Biosensors and Bioelectronics 47 (2013) 559–565

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

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

Jiawang Ding [a,b,c], Wei Qin [a,b,*]

[a] Key Laboratory of Coastal Zone Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research (YIC), Chinese Academy of Sciences (CAS), Yantai, Shandong 264003, PR China
[b] Shandong Provincial Key Laboratory of Coastal Zone Environmental Processes, YICCAS, Yantai, Shandong 264003, PR China
[c] University of Chinese Academy of Sciences, Beijing 100049, PR China

ARTICLE INFO

Article history:
Received 24 December 2012
Received in revised form
13 March 2013
Accepted 26 March 2013
Available online 6 April 2013

Keywords:
Nucleases
Hydroxyl radicals
Potentiometry
Protamine
Polyion sensors

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,

* Corresponding author at: Key Laboratory of Coastal Zone Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research (YIC), Chinese Academy of Sciences (CAS), Yantai, Shandong 264003, PR China. Tel.: +86 535 2109156; fax: +86 535 2109000.
E-mail address: wqin@yic.ac.cn (W. Qin).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.03.066

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

as the protamine concentration to achieve half of the maximum EMF response ($EMF_{1/2,\ max}$).

For the direct detection mode, the substrate solution with a total volume of 3.0 mL containing 90 μL of 1 mg/mL protamine and 30 μL of 100 μM ssDNA3 for S1 nuclease, or 75 μL of 1 mg/mL protamine and 40 μL of 50 μM dsDNA for DNase I, was incubated for 10 min at room temperature. Various amounts of enzyme were incubated in the substrate solution at 37 °C for 20 min. The enzyme cleaves the DNA within the complex into smaller fragments. Thus, free protamine is generated and can be detected potentiometrically via the protamine-sensitive membrane electrode. Since free protamine generated is not high enough to reach an equilibrium response during 10 min, the potential difference (ΔE) between the baseline and the potential measured at 10 min was used for quantification of the enzyme activity.

2.5. Assays for DNA cleavage by hydroxyl radicals

The Fenton solution contains 2.5 mM ascorbic acid, 5 mM Fe(II), 10 mM EDTA, and 50 mM hydrogen peroxide (Jain and Tullius, 2008). For the potentiometric titration mode, experiments were performed in a 3.0 mL Tris–HCl buffer solution (50 mM, pH 7.4) containing 30 μL of 100 μM ssDNA3 and 120 μL of the Fenton solution, which was incubated for 5 min at room temperature. For the direct detection mode, the substrate solution containing 90 μL of 1 mg/mL protamine and 30 μL of 100 μM ssDNA3 was incubated for 10 min at room temperature. A solution with a total volume of 3.0 mL containing 120 μL of the Fenton solution and 120 μL of the substrate solution was incubated for 5 min at room temperature before measurement. Other detection procedures were the same as mentioned above.

3. Results and discussion

3.1. Characteristics of the polycation-sensitive membrane electrode

Over the past several years, significant progress has been made in the development of polyion sensors for direct and titration-based measurements of protamine or heparin in clinical samples (Ye and Meyerhoff, 2001). In this paper, a traditional protamine-sensitive membrane was constructed with addition of a lipophilic salt ETH 500 to decrease the membrane resistance. The electrochemical impedance spectroscopy shows that the bulk resistance of the membrane is ca. 0.37 MΩ (Fig. 1b), which is much smaller than that of the membrane without ETH 500 (Fig. 1a). ETH 500, as a kind of ionic liquid, may account for the large change in the apparent bulk value. In addition, ETH 500 has an important selectivity-modifying effect by influencing the activity coefficients in the membrane (Chen et al., 2012). Indeed, the polymeric membrane shows an increase of ca. 10 mV in the potential equilibrium response as compared to that of the conventional membrane without ETH 500.

