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Direct detection of double-stranded DNA (dsDNA) using zinc finger proteins (ZFPs) is of great importance in biomedical applications such as identifying pathogens and circulating DNAs. However, its sensitivity is still not sufficiently high because limited signalling labels can be conjugated or fused. Herein, we report sensitive and direct detection of dsDNA using (i) alkaline phosphatase (ALP) as a fast catalytic label conjugated to ZFPs along with (ii) electrochemical measurement of ALP product (L-ascorbic acid) at indium-tin oxide electrode with a high signal-to-background ratio. ALP is simply conjugated to a ZFP through lysine residues in a ZFP purification tag, a maltose binding protein (MBP). Sandwich-type electrochemical detection of dsDNA allows a detection limit of ca. 100 fM without using DNA amplification.

Sensitive and sequence-specific detection of double-stranded DNAs (dsDNAs) is of great importance in biomedical applications such as identifying pathogens[1,2] and circulating DNAs.[3,4] Two common methods have been used to achieve this goal: nucleic acid amplification [such as polymerase chain reaction (PCR)][5,6] and DNA denaturation/hybridization.[7] The detection based on nucleic acid amplification allows high sensitivity, while the detection based on DNA denaturation/hybridization allows high sequence specificity. However, both detection methods require complicated detection procedures, and it is not easy to obtain both high sensitivity and high sequence specificity simultaneously. Moreover, DNA denaturation/hybridization is not efficient, because denatured single-stranded DNAs (ssDNAs) are often rehybridized with themselves rather than hybridized with their complementary probes.

Sequence-specific direct detection of dsDNAs[8] has also been developed, such as methods using DNA-binding zinc finger proteins (ZFPs)[9–15] and triplex-forming oligonucleotides.[16–18] Such methods enable us to obviate a time-consuming procedure of DNA denaturation and hybridization by directly detecting dsDNA. A zinc finger (ZF) is one of the most common DNA-binding domains. The Cys2-His2 ZF domain consists of 30 amino acids folded into a ββα structure. Its structure is stabilized by the chelation of a zinc ion bound to two cysteines and two histidines.[19] Natural and engineered ZFPs can be fused to various functional domains, which enables diverse applications such as gene regulation[19] and DNA diagnostics.[8,9] The sensitivity in ZFP-based methods could be improved depending on their signalling labels. Alkaline phosphatase (ALP) and horseradish peroxidase (HRP) are employed as common labels in enzyme-linked immunosorbent assays (ELISAs) to obtain high sensitivity.[20,21] In ZFP-based methods, two types of enzyme labels were used instead of ALP and HRP: (i) a label that forms a full-length active enzyme (e.g., β-lactamase and green fluorescent protein) by sequence-enabled reassembly (SEER) of two split enzymatic fragments upon ZFP binding to dsDNA[9–11] and (ii) a full-length enzyme that is fused with ZFPs (e.g., luciferase and glucose dehydrogenase).[13–15] A couple of studies determined detection limit, which could be lowered by using an alternative detection method. The colorimetric detection of dsDNA on the ZFP array using a formation of β-lactamase by SEER provided a detection limit of 50 fmol.[9] The use of ALP and HRP in ZFP-based methods was limited because the conjugation of ALP or HRP to ZFPs and their fusion with ZFPs are not efficient.[13] Cysteine and lysine residues commonly used for chemical conjugation participate in the scaffold formation of ZFPs. As a result, the chemical conjugation using those residues might affect a binding ability of ZFPs.[13]

Fast and simple DNA detection methods for point-of-care testing (POCT) have been intensively investigated. Of many detection methods, electrochemical detection is primarily chosen, especially for obtaining a high sensitivity along with a miniaturized system.[22,23] Although various sensitive electrochemical DNA sensors using DNA hybridization have been developed,[22,23] they are not practically applicable to dsDNA detection. Therefore, a sensitive

electrochemical method that enables direct detection of dsDNA is highly required for POCT.

