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Highly selective and sensitive method for cysteine detection based on fluorescence resonance energy transfer between FAM-tagged ssDNA and graphene oxide

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ABSTRACT

In this work, a new platform for effective sensing cysteine (Cys) was developed based on fluorescence resonance energy transfer (FRET) between FAM-tagged single-stranded DNA (FAM-ssDNA) and graphene oxide (GO). Due to the noncovalent assembly between FAM-ssDNA and GO, fluorescence quenching of the FAM took place because of FRET. This method relied on the competitive ligation of Ag^+ by Cys and "cytosine–cytosine" (C–C) mismatches in a FAM-labeled DNA strand of the self-hybridizing strand. At first, enough amount of Ag^+ was introduced to bind "C–C" mismatches and form double-stranded DNA (dsDNA), which had weak affinity to GO and kept FAM away from GO surface. However, the presence of Cys removed Ag^+ away from "cytosine–Ag^+–cytosine" (C–Ag^+–C) base pairs, leading to the formation of ssDNA again and FRET, and then fluorescence of the FAM-ssDNA was efficiently quenched. The fluorescence intensity decrease was found to be proportional to the increase of concentration of Cys in both aqueous buffer (2–200 nM) and human serum (5–200 nM), and the sensitivity of the proposed method towards Cys was much higher than that of other reported assays for Cys.

1. Introduction

Graphene has attracted ever-increasing attention because of its special physical and chemical properties as a novel two-dimensional nanomaterial [1–4]. This single-layer carbon nanomaterial is as thick as only one carbon atom, but possesses a large two-dimension plane, where almost all of the carbon atoms are in sp^2 hybridization and consequently this structure makes it rich in π electrons [5]. Due to this distinct feature of graphene, dye-labeled single-stranded oligonucleotides could be easily adsorbed onto it by π–π stacking interactions between nucleotide bases and graphene, leading to FRET caused by the shortened distance between dye and graphene and the immediate quenching of fluorescence of dye-tagged ssDNA [6–15]. Therefore, quantitative fluorescent detection of DNA has been realized relying on this phenomenon and single base mismatch was distinguished successfully in this way [6]. The unique structure of graphene has made it more efficient when applied in sensing multiple molecules, for example, simultaneous quenching of multiple DNA probes labeled with different fluorophores could be achieved to sense multiple DNA targets [7]. In order to improve the water-solubility, graphene is usually oxidized before using in fluorescence analysis [6–15]. Oxidized graphene, with many oxidation functional groups, including carboxyl group, hydroxyl group and so on, has exhibited its superiority in many analytical methods [8,10]. Up to now, GO has showed excellence as efficient quenching agent in fluorescence-based methods of quantitative detection of biological molecules and metal ions [8–15], ranging from thrombin, ATP, to heavy metal ions such as Hg^{2+}, Ag^+ and so forth. These previously reported works showed that GO had its incomparable superiorities in low cost, short assay time, low background and high sensitivity.

Some metal ions are able to selectively react with some mismatched base pairs [16–21]. In this way, metal ions and mismatches can form some kinds of special base pairs. For example, a strong interaction exists between Hg^{2+} and thymine bases, and then Hg^{2+} offsets the thymine–thymine mismatch to form "thymine–Hg^{2+}–thymine" (T–Hg^{2+}–T) base pairs [16]. Similarly, Ag^+ has been found to selectively bind cytosine and form "cytosine–Ag^+–cytosine" (C–Ag^+–C) base pairs [17]. Even though many metal ion-based sensors have been established, current research interests and many works are still focused on these kinds of special bases, especially in the field of developing new sensors and methods [18–21].

Cys, a member of the commonly-seen amino acids in body, synthesized from methionine and serine in blood, plays a vital role in nutrition in human body. However, it is unstable and can be easily

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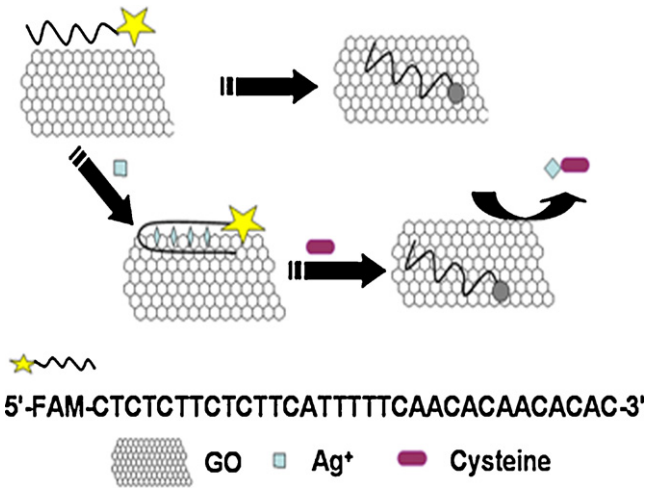

Scheme 1. Schematic representation of fluorescent detection of Cys on GO platform by using Ag^+ as the mediator.

changed into cystine. Cys has important physiological functions, such as anti-oxidation, anti-aging and detoxification [22,23]. Cys deficiency could be involved in many syndromes, such as edema, lethargy, liver damage, muscle and fat loss, slowed growth, and skin lesion [24]. Therefore, it is meaningful to develop new methods to detect Cys due to its important role in human body.

