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Styryl quinolinium/G-quadruplex complex for dual-channel fluorescent sensing of Ag^+ and cysteine

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ABSTRACT

Based on the disruption and recovery of fluorescence of styryl quinolinium (SQ) dye/G-quadruplex complex, a label-free method for dual-channel detection of Ag^+ and cysteine is developed. The above sensing system exhibits high sensitivity and good selectivity for Ag^+, even in the presence of other metal ions (such as Na^+, Mg^{2+}, Mn^{2+}, Ba^{2+}, Ca^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+} and Cu^{2+}). Due to cysteine (Cys) is a strong Ag^+-binder and competes with quadruplex-forming G-rich oligonucleotides for Ag^+ binding, and results in the reformation of G-quadruplex structure and the recovery of fluorescence. Thus SQ dye/G-quadruplex complexes are not only a potential primary sensor toward Ag^+, but also a secondary sensor toward Cys. Neither the Ag^+-sensing nor the Cys-sensing systems required labeled oligonucleotides. Under the optimal conditions, this method is capable of detection of Ag^+ in the range of 0.1–15.0 μM with a detection limit of 26.0 nM. In addition, the proposed method allows a good response to cysteine with a linear range of 0.008–10 μM, and the limit of detection is found to be 2.0 nM. Moreover, this method has been applied successfully to the sensing of cysteine in human urine with satisfactory recovery.

1. Introduction

Cysteine (Cys) is an important amino acid and is involved in all kinds of important cellular functions. The deficiency or excess accumulation of Cys is harmful to living system [1–3]. Cysteine also has particular characteristics: it possesses a thiol group, which can bond strongly to silver. The silver ion has antimicrobial activities and it can be used in curative and preventive health care [4–7]. Therefore, the detection and sensing of silver and Cys are certainly challenging and intriguing to the current researchers.

Styryl dyes are widely used as fluorescent probes for cellular organelles or macromolecules in medicinal analysis, such as nuclear-selective staining [8–10]. The mechanism of nuclear staining of these dyes may involve an increase of their fluorescence intensity upon binding to DNA [11]. However, there are few reports available in the literature in which styryl dye could be specific binding to G-quadruplexes. G-quadruplexes are unique higher-order structures formed by G-rich nucleic acid sequences and based on stacked arrays of G-quartets connected by Hoogsteen-type base pairing [12–14]. 4-(4-(Dimethylamino) styryl)-N-methylquinolinium iodide (SQ), one member of styryl family, was reported to use for staining living nerve terminals [15]. However, we found that the presence of G-quadruplex, especially intramolecular G-quadruplex, may greatly enhance the fluorescence intensities of SQ dye, which could be used as a label-free fluorescent sensor.

Though there are several receptors to recognize Ag^+ and Cys individually [16–19], to the best of our knowledge, there is no report of any styryl molecular system that would detect Ag^+ and Cys selectively. The SQ dye appears to be nontoxic, inexpensive, and easy to use and shows a remarkable fluorescence response selective for intramolecular G-quadruplex DNA. The free SQ dye alone has no fluorescence in solution; however, it has strong fluorescent signal after binding to intramolecular G-quadruplex to form SQ dye/G-quadruplex complexes. In this paper, we have developed an Ag^+ and Cys detection method based on the formation of SQ dye/G-quadruplex complexes. The working mechanism of this method was based on the disruption and recovery of fluorescence signal of SQ/G-quadruplex complex using Ag^+ and Cys (see Scheme 1). Under the optimal conditions, high selectivity, sensitivity and wide liner range for label-free Ag^+ and Cys detection have been achieved by this method.

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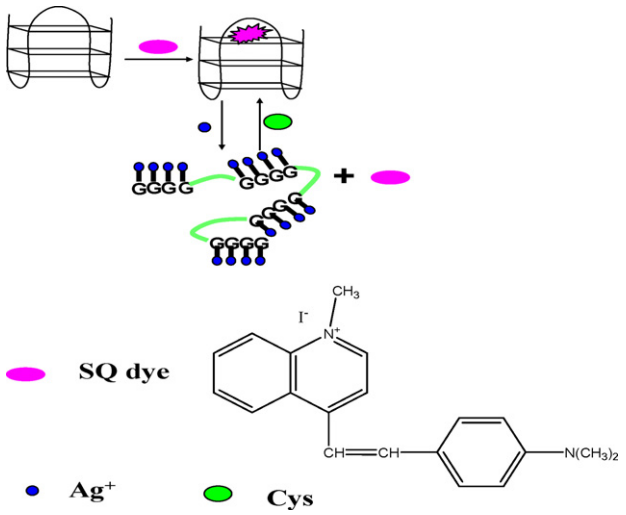

Scheme 1. Schematic illustration of Ag^+ and Cys sensors based on the disruption and recovery of the fluorescence of SQ/G-quadruplex complex.

