ChemComm

Cite this: *Chem. Commun.*, 2011, **47**, 7689–7691

www.rsc.org/chemcomm

COMMUNICATION

Tetrahedron-structured DNA and functional oligonucleotide for construction of an electrochemical DNA-based biosensor†

Nan-Nan Bu, Chun-Xia Tang, Xi-Wen He and Xue-Bo Yin*

Received 21st March 2011, Accepted 17th May 2011
DOI: 10.1039/c1cc11628b

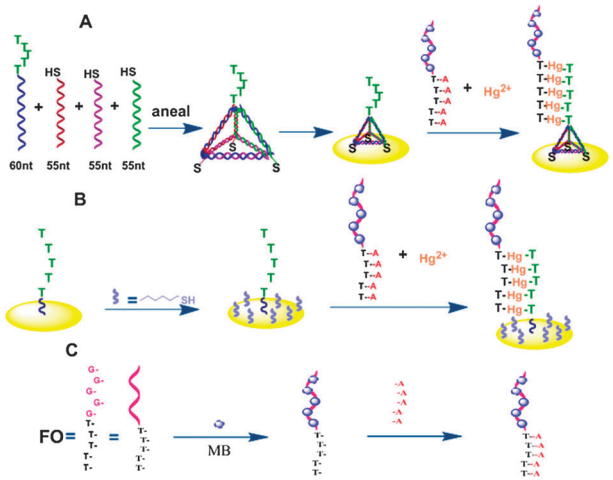
Tetrahedron-structured DNA (ts-DNA) in combination with a functionalized oligonucleotide was used to develop a "turn-on" biosensor for Hg^{2+} ions. The ts-DNA provided an improved sensitivity and was used to block the active sites.

Mercury is one of the most toxic elements and has an important environmental and biological effect,[1] but we have little knowledge of the metal ions transfer mechanism into cells, speciation, and beneficial or deleterious functions.[1b] Atomic (mass) spectrometry can only provide the elemental information.[2] However, "metal ion biosensor revolutionize biochemistry, cellular biology, and related fields"[1b] with a sensitive and simple setup. Functional nucleic acids provide "an excellent alternative" in detecting metal ions because of their selectivity and affinity to some metal ions, such as Hg^{2+}, Ag^{+}, Pb^{2+}, and Cu^{2+}.[1b,3] Electrochemical transducers are simple and cost-effective for DNA-based ion sensors.[3,4] To this end, linear single-stranded DNA (ss-DNA) is immobilized onto the electrode surface to recognize the target.[3,4] The active molecules attached chemically to the end of the DNA generate the signal related to the metal ion content.[3,4]

The DNA strand must be maintained in a perpendicular orientation for efficient hybridization to its target.[5,6] The coverage of DNA strands on the electrode surface is also a critical factor influencing the performance of DNA biosensors. A high density of DNA strands resulted in a decreased signal output because each DNA strand needs enough space for interaction with its target; the low coverage of DNA shows a low capacity for the analytes.[6] Therefore, an optimal density and even distribution of the DNA strands on the electrode surface is needed, but it is difficult to control linear ss-DNA.

Electrochemically active molecules are attached chemically to linear DNA for the signal related to the target concentration, but the labeling procedure is time-consuming and labor-intensive. False positive signals may generate in the absence of the target.[5] Moreover, decrease in the binding affinities of chemically labeled DNA strands for their target was also observed.[7] Thus, it is important to design an alternative approach that avoids oligonucleotide labeling and decreases the background signal for the development of electrochemical metal ion sensors.

In this work, a tetrahedron-structured DNA (ts-DNA)[8] was used to construct a Hg^{2+} ion biosensor in combination with a functional oligonucleotide (FO) for the integration of methylene blue (MB) and the formation of stable thymine-Hg^{2+}-thymine complex (T-Hg^{2+}-T).[9] Compared with linear single stranded DNA (ss-DNA), ts-DNA provided an improved sensitivity for Hg^{2+} ions and it was unnecessary to block active sites with an alkanethiol monolayer. FO, with the sequence of T_5-G_5, is used to integrate with an electrochemical active probe, MB and to identify Hg^{2+} ions *via* the formation of T-Hg^{2+}-T. With FO, the chemical labeling probe molecule is not required. The ts-DNA was immobilized onto the gold electrode surface and T_5 DNA section was introduced for Hg^{2+} ion identification (Scheme 1A). After incubation with Hg^{2+} ions and FO integrated with MB, the T-Hg^{2+}-T complex formed. The signal from MB was monitored to quantify Hg^{2+} ions. As a comparison, the biosensor with linear ss-DNA as an identification unit was also present, shown in Scheme 1B.