Numerous methods have been developed to detect Cys, including electrochemical analysis [24–26], chromatography [27] and colorimetric assay [22,28–30]. As a highly sensitive, rapid and non-destructive technique, fluorescence analysis has also been extensively utilized for Cys determination [31,32]. Herein, we report a novel FRET-based method for Cys built on GO platform. Scheme 1 shows the illustration of fabrication of the assay for Cys on GO. A FAM-labeled Ag^+ specific oligonucleotide, rich in cytosine, was employed as the fluorescent probe in sensing targets. The conformation of the probe changed from random coil ssDNA to straight stiff dsDNA with "C–Ag^+–C" base pairs followed by the addition of Ag^+ [33]. However, the state of DNA changed again when Cys was subjected to the sensing system, because Cys could move Ag^+ away from the "C–C" mismatches with its active sulfhydryl group [34]. In this work, the excellent quenching performance of GO was combined with Ag^+-mediated base pairs "C–Ag^+–C" to sensitively and selectively detect Cys. When FAM-labeled DNA probe was in the state of random coil ssDNA, FRET from FAM-ssDNA to GO occurred, because the distance from the energy donor FAM to the energy acceptor GO was shortened by π–π stacking interactions between nucleotide bases and GO, but when the DNA probe was in formation of dsDNA, the energy donor FAM was far away from the surface of GO and FRET disappeared. So at first, the introduction of enough amount of Ag^+ led to a large degree of fluorescence recovery, then Cys was applied to move Ag^+ away to make the fluorescence of the probe quenched, and the degree of fluorescence quenching was closely related to the amount of added Cys. Compared with other reported methods, the proposed method has some advantages of simple instrument, low background and high sensitivity both in aqueous buffer and serum.

2. Materials and methods

2.1. Materials

(5′6-FAM-CTCTCTTCTCTTCATTTTTCAACACAACACAC-3′), FAM-labeled Ag^+-specific oligonucleotides, were purchased from Sangon Biotech (Shanghai, China) Co. Ltd. and purified by HPLC. $AgNO_3$, Cys and other 21 inspected species (including the other 19 commonly used amino acids, cystine and glutathione) were of analytical grade and prepared with ultrapure water from a Millipore system (18.2 MΩ resistivity). Tris-(2-carboxyethyl) phosphine (TCEP) was added in the Cys stock solution to activate the sulfhydryl group before the experiments [35]. Human serum was obtained from Zhongnan Hospital in Wuhan (Hubei, China). All the experiments were carried out in HEPES buffer (10 mM HEPES, 50 mM $NaNO_3$, pH 7.4).

2.2. Instrumentation

The morphology of GO was characterized by a JEM-2100 transmission electron microscope (TEM) operated at 200 kV. A confocal laser micro-Raman spectrometer (HR800UV, JOBIN YVON) was used to obtain the Raman spectra of GO with excited line being set at 633 nm. Subsequently, FT-IR spectrum of GO was collected with a Nicolet 5700 Fourier transform infrared spectrometer. Finally, fluorescence spectrometer (F-4600, Hitachi Co. Ltd., Japan) with a Xenon lamp excitation source was employed to record fluorescence spectra. The excitation was set at 493 nm and the emission was monitored at 520 nm.

2.3. Preparation of GO

GO was prepared from graphene powder based on Hummer's method with some modifications [8,36,37]. Generally, 3 g graphene powder was mixed into a hot solution consisting of concentrated 12 mL H_2SO_4, 2.5 g $K_2S_2O_8$, and 2.5 g P_2O_5. This mixture was allowed to react for 5 h, then diluted gradually with 0.5 L deionized water and kept at 80 °C for 12 h. After the mixture was filtrated, washed with water and dried overnight at 60 °C, the powder obtained was added into 120 mL concentrated H_2SO_4, followed by the gradual addition of 15 g $KMnO_4$ under continuous stirring in ice bath. The mixture was stirred at 40 °C for 0.5 h and 90 °C for 1.5 h, respectively. Subsequently, the mixture was diluted with 250 mL water and reacted for 0.5 h at 105 °C, followed by the addition of 0.7 L water and 20 mL of 30% H_2O_2 after stirring for 2 h. Afterwards, the above mixture was filtrated and washed with diluted HCl (V_{HCl}/V_{water} = 1:10) and water several times, respectively. For further purification, the obtained GO powder was dialyzed in GO dispersion to remove the residue, and then exfoliated under sonication for over 4 h (120 W). Finally, the obtained solution was centrifuged to remove the unexfoliated graphite oxide and get the homogeneous GO suspension (0.5 mg/mL).

2.4. Fluorescence spectra measurements

2.4.1. Selection of optimal Ag^+ concentration

FAM-ssDNA solution (500 nM) was incubated for 5 min at 90 °C and then cooled down slowly to the room temperature. Different volumes (0–150 μL) of Ag^+ solution (1 μM) were mixed with 20 μL 500 nM of FAM-ssDNA in 1.5 mL test tube respectively, and different volumes of HEPES buffer were added to make each solution 985 μL, then these mixtures were allowed to react for 0.5 h at 30 °C. After that, 15 μL GO aqueous solution (0.5 mg/mL) was added into each mixture to make the final volume 1 mL and kept at 30 °C for 10 min. Finally the fluorescence spectra were measured at room temperature.