2. Experimental

2.1. Materials and reagents

Purified oligonucleotides (listed in Table 1) were purchased from Sangon Biotech Co. Ltd. (Shanghai). The concentration of the oligonucleotides was represented as single-stranded concentration determined by the absorbance at 260 nm and the molar extinction coefficient. SQ dye, amino acids and metal salts $AgNO_3$, Na_2SO_4, $CaCl_2$, $PbCl_2$, $MnSO_4$, $BaCl_2$, $MgSO_4$, $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Hg(NO_3)_2$ and $CuSO_4$ were obtained from Sigma–Aldrich (St. Louis, MO). All chemical reagents were of analytical grade and used as received without further purification. The water was purified using a Millipore filtration system.

2.2. Reparation of SQ/G-quadruplex complexes

Oligonucleotide solutions (2.0 μM) were prepared in Tris–HAc buffer (50 mM, pH = 5.5) containing KAc (0.06 mM). 4.0 μM of SQ dye was added into the solution incubating for 10 min to form SQ dye/G-quadruplex complexes.

2.3. Fluorescence spectroscopy

All fluorescence measurements were made using Cary Eclipse spectrofluorimeter. The SQ/G-quadruplex complexes solution prepared as above. The spectra were excited at 579 nm and monitored from 600 to 800 nm.

2.4. Ag^+ detection

Different concentrations of Ag^+ were added to the SQ/G-quadruplex complex solution prepared as above. The mixture was allowed to incubate for 80 min.

2.5. Cys detection

To the SQ/G-quadruplex complex solution prepared as above, an indicated concentration of Ag^+ (20 μM) was added and the mixture was allowed to incubate for 80 min. To this solution was added different concentrations of Cys, and the mixture was incubated for 10 min.

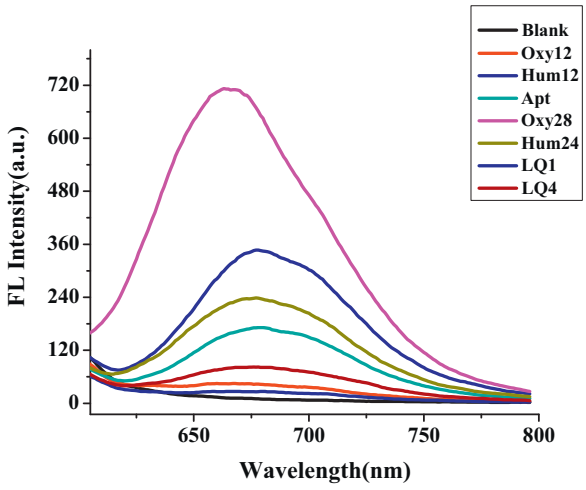

Fig. 1. Fluorescence spectra of 4.0 μM SQ with different G-quadruplexes at 2.0 μM.

3. Results and discussion

3.1. Selection of G-quadruplex

To investigate the fluorescence response of SQ to different G-quadruplexes (listed in Table 1), the fluorescence spectra of SQ in the presence of Oxy12, Hum12, Apt, Oxy28, Hum24, LQ1 and LQ4 are measured. The results (Fig. 1) show that the fluorescence intensity of SQ is enhanced greatly upon binding to different G-quadruplex DNAs, whereas it exhibits no fluorescence when free in solution. Among these G-quadruplexes, the fluorescence intensity of SQ/Oxy28 complex is always higher than that of other SQ/G-quadruplex complexes, and its fluorescence spectra exhibited a marked blue shift relative to that of other SQ/G-quadrulex complexes. The large intensity with Oxy28 is suggstive of a strong association of SQ with the Oxy28 G-quadruplex molecular. The association may restrict the motion of methine-bridge of SQ. As a result, SQ relaxes efficiently through the radiative pathway and gives strong fluorescence [20]. At the same time, the blue shift relative to other complexes may provide a opportunity to reduce the background interference from other G-quadruplex complexes. Thus Oxy28 is used in subsequent experiments.

3.2. Optimization of influencing factors

The effect of pH (from 3.5 to 8.5) on the enhanced fluorescence intensity is investigated in Tris-HAc buffer, and the experimental results reveal that the maximum enhanced intensity of SQ by Oxy28 is found at pH 5.5.

The effect of SQ concentration on the enhanced fluorescence intensity is investigated at pH 5.5 in the Tris–HAc buffer solution with the presence of 2.0 μM Oxy28 (see Fig. 2). The results show that maximum fluorescence intensity occurred when the concentration is 4.0 μM. However, the fluorescence intensity decreased clearly when the SQ concentration is larger than 4.0 μM, which may be explained by that the binding of SQ to Oxy28 was overload and SQ formed aggregates in aqueous solution [21], resulting in the decreasing of fluorescence intensity. In subsequent studied, 4.0 μM is recommended.

An appropriate concentration of K^+ is crucial for G-quadruplex folding and SQ/G-quadruplex complex formation, whereas the disruption of G-quadruplex structures by Ag^+ will be weakened if the K^+ concentration becomes too high. Hence, the K^+ concentration on the enhanced fluorescence intensity is investigated, and the results