Scheme 1 Illustration of the electrochemical Hg^{2+} ion sensor with an oligonucleotide strand for Hg^{2+}-identification and probe introduction. (A) The ts-DNA[8] and (B) the linear ss-DNA containing Hg^{2+}-identification section for the construction of the Hg^{2+} sensors. (C) Modification of functional oligonucleotide by introduction of methylene blue and hybridizing to A_5.

Research Center for Analytical Sciences, College of Chemistry, Nankai University, Tianjin, 300071, P.R. China.
E-mail: xbyin@nankai.edu.cn; Fax: +86-22-23502458
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1cc11628b

Scheme 1C shows the modification to FO strand for sensing Hg^{2+} ions.

Fig. 1 presents the comparison of the differential pulse voltammetry (DPV) signals obtained using either ts-DNA or ss-DNA to introduce the Hg^{2+} identification section. 10 nM Hg^{2+} ions produced the signal of 0.577 μA using linear ss-DNA, but 0.822 μA with ts-DNA. Moreover, 20 nM Hg^{2+} ions produced a signal a little different from that of 10 nM Hg^{2+} with ss-DNA. The high surface density of T_5 may result in the self-assembly of Hg^{2+} and neighboring DNA strands at the electrode surface.[9a] Oligonucleotide length also influences the DNA assembly on the substrate and recognition to its target.[6,10] Short ss-DNA (less than ten bases) formed self-protected interactions and prevented efficient hybridization with a decreased sensitivity,[6,10] and DNA became inactive as they became more densely packed.[6] Therefore, the linear ss-DNA with five bases was ascribed for the low efficiency in terms of the strand length and uncontrollable surface density.

Improved signal intensity was observed with ts-DNA strategy. 0.2 nM Hg^{2+} ions is detectable as shown in Fig. 1Ab. Each thread of the tetrahedron DNA contains 17 base pairs with lengths of about 5.8 nm. Thus, ts-DNA provided the space enough to form the T-Hg^{2+}-T complex. The ds-DNA in ts-DNA did not affect the electron transfer because the double strand was validated to have high conductivity.[9e,11]

An alkanethiol monolayer is often used to block the active site of the gold electrode and keeps the DNA strands in a perpendicular orientation for the development of linear DNA-based biosensors.[4,12] A comparison including or omitting 2-mercaptoethanol (MCE) passivation is shown in Fig. 2. MCE passivation is clearly critical for the sensitivity of linear ss-DNA-based biosensor (Fig. 2B), where a low signal intensity is observed in the absence of the thiol monolayer (7.31 and 4.86 μA for ss-DNA with and without the thiol layer, respectively). However, the ts-DNA mode produced a comparably large amperometric signal with and without the MCE monolayer. This effect was ascribed to the high rigidity of ts-DNA that allowed the Hg^{2+} identification section to stay at the electrode surface with highly ordered perpendicular orientation. The space for one ts-DNA molecule also avoids the interaction between the neighboring Hg^{2+} identification strands. In contrast, linear DNA tends to lie flat on the surface in the absence of MCE treatment,[5] leading to low hybridization efficiency. Moreover, as shown in Fig. 1a, the background signals were 0.378 and 0.493 μA for ts-DNA- and ss-DNA-modes, respectively. The low background was also observed

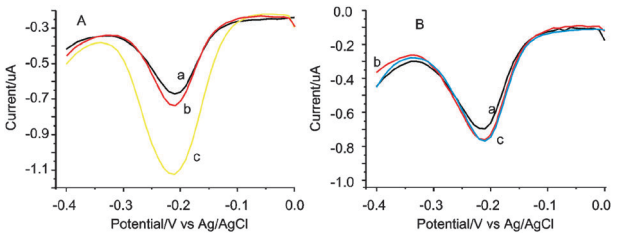


Fig. 1 The comparison of signals with (A) ts-DNA or (B) ss-DNA to introduce the Hg^{2+} ion identification section. The concentration in (A): (a) 0, (b) 0.2, and (c) 10 nM as well as that in (B): (a) 0, (b) 10, and (c) 20 nM.