2.4.2. Assay for Cys in aqueous buffer

FAM-ssDNA solution (500 nM) was incubated for 5 min at 90 °C and then cooled down slowly to the room temperature. 150 μL Ag^+ solution (1 μM) was mixed with 20 μL 500 nM of FAM-ssDNA in 1.5 mL test tube and incubated for 0.5 h at 30 °C, followed by addition of 15 μL GO solution (0.5 mg/mL) and reaction for 10 min,

then different volumes (0–200 μL) of Cys (1 μM) were transferred into the above system and reacted for over 2 h, finally appropriate aliquot of HEPES buffer was introduced to make each reaction solution 1 mL. After that, the fluorescence measurements were done at room temperature. To confirm the selectivity of the method towards Cys, the other 21 inspected species at the concentration of 1 μM instead of 150 nM Cys were used to react with above system and performed in the same way. All the above experiments were conducted in HEPES buffer (10 mM, 50 mM $NaNO_3$, pH 7.4).

2.4.3. Detection of Cys in serum

20 μL FAM-ssDNA (500 nM) was incubated with 150 μL Ag^+ solution (1 μM) for 30 min at 30 °C, and reacted for 10 min after the addition of 15 μL GO solution (0.5 mg/mL). Later, 10 μL of human serum was mixed in the above solution. Subsequently, different volumes (0–200 μL) of 1 μM of Cys were transferred into the above system and reacted for over 2 h, finally appropriate aliquot of HEPES buffer was introduced to make each reaction solution 1 mL, and then fluorescence spectra were monitored at room temperature.

3. Results and discussion

3.1. Characterization of single-layer GO

GO was synthesized according to the Hummer's method with some modifications. To confirm the successful formation of GO sheet, the as-prepared GO was characterized by TEM. As shown in Fig. 1A, the sheet width of GO was approximately 0.3–0.5 μm exhibiting relatively narrow size distribution. The chemical structure of GO was determined by both FT-IR and Raman spectroscopy. The Raman spectrum measured with a confocal laser micro-Raman spectrometer was demonstrated in Fig. 1B, two main Raman peaks reflected the characteristics of GO. The strong Raman peak at 1591 cm^{-1}, the G band, indicated the vibration of carbon atoms in the form of sp^2 hybridization and mainly caused by the unoxidized carbon atoms in the GO plane. The other strong peak at 1334 cm^{-1}, the D band, was considered as the vibration of carbon atoms in the form of sp^3 hybridization, which could be attributed to the oxidized carbon atoms in GO structure. As the Raman spectra were consistent with what was discussed in a previously reported literature [8], it was possible that we had successfully prepared GO in this work. The existence of sp^2 hybridized carbon atoms provided an opportunity for the adsorption of dye-labeled ssDNA onto the surface of GO by the π–π stacking interactions and FRET between dye and GO. In addition, the information of successful synthesis of GO was further provided by a Nicolet 5700 FT-IR spectrometer. The FT-IR spectra was shown favorably in Fig. 1C: the broad and intense peak at 3400 cm^{-1} represented the O—H stretching vibration, while the peak at 1732 cm^{-1} attributed to the stretching vibration of C=O, the peak at 1624 cm^{-1} indicated the vibration of the unoxidized graphene skeleton C=C and the absorbed H_2O, the peaks at 1379 cm^{-1} and 1228 cm^{-1} were caused by C—OH, the coupling vibrations of O—H in-plane bending and C—O stretching, and the peak at 1061 cm^{-1} indicated the C—O stretching vibration. This FT-IR result was also well in agreement with a literature that reported recently [36]. It could be concluded from the FT-IR spectrum that some hydrophilic groups such as —OH, —COOH were acquired, which made GO water-soluble and dispersed well in water.

3.2. FRET between FAM-tagged ssDNA and GO

GO is a kind of nanomaterial with wide two-dimensional surface and favorable water solubility. Owing to the π–π stacking interactions between nucleotide bases and GO, FAM-labeled ssDNA

