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A novel label-free optical cysteine sensor based on the competitive oxidation reaction catalyzed by G-quadruplex halves

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ABSTRACT

A sensitive and selective colorimetric detection method for Cysteine (Cys) was established in this paper. The detection mechanism is based on the oxidation of Cys by H_2O_2, which prevents the catalysis of the 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)–H_2O_2 reaction by G-quadruplex halves. With the addition of Cys, the amount of the blue-green-colored free-radical cation ($ABTS^{\cdot+}$) was reduced. The absorbance of $ABTS^{+}$ at 421 nm weakened as the color of the solution changed from blue-green to colorless. The concentration of Cys can be determined by monitoring this competitive reaction with the naked eye or using a UV–vis spectrometer. The calibration curve showed that the net absorption value at 421 nm linearly increased over the Cys concentration range of 0.005–100 μM with a detection limit of 5 nM. Furthermore, amino acids other than Cys cannot mediate the color change under the identical conditions because of the absence of thiol groups, thereby suggesting the selectivity towards Cys of the proposed method. The optical sensor is high selective, which is important for the determination of Cys in serum samples. The assay shows great potential for its practical application as a disease-associated indicator which could satisfy the need for amino acid determination in fields such as food processing, biochemistry, pharmaceuticals, and clinical analysis.

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1. Introduction

Cysteine (Cys) is a non-essential, thiol-containing amino acid in many proteins of the human body that is crucial to the regulation of several biological processes such as redox reaction, protein synthesis, metabolism and detoxification (Gazit et al., 2003, 2004; Nikiforova et al., 2002; Shahrokhian, 2001). Cys is an essential biological molecule in the physiological conditions that is required for the growth of cells and tissues. Abnormal levels of Cys have been implicated in a variety of diseases. A Cys deficiency could be accompanied by a number of symptoms such as slow growth, muscle and fat loss, hair depigmentation, weakness, edema, skin lesions, liver damage, lethargy, Alzheimer's disease, Parkinson's disease and acquired immune deficiency syndrome (Goodman et al., 2000; Lang et al., 2000; Shahrokhian, 2001). In view of the significance of Cys in health care, Cys is widely employed in the formulation of drugs and food supplements. Thus, the careful analytical quantification of this amino acid with high specificity and sensitivity is in demand.

Various methods based on analytical techniques have been reported for the determination of Cys, including high-performance liquid chromatography (HPLC), UV–vis spectroscopy (Lee et al., 2008; Li et al., 2005; Rusin et al., 2004; Wang et al., 2005), Fourier-transform infrared spectroscopy (Sato et al., 2005), mass spectrometry (Burford et al., 2003), fluorescence spectroscopy (Kwon et al., 2012; Lim et al., 2010; Shiu et al., 2010; Zhu et al., 2010), fluorescence-coupled HPLC (Tcherkas and Denisenko, 2001), and electrochemical voltammetry (Shahrokhian, 2001; Tseng et al., 2006). These approaches usually require sophisticated instrumentation, involve cumbersome laboratory procedures, and have poor throughput, thereby limiting the scope of their practical applications. Thus, there is an intense demand for a simple, rapid sensor for the detection of Cys with high sensitivity and specificity.

To today, optical biosensors, especially the colorimetric biosensors, have quickly become the methods of choice for several biosensing applications. Although various reports have described the applications of nanoparticles colorimetry (Ai et al., 2009; Medley et al., 2008; Xu et al., 2009), their practical use has been limited by factors such as high cost. Furthermore, their applications in certain cases are unstable in aqueous environments.

Recently, a series of G-quadruplex-based DNAzymes that specifically bind to hemin and show peroxidase-like activity have been selected via the systematic evolution of ligands by exponential enrichment process. Since the complexes of the hemin–G-quadruplex (G-quadruplex halves) were first reported by Travascio et al. (1998), DNAzymes have been widely used in the design of chemosensors and biosensors (Golub et al., 2011; Li et al., 2008, 2009b, 2009d; Lu et al., 2008, 2009; Xu et al., 2011;

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Zhu et al., 2011). Several Cys detection methods that use G-quadruplex halves have been reported (Jia et al., 2011; Li et al., 2009c; Zhou et al., 2009). These methods are mostly based on the destruction of G-quadruplex structures by metal ions to form DNA–metal base pairs (e.g., C–Ag^+–C or T–Hg^{2+}–T base pairs). These base pairs strongly inhibit the peroxidase activity of G-quadruplex halves, and consequently "turn off" of the signal. While due to Ag^+ or Hg^{2+} preferentially binding to Cys, G-quadruplexes are reformed after the addition of Cys, which then increases the peroxidase activity that is detected by the sensing system. However, these sensors have several shortcomings such as the environment pollution caused by heavy metal ions and the complexity of the detection process.

Herein, we introduce a simple and highly selective method for ultrasensitive colorimetric Cys detection that utilizes G-quadruplex halves. G-quadruplexes halves can effectively catalyze the H_2O_2-mediated oxidation of 2,2-azinobis (3-ethylbenzothiozoline)-6-sulfonic acid (ABTS) into the blue-green-colored radical $ABTS^{\cdot+}$, which is accompanied by an increased absorption signal at 421 nm. If Cys is present in solution at the beginning of the assay, the amino acid will be oxygenized by H_2O_2 because of its sulfhydryl (–SH) content, resulting in disturbing the ABTS–H_2O_2 reaction. During this process, the production of $ABTS^{\cdot+}$ will be reduced. Thus, the solution color fades and the absorbance at 421 nm weakens, which is the basis of this colorimetric assay. The changes in the absorbance signal were recorded, which can be observed by the naked eye. Consequently, a simple, rapid, pollution-free and highly specific Cys detection method was developed.