In electrochemical detection, ALP is most commonly used as an enzyme label because the difference in formal potentials between ALP substrate and product is sufficient enough to differentiate two chemicals electrochemically.[24,25] In recent years, we have shown that electrochemical biosensors using ALP labels combined with indium-tin oxide (ITO) electrodes facilitates high signal-to-background ratios, i.e., very low detection limits[25–28] because ITO electrodes show low and flat capacitive background currents.

In this study, we report a sandwich-type electrochemical method that allows sensitive and direct detection of dsDNA. ALP and biotin are chemically conjugated to a detection probe ZFP and a capture probe ZFP, respectively, with the help of lysine groups of a maltose binding protein (MBP) fused to engineered ZFPs. The specific bacterial dsDNA sequence is recognized by engineered ZFPs. This sequence-specific recognition is then converted to electrochemical signal through enzymatic amplification at the optimal condition determined in this study.

Experimental

Chemicals and solutions

Streptavidin, biotin, ALP, L-ascorbic acid (AA), trichloroethylene, ethanol, H_2O_2, and NH_4OH were obtained from Aldrich, and L-ascorbic acid phosphate (AAP) was obtained from Wako (Osaka, Japan). Sulfosuccinimidyl-4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate (sulfo-SMCC), *N*-succinimidyl-S-acetylthiopropionate (SATP), and EZ-link sulfo-NHS-LC-LC-biotin were obtained from Thermo Scientific, Inc. All reagents for preparing buffer solutions were obtained from Aldrich. ssDNA was obtained from Genotech (Daejeon, Korea).

Tris buffer containing Zn^{2+} (TZ buffer, pH 8.5) consisted of 100 mM tris, 90 mM KCl, 0.1 mM $ZnCl_2$, and 1 mM $MgCl_2$. TBSB buffer (pH 8.0) contained 50 mM tris, 0.138 M NaCl, 0.0027 M KCl, 1% (w/v) bovine serum albumin. HEPES buffer (pH 7.5) contained 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 90 mM KCl, 0.1 mM $ZnCl_2$, and 1 mM $MgCl_2$. Washing buffer was TZ buffer containing 0.05% Tween-20.

Cloning, expression, and purification of ZFPs

One pair of ZFPs (rrsA125 and rrsA160) was used in this study, which was designed to detect a non-pathogenic strain of *E. coli*.[9] Six finger ZFPs rrsA125 and rrsA160 were capture and detection probes, respectively. ZFPs were constructed by the modular assembly method using the Barbas set of modules in a modified Sp1C framework.[29] ZFPs (rrsA125 and rrsA160) were subcloned between the BamHI and HindIII sites of pMAL-c2X full-length β-lactamase, replacing the full-length β-lactamase. The vector enables bacterial expression of the proteins as fusions with an *N*-terminal maltose binding protein (MBP) as a purification tag. Proteins were expressed in *E. coli* BL21 (Invitrogen) upon induction with 0.3 mM isopropyl b-D-1-thiogalactopyranoside at an OD600 of 0.6–0.8 for 5 h at 37 °C. Cells were pelleted and resuspended in Zinc Buffer A (ZBA: 100 mM Tris base, 90 mM KCl, 1 mM $MgCl_2$ and 100 mM $ZnCl_2$ at pH 7.5) including 5 mM dithiothreitol (DTT) and 50 mg/ml RNase A. After sonication, proteins in cell lysates were applied to an amylose resin column pre-equilibrated with ZBA containing 5 mM DTT, washed with ZBA containing 2 M NaCl and ZBA containing 1 mM tris(2-carboxyethyl)phosphine (TCEP), and eluted in ZBA containing 10 mM Maltose and 1 mM TCEP. Concentration and purity were assessed by Coomassi-stained polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS–PAGE) using bovine serum albumen (BSA) standards. Purified protein was stored in ZBA containing 1 mM TCEP at –20 °C until use.

