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# Functional nucleic acid-based electrochemiluminescent biosensor for interaction study and detection of $Ag^+$ ions and cysteine†

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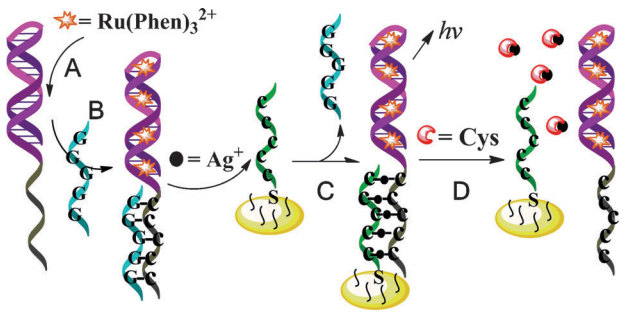
**An electrochemiluminescent biosensor was designed for the detection of $Ag^+$ ions and cysteine as well as their interaction study. To this end, a functional nucleic acid was designed for target recognition and probe intercalation.**

Nucleic acids have long been considered as a kind of molecule with genetic information. However, the functionality of nucleic acids has attracted extensive attention for sensing purposes.[1] DNAzymes, as specific DNA molecules, possess catalytic activities toward specific substrates.[2] Aptamers are antibody-like nucleic acids for molecular recognition from organic small molecules, proteins, cells, to bio-tissues.[3] Although the negatively charged DNA can bind positively charged metal ions, some metal ions selectively bind to some bases to form stable metal-mediated DNA duplexes.[4] For example, $Ag^+$ is capable of selectively coordinating cytosine (C) bases to form stable C–$Ag^+$–C complexes,[5] and $Hg^{2+}$ interacts specifically with thymine–thymine (T–T) mismatches.[6]

Some studies[6a,7] have indicated that single stranded- and double stranded-DNA (ssDNA and dsDNA) can integrate some small molecules through electrostatic interactions or intercalation into the grooves of dsDNA. $Ru(phen)_3^{2+}$ (phen = 1,10-phenanthroline) is used to develop electrochemiluminescent (ECL) biosensors through its intercalation into the grooves of dsDNA.[6a,7a–c,f] While the chemical labeling of the DNA strand is not needed with the intercalation strategy, a high efficiency of probe introduction is achieved because more than one signal molecule can be tagged onto a single DNA strand.[6a,7a–c,f]

$Ag^+$ ions are often detectable in aquatic ecosystems because of their release from the electrical and imaging industries and pharmacy.[8] Their high toxicity has a significant impact on the aquatic organisms.[8] Cysteine (Cys) is an important species with vast scale distribution and plays an important role in biological systems as a bio-catalytic centre, in post-translational modification, and metal binding for detoxification.[9] The study of the interaction between metal ions and amino acids is also interesting for understanding the possible detoxification mechanism of metal ions.[9] Although previous works[5] have made great contributions in the sensing of Cys and $Ag^+$, it is still a challenge to improve the detection sensitivity with new methodology. Electrochemical and related methods are of particular interest due to their simple setup, low cost and rapid detection, but only a few assays have been developed for $Ag^+$ ions and Cys with functional nucleic acids.[5h] ECL, especially ruthenium complex-based, has been validated with merits, including simple setup, high sensitivity and selectivity, and broad linear range.[10] These characteristics make ECL of great promise for probing various species, but to our knowledge no ECL method has been found for the detection of Cys and $Ag^+$ ions with a functional nucleic acid as the platform.

In this work, an ECL sensing platform was developed for the detection of $Ag^+$ ions and Cys as well as their interaction study. To this end, a functional oligonucleotide (FO) was designed with an intermolecular duplex for intercalation of $Ru(phen)_3^{2+}$, and a multi-cytosine section for $Ag^+$ ion recognition. The incubation of FO and $Ru(phen)_3^{2+}$ achieved the intercalation of $Ru(phen)_3^{2+}$ into the intermolecular duplex (Scheme 1A). To avoid the self-assembly between cytosine-rich sections in different FOs and $Ag^+$ ions, a G–C duplex was preformed with the $Ag^+$ ion recognition section




**Scheme 1** Schematic illustration of the functional oligonucleotide (FO)-based biosensor for $Ag^+$ ions and cysteine (Cys). (A, B) Intercalation of $Ru(phen)_3^{2+}$ into the duplex of FO and formation of G–C duplex with an $Ag^+$ ion recognition section; (C) formation of C–$Ag^+$–C complex *via* the incubation of $Ag^+$ ions and FO at a $C_5$-modified electrode for sensing $Ag^+$ ions; (D) the dissociation of the C–$Ag^+$–C complex with Cys, and the decreased ECL emission was used to quantify Cys.