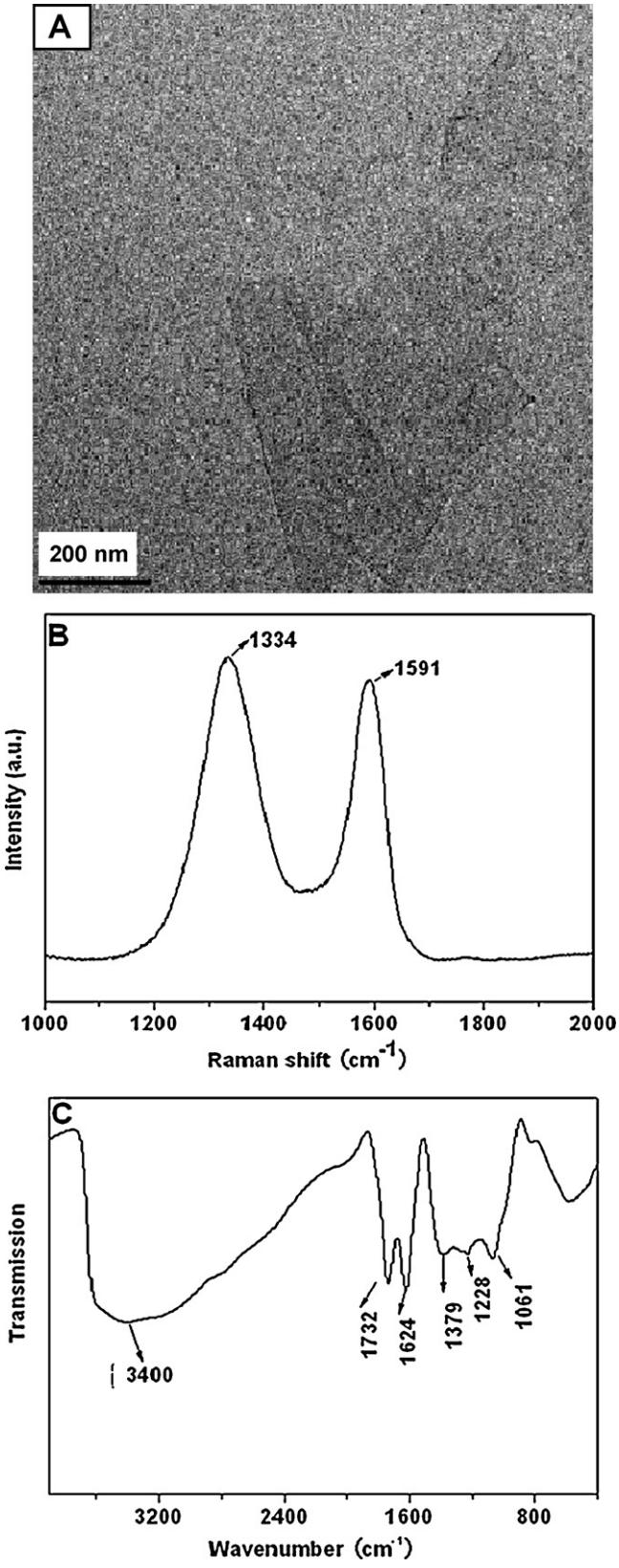

Fig. 1. High resolution transmission emission microscope (HRTEM) image of as-prepared GO (A), Raman spectra of GO (B), and FT-IR spectrum of GO (C).

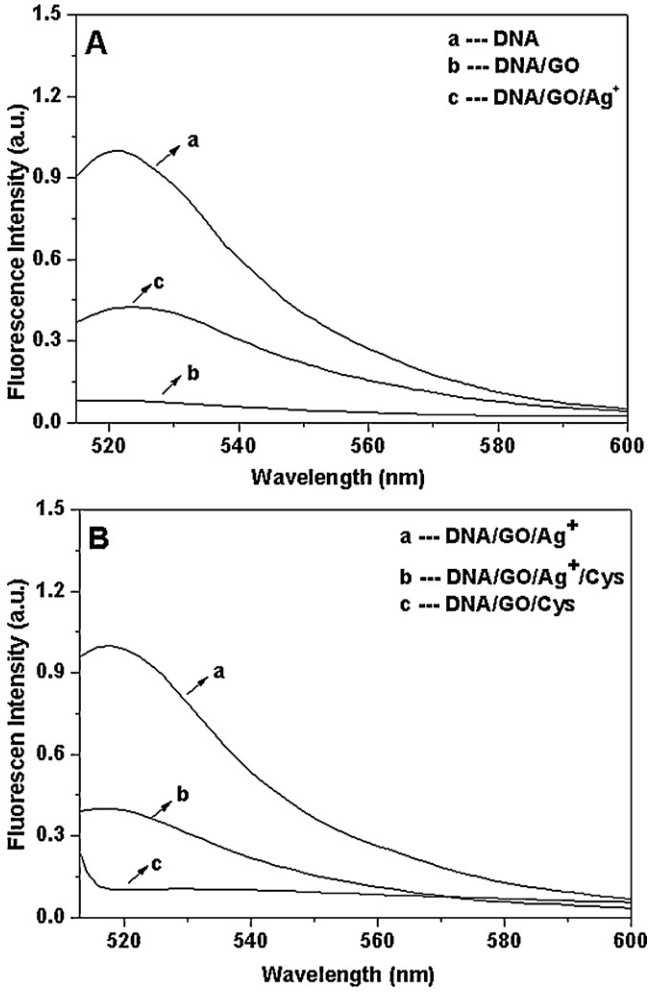

Fig. 2. (A) Fluorescence spectra of before (curve a) and after (curve b) GO (7.5 μg/mL) quenched 10 nM FAM-labeled ssDNA, and the change of fluorescence intensity after addition of Ag^+ (100 nM) (curve c). (B) Fluorescence spectra of FAM-ssDNA/GO (10 nM/7.5 μg/mL) in the absence (curve a) and presence (curve b) of Cys (150 nM) after reacted with Ag^+ (150 nM), and the fluorescence change of FAM-ssDNA/GO (10 nM/7.5 μg/mL) with Cys (150 nM) addition (curve c). All these fluorescence spectra were carried out in HEPES buffer (10 mM, 50 mM $NaNO_3$, pH 7.4) at 25 °C. The fluorescence was excited at 493 nm, and given in normalized form.