Preparation of ds target DNA

The sequence of ssDNA oligonucleotide used to form target dsDNA was 5'-GAC ***TGG GAA ACT GCC TGA TGG AGG GGG ATA ACT ACT GGA*** GGG TTTT CCC ***TCC AGT AGT TAT CCC CCT CCA TCA GGC AGT TTC CCA*** GTC-3'. ZFP binding site is shown in bold and italic. The sequence of nontarget ssDNA was 5'-HS(C6)-AAA AAA AAA AAA AAA AAA AAA TTA GAG AAA ATG TT-3'. dsDNA was prepared by heating autoclaved water containing 100 nM ssDNA at 95 °C for 10 min, and then slowly cooling down at room temperature for 6 h to form hairpins containing a four-thymidine loop. The resulting DNA solution was diluted with TZ buffer.

Chemical conjugation

To maintain ZFP structure during chemical conjugation, Zn^{2+}-containing HEPES buffer was used. The ALP-conjugated ZFP was prepared by cross-linking the amine group of ALP and the amine group of a MBP of the detection probe ZFP rrsA160. 1 mL of HEPES buffer containing 100 μg/mL detection probe ZFP and 50 μL of HEPES buffer containing 1 mg/mL sulfo-SMCC were mixed and incubated for 30 min at room temperature. Sulfo-SMCC-conjugated ZFP was filtered by centrifugation for 20 min at 12,000 rpm. Afterward, the filtrate was dissolved in 1 mL of HEPES buffer. 1 mL of HEPES buffer containing 200 μg/mL ALP and 10 μL of HEPES buffer containing 2 mg/mL SATP were mixed, and the mixture was then incubated for 30 min at room temperature. The resulting solution was mixed with 20 μL of a deacetylation solution (HEPES buffer) containing 0.012 g/mL ethylenediaminetetraacetic acid and 0.044 g/mL hydroxylamine·HCl, for 2 h at room temperature, and the mixture was then filtered by centrifugation for 20 min at 12,000 rpm. The filtrate was dissolved in 1 mL of HEPES buffer. The solution containing sulfo-SMCC-conjugated ZFP and the solution containing SATP-conjugated ALP were mixed at a molar ratio of 1:1 for 2 h at room temperature. To filter the ALP-conjugated ZFP, the final mixture was centrifuged for 20 min at 12,000 rpm. The filtrate was dissolved in 1 mL of TZ buffer.

The biotin-conjugated ZFP was obtained by cross-linking EZ-link sulfo-NHS-LC-LC-biotin and the amine group of a MBP of the capture probe ZFP rrsA125. 500 μL of HEPES buffer containing 100 μg/mL ZFP was mixed with 10 μL of 2 mM EZ-link sulfo-NHS-LC-LC-biotin and incubated for 2 h at 4 °C, and the mixture was then filtered by centrifugation for 20 min at 12,000 rpm. The filtrate was dissolved in 1 mL of TZ buffer.

Preparation of sensing layers and DNA binding

ITO electrodes were obtained from Corning (Daegu, Korea). The ITO electrodes (1 cm × 2 cm each) were pretreated by dipping them in a mixed solution of water, H_2O_2 (30%), and NH_4OH (30%) at a volume ratio of 5:1:1 for 1 h at 70 °C after cleaned with trichloroethylene, ethanol, and distilled water under sonication for 15 min.[30] To obtain streptavidin-modified ITO electrodes, the electrodes were treated with 70 μL of carbonate buffer (50 mM, pH 9.6) containing 10 μg/mL streptavidin for 2

h at 20 °C. Afterward, the electrodes were treated with 70 μL of TBSB buffer for 30 min at 4 °C. To immobilize biotin-conjugated ZFP rrsA125, the modified electrodes were treated with 70 μL of TZ buffer containing 10 μg/mL biotin-conjugated ZFP and then washed with TZ buffer and washing buffer. For the binding of target dsDNA to the capture probe immobilized on the electrodes, 70 μL of TZ buffer containing different concentrations of dsDNA was applied onto the capture-probe-immobilized electrodes. This state was maintained for 30 min at 4 °C, and the electrodes were then washed with TZ buffer and washing buffer, sequentially. Subsequently, 70 μL of TZ buffer containing 10 μg/mL ALP-conjugated ZFP rrsA160 was dropped onto the target-treated electrodes, and the dropped state was maintained for 30 min at 4 °C, followed by washing with TZ buffer and washing buffer, sequentially.

