Talanta 83 (2011) 937–942

Contents lists available at ScienceDirect

Talanta

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

Electrochemical detection of nicotinamide adenine dinucleotide based on molecular beacon-like DNA and *E. coli* DNA ligase

Xiaoxiao He, Xiaoqi Ni, Yonghong Wang, Kemin Wang*, Lixin Jian

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Biology, College of Chemistry & Chemical Engineering, Hunan University, Key Laboratory for Bio-Nanotechnology and Molecule Engineering of Hunan Province, Changsha 410082, PR China

ARTICLE INFO

Article history:
Received 10 August 2010
Received in revised form 20 October 2010
Accepted 29 October 2010
Available online 5 November 2010

Keywords:
E. coli DNA ligase
Molecular beacon-like DNA
Electrochemical assay
NAD^+ detection

ABSTRACT

An electrochemical method for nicotinamide adenine dinucleotide (NAD^+) detection with high sensitivity and selectivity has been developed by using molecular beacon (MB)-like DNA and *Escherichia coli* DNA ligase. In this method, MB-like DNA labeled with 5′-SH and 3′-biotin was self-assembled onto a gold electrode in its duplex form by means of facile gold–thiol chemistry, which resulted in blockage of electronic transmission. It was eT OFF state. In the presence of NAD^+, *E. coli* DNA ligase was activated, and the two nucleotide fragments which were complementary to the loop of the MB-like DNA could be ligated by the NAD^+-dependent *E. coli* DNA ligase. Hybridization of the ligated DNA with the MB-like DNA induced a large conformational change in this surface-confined DNA structure, which in turn pushed the biotin away from the electrode surface and made the electrons exchange freely with the electrode. Then the generated electrochemical signals can be measured by differential pulse voltammetry (DPV). Under optimized conditions, a linear response to logarithmic concentration of NAD^+ range from 3 nM to 5 μM and a detection limit of 1.8 nM were obtained. Furthermore, the proposed strategy had sufficient selectivity to discriminate NAD^+ from its analogues.

1. Introduction

Nicotinamide adenine dinucleotide (NAD^+) is a ubiquitous biological molecule that participates in many biological processes, including the regulation of energy metabolism [1,2], DNA repair [3], and transcription [4,5]. For example, NAD^+ is required in several important signaling pathways in mammalian cells, including poly(ADP-ribosyl)ation in DNA repair [3], G-protein-coupled signaling [6]. NAD^+ can also dominate some significant life process correlated with the metabolism in many organisms [7–9]. It has been confirmed that the NAD^+ level played the pivotal role in the yeast cell senile regulation [10,11]. Furthermore, recent studies showed that the change in NAD^+ level has been found to be closely related to many diseases; Kelley et al. [12] found that the diabetes had lower ability to regulate the NAD^+ level. Torabi et al. [13] found that NAD^+ concentration in cancer tissues was higher than that in normal tissues. Murray et al. [14] reported that HIV-1 infection of human cells *in vitro* leaded to significant decreases in the intracellular concentration of NAD^+. Therefore, the determination of NAD^+ is essential for biochemical study as well as clinic diagnosis.

Many methods have been carried out to detect NAD^+ both *in vitro* and in vivo, such as UV absorbance in combination with HPLC separation [15], fluorescence of a NAD^+ derivative [16–18], chemiluminescence [19], nuclear magnetic resonance (NMR) spectrometry [10,20], capillary electrophoresis [21–23], electrospray ionization mass spectrometry (ESI-MS) [24]. For example, Yamada et al. [25] developed a liquid chromatographic–tandem mass spectrometric method that simultaneously measured cellular NAD^+ and related compounds. Xie et al. [23] reported an in-capillary enzymatic cycling assay that was developed to determine the NAD^+ and NADH contents of a single cell. Unfortunately, these approaches are complex, time consuming, they also need expensive, high-power consumption, cumbersome equipments or expensive light sources. Furthermore, these methods are not sensitive or specific enough. Therefore, other avenues need to be explored to have a convenient, rapid, high specific and sensitive method to assay the negligible amount of NAD^+.

As all we known, some methods for target biomolecules detection based on the biomolecules-dependent enzymatic reaction have been gaining wide attention in recent years. The target biomolecules, as a substrate or an important factor of enzyme, can be indirectly detected through monitoring the biomolecules-dependent enzymatic reactions [26]. The methods display high selectivity because the enzymatic reaction cannot occur in the absence of certain biomolecule. For example, ATP-dependent luciferase–luciferin reaction for ATP detection [27,28], pyrrolo quinoline quinone (PQQ)-dependent redox enzyme for PQQ detection [29], NADP-dependent diaphorase reaction for NADP detection

* Corresponding author. Tel.: +86 731 88821566; fax: +86 731 88821566.
E-mail address: kmwang@hnu.cn (K. Wang).