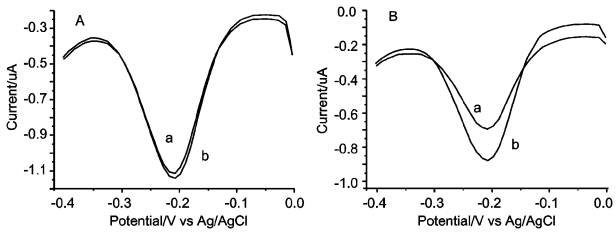


Fig. 2 Comparison of (A) ts-DNA-based sensors for 10 nM Hg^{2+} ions and (B) ss-DNA-based sensors for 100 nM Hg^{2+} ions in the absence (a) and the presence (b) of 2-mercaptoethanol passivation.

even if no MCE passivating layer was used in the case of the ts-DNA mode. Therefore, ts-DNA at the electrode surface acted as an interface for the low background and eliminated the passivation with the alkanethiol monolayer. Although interaction between Hg^{2+} and thiol was applied to regenerate the DNA-based Hg^{2+} sensor,[9b] the use of ts-DNA made the present sensor simple and the newly prepared sensor is as simple as the regeneration of a sensor.

The electrochemical signals were recorded with varying concentrations of Hg^{2+} ions using ts-DNA or ss-DNA to introduce T_5 (Fig. 3). A small response was observed with no Hg^{2+}, perhaps due to non-specific adsorption of the MB. Higher concentrations of Hg^{2+} produced higher signals. The sigmoid response between signal and concentration (Fig. 3B and D) suggested that the binding of Hg^{2+} ion to the electrode surface was a cooperative process.[13] Using linear ss-DNA, a concentration-dependent response was observed from 20 nM to 1 μM with a detection limit of 10 nM. The concentration-dependent response ranged from 100 pM to 20 nM with ts-DNA. The detection limit was 100 pM, which was 100-fold lower than that obtained with the ss-DNA-based sensor. Therefore, the ts-DNA-based sensor demonstrated excellent sensitivity. The sensitivity improvement with ts-DNA may be ascribed to the low background and the improved formation of T-Hg^{2+}-T. Moreover, the present sensor shows a good reproducibility as a precision (RSD, $n = 5$) of 1.7% is obtained as shown in Fig. S5 (ESI†).

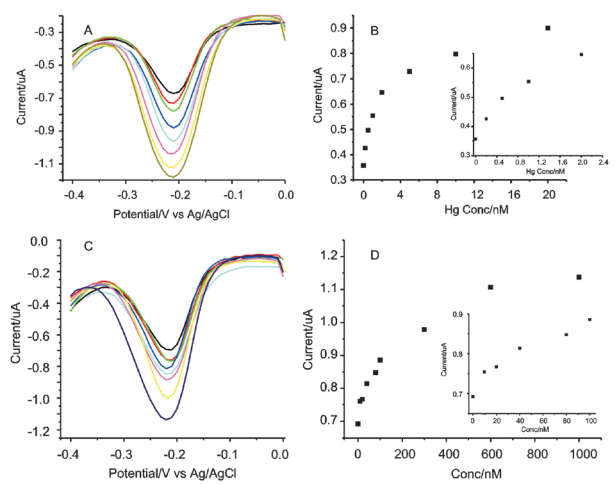


Fig. 3 Sensitivity of the Hg^{2+} biosensors. The ts-DNA (A and B) and ss-DNA (C and D). (A, C) Changes in the electrochemical response with separated determination of each concentration; (B, D) EC profile for varying concentrations of Hg^{2+}.

A comparison of the detection limits of various Hg^{2+} ion sensors based on T-Hg^{2+}-T is listed in Table S1†. The detection limit obtained with ts-DNA was lower than that of most of the previous reports. Although some sensors provided much lower detection limits,[9e] this work applied ts-DNA as a simple protocol for sensitivity improvement. In addition, the integration of MB onto the guanine bases also eliminated the time-consuming covalent derivatization to DNA strands. While it was as simple as the electrochemical impedance spectroscopy method,[14] this sensor did not suffer from an increase in resistance from the negatively charged DNA strand.