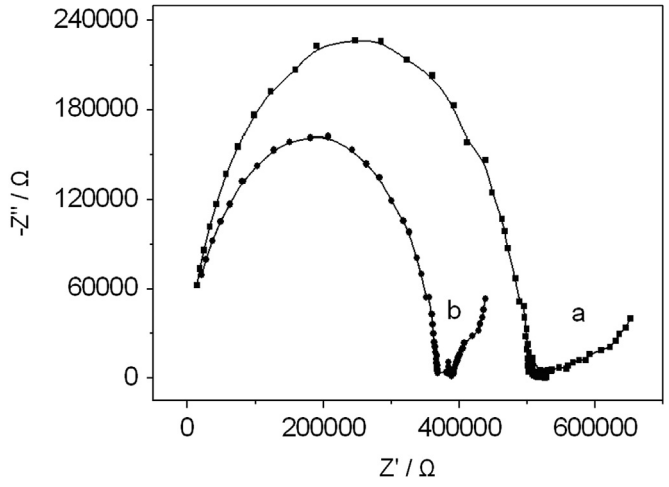


Fig. 1. Impedance spectra of the protamine-sensitive membrane electrode in the absence (a) and presence (b) of 1 % ETH 500 in the membrane. The open circuit potential of the electrode was used for impedance measurements.

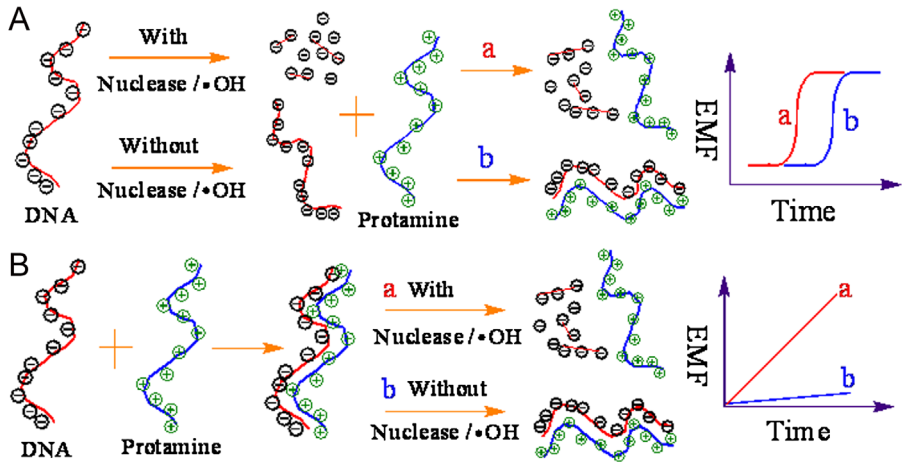
3.2. Mechanism of potentiometric sensing of nucleases and hydroxyl radicals

Interactions between polyelectrolytes, especially single-stranded nucleic acids, and oppositely charged water-soluble conjugated polymers have been widely used for the development of fluorescence-based sensors (Feng et al., 2010). Herein, we develop a label-free potentiometric sensor based on a new biocomplex. Owing to the electrostatic interactions between the positively charged guanidinium groups of protamine and the negatively charged phosphate groups of DNA, these two species tend to form a biocomplex (Prieto et al., 1997). The potential response of the biocomplex on the polycation-sensitive membrane electrode is negligible due to the inefficient extraction of the macromolecular complex into the transduction membrane. Potentiometric sensing strategies for nuclease activities and oxidative damage of DNA via titration (Scheme 1A) or direct detection (Scheme 1B) using protamine as an indicator are designed. For the potentiometric titration mode, the enzymatic cleavage of DNA or oxidative DNA damage could dramatically affect the electrostatical interactions between DNA and protamine and thus shift the DNA–protamine titration curve. Therefore, the cleavage of DNA by nucleases or hydroxyl radicals can be monitored by the endpoint of the titration. For the direct detection mode, a biocomplex of protamine and a DNA molecule is used as a substrate. The nuclease of interest or hydroxyl radicals cleave the DNA within the complex into smaller fragments. Thus, free protamine is generated and can be detected potentiometrically via the protamine-sensitive membrane electrode. By monitoring the potential change, this strategy may offer a sensitive platform for label-free detection of nuclease activities and hydroxyl radicals.