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and a $G_5$ oligonucleotide (Scheme 1B). After a thiolated $C_5$ oligonucleotide was immobilized onto the electrode surface, a C–$Ag^+$–C complex formed between FO and the immobilized $C_5$ sequence in the presence of $Ag^+$ ions (Scheme 1C). An ECL emission was observed and was employed to monitor $Ag^+$ ion concentration when a potential was applied with tripropylamine (TPA) as co-reactant. The strong interaction between Cys and $Ag^+$ ions can dissociate the C–$Ag^+$–C complex. Then, the FO was released from the electrode surface as shown in Scheme 1D. Correspondingly, the decreased emission was used to quantify the content of Cys. Electrochemical and fluorescent investigations into the present biosensor are illustrated in Fig. S1–S3.†

Fig. 1 shows that the ECL emission was recorded with different $Ag^+$ concentrations. Almost zero signal was observed when no $Ag^+$ ions were present and the signal increased as the $Ag^+$ concentration increased. The results also suggested the formation of C–$Ag^+$–C complex and the intercalation of the $Ru(phen)_3^{2+}$ probe into the duplex of FO. The concentration-dependent responses existed over the range of 10 pM–100 nM. The sigmoid shape (Fig. 1B) illustrated that the introduction of $Ag^+$ ions was a cooperative process similar to that in $Hg^{2+}$ ion biosensors.[6a,g] The detection limit was 5 pM (S/N = 3), which was lower than that obtained by fluorescent and colorimetric methods (Table S1†) because of the "turn-on" mode and the high sensitivity of ECL. The selectivity and reproducibility for $Ag^+$ is illustrated as shown in Fig. S4 and S5A.†

The detoxification of metal ions in bio-tissue is often achieved through the formation of metal–sulfur complexes.[11] As reported previously,[5] Cys, as an amino acid containing sulfur, can bind to $Ag^+$ strongly for the dissociation of the C–$Ag^+$–C complex. Thus, the $Ag^+$ detection technique can be modified for sensing Cys. As a "turn-off" biosensor, the higher the basic signal, the broader the responding ranges for Cys. Thus, 100 nM $Ag^+$ ions, as the highest concentration (Fig. 1), were selected for the detection of Cys with the maximum signal window and broad responding range.

To study the feasibility of the biosensor for Cys, 10 μL of 100 nM $Ag^+$ ions and 5 μL of FO integrated $Ru(phen)_3^{2+}$ probe were first incubated on a $C_5$-modified electrode. Then, various concentrations of Cys were dropped onto the above electrode surface and the ECL emission was measured. As shown in Fig. 2, the ECL emission decreased as the Cys concentration increased, and emission became almost zero when the Cys concentration was higher than 50 nM, indicating that most of the $Ag^+$ ions were released and therefore no $Ru(phen)_3^{2+}$ probe existed at the electrode surface. A concentration-dependent response was seen with the Cys concentration ranging from 100 pM to 50 nM, indicating that the sensing system can be used to quantify Cys. The detection limit was 50 pM for Cys, which was much lower than the previous sensors based on the $Ag^+$–cytosine interaction (Table S1†). As shown in Fig. S5B,† the biosensor has a reproducibility with a RSD of 5.0% ($n$ = 6) for Cys.

Because Cys contains a carboxylic acid group, an α-amine group, and a side-chain thiol group, to test the interaction site of Cys and $Ag^+$ in the C–$Ag^+$–C complex, the response from various amino acids at the present biosensor was monitored. As shown in Fig. 3A, phenylalanine (Phe), proline (Pro), glutamic acid (Glu), arginine (Arg), tryptophan (Trp), asparagine (Asn), histidine (His), and glycine (Gly) have almost negligible ECL changes at their 10 nM level. All of those amino acids have a carboxylic acid group and an α-amine group, similar to those in Cys molecules. Thus, we can conclude that the ECL decrease and the interaction between Cys and C–$Ag^+$–C are not from the carboxylic acid and amine groups in Cys.

Glutathione (GSH) has a similar ECL response to Cys at the present biosensor, as shown in Fig. 3B. GSH is a tripeptide containing Glu, Cys and Gly and the thiol group in GSH is free.[11] So, we consider that the thiol in Cys plays an important role in the response of the biosensor to Cys. To validate this conclusion, the response of glutathione disulfide (GSSG) was tested and a negligible ECL response was observed at its 10 nM level (Fig. 3A). GSSG is a disulfide formed from two GSH molecules with a disulfide bond.[12] Thus, the side-chain thiol group in Cys corresponded to the dissociation of C–$Ag^+$–C and was the active site for detoxification of metal ions. Using the ECL response of different species (Fig. 3), the stability order of different $Ag^+$ complexes was obtained as followed: $Ag^+$–Cys > C–$Ag^+$–C > $Ag^+$–amino acids without thiol. Because only the amino acids containing thiol groups can dissociate the $Ag^+$–DNA complex, the results validate the importance of the thiol group in detoxicity of metal ions.