The selectivity of the biosensor for Hg^{2+} ions was tested by recording the signal from 10 nM Hg^{2+} ions in the presence and absence of competing metal ions (Fig. S6†). The signals produced by 10 μM of nine metal ions were at least 10-fold less than the signal produced by 10 nM Hg^{2+} ions. Consequently, selectivity was at least 10 000-fold higher for Hg^{2+} ion than the competing metal ions. The signal produced from the mixture of 10 nM Hg^{2+} with 1 μM of each of the competing ions was essentially similar to that from the standard solution because these ions cannot be inserted into the T–T base pair. Because of its high selectivity for Hg^{2+} ions, the biosensor did not need a chelating reagent to eliminate the interferences.[15] The modified electrode was thoroughly cleaned before determination, and therefore, the sensor did not suffer from the environmental interferences encountered during fluorescence sensing of Hg^{2+}.[13,16]

The practicability of the biosensor was validated with the determination of Hg^{2+} ions in natural water samples, including tap water, lake water and river water. The results obtained with the present biosensor were compared with those obtained by cold vapor atomic absorption spectroscopy (CV-AAS). No Hg^{2+} was detected in the tap water, but the concentration of Hg^{2+} in the lake water and river water were 3.03 and 1.83 nM, respectively. The results for three water samples were in good agreement with those obtained by CV-AAS (Table S2†), showing the practicability of the proposed method for real samples. The recoveries ranged from 95.4 to 109% for the sample spiked with Hg^{2+} ions and indicated that no interference was associated with these sample matrices. In comparison with the CV-AAS method, the proposed biosensor requires a low sample volume (10 μL) to determine Hg^{2+} with a simple and compact procedure.

In summary, tetrahedron-structured DNA (ts-DNA)-based platform was used to immobilize nucleic acid probes on gold surfaces for the detection of Hg^{2+} ions in combination with the integration of methylene blue (MB) on guanine bases. Compared with linear single stranded-DNA (ss-DNA) biosensor, the ts-DNA-based electrochemical system provides several unique features. Firstly, ts-DNA can be prepared rapidly and reliably, and assembled readily with ordered orientation onto gold surfaces in a single step. Secondly, passivation using an alkanethiol monolayer is unnecessary. Thirdly, the ts-DNA-based biosensor exhibits an excellent sensitivity and selectivity for detecting Hg^{2+} ion. With the various targets for DNA strand, the merits by use of ts-DNA and the integrated probe molecule could easily be extended to the determination of other analytes.[3]

This work is supported by NSF of China (No. 21075068), the National Basic Research Program of China (973 Program, Nos. 2011CB707703 and 2007CB914100) and the Tianjin Natural Science Foundation (No. 11JCZDJC22200).