0039-9140/$ – see front matter.
doi:10.1016/j.talanta.2010.10.051

Table 1
Sequence of oligonucleotides.

Oligonucleotide	Sequence (5′–3′)
MB-like DNA	HS-$(CH_2)_6$-CCTCTCCGTGTCTTG TACTTCCCGTCAGAGAGG-biotin
Oligo A	p-TACAAGACAC
Oligo B	GACGGGAAG

[30]. NAD^+, as an important biomolecule, is also prerequisite in many enzymatic reactions. Enzymes that utilize NAD^+ as a substrate include some NAD^+-dependent DNA ligases [31], NAD^+-dependent oxidoreductases [32,33], poly(ADP-ribose) polymerase (PARP) [3] and the recently characterized Sir2p family [34], NAD^+-dependent deacetylases [35,36]. Therefore, it is highly desirable to develop NAD^+ detection method by combination NAD^+-dependent enzymatic reaction with bioluminescence, fluorescence, electrochemistry or other analytical technologies.

Recently, our group has developed a kinase-based ATP fluorescence assay using molecular beacon [37] and ligase-based ATP electrochemical assay using molecular beacon (MB)-like DNA [38], respectively. Both of the two methods took advantages of the enzymatic reaction mediated conformation change of DNA in the presence of ATP. They both demonstrated simple, fast and enough selectivity to discriminate ATP from its analogues. By comparison with kinase-based ATP fluorescence assay, the ligase-based ATP electrochemical assay promoted the detection limit with 400 times without employing cumbersome and expensive optics, light sources, or photodetectors. *Escherichia coli* (*E. coli*) DNA ligase is an NAD^+-dependent enzyme that catalyzes the formation of phosphodiester bonds between complementary 3′-hydroxyl and 5′-phosphoryl termini of dsDNA. Here, we developed an electrochemical method for NAD^+ detection by using MB-like DNA and *E. coli* DNA ligase. The electrochemical properties of MB-like DNA modified electrode and experimental conditions were studied in detail.

2. Experimental

2.1. Reagents and materials

E. coli DNA ligase was purchased from Takara Biotechnology Co., Ltd. (Dalian, China). Mercaptohexanol (MCH), nicotinamide adenine dinucleotide (NAD^+) and the reduced form of nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADP), nicotinamide adenine dinucleotide phosphate hydride (NADPH), adenosine-5′-triphosphate (ATP) and adenosine monophosphate (AMP) were obtained from Sigma–Aldrich (Shanghai) Trading Co., Ltd. (Shanghai, China). All other chemicals were obtained from Reagent & Glass Apparatus Corporation of Changsha and were used without further purification (Changsha, China). The solutions in the experiments were prepared with ultrapure water (Milli-Q 18.2 MΩ cm, Millipore (Shanghai) Trading Co., Ltd., Shanghai, China). All synthetic oligonucleotides were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). The sequences of oligonucleotides used in this work are listed in Table 1.

2.2. Instruments

The electrochemical signals were measured at room temperature by using a CHI660A electrochemical workstation (Shanghai Chenhua Instrument Corporation, Shanghai, China). The electrochemical cell consisted of a three-electrode system, with a gold or modified gold disk electrode (2.0 mm in diameter, CHI 101; CH Instrument) as the working electrode, a platinum foil as counter electrode, and a saturated calomel electrode (SCE) as the reference electrode, respectively. All the potentials in this paper were with respect to SCE. And all solutions were deaerated with high-purity nitrogen and kept under a nitrogen atmosphere throughout the electrochemical measurements.

2.3. Experimental details

2.3.1. Experiment principle

The principle for electrochemical detection of NAD^+ based on MB-Like DNA and *E. coli* DNA ligase was demonstrated in Fig. 1. As elucidated in Fig. 1(a), the MB-like DNA dually labeled with 5′-SH and 3′-biotin was self-assembled onto gold electrodes in a stem–loop structure via gold–sulfur affinity. We reasoned that the biotin tagged on the end of the DNA approached the electrode surface and the MB-like DNA occupied a large area, thus the electrode could not efficiently exchange electrons with the solution. It was eT OFF state. When the two nucleotide fragments (oligo A and oligo B) which were complementary to the loop of the MB-like DNA were added, the MB-like DNA was hybridized with the two half-match oligos to form a nick. However, it was still eT OFF state because there was no ligation between the two oligos. In the presence of NAD^+, the *E. coli* DNA ligase would be activated and catalyzed the ligation reaction to form the whole-match oligo to open MB-like DNA completely, which in turn released the biotin from the electrode surface and induced the change of the DNA from a stem–loop to an extended structure (Fig. 1(b)). Therefore, the ferricyanide could be allowed to easily access to the surface, and the electrons exchanged freely with the electrode. It generated measurable electrochemical signals (eT ON). The DPV was then adopted to determine the concentration of NAD^+. On the contrary, *E. coli* DNA ligase cannot be activated to catalyze the two nucleotide fragments juncture if the DNA ligation solution was absent of NAD^+. Thus the ligation reaction still could not happen, and it remained eT OFF state.