Electrochemical measurement

Teflon electrochemical cells consisted of an ITO working electrode, an Ag/AgCl reference electrode (3 M NaCl), and a Pt counter electrode. The exposed geometric area of the ITO electrodes was ca. 0.28 cm^2. The electrochemical measurements were carried out with CHI 708C (CH instruments, Inc., Austin, TX, USA). Data for direct dsDNA detection was obtained after an incubation period of 10 min for an enzymatic reaction at 37 °C.

Results and Discussion

Fig. 1 shows a schematic diagram of the direct electrochemical detection of dsDNA using ALP-conjugated ZFP rrsA125 and biotin-conjugated ZFP rrsA160. Engineered ZFPs rrsA125 and rrsA160 previously showed high specificity with strong binding affinities (1.3 nM and 7.4 nM, respectively).[9] This pair of ZFPs binds to the continuous sites of 36 bp on their target DNA. ALP was conjugated to a lysine residue of the MBP fused with the detection probe ZFP rrsA160, and biotin was also conjugated to a lysine residue of the MBP fused with the capture probe ZFP rrsA125. When ALP was fused to the C-terminal of ZFP rrsA160, the yield (3 μM) was low, compared to the ZFP with no fusion (14 μM). Moreover, when the fused ALP was applied to direct detection of dsDNA, a concentration-dependent change in electrochemical signals was not observed significantly.

The biotin-conjugated ZFP rrsA125 was immobilized on the streptavidin-modified ITO electrode, and captured the target dsDNA. The ALP-conjugated ZFP rrsA160 was applied to the bound complex of ZFP rrsA125 and target DNA to bind to the remaining continuous target site on the captured dsDNA. ITO electrodes were used to harness their low and reproducible electrochemical backgrounds.[25,26] Streptavidin was directly adsorbed on the ITO electrode to minimize nonspecific binding of proteins and DNAs, and to immobilize the biotin-conjugated ZFP rrsA125. The streptavidin-modified surface allows less nonspecific binding of DNAs than the avidin-modified surface.[31]

In order to obtain a high signal-to-background ratio in electrochemical detection using an ALP label, (i) the formal potential of an ALP substrate should be much higher than that of an ALP product and/or (ii) the electrochemical oxidation of an ALP substrate should be much faster than that of an ALP product. In many electrochemical biosensors, *p*-aminophenyl phosphate (APP) and *p*-aminophenol (AP) are used as an ALP substrate and an ALP product, respectively.[24,26] However, the difference in formal potential between APP and AP is not sufficiently large and the electrochemical oxidation of AP occurs slowly at the low electrocatalytic ITO electrode. To overcome this problem, partial modification of the ITO electrode with highly electrocatalytic materials such as a gold nanoparticle[32] and graphene[33] is required, or a different couple of an ALP substrate and product should be used.[25,34] AAP and AA were chosen for an ALP substrate and product couple, because the difference in formal potential is great and AA oxidation occurs sufficiently fast above 0.1 V at a bare ITO electrode.[25] Once the ALP label converts AAP into AA during a 10-min incubation, AA is accumulated near the ITO electrode. After the incubation, the accumulated AA is oxidized to dehydroascorbic acid, generating electrochemical signal.