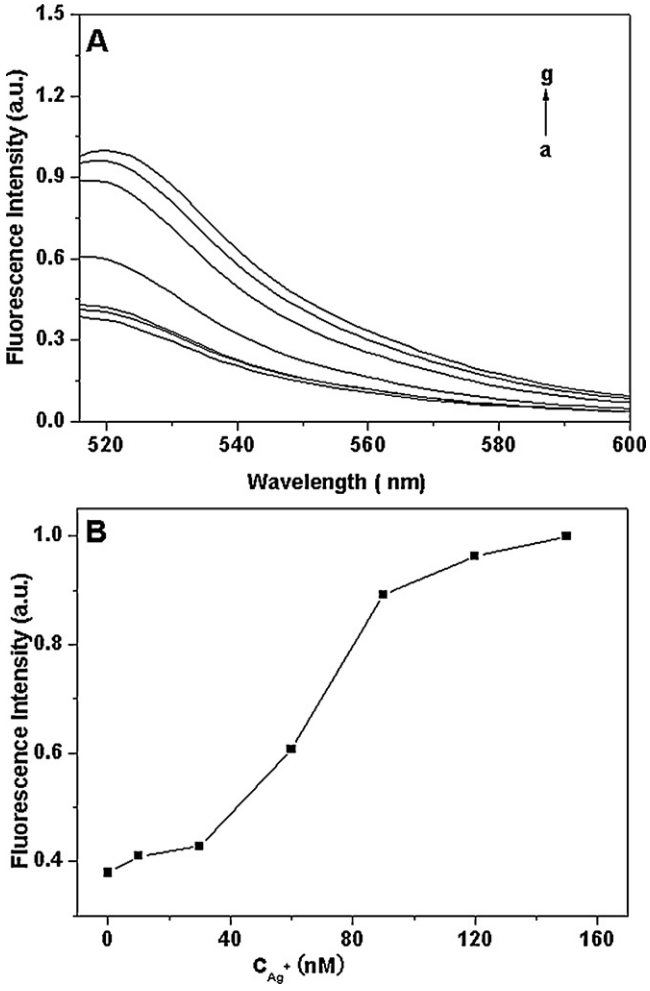

Fig. 3. (A) Fluorescence emission spectra of 10 nM FAM-ssDNA after incubation with different concentrations of Ag^+ (a–g: 0 nM, 10 nM, 30 nM, 60 nM, 90 nM, 120 nM, 150 nM) and then mixed with GO (7.5 μg/mL). (B) The fluorescence intensity at 520 nm changed against Ag^+ concentration. The fluorescence spectra was excited at 493 nm, and given in normalized form. All were in HEPES buffer (10 mM, 50 mM $NaNO_3$, pH 7.4) at 25 °C.

was absorbed rapidly when approached the surface of GO. Therefore, the fluorescence of FAM was efficiently quenched due to FRET between FAM and GO. The fluorescence of 10 nM FAM-ssDNA was quenched at least 90% by 7.5 μg/mL GO (Fig. 2A, curve b), compared with the original intensity (Fig. 2A, curve a), indicating the strong quenching effect of GO on the fluorescence of dye. The FAM-ssDNA we used was rich in cytosine, which could capture Ag^+ by forming "C–Ag^+–C" base pairs. In the presence of 100 nM Ag^+, the random coil ssDNA changed its conformation to rigid dsDNA, thus weakening the π–π stacking interactions between DNA nucleotide bases and GO due to the hydrophilic force of the phosphoric skeleton in dsDNA, finally leading to the recovery of fluorescence (Fig. 2A, curve c). In this way, 150 nM Ag^+ was used as the mediator to obtain a strong fluorescence at first (Fig. 2B, curve a). However, the binding affinity of Ag^+ for Cys was much stronger than that for "C–C" mismatches [29,30], and therefore Cys would remove Ag^+ from the "C–Ag^+–C" complex by working with its active sulfhydryl group and thus dsDNA switched to ssDNA again. Hence, the addition of 150 nM Cys led to the occurrence of FRET and the fluorescence quenching of FAM-ssDNA (Fig. 2B, curve b). In order to prove that the presence of Cys would not directly affect the fluorescence of FAM-ssDNA, a control experiment was given that only 150 nM Cys was introduced after the addition of GO, and the result indicated that Cys made no contribution to the system when Ag^+ was absent (Fig. 2B, curve c), which implied that Ag^+ was essential as the mediator.

3.3. Optimization of concentration of Ag^+

It's important to carefully optimize the concentration of Ag^+ as this is crucial in the detection of cysteine. Too low concentration of Ag^+ could lead to narrower detection range of cysteine, while too high of it, having excess free Ag^+ to bind to cysteine precedingly, would result in a lack of sensitivity in Cys determination. In this regard, the optimization of Ag^+ concentration was performed (Fig. 3). As could be seen in Fig. 3A, the fluorescence intensity increased gradually with the increasing concentration of Ag^+, and a platform of fluorescence intensity was observed when the concentration of Ag^+ reached 150 nM (Fig. 3B). Therefore, an optimized concentration of 150 nM Ag^+ was chosen in the experiments.

3.4. Quantitative analysis of Cys in aqueous buffer

As shown in Fig. 4, a new sensitive assay for Cys was successfully designed. The fluorescence intensity was dependent on the concentration of Cys over a range of 2–200 nM when the