2.3.2. MB-like DNA probe immobilization on the electrode

Prior to MB-like DNA probe immobilization, the gold electrode was polished with 0.3 and 0.05 μm alumina powder, respectively. The polished electrode was then cleaned ultrasonically sequentially in ultrapure water, ethanol, and ultrapure water for 10 min each, followed by electrochemically cleaning in 0.1 M H_2SO_4 by potential scanning between −0.2 and 1.6 V until a reproducible cyclic voltammogram was obtained. The clean electrode was rinsed with ultrapure water and dried in a mild nitrogen stream. 20 μL of 1.5 μM MB-like DNA solutions was placed on the cleaned gold electrode that was held upside down and kept 2 h at room temperature. During the modification process, the electrode dropped with MB-like DNA solution was further covered with a plastic cap to avoid the solution evaporation. After being exposed to a 1 mM mercaptohexanol (MCH) solution (in 0.1 M PBS buffer, pH 7.4) for 20 min to block the remaining bare region [38], the resulting electrode was ready for the NAD^+ detection.

2.3.3. Detection of NAD^+

By immersing the MB-like DNA/MCH modified electrodes into a 20 μL freshly prepared DNA ligation solution (containing NAD^+ with certain concentration, 20 U *E. coli* DNA ligase, 1 μM oligo A, 1 μM oligo B, 5 mM $MgCl_2$ and 20 mM Tris–HCl, respectively), the ligation reaction of the two oligonucleotides and subsequent hybridization reactions of the ligated DNA fragments to the MB-like DNA probes were conducted in a moist hybridization chamber at 40 °C for 2 h. Thereafter the electrodes were thoroughly rinsed with ultrapure water to remove any nonspecifically bound species. The electrodes were subsequently dried with nitrogen purge. Then, a three-electrode electrochemical cell was used to detect the electrochemical signals by DPV. All electrochemical measurements were performed in 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and 0.4 M KNO_3 at room temperature.

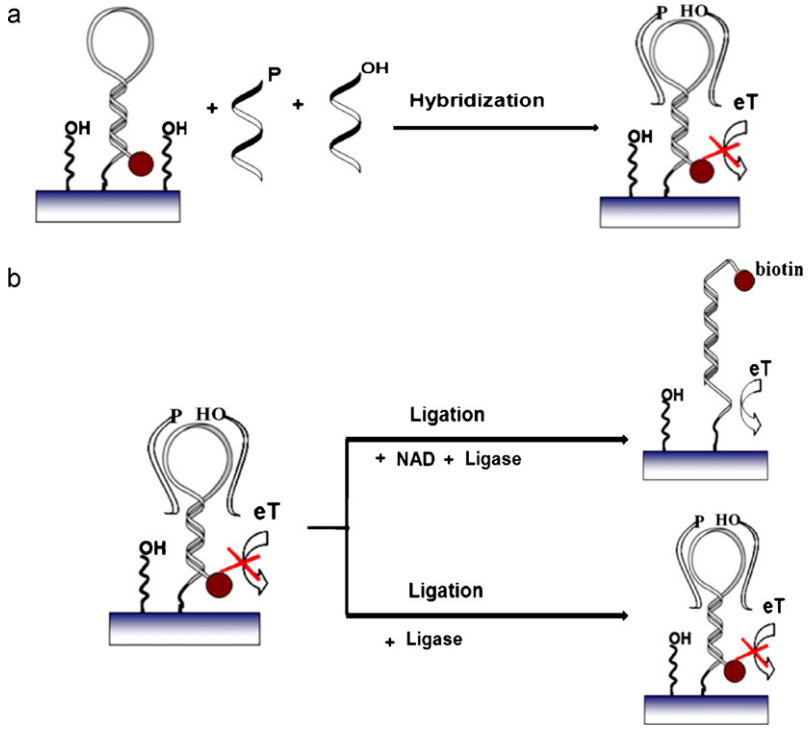

Fig. 1. (a and b)Principle of electrochemical detection of NAD^+ based on molecular beacon-like DNA and *E. coli* DNA ligase.