3.3. Optimization of the DNA length and concentration

The effect of DNA length on analytical properties of the titration curves was investigated by using several ssDNA with different lengths varying from 8 to 42 mer. As shown in Fig. 2A, the EMF response curves are shifted to higher mass concentrations with increase in DNA length at the same DNA concentration. These titration curves can be utilized to determine the stoichiometry for the interactions between the DNA molecules with different lengths and protamine. It can be seen that the neutralization stoichiometries increase with the length of DNA, indicating an increase in the number of protamine binding sites per mole of DNA (see Table S1 in the Supporting information). In principle, longer DNA lengths lead to larger signal changes. However, the optimal DNA length depends on discrepancy in potential changes before and after the enzymatic digestion or oxidative damage of DNA. With longer DNA, its fragments generated by enzymatic digestion can still effectively interact with protamine, especially with low enzyme activities; on the other hand, shorter DNA could induce weak interaction with protamine. Thus, the 27 mer DNA was employed in the subsequent work.

The potential response may also be affected by the DNA concentration. As shown in Fig. 2B, the EMF response curves are shifted to higher mass concentrations in the presence of DNA compared to that of the buffer alone. This shift will be larger with increase in the DNA concentration. It should be noted that higher concentrations of DNA cause larger changes in the potential



Scheme 1. Schematic illustration of (A) the potentiometric titration mode and (B) the direct detection mode for nucleases and oxidative damage of single-stranded DNA.

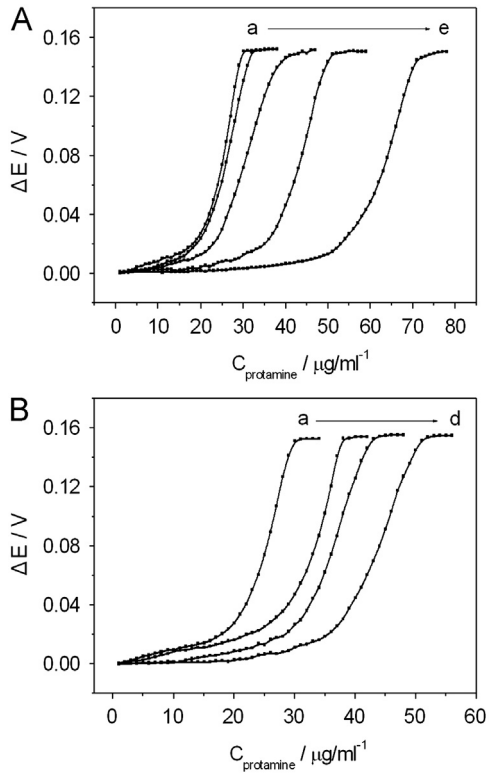


Fig. 2. Potentiometric titrations of (A) 0.0 (a), and 2.67 μM of 8 mer (b), 15 mer (c), 27 mer (d), and 42 mer DNA (e), and (B) 0.0 (a), 0.67 (b), 1.33 (c), and 2.67 μM 27 mer DNA (d) with 1.0 mg/mL protamine.

response, but on the other hand would induce lower sensitivity for nucleases detection. On the contrary, lower concentrations of DNA are more sensitive for nucleases detection, but with a narrow concentration range. Considering a compromise between wide response range and high sensitivity, 5.0 and 4.2 μM were employed for titration of ssDNA3 and dsDNA, respectively.