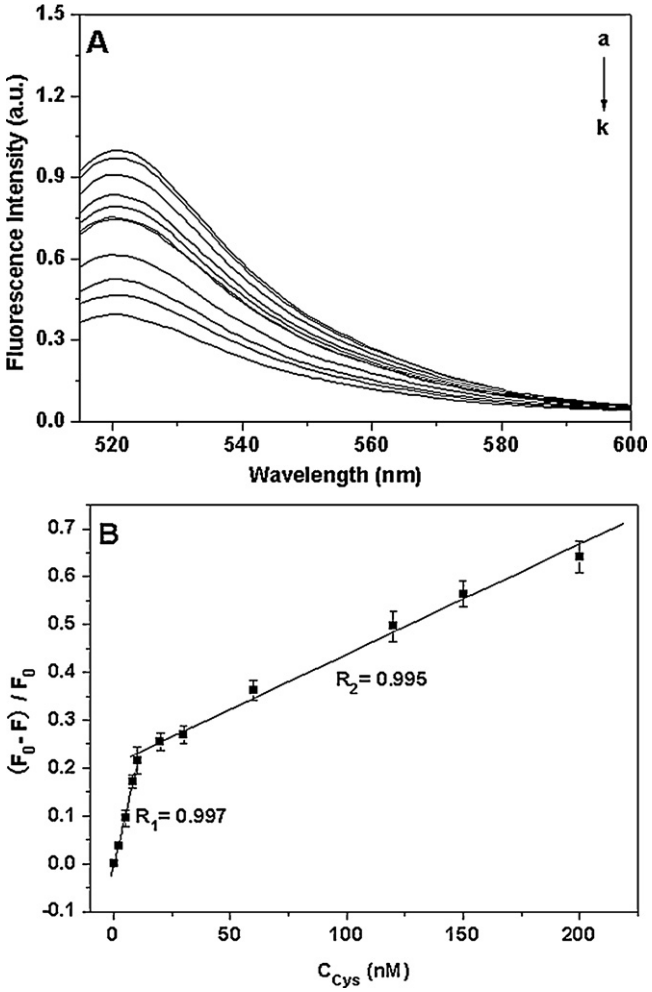

Fig. 4. (A) The normalized fluorescence spectra of 10 nM FAM-ssDNA upon incubation with 150 nM Ag^+ and mixed with GO (7.5 μg/mL), then reacted with a series of concentrations of Cys (a–k: 0 nM, 2 nM, 5 nM, 8 nM, 10 nM, 20 nM, 30 nM, 60 nM, 120 nM, 150 nM, 200 nM). (B) Calibration curve for Cys detection in buffer, data were presented as average ± sd from three independent measurements. All these experiments were operated at 25 °C and the fluorescence excitation and emission wavelengths were 493 nm and 520 nm, respectively.

concentration of Ag^+ was set at 150 nM. Fig. 4B showed that the fluorescence intensity deceased rapidly as the concentration of Cys increased from 2 nM to 10 nM (R_1 = 0.997). However, it existed another linear relationship (R_2 = 0.995) when the concentration of Cys changed from 10 nM to 200 nM, where the fluorescence intensity decreased more slowly as the Cys concentration increased. The reason for this phenomenon was probably that FAM-labeled DNA in the state of random coil single strand was corresponding rare when Cys was less than 10 nM in the system, and the vast plane structure of GO could strongly adsorbed these random coil single-strand FAM-DNA onto its surface as soon as possible, which attributed to the weak adsorption competition among these free FAM-ssDNA; When Cys exceeded 10 nM in the system, free FAM-DNA in the state of random coil single strand became much more, adsorption competition among them became more intense, so the fluorescence changed more slowly as the Cys concentration increased. This method could be applied to detect as low as 2 nM Cys in aqueous buffer, and the linear detection range was wide, ranging from 2 nM to 200 nM. From this perspective, the proposed method towards Cys has its own uniqueness compared with some recently reported ones [24–26,29–31,38–40], that is, the present method is more effective to detect Cys down to trace levels.

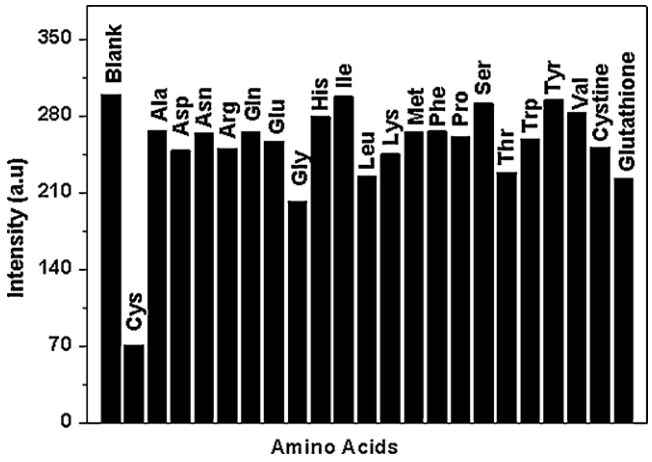

Fig. 5. Selectivity of GO-based method for Cys. A Cys concentration of 150 nM was tested, while the concentration of other 21 inspected species was 1 μM.

3.5. Selectivity of the method towards Cys

For determination of Cys in biological samples, the main interference of other SH-containing compounds may come from cystine and glutathione, because they always coexist with cysteine [23]. The results displayed in Fig. 5 showed that neither cystine nor glutathione influenced the detection of Cys in biological samples. Furthermore, the effects of the other 19 commonly used amino acids should be taken into account. Typically, under optimum experimental conditions, the Cys detection system was incubated with 150 nM Cys and 1 μM other 19 amino acids in HEPES buffer, respectively. As shown in Fig. 5, comparing with the blank system, the fluorescence of the system for the other 19 commonly used amino acids changed very little, while a significant fluorescence decrease was observed for Cys. This suggests that the binding affinity of Ag^+ to a "C–C" mismatch site appears to be much stronger than that to all amino acids except for Cys. The results have thus clearly illustrated the specificity of the method for Cys.