2.3.4. Regeneration of the MB-like DNA modified electrode

The procedure was performed by immersing the used electrodes into ultrapure water at 95 °C for 5 min to remove the nucleotide which was complementary to the loop of MB-like DNA. Then, the electrodes were transferred to the ice-cold PBS buffer solution to refold the MB-like DNA probe again. The regenerated electrode was reused for detecting the target of NAD^+ using the same procedure as in Section 2.3.3.

3. Results and discussions

3.1. Characterization and feasibility investigation of the electrochemical assay

DPV had been performed to investigate the feasibility of this electrochemical assay. Fig. 2(a) presented the DPV of bare gold electrode, the MB-like DNA/MCH modified gold electrode, the MB-like DNA/MCH modified electrode treated with ligation solution without NAD^+ (20 U *E. coli* DNA ligase, 1 μM oligo A, 1 μM oligo B and ligation buffer) and the MB-like DNA/MCH modified electrode treated with ligation solution (3 μM NAD^+, 20 U *E. coli* DNA ligase, 1 μM oligo A, 1 μM oligo B and ligation buffer) in 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and 0.4 M KNO_3, respectively. The DPV for the bare gold electrode (curve 1) exhibited a high currents signal, which indicated the electrons exchanged freely with the electrode. When gold electrode was modified with MB-like DNA/MCH, a weak currents signal was obtained (curve 2) due to the electronic transmission block came from the immobilized MB-like DNA/MCH. Obvious peak current was obtained after the modified electrode was treated with NAD^+ ligation solution (curve 3), this was due to the biotin molecules were away from the electrode after NAD^+-induced the currents from a signal-off to a signal-on state, which was in good agreement with the assay principle. When the modified electrode was treated using ligation solution without NAD^+, it showed a weak current signal (curve 4). The reason for this was that the *E. coli* DNA ligase would not catalyze ligation reaction in the absence of NAD^+.

As an effective method to probe the interface properties of modified electrodes, electrochemical impedance spectroscopy (EIS) was also used to evaluate the interfacial electron-transfer state at different stages of assay preparation. As displayed in Fig. 2(b), at the bare Au electrode, very small electron-transfer resistance (R_{et}), which was measured as the radius of the semicircle in the EIS, was presented (curve 1), suggesting a free electron-transfer process at such electrode. Compared with the bare Au electrode, the MB-like DNA/MCH-modified electrode showed a larger R_{et} (curve 2). It was mainly due to the reason that a large amount of biotin modified MB-like DNA would form a big barrier to prevent the $[Fe(CN)_6]^{3-/4-}$ anions from getting access to the electrode surface. After treated with the ligation solution containing NAD^+, the MB-like DNA/MCH-modified electrode showed a relatively small R_{et} (curve 3) relative to curve 2. This was attributed to fact that the MB-like DNA was opened in the presence of NAD^+, which induced the biotin away from the electrode surface. Therefore, the electrochemical impedance decreased. While the MB-like DNA/MCH-modified electrode was treated with ligation solution without NAD^+, there was also a large semicircle domain (curve 4). The reason for this was that the *E. coli* DNA ligase could not catalyse two nucleotide fragments ligation reaction in the absence of NAD^+. Therefore, the DPV results and interface characterization by EIS indicated the electrochemical assay could be used to detect NAD^+.

3.2. Optimization of experimental conditions

3.2.1. Effect of surface-modifying time of MB-like DNA

DNA probe surface-modifying time is an important parameter in optimizing the signal gain of electrochemical DNA (E-DNA) sensors. We employed the concentration of 1.5 μM MB-like DNA to fabricate the modified electrode according to the previous report [38]. As can be seen from the current signal of MB-like DNA gold electrode (Fig. 3(a)), the current signal was decreased