2. Experimental section

2.1. Materials

The 26-mer oligonucleotide (GGTGGTGGTGGTTGTGGTGGTGG-TGG) (AGRO100) was purchased from the Shanghai Sangon Biotechnology Co. (Shanghai, China) and used without further purification. Potassium acetate and H_2O_2 were produced by the Tianjin Kaitong Chemicals Company (Tianjin, China). Triton X-100, L-Cys (Cys), glutathione, 2,2-azinobis (3-ethylbenzothiozoline)-6-sulfonic acid (ABTS), hemin and all other amino acids were obtained from the Shanghai Chemical Reagent Company (Shanghai, China). Double-distilled water was used throughout this study. All chemical reagents were of analytical grade and used without further purification. Prior to use, AGRO100 was dissolved in 25 mM Tris–Ac buffer (pH 8.0). The stock solution of hemin (5 mM) was prepared in DMSO, stored in the dark at −20 °C, and diluted to the required concentration with an aqueous buffer.

2.2. Preparation of G-quadruplex halves

The AGRO100 solution was heated to 90 °C for 5 min and cooled slowly to room temperature. An equal volume of the low-salt buffer (25 mM Tris–Ac, pH 8.0; 0.2 mM KAc, 0.05% (w/v) Triton X-100) was then added. The mixture was incubated at room temperature for 40 min, which was followed by the addition of an equal volume of hemin and further incubation for 1 h to form the hemin–AGRO100 complex. Finally, the as-prepared hemin–G-quadruplex complexes (G-quadruplex halves) were diluted to the appropriate concentrations using the low-salt buffer.

2.3. Colorimetric assay of Cys

Cys (from 5 nM to 10 mM) and other amino acids (15 μM) were separately added to an equal volume of the hemin–G-quadruplex complex solution. The mixture was shaken at room temperature for 5 min before the 2.8 mM ABTS solution was added. Catalytic reactions were initiated by the addition of 1.25 mM H_2O_2. After 5 min, the mixed solutions were separately transferred into a 1 mL quartz cuvette. The detected absorbance of the system could reach its maximum value at 5 min. The absorption spectrum of the reaction product $ABTS^+$ and the kinetic absorbance–time curves were recorded on a 2450 UV–vis spectrophotometer (Shimadzu, Japan) by monitoring the colorimetric absorbance values of $ABTS^+$ at 421 nm. Equal inaccessible time gaps between sample mixing and absorbance measurement were guaranteed to improve the data set consistency. The absorbance at 421 nm was used for the quantitative analysis. To briefly and clearly investigate the results, $\Delta A_{421\ nm}$ was defined as $\Delta A_{421\ nm}=A_0-A_m$. Where A_0 is the absorbance of the catalyzed ABTS–H_2O_2 reaction without Cye incubation at 421 nm and A_m is the measured absorbance at 421 nm.

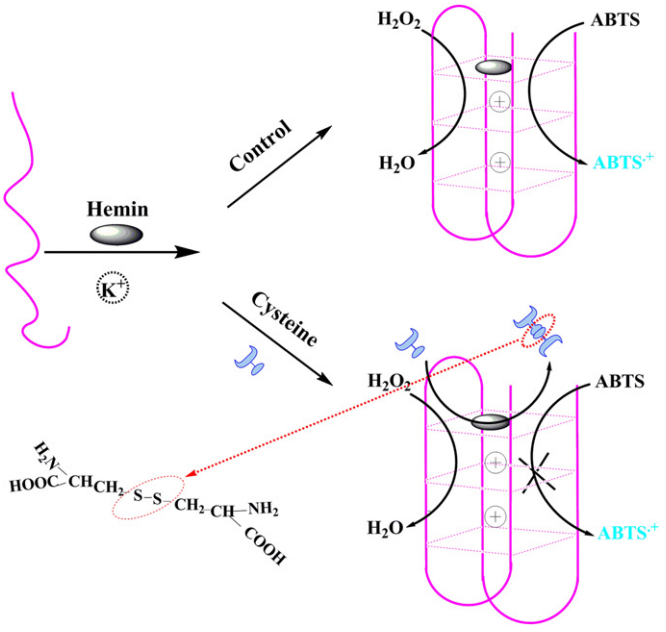
2.4. Sample preparation

Human serum samples were pretreated according to a previous report (Alothman et al., 2010) with slight modifications. Briefly, 2 mL human blood was centrifuged at 4000 rpm for 15 min at room temperature. To remove serum protein, 1.2 mL acetonitrile was added, followed by spiking with different concentrations of Cys. After vortexing for 30 s, the mixture was centrifuged at 12,000 rpm for 10 min to remove the serum protein residues. The supernatant was taken carefully and used for detection.

3. Results and discussion

3.1. Detection principle

Scheme 1 illustrates the mechanism of the designed Cys sensor. Wang's group (Li et al., 2009a, 2009c) previously reported that AGRO100 (a 26-mer single-stranded DNA oligo) had the lowest dissociation constant (K_d=129 nM) and the highest initial catalytic rates (v=15.79 μM/min), when it was bound to hemin in the hemin–G-quadruplex complexes. As shown in Scheme 1,



Scheme 1. Schematic representation of the optical method for detecting Cys based on competitive oxidation reaction catalyzed by G-quadruplex halves.

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