To compare electrochemical behaviors of AAP and AA, cyclic voltammograms were obtained at bare ITO electrodes in TZ buffer (pH 8.5) (Fig. 2). The cyclic voltammogram in the presence of AAP (curve ii of Fig. 2) was almost identical to that in the absence of AAP (curve i of Fig. 2). It indicates that AAP oxidation is negligible up to 0.5 V. The cyclic voltammogram in the presence of AA (curve iii of Fig. 2) was much larger than that in its absence (curve i of Fig. 2). It clearly shows that a high electrochemical signal of AA can be obtained without the interference of an electrochemical background of AAP. In the presence of both AAP and ALP, a high electrochemical signal was obtained (curve iv of Fig. 2) because AAP was converted into AA by ALP.

To obtain concentration-dependent electrochemical data, cyclic voltammogram, chronoamperogram, or chronocoulogram can be used. In many cases, chronocoulogram allows more reproducible electrochemical signals and backgrounds.[25,28] Accordingly, chronocoulogram was used to obtain dsDNA dose-dependent data. Fig. 3a shows chronocoulograms obtained at an applied potential of 0.30 V at concentrations of zero and 1 nM. The charge value for 1 nM was much higher than that for zero. The charge ratio of the charge value for 1 nM to the charge value for zero (i.e., signal-to-background ratio) depends on the applied potential. High applied potentials increase electrochemical background levels, whereas low applied potentials decrease electrochemical signal levels. Among the four different levels of applied potentials (0.25, 0.30, 0.35, and 0.40V), an applied potential of 0.30 V allowed the highest signal-to-background ratio (Fig. 3b). Zn^{2+} plays a crucial role in the structural conformation of ZFPs, thus binding of ZFPs to target dsDNA. In Fig. 3c, the charge values for zero and 1 nM in the presence of Zn^{2+} were compared with those in the absence of Zn^{2+}. The charge value for 1 nM in the absence of Zn^{2+} was significantly lower than that in the presence of Zn^{2+}. This result clearly indicates that the presence of Zn^{2+} is important for direct binding of ZFPs to dsDNA. To investigate the effect of nontarget ssDNA on the measurement value, we compared the chronoamperometric charge values for zero and 1 nM dsDNA in the absence and presence of 1 nM nontarget ssDNA. In Fig. 3c, the charge value for zero dsDNA in the presence of nontarget ssDNA was similar to that for zero dsDNA in the absence of nontarget ssDNA. The charge value for 1 nM dsDNA in the presence of nontarget ssDNA was slightly lower than that for 1 nM dsDNA in the absence of nontarget ssDNA. These results indicated that the effect of nontarget ssDNA on the electrochemical measurement value is not significant.

Fig. 4a shows chronocoulograms obtained in various concentrations of target dsDNA. As the concentration of dsDNA increased, the charge curve was increased, indicating

DNA-dose dependent signal increase. The concentration data in Fig. 4b corresponds to the charge value measured at 100 s in the chronocoulograms. Fig. 4b indicates that dsDNA can be detected in a wide range of concentrations. The calculated detection limit was approximately 100 fM, suggesting that the detection method is highly sensitive.

Conclusions

In this study, we have demonstrated sensitive and direct detection of specific bacterial dsDNA sequences using (i) the ZFP labelled with ALP possessing fast enzyme kinetics and (ii) electrochemical measurement of AA at the ITO electrode with a high signal-to-background ratio. ALP was simply conjugated to the ZFP through lysine residues of a ZFP purification tag, MBP. Sandwich-type electrochemical detection of dsDNA allowed a detection limit of ca. 100 fM. Thus, we were able to achieve high sensitivity for non-PCR-based dsDNA detection utilizing engineered ZFPs. Our data suggest that this electrochemical method could be a promising approach for ultrasensitive and sequence-specific detection of dsDNA without PCR, aiming at developing a POCT device. With this advantageous feature, our future study will be directed to combine this electrochemical detection system with multiple signal amplification such as enzymatic amplification plus redox cycling.