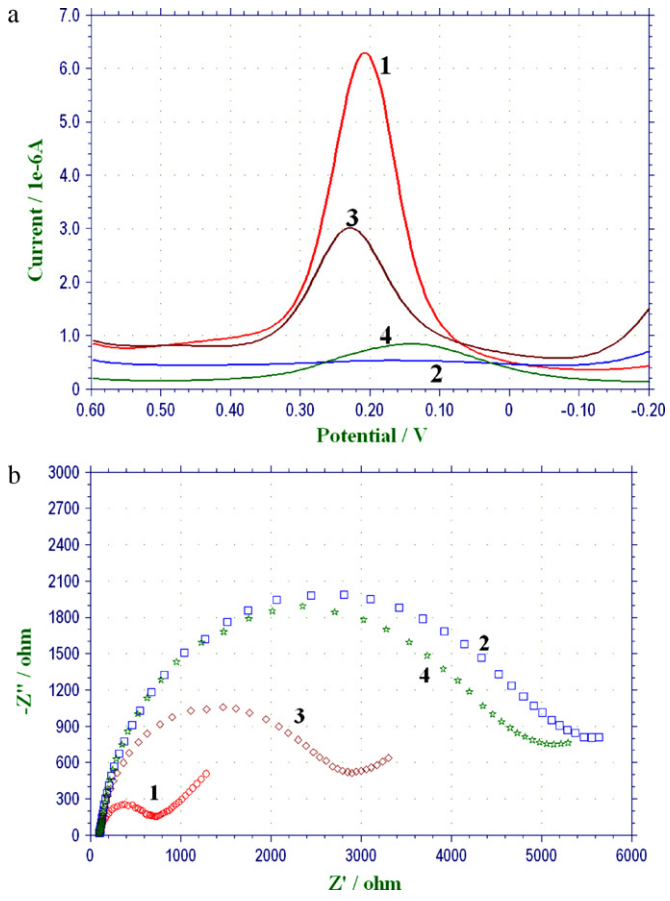

Fig. 2. Differential pulse voltammograms (DPV) (a) and Nyquist plots (b) of bare gold electrode (1), MB-like DNA/MCH modified gold electrode (2), MB-like DNA/MCH modified electrode treated with ligation solution (3), and MB-like DNA/MCH modified electrode treated with ligation solution without NAD^+ (4). Supporting electrolyte, 10 mM PBS (pH 7.4) containing 0.5 mM $[Fe(CN)6]^{3-/4-}$ and 0.4 M KNO_3. DPV, pulse amplitude 50 mV; pulse period, 0.2 s. Nyquist plots in electrolyte at 210 mV; frequency range 0.1–100 kHz; ac amplitude 5 mV.

with increasing surface-modifying time. As the surface-modifying time was increased to 60 min, the current signal reached a relative minimum value. Further increase of the surface-modifying time would not decrease the current signal. Thus 60 min was chosen as the appropriate surface-modifying time for the following experiments.

3.2.2. *Effect of different ligation temperature*

To set an optimum ligation temperature is also very important to obtain accurate results. Fig. 3(b) showed the effect of ligation temperature on response current. The response current increased with increasing temperature from 25 to 40 °C. At high temperature, the enzyme is easy to be denatured, and lower temperature will result in difficulty of MB-like DNA conformational change. Therefore, 40 °C was chosen as the optimum temperature for the ligation of the assay.

3.2.3. *Effect of different ligation time*

When the targets and two nucleotide fragments reach the MB-like DNA at the surface of the electrode, it would take time for opening the stem–loop structure of the MB-like DNA [39]. As shown in Fig. 3(c), it was demonstrated the electrochemical signal increased dramatically and then tended to change only slightly when the ligation time was increased from 10 to 180 min. So a ligation time of 120 min was selected for the following NAD^+ detection.

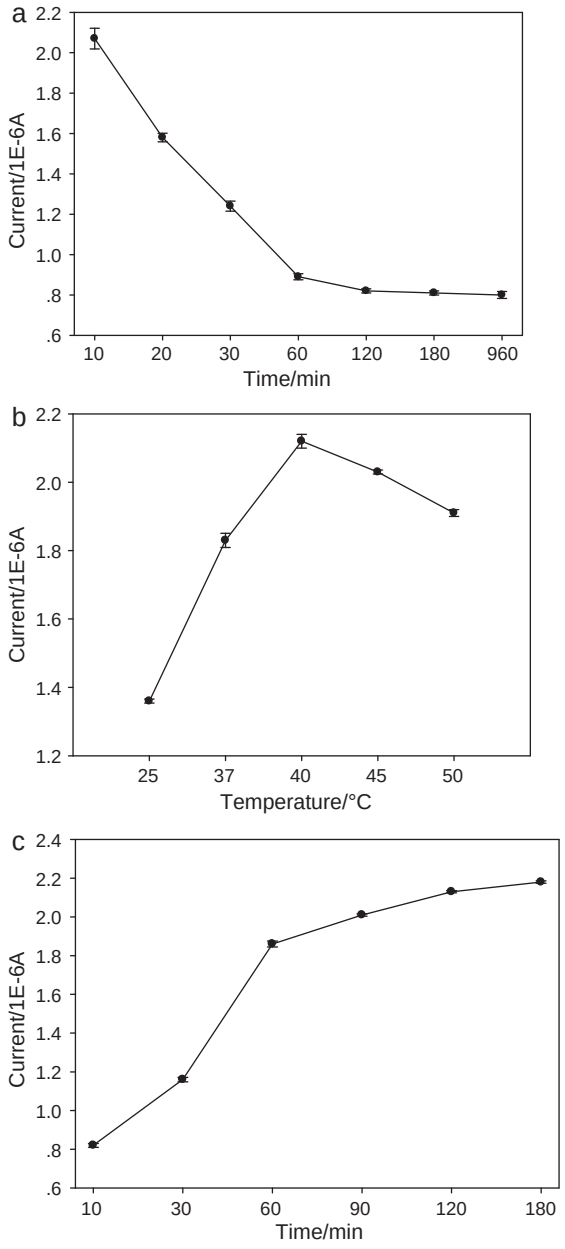

Fig. 3. Optimization of experimental conditions: (a) effect of surface modification time of MB-like DNA: 10–960 min. (b) Effect of hybridization temperature: 25–50 °C. (c) Effect of hybridization time: 10–180 min.