The determination of Cys in serum samples and the resulting comparison between the present biosensor and electrospray ionization-mass spectrometry (ESI-MS) were used to validate the practicability of the biosensor for real samples. Mass flow charts from standard and spiked samples are shown in Fig. S6.† As shown in Table 1, the recovery ranged from 107 to 110%. The sample spiked with 2.15 μM Cys was

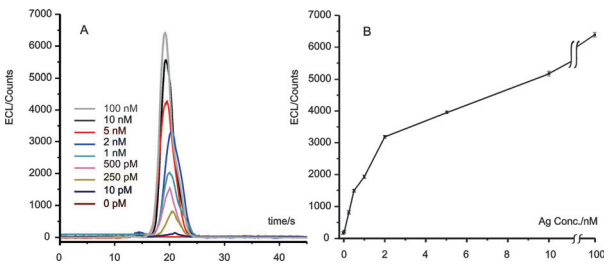


**Fig. 1** Sensitivity of the biosensor for $Ag^+$ ions. (A) ECL emission in the presence of $Ag^+$ ions with varying concentrations. (B) ECL profile for $Ag^+$ ions with varying concentrations.

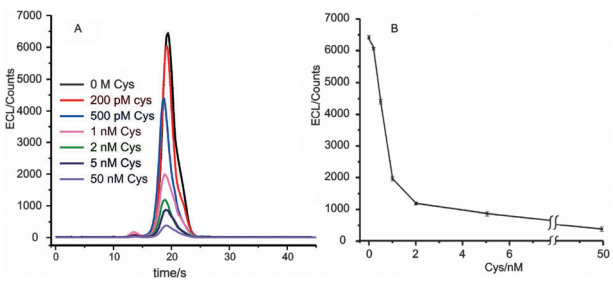


**Fig. 2** Sensitivity of the biosensor for Cys. (A) ECL profiles in the presence of varying concentrations of Cys. (B) ECL signals for varying concentrations of Cys. 10 μL of 100 nM $Ag^+$ ions and 5 μL of FO integrated $Ru(phen)_3^{2+}$ probe for Cys determination.

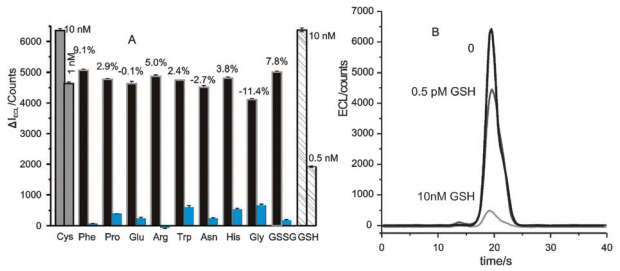


**Fig. 3** The ECL response of Cys, some amino acids, glutathione (GSH), and glutathione disulfide (GSSG). (A) The ECL change with 1.0 and 10 nM Cys and 0.5 and 10 nM GSH. The ECL change from amino acids and GSSG with and without 1.0 nM Cys. (B) ECL profile from 0, 0.5 and 10 nM GSH. The percentage number in (A) shows the relative error for determination of 1.0 nM Cys containing 10 nM interfering amino acids.

**Table 1** Analytical results of Cys in serum obtained using the present electrochemiluminescent biosensor and by electrospray ionization-tandem mass spectrometry (ESI-MS)

| | | By present ECL biosensor[a] | | By ESI-MS[b] | |
|---|---|---|---|---|---|
| | Added conc./μM | Conc. found/μM | Recovery (%) | Conc. found/μM | Recovery (%) |
| Serum | 0 | 0 | — | 0 | — |
| | 2.15 | 2.34 | 109 | 2.42 | 112 |
| | 2.00 | 2.14 | 107 | — | — |
| | 0.500 | 0.551 | 110 | — | — |

[a] 1000 times dilution to serum. [b] 100 times dilution to serum.

analyzed by the present biosensor and ESI-MS. The values obtained by the two methods were consistent with each other. This implied that no significant interference was encountered and that the present biosensor has promising features for the analysis of biological samples. The other merit of this biosensor is the small sample consumption (only 10 μL), making the present method of diagnostic potential with scarce samples.

In conclusion, a simple, sensitive and selective ECL assay for $Ag^+$ ions and cysteine was reported with a functional oligonucleotide designed for $Ru(phen)_3^{2+}$ intercalation and $Ag^+$ ion recognition. Its performance was validated by the analysis of serum samples through the comparison with ESI-MS and recovery experiments. Using the different ECL responses from various species (Cys, amino acids, GSH, and GSSG), the biosensor was also used to study the interaction between $Ag^+$ ions and different ligands. A comparison with other detection strategies (Table S1†) validated the merits of the electrochemical and related methods, although few works were found for the development of an ECL biosensor for $Ag^+$ ions and Cys. Artificial design of a functional oligonucleotide will open a new field for metal detection and the study of metal ion–biospecies interaction through electrochemical and related methods.[4a]

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