3.4. Potentiometric titrations for nucleases and hydroxyl radicals

S1 nuclease is an ssDNA specific endonuclease that has been widely used in removing single-stranded overhangs from DNA fragments to yield blunt ends, mapping of RNA transcripts, and probing the structures of purified DNA (Panayotatos and Wells, 1981). The reaction time of the cleavage of ssDNA by S1 nuclease was optimized with a fixed DNA concentration. Experiments show that increasing the S1 nuclease activity leads to a higher cleavage reaction rate (Fig. S1 in the Supporting information). In addition, with increase in reaction time, the mass shift is more obvious and then tends to an almost constant value. It should be noted that it is possible to detect the enzyme activities within 5 min. But the linear response range is narrow. Therefore, 20 min was selected for the further study as a compromise between wide response range and short analysis time.

For the potentiometric titration mode, the ssDNA cleavage by S1 nuclease was determined using the protamine-sensitive membrane electrode. In the absence of S1 nuclease, the EMF response curve can be shifted to a longer response time (i.e., a higher mass concentration) in the presence of DNA compared to that of the buffer alone, and this shift is attributed to the interaction between DNA and protamine. The addition of S1 nuclease to the incubation mixture decreases the mass shift, indicating the effective digestion of the DNA. As shown in Fig. 3A, the mass shift is reduced with increasing the activity of S1 nuclease. The endpoint of the titration curves was used for S1 nuclease quantification. The detection limit was calculated to be 0.27 U/mL at a signal-noise ratio (S/N) of 3. With longer analysis time, the detection limit achieved by the label-free potentiometric method is comparable to or better than those obtained by spectrophotometric and fluorometric S1 nuclease assays (Table 1). Moreover, the label-free potentiometric sensing mode offers additional advantages over previous reports such as resistance to color and turbid interferences.

To illustrate whether our new sensing strategy is applicable to other nucleases, the detection of nonrestriction nuclease activity was carried out. DNase I is a nonrestriction nuclease that degrades dsDNA in a nonspecific manner when Ca^{2+} or Mn^{2+} ions are present, producing 3′-OH oligonucleotides, and is extensively used in probing genomic DNA, removing the DNA template after in vitro transcription and nick translation (Anderson, 1981; Campbell and Jackson, 1980). The potentiometric assay for DNase I was investigated via titration. As expected, the addition of DNase I to the substrate solution decreases the mass shift of the potential response curve. Moreover, the mass shift is reduced with increasing the activity of DNase I (Fig. 3B). The concentration of protamine infused is proportional to the activity of DNase I from 1 to 10 U/mL. The detection limit is 0.39 U/mL ($S/N=3$), which is comparable with those of the common DNase assays (Pu et al., 2010; Zhao et al., 2008; Jeltsch et al., 1993).

The damage of the single-stranded DNA by hydroxyl radicals was also tested via titration (Fig. 3C). The addition of the Fenton

Table 1
Comparison of the detection limits and enzyme-catalyzed reaction times of various label-free sensing methods for S1 nuclease.

Detection method	Detection limit (U/mL)	Reaction time (min)	Reference
Fluorescence assays based on cationic conjugated polymer/DNA complexes	2.8×10^{-3}	50	Feng et al., 2007
Fluorescence assays based on conjugated polymer and DNA/intercalating dye complex	2.6×10^{-3}	60	Pu et al., 2010
Optical sensing based on light scattering of carbon nanotubes	5×10^{-3}	60	Zhao et al., 2011
Visual detection based on positively-charged gold nanoparticles as colorimetric probes	Not shown	30	Cao et al., 2011
Fluorescence assays based on double-strand DNA-templated formation of copper nanoparticles	0.30	60	Hu et al., 2013
Potentiometric sensing using protamine as an indicator	0.27	20	This work