3.3. *Detection of NAD^+*

The detection performance of the NAD^+ assay was evaluated by exposing the sensor to a series of NAD^+ concentrations under the same experimental condition. The electrochemical response to different concentrations of NAD^+ was illustrated in Fig. 4. As can be seen, one observed dynamically increased DPV peak currents in the presence of increasing target concentration within the range from 0.5 nM to 25 μM. Linear relationship between the peak current and the logarithmic concentration of NAD^+ was observed in the range of 3–5000 nM with a correlation factor 0.987. The linear regression equation was $y = 0.7403x + 0.3146$ (here, x was the logarithmic concentration of NAD^+ (nM), and y was the response peak current). Meanwhile, the detection limit was 1.8 nM as calculated according to the rule of three times standard deviation over the background signal. The results indicated that the present method could success-

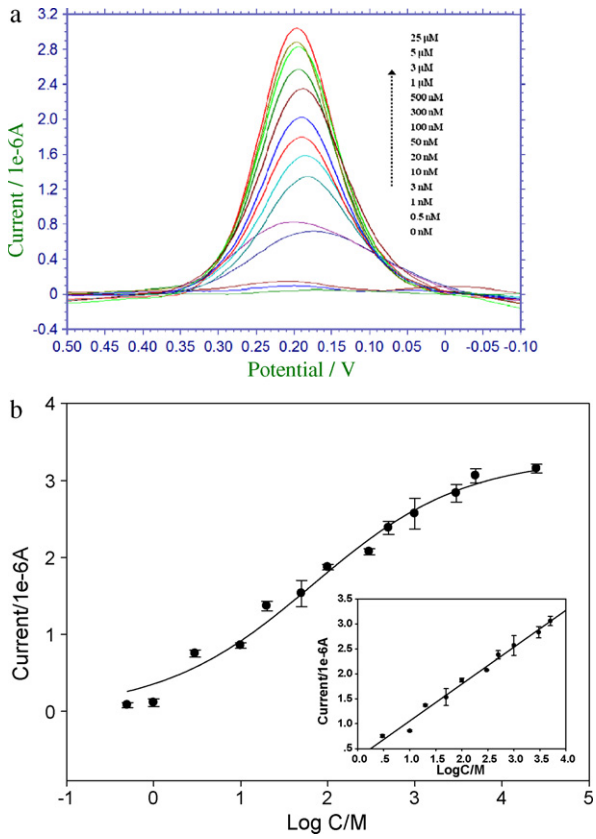

Fig. 4. (a) Differential pulse voltammograms of modified gold electrode incubated with different concentrations of NAD^+ (from bottom to top: 0 nM to 25 μM). The LOD base on S/N = 3 is 1.8 nM. (b) The response curve obtained with different logarithmic concentrations of NAD^+. Inset showed linear relationship between the current and the logarithm of the NAD^+ concentration from 3 nM to 5 μM.

fully detect the NAD^+ with high sensitivity. The detection limit of this electrochemical NAD^+ assay based on molecular beacon-like DNA and *E. coli* DNA ligase was 3–4 orders of magnitude lower than that of reported dehydrogenase-based NAD^+ amperometric method [40]. Moreover, under the optimum conditions, the RSD of this method ($n = 5$) was found to be 4.1%, the regression equation was $Y = 3.2048/(1 + \exp((1.7607 - x)/0.8257))$ (x was the logarithmic concentration of NAD^+; Y was the peak current, 10^{-6} A; $n = 5$, $R^2 = 0.9908$). It was shown that this biosensor had an acceptable reproducibility for the detection of NAD^+.

