy will provide a promising platform for high-throughput screening of nucleases, reactive oxygen species and the drugs with potential inhibition abilities.

1. Introduction

Nucleases, which belong to the class of enzymes called hydrolases, are capable of cleaving DNA into mono- or oligonucleotide fragments. The cleavage of DNA by nucleases such as endonucleases and exonucleases has been shown to play a critical role in biological processes involving replication, recombination, DNA repair, molecular cloning, genotyping, and mapping (Roberts, 1990; Pingoud and Jeltsch, 2001; Linn and Roberts, 1982). Traditional methods such as gel electrophoresis, radioactive labeling, high performance liquid chromatography and enzyme-linked immunosorbent assays (ELISA) are commonly used for nucleases (McLaughlin et al., 1987; Alves et al., 1989; Jeltsch et al., 1993). While these methods generally have high accuracy, their routine laboratory practice is restricted due to their laboriousness or complicated conjugated chemistries (e.g., substrate labeling). In recent years, optical sensors have been extensively used for nuclease assays. Colorimetric sensors based on the conformational change of polythiophene (Tang et al., 2006), self-assembly of a alkynylplatinum(II) terpyridyl complex (Yu et al., 2009) or aggregation state change of gold nanoparticles (Xu et al., 2007; Shen et al., 2009) have been described for nucleases. These methods are convenient to use, but suffer from problems of low sensitivity and interference from nonspecific aggregation. Fluorescent sensors based on G-quadruplex-binding fluorescent probes (Leung et al., 2011), molecular beacons, conjugate polyelectrolytes and quantum dots (Pu et al., 2010; Hu et al., 2010; Wang et al., 2008; Suzuki et al., 2008; Huang et al., 2008) have also been developed, which provide higher detection sensitivity than colorimetric methods. However, these fluorescent sensors are compromised by either probe labeling or interferences from the cleavage buffer or turbid media. In addition, some fluorescent sensors cannot be used for restriction nucleases with dsDNA substrates (Tang et al., 2006). Electrochemical sensors have advantages of rapid response,

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ease of use, low cost and resistance to turbid interferences. Researchers have reported an amprometric sensor for DNase using a ferrocenyloligonucleotide-immobilized electrode (Sato et al., 2008, 2009). However, the incorporation of redox tags makes its regular usage difficult. Hence, the search for general and label-free electrochemical strategies for nucleases including endonucleases and exonucleases is highly required.

In recent years, oxidative damage of DNA by reactive oxygen species (ROS), such as hydroxyl, alkoxyl, and peroxyl radicals and singlet oxygen, has been linked to cancer, aging, and neurological diseases (Mugweru et al., 2004). Therefore, extensive efforts have been made to measure the damage of DNA by reactive oxygen species. The same methods as for enzymatic cleavage are presently available, including but not limited to gel electrophoresis, HPLC, fluorescence resonance energy transfer techniques based on doubly labeled DNA probes (Fitzsimons and Barton, 1997; Natrajan et al., 1990; Hashimoto et al., 2001), and direct visualization via gold nanoparticles or conjugated polymers (Tang et al., 2006; Shen et al., 2009). There is still a significant interest in seeking more sensitive and convenient strategies for sensing the cleavage of DNA by ROS.

Potentiometry with ion-selective electrodes (ISEs) represents an attractive tool for trace analysis because of their low detection limit, and independence of sample volume and sample turbidity (Bakker and Pretsch, 2007; Bobacka et al., 2008; Malon et al., 2006). Polymeric membrane ISEs have been used for sensitive detection of enzymes such as horseradish peroxidase (Nagy et al., 1973), alkaline phosphatase (Rozum and Koncki, 2008), butyrylcholinesterase (Ding and Qin, 2009a, 2009b) and urease (Koncki, 2007). In those enzymatic assays, the detectable ions are restricted to singly charged reagents. Enzymatic assays for proteases and ribonucleases have also been proposed by using a polyanion-sensitive electrode as a detector (Abd-Rabboh et al., 2003; Esson and Meyerhoff, 1997). However, the voltage changes of DNA on the polyanion-sensitive electrode have been found rather small due to the poor extraction of these hydrophilic phosphate-rich polyanions into the sensing membranes. In contrast, DNA molecules can be indirectly measured by using a polycation-sensitive membrane electrode with protamine as an indicator which binds electrostatically to DNA (Ding et al., 2012).

In this work, we demonstrate for the first time a label-free potentiometric method to detect nuclease activities and oxidative damage of DNA molecules using a polycation-sensitive membrane electrode. Protamine, a linear polyionic species, is used as an indicator to report the cleavage of DNA by nucleases such as restriction and nonspecific nucleases, and the damage of DNA induced by hydroxyl radicals. It will be shown that the cleavage of DNA by nucleases and the damage of DNA induced by hydroxyl radicals can effectively prevent the DNA from electrostatically interacting with the protamine domain, which could be sensitively detected via the potentiometric titrations or the direct measurements.