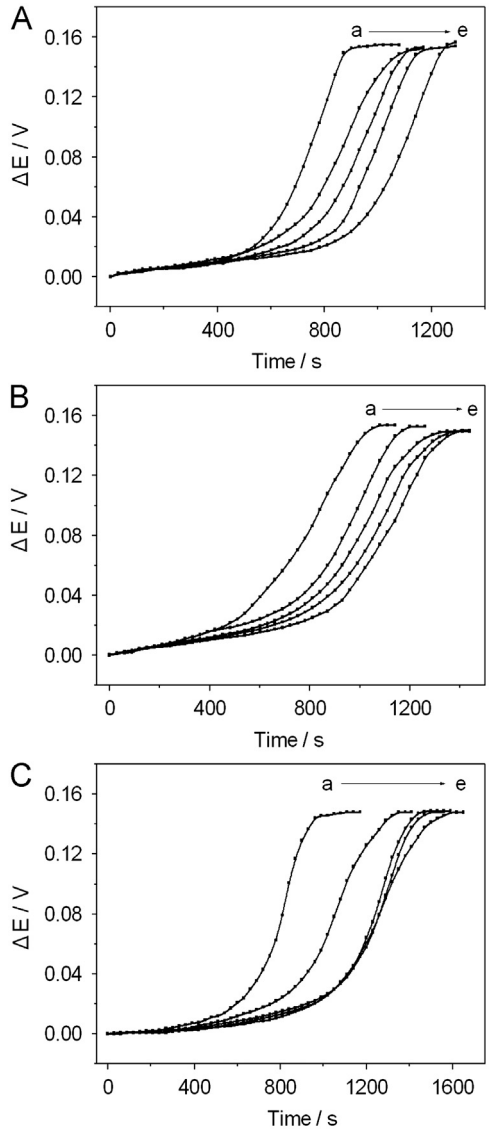


Fig. 3. Potentiometric titrations of (A) 0.0 μM DNA (a), 1.0 μM DNA in the presence of 10 (b), 5 (c), and 1 U/mL S1 nuclease (d) and 1.0 μM DNA alone (e), (B) 0.0 μM DNA (a), 0.84 μM DNA in the presence of 10 (b), 5 (c), and 1 U/mL DNase I (d), and 0.84 μM DNA alone (e), and (C) 0.0 μM DNA (a), 1.0 μM DNA in the presence of a Fenton reaction mixture (b), 2.5 mM ascorbic acid, 5 mM Fe(II), and 10 mM EDTA (c), 50 mM hydrogen peroxide (d), and 1.0 μM DNA alone (e) with 1.0 mg/mL protamine.

reaction mixture causes the damage of ssDNA, which leads to the mass shift of the potential response curve. The control experiments show that H_2O_2, ascorbic acid or Fe^{2+} itself has nearly no effect on the potential response of the electrode. This phenomenon could be monitored by the polycation-sensitive membrane electrode, and used to sensitively detect ·OH. Previous reports have shown that dsDNA can also be oxidatively damaged by ·OH leading to the generation of free bases or different-sized length fragments (Oliveira et al., 2012; Zhang et al., 2010). Therefore, the proposed potentiometric assay could be used for the detection of oxidative damage of dsDNA by hydroxyl radicals.

3.5. Direct potentiometric detection of nucleases and hydroxyl radicals

Although nucleases or hydroxyl radicals can be sensitively detected by the potentiometric titration with protamine, the entire titration process might be time-consuming. Therefore, an alternative potentiometric assay was performed via the direct detection mode (Scheme 1B).

For substrate preparation, different mass stoichiometries of DNA:protamine can be employed. The stoichiometry plays an important role in the potential response. Thus, different mass stoichiometries were tested to reduce the background signal. Experiments show that no significant potential response could be observed using a mass stoichiometry of 1:3.5 (DNA:protamine). Therefore, this mass ratio was employed for the enzymatic assays.