3.4. Selectivity of the electrochemical assay

The ability to be specific to the analytes is very critical for an ideal biosensor strategy. The selectivity of the target biomolecules detection based on monitoring the biomolecules involved enzymatic reaction generally attributed to the fact that the enzymatic reaction cannot occur in the absence of target biomolecules [27–30]. This correlation was also observed for the NAD^+ electrochemical assay reported here. As exhibited in Fig. 5, the selectivity of this NAD^+ electrochemical assay was investigated by comparing the current signal change of samples containing NAD^+ with that of NADH, NADP, NADPH, ATP and AMP, respectively. The current signal change was measured individually after NAD^+, NADH, NADP, NADPH, ATP and AMP with the same concentration of 3 μM in the samples. It was clearly demonstrated that a significant increase induced only by the interaction of the MB-like DNA and the NAD^+ ligation solution was observed compared to the other five analogues samples, obviously indicating that the proposed strategy had sufficient selectivity in NAD^+ detection, and was able to discriminate NAD^+ from its analogues. In addition, when NADH, NADP, NADPH, ATP and AMP were coexisted in NAD^+ with the same concentration of 3 μM, the signal did not changed much compared with NAD^+. The result indicated that this assay would hopefully be used to perform the analysis of real samples.

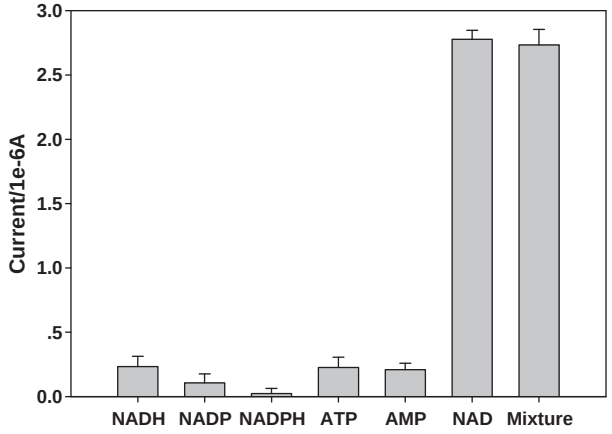

Fig. 5. Selectivity of the NAD^+ detection assay. The concentration of NAD^+, NADH, NADP, NADPH, ATP, AMP is 3 μM, respectively. And the mixture was composed of NAD^+, NADH, NADP, NADPH, ATP and AMP with the same concentration of 3 μM.

3.5. Sensor regeneration

The regeneration of the E-DNA is important for the E-DNA applications [41]. Here the sensing interface regeneration in our experiments was performed by immerging in hot water. We have successfully recovered up to ~81% of the original signal under identical conditions after ten rounds of regeneration. This means that the sensor can be completely regenerated for repeated usage.

4. Conclusions

In summary, we have developed a novel electrochemical method for NAD^+ detection using NAD^+-dependent *E. coli* DNA ligation reaction and MB-like DNA. The method takes advantage of the sensitive MB-like DNA conformation change when the two nucleotide fragments which are complementary to the loop of the MB-like DNA are ligated by the *E. coli* DNA ligase in the presence of NAD^+, and accompanying measurable electrochemical signals. By using of the NAD^+-dependent enzymatic reaction, the sensitivity is substantially enhanced and the assay system is furnished with high selectivity and cost-efficiency. This method is capable of selectively determining NAD^+ from other five NAD^+ analogues. Detection limit of NAD^+ can reach down to 1.8 nM. Furthermore, by employing this platform, various kinds of biomolecules can be detected based on the biomolecules-dependent enzymatic reaction.

Acknowledgements

This work was supported by Program for Innovative Research Team of Hunan National Science Foundation (10JJ7002), International Science & Technology Cooperation Program of China (2010DFB30300), and National Science Foundation of PR China (90606003, 20775021).

References

[1] P. Aksoy, T.A. White, M. Thompson, E.N. Chini, Biochem. Biophys. Res. Commun. 345 (2006) 1386.
[2] C. Canto, Z. Gerhart-Hines, J.N. Feige, M. Lagouge, L. Noriega, J.C. Milne, P.J. Elliott, P. Puigserver, J. Auwerx, Nature 458 (2009) 1056.