Notes and references

1 (*a*) K. Leopold, M. Foulkes and P. Worsfold, *Anal. Chim. Acta*, 2010, **663**, 127–138; (*b*) S.-F. Torabi and Y. Lu, *Faraday Discuss.*, 2011, **149**, 125–135.
2 (*a*) N. Ferrúa, S. Cerutti, J. A. Salonia, R. A. Olsina and L. D. Martinez, *J. Hazard. Mater.*, 2007, **141**, 693–699; (*b*) I. Bontidean, A. Mortari, S. Leth, N. L. Brown, U. Karlson, M. M. Larsen, J. Vangronsveld, P. Corbisier and E. Csöregi, *Environ. Pollut.*, 2004, **131**, 255–262; (*c*) X. P. Zhu and S. D. Alexandratos, *Microchem. J.*, 2007, **86**, 37–41.
3 (*a*) G. H. Clever, C. Kaul and T. Carell, *Angew. Chem., Int. Ed.*, 2007, **46**, 6226–6236; (*b*) I. Willner and M. Zayats, *Angew. Chem., Int. Ed.*, 2007, **46**, 6408–6418.
4 (*a*) D. Li, S. P. Song and C. H. Fan, *Acc. Chem. Res.*, 2010, **43**, 631–641; (*b*) S. Song, L. Wang, J. Li, J. Zhao and C. H. Fan, *TrAC, Trends Anal. Chem.*, 2008, **27**, 108–117; (*c*) J. W. Liu, Z. H. Cao and Y. Lu, *Chem. Rev.*, 2009, **109**, 1948–1998.
5 (*a*) Y. Xiao, T. Uzawa, R. J. White, D. DeMartini and K. W. Plaxco, *Electroanalysis*, 2009, **21**, 1267–1271; (*b*) Y. Xiao, A. A. Lubin, A. J. Heeger and K. W. Plaxco, *Angew. Chem., Int. Ed.*, 2005, **44**, 5456–5459.
6 R. J. White, N. Phares, A. A. Lubin, Y. Xiao and K. W. Plaxco, *Langmuir*, 2008, **24**, 10513–10518.
7 O. M. Sadik, A. O. Aluoch and A. Zhou, *Biosens. Bioelectron.*, 2009, **24**, 2749–2765.
8 H. Pei, N. Lu, Y. L. Wen, S. P. Song, Y. Liu, H. Yan and C. H. Fan, *Adv. Mater.*, 2010, **22**, 4754–4758.
9 (*a*) X. Zhu, L. F. Chen, Z. Y. Lin, B. Qiu and G. N. Chen, *Chem. Commun.*, 2010, **46**, 3149–3151; (*b*) D. H. Han, Y. R. Kim, J. W. Oh, T. H. Kim, R. K. Mahajan, J. S. Kim and H. Kim, *Analyst*, 2009, **134**, 1857–1862; (*c*) Z. Q. Zhu, Y. Y. Su, J. Li, D. Li, J. Zhang, S. P. Song, Y. Zhao, G. X. Li and C. H. Fan, *Anal. Chem.*, 2009, **81**, 7660–7666; (*d*) Y. Xu, L. Yang, X. Y. Ye, P. G. He and Y. Z. Fang, *Electroanalysis*, 2006, **18**, 1449–1456; (*e*) C. X. Tang, Y. Zhao, X. W. He and X. B. Yin, *Chem. Commun.*, 2010, **46**, 9022–9024; (*f*) J. S. Lee, M. S. Han and C. A. Mirkin, *Angew. Chem., Int. Ed.*, 2007, **46**, 4093–4096; (*g*) S. J. Liu, G. H. Nie, J. H. Jiang, G. L. Shen and R. Q. Yu, *Anal. Chem.*, 2009, **81**, 5724–5730; (*h*) X. F. Liu, Y. L. Tang, L. H. Wang, J. Zhang, S. P. Song, C. H. Fan and S. Wang, *Adv. Mater.*, 2007, **19**, 1471–1474.
10 A. Singh, S. Snyder, L. Lee, A. P. R. Johnston, F. Caruso and Y. G. Yingling, *Langmuir*, 2010, **26**, 17339–17347.
11 (*a*) T. L. Pittman and W. J. Miao, *J. Phys. Chem. C*, 2008, **112**, 16999–17004; (*b*) Y. C. Huang, B. Ge, X. D. Sen and H.-Z. Yu, *J. Am. Chem. Soc.*, 2008, **130**, 8023–8029.
12 (*a*) E. Paleček, *Electroanalysis*, 2009, **21**, 239–251; (*b*) A. Sassolas, L. J. Blum and B. D. Leca-Bouvier, *Electroanalysis*, 2009, **21**, 1237–1250.
13 J. W. Liu and Y. Lu, *Angew. Chem., Int. Ed.*, 2007, **46**, 7587–7590.
14 K. J. Odenthal and J. J. Gooding, *Analyst*, 2007, **132**, 603–610.
15 T. Li, S. J. Dong and E. K. Wang, *Anal. Chem.*, 2009, **81**, 2144–2149.
16 (*a*) A. Ono and H. Togashi, *Angew. Chem., Int. Ed.*, 2004, **43**, 4300–4302; (*b*) C. K. Chiang, C. C. Huang, C. W. Liu and H. T. Chang, *Anal. Chem.*, 2008, **80**, 3716–3721; (*c*) S. Yoon, E. W. Miller, Q. W. He, P. H. Do and C. J. Chang, *Angew. Chem., Int. Ed.*, 2007, **46**, 6658–6661; (*d*) Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami and A. Ono, *J. Am. Chem. Soc.*, 2006, **128**, 2172–2173; (*e*) D. Li, A. Wieckowska and I. Willner, *Angew. Chem., Int. Ed.*, 2008, **47**, 3927–3931; (*f*) R. Freeman, T. Finder and I. Willner, *Angew. Chem., Int. Ed.*, 2009, **48**, 7818–7821.