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Notes and references

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1 O. Lazcka, F. J. Del Campo and F. X. Muñoz, *Biosens. Bioelectron.*, 2007, **22**, 1205.
2 V. Velusamy, K. Arshak, O. Korostynska, K. Oliwa and C. Adley, *Biotechnol. Adv.*, 2010, **28**, 232.
3 E. Heitzer, P. Ulz and J. B. Geigl, *Clin. Chem.*, 2015, **61**, 112.
4 M. van der Vaart and P. J. Pretorius, *Clin. Biochem.*, 2010, **43**, 26.
5 B. Schweitzer and S. Kingsmore, *Curr. Opin. Biotechnol.*, 2001, **12**, 21.
6 P. T. Monis and S. Giglio, *Infect. Genet. Evol.*, 2006, **6**, 2.
7 D. R. Call, M. K. Borucki and F. J. Loge, *J. Microbiol. Methods*, 2003, **53**, 235.
8 I. Ghosh, C. I. Stains, A. T. Ooi and D. J. Segal, *Mol. Biosyst.*, 2006, **2**, 551.
9 M.-S. Kim, G. Stybayeva, J. Y. Lee, A. Revzin and D. J. Segal, *Nucleic Acids Res.*, 2011, **39**, e29.
10 C. I. Stains, J. R. Porter, A. T. Ooi, D. J. Segal and I. Ghosh, *J. Am. Chem. Soc.*, 2005, **127**, 10782.
11 A. T. Ooi, C. I. Stains, I. Ghosh and D. J. Segal, *Biochem.*, 2006, **45**, 3620.
12 Y. Osawa, K. Ikebukuro, H. Motoki, T. Matsuo, M. Horiuchi and K. Sode, *Nucleic Acid Res.*, 2008, **36**, e68.
13 K. Abe, T. Kumagai, C. Takahashi, A. Kezuka, Y. Murakami, Y. Osawa, H. Motoki, T. Matsuo, M. Horiuchi, K. Sode, S. Igimi and K. Ikebukuro, *Anal. Chem.*, 2012, **84**, 8028.
14 W. Yoshida, A. Kezuka, Y. Murakami, J. Lee, K. Abe, H. Motoki, T. Matsuo, N. Shimura, M. Noda, S. Igimi and K. Ikebukuro, *Anal. Chim. Acta*, 2013, **801**, 78.
15 J. Lee, A. Tatsumi, K. Abe, W. Yoshida, K. Sode and K. Ikebukuro, *Anal. Methods*, 2014, **6**, 4991.
16 A. Patterson, F. Caprio, A. Vallée-Bélisle, D. Moscone, K. W. Plaxco, G. Palleschi and F. Ricci, *Anal. Chem.*, 2010, **82**, 9109.
17 H. Zheng, X. Ma, L. Chen, Z. Lin, L. Guo, B. Qiu and G. Chen, *Anal. Methods*, 2013, **5**, 5005.
18 P. P. Chan and P. M. Glazer, *J. Mol. Med.*, 1997, **75**, 267.
19 T. D. Camenisch, M. H. Brilliant and D. J. Segal, *Mini Rev. Med. Chem.*, 2008, **8**, 669.
20 F. W. Scheller, C. G. Bauer, A. Makower, U. Wollenberger, A. Warsinke and F. F. Bier, Immunoassays Using Enzymatic Amplification Electrodes. In Biomolecular Sensors; E. Gizeli, C. R. Lowe, Eds.; Taylor & Francis Ltd.: New York, 2002, pp 207−238.
21 A. Warsinke, *Adv. Biochem. Eng./Biotechnol.*, 2008, **109**, 155−193.
22 E. Paleček and M. Bartošík, *Chem. Rev.*, 2012, **112**, 3427.
23 T. G. Drummond, M. G. Hill and J. K. Barton, *Nature Biotechnol.*, 2003, **21**, 1192.
24 M. S. Wilson and R. D. Rauh, *Biosens. Bioelectron.*, 2004, **20**, 276.
25 M. R. Akanda, M. A. Aziz, K. Jo, V. Tamilavan, M. H. Hyun, S. Kim and H. Yang, *Anal. Chem.*, 2011, **83**, 3926.
26 J. Das, K. Jo, J. W. Lee and H. Yang, *Anal. Chem.*, 2007, **79**, 2790.
27 H. Yang, *Curr. Opin. Chem. Biol.*, 2012, **16**, 422.
28 G. Dutta, S. Kim, S. Park and H. Yang, *Anal. Chem.*, 2014, **86**, 4589.
29 M. S. Bhakta and D. J. Segal, *Methods Mol. Biol.*, 2010, **649**, 3.
30 M. Choi, K. Jo and H. Yang, *Bull. Korean Chem. Soc.*, 2013, **34**, 421.
31 M. A. Aziz, K. Jo, J.-A. Lee, M. R. H. Akanda, D. Sung, S. Jon and H. Yang, *Electroanalysis*, 2010, **22**, 2615.
32 M. A. Aziz, S. Patra and H. Yang, *Chem. Commun.*, 2008, 4607.
33 S. Park, A. Singh, S. Kim and H. Yang, *Anal. Chem.*, 2014, **86**, 1560.
34 M. A. Aziz, K. Jo, M. A. Qaium, C.-H. Huh, I. S. Hong and H. Yang, *Electroanalysis*, 2009, **21**, 2160.