[3] A. Wilkinson, J. Day, R. Bowater, Mol. Microbiol. 40 (2001) 1241.
[4] R.M. Anderson, K.J. Bitterman, J.G. Wood, O. Medvedik, H. Cohen, S.S. Lin, J.K. Manchester, J.I. Gordon, D.A. Sinclair, J. Biol. Chem. 277 (2002) 18881.
[5] J. Rutter, M. Reick, L.C. Wu, S.L. McKnight, Science 293 (2001) 510.
[6] D. Corda, M.D. Girolamo, EMBO J. 22 (2003) 1953.
[7] Q.H. Zhang, D.W. Piston, R.H. Goodman, Science 295 (2002) 1895.
[8] J.S. Smith, J.D. Boeke, Genes Dev. 11 (1997) 241.
[9] J.J. Sandmeier, I. Celic, J.D. Boeke, J.S. Smith, Genetics 160 (2002) 877.
[10] R.M. Anderson, M.L. Esteves, A.R. Neves, M. Latorre-Esteves, S. Lavu, O. Medvedik, C. Taylor, K.T. Howitz, H. Santos, D.A. Sinclair, Science 302 (2003) 2124.
[11] S.G. Lin, P.A. Defossez, L.G. Guarente, Science 289 (2000) 2124.
[12] D.E. Kelley, J. He, E.V. Menshikova, V.B. Ritov, Diabetes 51 (2002) 2944.
[13] F. Torabi, K. Ramanthan, P.O. Larsson, L. Gorton, K. Svanberg, Y. Okamoto, B. Danielsson, M. Khayyami, Talanta 50 (1999) 787.
[14] M.F. Murray, M. Nghiem, A. Srinivasan, Biochem. Biophys. Res. Commun. 212 (1995) 126.
[15] M.R. Litt, J.J. Potter, E. Mezey, M.C. Mitchell, Anal. Biochem. 179 (1989) 34.
[16] K.S. Putt, P.J. Hergenrother, Anal. Biochem. 326 (2004) 78.
[17] F. Joubert, H.M. Fales, H. Wen, C.A. Combs, R.S. Balaban, Biophys. J. 86 (2004) 629.
[18] T. Koshida, T. Arakawa, T. Gessei, D. Takahashi, H. Kudo, H. Saito, K. Yano, K. Mitsubayashi, Sens. Actuators B 146 (2010) 177.
[19] D.C. Williams III, W.R. Seitz, Anal. Chem. 48 (1976) 1478.
[20] A.R. Neves, R. Ventura, N. Mansour, C. Shearman, M.J. Gasson, C. Maycock, A. Ramos, H. Santos, J. Biol. Chem. 277 (2002) 28088.
[21] R.J.O. Cosford, W.G. Kuhr, Anal. Chem. 68 (1996) 2164.
[22] T.M. Casey, K.G. Dufall, P.G. Arthur, Eur. J. Biochem. 261 (1999) 740.
[23] W.J. Xie, A.S. Xu, E.S. Yeung, Anal. Chem. 81 (2009) 1280.
[24] D.H. Kim, B.N. Marbois, K.F. Faull, C.D. Eckhert, J. Mass Spectrom. 38 (2003) 632.
[25] K. Yamada, N. Hara, T. Shibata, H. Osago, M. Tsuchiya, Anal. Biochem. 352 (2006) 282.
[26] J.L. Zhou, P.P. Nie, H.T. Zheng, J.M. Zhang, Chin. J. Anal. Chem. 37 (2009) 617.
[27] Z.Q. Wang, P.G. Haydon, E.S. Yeung, Anal. Chem. 72 (2000) 2001.
[28] T. Kamidate, K. Yanashita, H. Tani, A. Ishida, M. Notani, Anal. Chem. 78 (2006) 337.
[29] D.X. Shen, M.E. Meyerhoff, Anal. Chem. 81 (2009) 1564.
[30] J. Madoz, J. Fernandez-Recio, C. Gomez-Moreno, V.M. Fernandez, Bioelectrochem. Bioenerg. 47 (1998) 179.
[31] L.F. Liu, Z.W. Tang, K.M. Wang, W.H. Tan, J. Li, Q.P. Guo, X.X. Meng, C.B. Ma, Analyst 130 (2005) 350.
[32] P.E. Smith, J.J. Tanner, J. Am. Chem. Soc. 121 (1999) 8637.
[33] H.J. Zhou, Z.P. Zhang, P. Yu, L. Su, T. Ohsaka, L. Mao, Langmuir 26 (2010) 6028.
[34] S.J. Lin, L. Guarente, Curr. Opin. Cell Biol. 15 (2003) 241.
[35] J. Landry, R. Sternglanz, Methods 31 (2003) 33.
[36] M.G. Li, B.J. Petteys, J.M. McClure, V. Valsakumar, S. Bekiranov, E.L. Frank, J.S. Smith, Mol. Cell. Biol. 30 (2010) 3329.
[37] C.B. Ma, X.H. Yang, K.M. Wang, Z.W. Tang, W. Li, W.H. Tan, X.Y. Lv, Anal. Biochem. 372 (2008) 131.
[38] Y.H. Wang, X.X. He, K.M. Wang, X.Q. Ni, Biosens. Bioelectron. 25 (2010) 2101.
[39] Z.J. Wang, Y.H. Yang, K.L. Leng, J.S. Li, F. Zheng, G.L. Shen, R.Q. Yu, Anal. Lett. 41 (2008) 24.
[40] S.B. Saidman, M.J. Lobo-Castanon, A.J. Miranda-Ordieres, P. Tunon-Blanco, Anal. Chim. Acta 424 (2000) 45.
[41] S. Balamurugan, A. Obubuafo, S.A. Soper, D.A. Spivak, Anal. Bioanal. Chem. 390 (2008) 1009.