Fig. 4A shows the potentiometric responses of the protamine-sensitive membrane electrode to different S1 nuclease activities. In the absence of S1 nuclease, very low potential signal can be obtained with the electrode. With increase in the enzyme activity, the electrode exhibits higher potential responses. This is due to the free protamine generated from S1 nuclease digestion of the DNA molecules within the complex into smaller fragments. The enzyme activity can be detected by monitoring the potential difference (ΔE) between the baseline and the potential measured at 10 min. Fig. S2 illustrates a linear relationship between the potential difference and the enzyme activity of S1 nuclease in the range from 1 U/mL to 10 U/mL. The detection limit is 0.32 U/mL ($S/N=3$). Recently, researchers have reported more-sensitive optical nuclease-sensing systems (Zhao et al., 2011; Liu et al., 2011). Compared with their systems, the proposed potentiometric detection strategy is simple and needs neither labeling nor immobilization of DNA molecules. In addition, the sensitivity can be further improved by using a rotating electrode (Ye and Meyerhoff, 2001).

Direct detection for DNase I was also carried out to verify the generality of this method. Experiments show that an optimal mass stoichiometry is 1:2.3. As shown in Figs. 4B and S3, the potential difference was proportional to DNase I activity in the range of 1 to 10 U/mL with a detection limit of 0.45 U/mL ($S/N=3$). The detection limit is comparable with the ELISA method for DNase (Pu et al., 2010, Zhao et al., 2008; Jeltsch et al., 1993).

The qualitative estimation of the potentiometric assay for ·OH was also performed via direct detection. As indicated in Fig. 4C, the untreated substrate could form a biocomplex, leading to no obvious potential response. When the substrate is incubated in the Fenton solution for 5 min, a large potential response is generated on the electrode. These results show that the assay

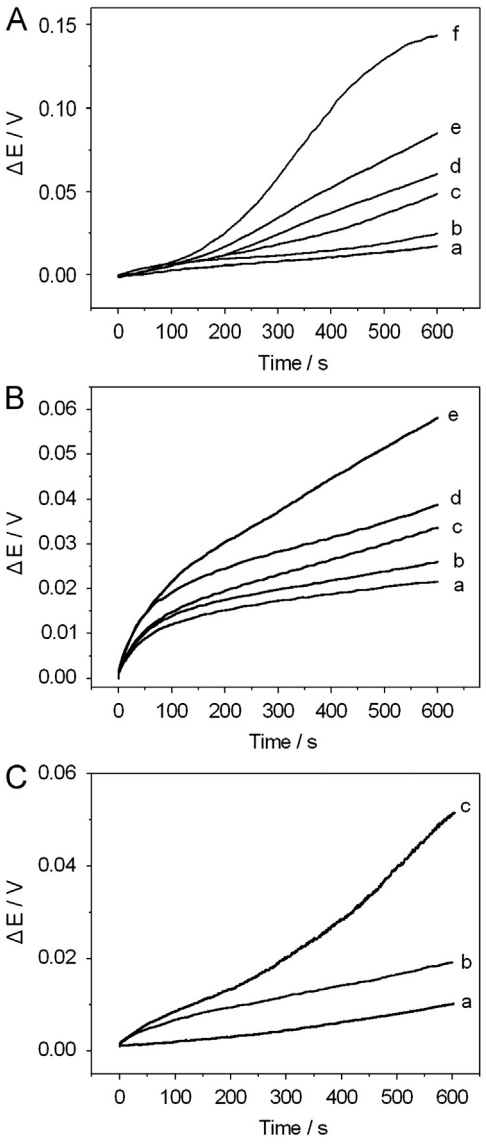


Fig. 4. Potentiometric responses of polycation-selective electrodes to (A) 0 (a), 1 (b), 5 (c), 8 (d), 10 U/mL of S1 nucleases in the presence of substrate (e) and 30 μg/mL protamine alone (f), and (B) 0 (a), 1 (b), 5 (c), 10 U/mL of DNase I in the presence of enzyme substrate (d) and 30 μg/mL protamine alone (e). (C) Potentiometric responses of the protamine-sensitive membrane electrode to the biocomplex before (a) and after oxidative damage (b), and to 30 μg/mL protamine alone (c).

can easily monitor the cleavage process of DNA by ·OH. As some antioxidants have the capabilities to scavenge ·OH, our assays can provide a rapid and convenient method to screen anti-oxidation natural products or drugs.