2. Materials and methods

2.1. Chemicals and materials

DNase I and S1 nuclease (the activity unit of nuclease used here is the traditional one defined by the classic digestion experiment) and all oligonucleotides were purchased from Sangon Biotechnology Inc. (Shanghai, China) and used without further purification. The ssDNA with the sequence ACCTG GGGGA GTATT GCGGA GGAAG GT and its complementary sequence TGGAC CCCCT CATAA CGCCT CCTTC CA were chosen as the dsDNA and used for the nonrestriction nuclease study. The single-stranded DNAs with different base lengths as follows are used for the restriction nuclease study:

ssDNA1 (8 mer): 5′-GGTTGGTG-3′

ssDNA2 (15 mer): 5′-GGTTGGTGTGGTTGG-3′

ssDNA3 (27 mer): 5′-ACCTG GGGGA GTATT GCGGA GGAAG GT-3′

ssDNA4 (42 mer): 5′-ACCTG GGGGA GTATT GCGGA GGAAG GTGGT TGGTG TGGTT GG-3′

2-Nitrophenyl octyl ether (o-NPOE), tetradodecylammonium tetrakis(4-chlorophenyl) borate (ETH 500), high molecular weight poly(vinyl chloride) (PVC) were purchased from Fluka AG (Buchs, Switzerland). Dinonylnaphthalene sulfonic acid (DNNS, 50 wt% solutions in heptane), protamine sulfate salt from herring, tetrahydrofuran (THF), and tris(hydroxymethyl)-aminomethane (Tris) were purchased from Sigma. Aqueous solutions were prepared with freshly deionized water (18.2 MΩ specific resistance) obtained with a Pall Cascada laboratory water system.

2.2. Membrane preparation

The membrane composition for the polycation-sensitive film was 1.0 wt% DNNS, 1.0 wt% ETH 500, 49.0 wt% o-NPOE and 49.0 wt % PVC. Membranes of ca. 200 μm thickness were obtained by casting a solution of 360 mg of the membrane components dissolved in 3.0 mL of THF into a glass ring of 36 mm diameter fixed on a glass plate and letting the solvent evaporate over night. Membrane thicknesses were visually measured with a CX31-32C02 Olympus microscope (Tokyo, Japan). For each ISE, a disk of 7 mm diameter was punched from the parent membrane and glued to a plasticized PVC tube (i.d. 6 mm, and o.d. 9 mm) with THF/PVC slurry. All the electrodes were conditioned overnight in 50 mM pH 7.4 Tris–HCl buffer solution containing 0.12 M NaCl, which is identical to the inner filling solution.

2.3. Experimental set-up

All the measurements were carried out at 20 ± 2 °C using a CHI 760C electrochemical workstation (Shanghai Chenhua Apparatus Corporation, China) with an ion-selective electrode and an Ag/AgCl reference electrode. Measurements of electromotive force (EMF) were performed with stirring in the galvanic cell: Ag/AgCl/3 M KCl/sample solution/ISE membrane/inner filling solution/AgCl/Ag. A TJ-1A syringe pump controller (Longer Precision Pump Co., Ltd., Baoding, China) was used for titration. The AC impedance experiments with a conventional three-electrode system were carried out in a 50 mM pH 7.4 Tris–HCl buffer solution containing 0.12 M NaCl with frequencies ranging from 100 kHz to 0.01 Hz and an amplitude of 5 mV. The experimental data were fitted to the Warburg equivalent circuit.

2.4. Measurements of nuclease activities

For the potentiometric titration mode, a solution with a total volume of 600 μL containing 5.0 μM ssDNA3 and various amounts of S1 nuclease in buffer (30 mM CH_3COONa, 280 mM NaCl, and 1 mM $ZnSO_4$, pH 4.6), or containing 4.2 μM dsDNA and various amounts of DNase I in buffer (Tris–HCl 40 mM, $MgSO_4$ 10 mM, and $CaCl_2$ 1 mM, pH 8.0) was incubated at 37 °C. After 20 min, 20 μL of 0.1 M EDTA was added to the solution which was then heated at 70 °C for 5 min to stop the reaction. Potentiometric titration of the reaction mixture diluted to 3 mL with the buffer was carried out at room temperature by successive additions of 1.0 μL of 1.0 mg/mL protamine aqueous solution at a 0.1 min interval with a syringe pump. Titration curves were obtained by plotting the change in the EMF response vs. the concentration of protamine infused. The endpoint of the titration was determined