3.6. Detection of Cys in complex human serum matrix

A set of more meaningful Cys determination with the method was conducted in spiked human serum, which aimed at investigating the ability of the method used in complicated matrix. Serum is a recognized complicated biological matrix, which is similar with plasma without fibrinogen or other clotting factor. The present method not only responses effectively to Cys in aqueous buffer, but also shows its excellent analytical efficiency in human serum. A standard addition method was used to sense Cys in 100-fold diluted human serum. As shown in Fig. 6A, the fluorescence intensity decreased as the Cys concentration increased in human serum, and it exhibited a good linear correlation between fluorescence intensity and Cys concentration over the range of 5–200 nM (R = 0.992) (Fig. 6B). Serum brought a lot of complex matrix to our system, especially many kinds of proteins. These complicated materials in serum stabilized the fluorescence strength in some way and on the other hand, they might also participate in the nonspecific adsorption competition with each other onto the GO surface. All these reasons led to a comprehensive result that the fluorescence intensity quantitatively decreased with the concentration increase of Cys, and as low as 5 nM Cys in serum was determined. About 240–360 μM cysteine exists in healthy blood plasma [38,39], and our method could be used for detecting 2 nM in buffer and 5 nM in serum, suggesting that the proposed method has great potential for diagnostic purposes.

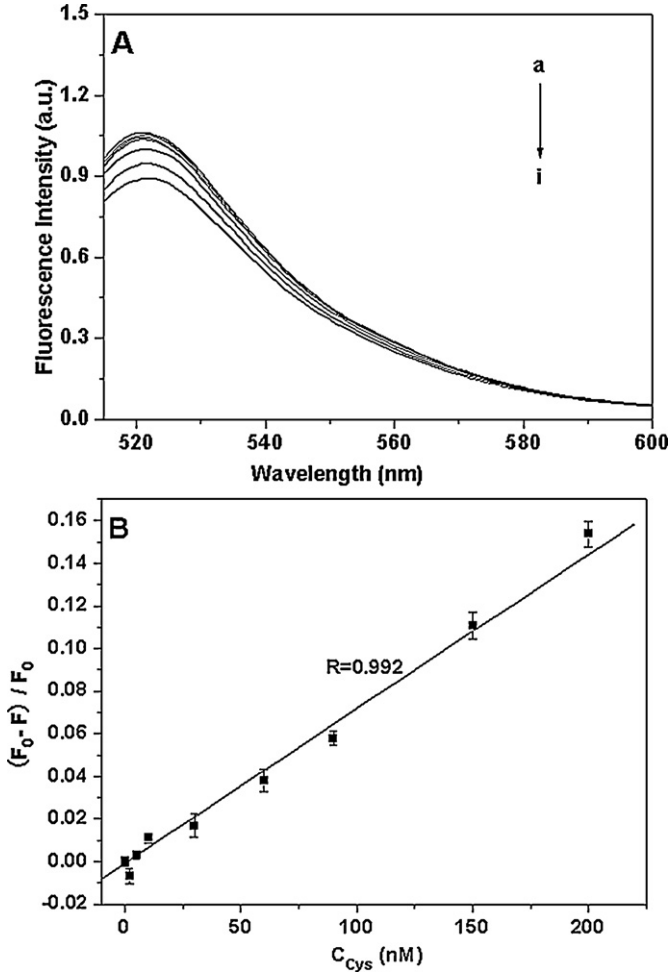

Fig. 6. (A) The normalized fluorescence emission spectra of FAM-ssDNA/Ag^+/GO (10 nM/150 nM/7.5 μg/mL) in the presence of different concentrations of Cys (a–i: 0 nM, 2 nM, 5 nM, 10 nM, 30 nM, 60 nM, 90 nM, 150 nM, 200 nM) in 100-fold diluted human serum. (B) Calibration curve for Cys detection in serum, data were presented as average ± sd from three independent measurements. Except for the addition of 100-fold diluted human serum, all other experimental conditions were performed the same as in buffer.

Several methods [23,32,38–40] for Cys detection have also been applied in serum, but few of them were able to accomplish quantitative analysis of Cys in serum, or complex separation process was needed. Therefore, the present method towards Cys based on GO has significant advantages over the others, such as convenient operation, no separating steps, and moreover the highest sensitivity was exhibited in this method, in which as low as 5 nM Cys was detectable in serum.

4. Conclusion

In summary, a novel platform for determination of Cys based on fluorescence quenching of FAM-ssDNA by GO has been constructed. In the absence of Cys, the FAM-labeled DNA was in the state of dsDNA owing to Ag^+ binding to "C–C" mismatches in the DNA sequence, and FAM was far away from the surface of GO. When Cys existed in the system, Ag^+ was efficiently extracted from dsDNA, leading to the formation of ssDNA, which assembled onto the GO by π–π stacking interaction and then FRET from FAM to GO appeared. Through the ingenious use of the outstanding electrochemical properties of GO, the proposed method has been applied to detect Cys with high sensitivity and good selectivity in both aqueous buffer and human serum. Therefore, the method towards Cys thus developed shows its potential use in Cys-related clinical diagnosis and research.