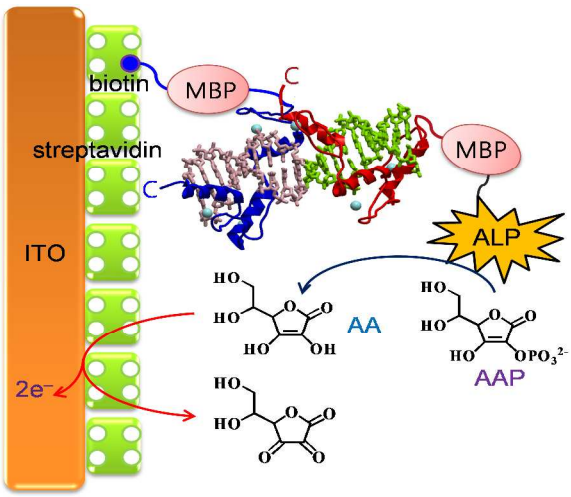

Fig. 1 Schematic representation of the direct electrochemical detection of dsDNA using an ALP-conjugated ZFP and a biotin-conjugated ZFP.

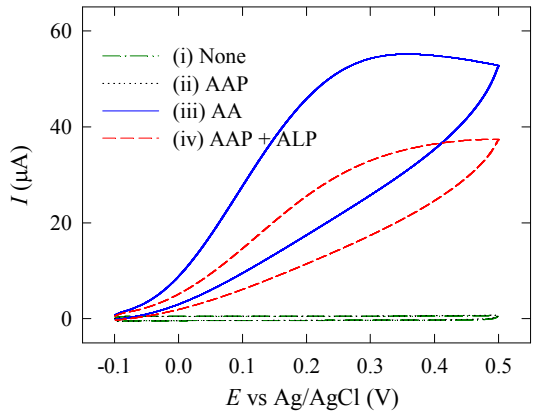

Fig. 2 Cyclic voltammograms obtained at bare ITO electrodes (at a scan rate of 50 mV/s) in TZ buffer (pH 8.5) containing (i) none, (ii) 1.0 mM AAP, (iii) 0.10 AA, or (iv) 1.0 mM AAP and 10 μg/mL ALP.