4. Conclusions

In summary, a novel, simple and general potentiometric method for nuclease activities and oxidative damage of single-stranded DNA molecules has been proposed. The potentiometric assays for monitoring nuclease activities and oxidative damage of single-stranded DNA have specific advantages over traditional spectrophotometric and fluorescence techniques such as rapidity, simplicity, and the ability to measure nucleases activities in turbid solutions (e.g., whole blood). In addition, our sensing scheme is versatile and holds great potential for high-throughput screening of nucleases, reactive oxygen species or the drugs with potential inhibition abilities.

Acknowledgments

This work was financially supported by the Instrument Developing Project of the Chinese Academy of Sciences (YZ201161), the National Natural Science Foundation of China (41176081, and 21207156), the Science and Technology Project of Yantai (2012132), and the Taishan Scholar Program of Shandong Province (TS20081159).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.03.066.

References

Abd-Rabboh, H.S.M., Nevins, S.A., Dúrúst, N., Meyerhoff, M.E., 2003. Biosensors and Bioelectronics 18, 229–236.
Alves, J., Ruter, T., Geiger, R., Fliess, A., Maass, G., Pingoud, A., 1989. Biochemistry 28, 2678–2684.
Anderson, S., 1981. Nucleic Acids Research 9, 3015–3027.
Bakker, E., Pretsch, E., 2007. Angewandte Chemie: International Edition 46, 5660–5668.
Bobacka, J., Ivaska, A., Lewenstam, A., 2008. Chemical Reviews 108, 329–351.
Cao, R., Li, B.X., Zhang, Y.F., Zhang, Z.N., 2011. Chemical Communications 47, 12301–12303.
Campbell, V.W., Jackson, D.A., 1980. Journal of Biological Chemistry 255, 3726–3735.
Chen, Y., Ding, J.W., Qin, W., 2012. Analyst 2012 (137), 1944–1949.
Ding, J.W., Chen, Y., Wang, X.W., Qin, W., 2012. Analytical Chemistry 84, 2055–2061.
Ding, J.W., Qin, W., 2009a. Chemical Communications 45, 971–973.
Ding, J.W., Qin, W., 2009b. Journal of the American Chemical Society 131, 14640–14641.
Esson, J.M., Meyerhoff, M.E., 1997. Electroanalysis 9, 1325–1330.
Feng, F.D., Tang, Y.L., He, F., Yu, M.H., Duan, X.R., Wang, S., Li, Y.L., Zhu, D.B., 2007. Advanced Materials 19, 3490–3495.
Feng, X.L., Liu, L.B., Wang, S., Zhu, D.B., 2010. Chemical Society Reviews 39, 2411–2419.
Fitzsimons, M.P., Barton, J.K., 1997. Journal of the American Chemical Society 119, 3379–3380.
Hashimoto, S., Wang, B., Hecht, S.M., 2001. Journal of the American Chemical Society 123, 7437–7438.
Hu, D., Huang, Z.Z., Pu, F., Ren, J.S., Qu, X.G., 2010. Chemistry-A European Journal 16, 2605–2610.
Hu, R., Liu, Y.R., Kong, R.M., Donovan, M.J., Zhang, X.B., Tan, W.H., Shen, G.L., Yu, R.Q., 2013. Biosensors and Bioelectronics 42, 31–35.
Huang, S., Xiao, Q., He, Z.K., Liu, Y., Tinnefeld, P., Su, X.R., Peng, X.N., 2008. Chemical Communications 44, 5990–5992.
Jain, S.S., Tullius, T.D., 2008. Nature Protocols 3, 1–9.
Jeltsch, A., Fritz, A., Alves, J., Wolfes, H., Pingoud, A., 1993. Analytical Biochemistry 213, 234–240.
Koncki, R., 2007. Analytica Chimica Acta 599, 7–15.
Leung, C.H., Shiu-Hin Chan, D., Yat-Wah Man, B., Wang, C.J., Lam, W., Cheng, Y.C., Fong, W.F., Wendy Hsiao, W.L., Ma, D.L., 2011. Analytical Chemistry 83, 463–466.
Linn, S.M., Roberts, R.J., 1982. Nucleases. Cold Spring Harbor Labortary Press, NY.
Liu, Z.D., Hu, P.P., Zhao, H.X., Li, Y.F., Huang, C.Z., 2011. Analytica Chimica Acta 706, 171–175.
Malon, A., Vigassy, T., Bakker, E., Pretsch, E., 2006. Journal of the American Chemical Society 128, 8154–8155.
McLaughlin, L.W., Benseler, F., Graeser, E., Piel, N., Scholtissek, S., 1987. Biochemistry 26, 7238–7245.
Mugweru, A., Wang, B., Rusling, J., 2004. Analytical Chemistry 76, 5557–5563.
Nagy, G., Von Storp, L.H., Guilbault, G.G., 1973. Analytica Chimica Acta 66, 443–455.
Natrajan, A., Hecht, S.M., Marel, G.A., Boom, J.H., 1990. Journal of the American Chemical Society 112, 3997–4002.
Oliveira, S., Carlos, B., Oliveira-Brett, A.M., 2012. Langmuir 28, 4896–4901.
Panayotatos, N., Wells, R.D., 1981. Nature 289, 466–470.
Pingoud, A., Jeltsch, A., 2001. Nucleic Acids Research 29, 3705–3727.
Prieto, M.C., Maki, A.H., Balhorn, R., 1997. Biochemistry 36, 11944–11951.
Pu, F., Hu, D., Ren, J.S., Wang, S., Qu, X.G., 2010. Langmuir 26, 4540–4545.
Roberts, R.J., 1990. Nucleic Acids Research 18, 2331–2365.
Rozum, B., Koncki, R., 2008. Talanta 77, 507–513.
Sato, S., Fujita, K., Kanazawa, M., Mukumoto, K., Ohtsuka, K., Waki, M., Takenaka, S., 2008. Analytical Biochemistry 381, 233–239.
Sato, S., Fujita, K., Kanazawa, M., Mukumoto, K., Ohtsuka, K., Takenaka, S., 2009. Analytica Chimica Acta 645, 30–35.