Acknowledgments

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References

[1] K.S. Kim, Y. Zhao, H. Jang, S.Y. Lee, J.M. Kim, J.H. Ahn, P. Kim, J.Y. Choi, B.H. Hong, Nature 457 (2009) 706–710.
[2] K.S. Novoselov, Z. Jiang, Y. Zhang, S.V. Morozov, H.L. Stormer, U. Zeitler, J.C. Maan, G.S. Boebinger, P. Kim, A.K. Geim, Science 315 (2007) 1379.
[3] H. Li, J. Chen, S. Han, W. Niu, X. Liu, G. Xu, Talanta 79 (2009) 165–170.
[4] R. Mas-Balleste, C. Gomez-Navarro, J. Gomez-Herrero, F. Zamora, Nanoscale 3 (2011) 20–30.
[5] C.H. Lui, L. Liu, K.F. Mak, G.W. Flynn, T.F. Heinz, Nature 462 (2009) 339–341.
[6] C.H. Lu, J. Li, J.J. Liu, H.H. Yang, X. Chen, G.N. Chen, Chem. Eur. J. 16 (2010) 4889–4894.
[7] S. He, B. Song, D. Li, C. Zhu, W. Qi, Y. Wen, L. Wang, S. Song, H. Fang, C. Fan, Adv. Funct. Mater. 20 (2010) 453–459.
[8] H. Dong, W. Gao, F. Yan, H. Ji, H. Ju, Anal. Chem. 82 (2010) 5511–5517.
[9] C.H. Lu, H.H. Yang, C.L. Zhu, X. Chen, G.N. Chen, Angew. Chem. Int. Ed. 48 (2009) 4785–4787.
[10] Y. Wang, Z. Li, D. Hu, C.T. Lin, J. Li, Y. Lin, J. Am. Chem. Soc. 132 (2010) 9274–9276.
[11] M. Zhang, B.C. Yin, X.F. Wang, B.C. Ye, Chem. Commun. 47 (2011) 2399–2401.
[12] Y. Wen, F. Xing, S. He, S. Song, L. Wang, Y. Long, D. Li, C. Fan, Chem. Commun. 46 (2010) 2596–2598.
[13] W. Wu, H. Hu, F. Li, L. Wang, J. Gao, J. Lu, C. Fan, Chem. Commun. 47 (2011) 1201–1203.
[14] H. Jang, Y.K. Kim, H.M. Kwon, W.S. Yeo, D.E. Kim, D.H. Min, Angew. Chem. Int. Ed. 49 (2010) 5703–5707.
[15] Y. Wen, C. Peng, D. Li, L. Zhuo, S. He, L. Wang, Q. Huang, Q.H. Xu, C. Fan, Chem. Commun. 47 (2011) 6278–6280.
[16] S.J. Liu, H.G. Nie, J.H. Jiang, G.L. Shen, R.Q. Yu, Anal. Chem. 81 (2009) 5724–5730.
[17] Y. Wang, J. Li, H. Wang, J. Jin, J. Liu, K. Wang, W. Tan, R. Yang, Anal. Chem. 82 (2010) 6607–6612.
[18] R.M. Kong, X.B. Zhang, L.L. Zhang, X.Y. Jin, S.Y. Huan, G.L. Shen, R.Q. Yu, Chem. Commun. (2009) 5633–5635.
[19] H. Urata, E. Yamaguchi, Y. Nakamura, S. Wada, Chem. Commun. 47 (2011) 941–943.
[20] Q. Li, X. Zhou, D. Xing, Biosens. Bioelectron. 26 (2010) 859–862.
[21] X.H. Zhou, D.M. Kong, H.X. Shen, Anal. Chem. 82 (2010) 789–793.
[22] O. Rusin, N.N. St. Luce, R.A. Agbaria, J.O. Escobedo, S. Jiang, I.M. Warner, F.B. Dawan, K. Lian, R.M. Strongin, J. Am. Chem. Soc. 126 (2004) 438–439.
[23] S. Huang, Q. Xiao, R. Li, H.L. Guan, J. Liu, X.R. Liu, Z.K. He, Y. Liu, Anal. Chim. Acta 645 (2009) 73–78.
[24] S. Shahrokhian, Anal. Chem. 73 (2001) 5972–5978.
[25] A. Salimi, S. Pourbeyram, Talanta 60 (2003) 205–214.
[26] A. Salimi, R. Hallaj, Talanta 66 (2005) 967–975.
[27] G. Chwatko, E. Bald, Talanta 52 (2000) 509–515.
[28] Z. Chen, S. Luo, C. Liu, Q. Cai, Anal. Bioanal. Chem. 395 (2009) 489–494.
[29] T. Li, L. Shi, E. Wang, S. Dong, Chem. Eur. J. 15 (2009) 3347–3350.
[30] D.M. Kong, L.L. Cai, H.X. Shen, Analyst 135 (2010) 1253–1258.
[31] S. Lim, J.O. Escobedo, M. Lowry, X. Xu, R. Strongin, Chem. Commun. 46 (2010) 5707–5709.
[32] F. Pu, Z. Huang, J. Ren, X. Qu, Anal. Chem. 82 (2010) 8211–8216.
[33] X.H. Zhou, D.M. Kong, H.X. Shen, Anal. Chim. Acta 678 (2010) 124–127.
[34] C. Zhao, K. Qu, Y. Song, C. Xu, J. Ren, X. Qu, Chem. Eur. J. 16 (2010) 8147–8154.
[35] C. David, S. Foley, M. Enescu, Phys. Chem. Chem. Phys. 11 (2009) 2532–2542.
[36] Y. Xu, H. Bai, G. Lu, C. Li, G. Shi, J. Am. Chem. Soc. 130 (2008) 5856–5857.
[37] W.S. Hummer, R.E. Offeman, J. Am. Chem. Soc. 80 (1958) 1339.
[38] Y.H. Bai, J.J. Xu, H.Y. Chen, Biosens. Bioelectron. 24 (2009) 2985–2990.
[39] A.A. Ensafi, S. Dadkhah-Tehrani, H. Karimi-Maleh, Anal. Sci. 27 (2011) 409.
[40] P. Kannan, S.A. John, Biosens. Bioelectron. 30 (2011) 276–281.