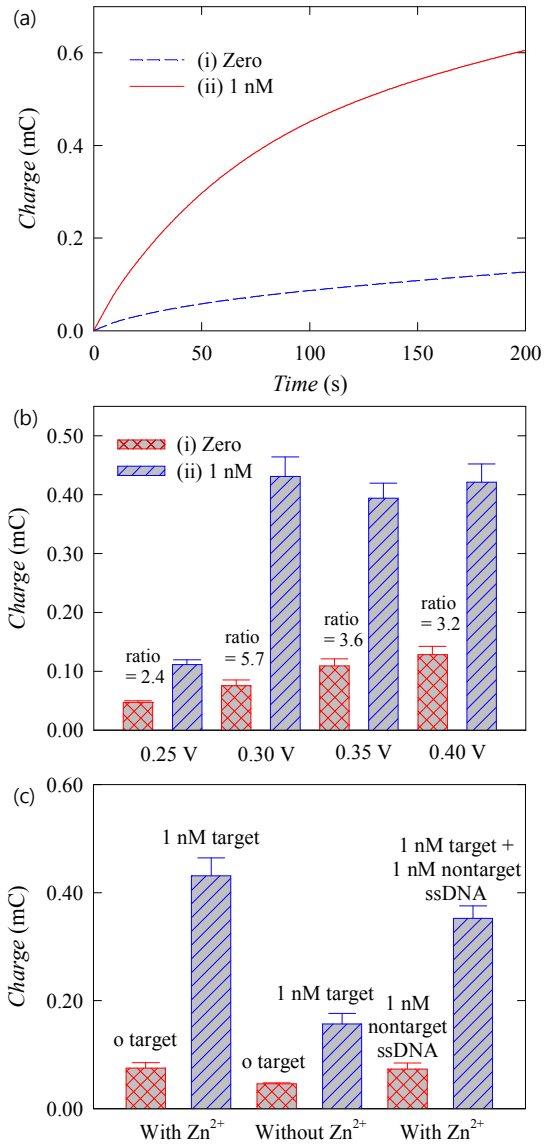

Fig. 3 (a) Chronocoulograms recorded at 0.3 V using the scheme of Fig. 1 after a 10-min incubation period in TZ buffer (pH 8.5) containing 1.0 mM AAP. Histograms for the dependence of the charge measured at 100 s in the chronocoulogram on (b) the applied potential and (c) Zn^{2+} and nontarget ssDNA. Data for two concentrations [(i) zero and (ii) 1 nM dsDNA] were compared.

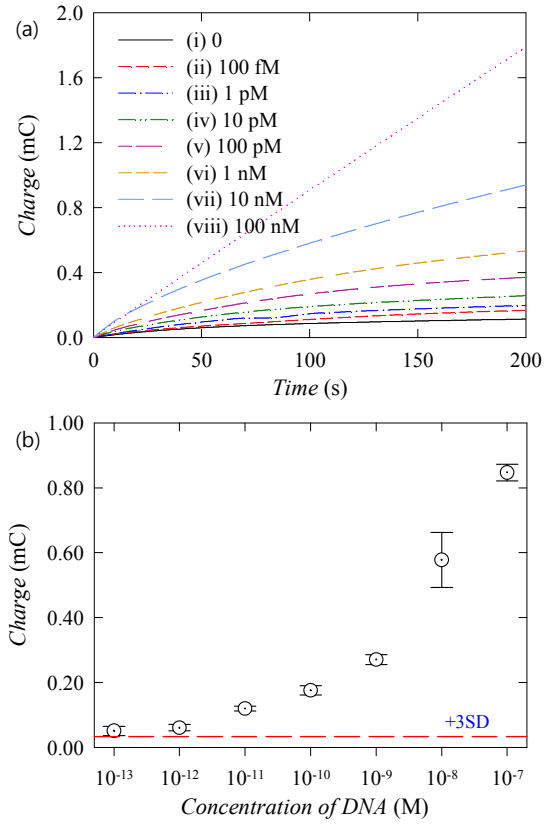

Fig. 4 (a) Chronocoulograms recorded at 0.30 V using the scheme of Fig. 1 for various dsDNA concentrations after a 10-min incubation period in TZ buffer (pH 8.5) containing 1.0 mM AAP. (b) Calibration plot: the concentration dependence of the charge at 100 s in panel a. Each experiment at the different concentrations was carried out with three different electrodes for the assay of the same sample. All charge data were subtracted by the mean value obtained from seven measurements at zero concentration. The dashed line corresponds to 3 times the charge standard deviation (SD) at zero concentration.