Shen, Q.P., Nie, Z., Guo, M.L., Zhong, C.J., Lin, B., Li, W., Yao, S.Z., 2009. Chemical Communications 45, 929–931.
Suzuki, M., Husimi, Y., Komatsu, H., Suzuki, K., Douglas, K.T., 2008. Journal of the American Chemical Society 130, 5720–5725.
Tang, Y.L., Feng, F.D., He, F., Wang, S., Li, Y.L., Zhu, D.B., 2006. Journal of the American Chemical Society 128, 14972–14976.
Wang, M., Zhang, D.Q., Zhang, G.X., Tang, Y.L., Wang, S., Zhu, D.B., 2008. Analytical Chemistry 80, 6443–6448.
Xu, X.Y., Han, M.S., Mirkin, C.A., 2007. Angewandte Chemie: International Edition 46, 3468–3470.
Ye, Q.S., Meyerhoff, M.E., 2001. Analytical Chemistry 73, 332–336.
Yu, C., Chan, K.H., Wong, K.M., Yam, V.W., 2009. Chemical Communications 45, 3756–3758.
Zhang, W., Yang, T., Li, W.J., Li, G.C., Jiao, K., 2010. Biosensors and Bioelectronics 25, 2370–2374.
Zhao, C., Qu, K.G., Song, Y.J., Ren, J.S., Qu, X.G., 2011. Advanced Functional Materials 21, 583–590.
Zhao, W., Lam, J.C., Chiuman, W., Brook, M.A., Li, Y.F., 2008. Small 4, 810–816.