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p-sulfonated calix[8]arene functionalized graphene as a "turn on" fluorescent sensing platform for aconitine determination

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ABSTRACT

This work reports a novel method for the determination of aconitine through the competitive host–guest interaction between p-sulfonated calix[8]arene (SCX8) and signal probe/target molecules by using SCX8 functionalized reduced graphene oxide (SCX8-RGO) as a receptor. Three dyes (ST, RhB, BRB) and aconitine were selected as the probe and target molecules, respectively. The formation of SCX8-RGO · ST, SCX8-RGO · RhB, and SCX8-RGO · BRB complexes greatly decreases the fluorescence emission of ST, RhB, and BRB. The aconitine/SCX8 complex possesses a higher binding constant than ST/SCX8, RhB/SCX8, and BRB/SCX8 complexes, thus the dye in the SCX8 cavity can be replaced by aconitine to revert the fluorescence emission of SCX8-RGO · dye, leading to a "switch–on" fluorescence response. The fluorescence intensity of SCX8-RGO · ST, SCX8-RGO · RhB, and SCX8-RGO · BRB complexes increased linearly with increasing concentration of aconitine ranging from 1.0 to 14.0 μM, 2.0–16.0 μM, and 1.0–16.0 μM, respectively. Based on the competitive host–guest interaction, the proposed detection method for aconitine showed detection limits of 0.28 μM, 0.60 μM, and 0.37 μM, respectively, and was successfully applied for the determination of aconitine in human serum samples with good recoveries from 95.1% to 104.8%. The proposed method showed high selectivity for aconitine beyond competitive binding analytes. In addition, the inclusion complex of the SCX8/aconitine was studied by the molecular docking and molecular dynamics simulation, which indicated that the phenyl ester group of the aconitine molecule was included into the SCX8 cavity.

1. Introduction

The exploitation of competitive binding for sensing applications has a long history, and the so-called competitive binding assay is extensively used in biochemistry, such as competitive binding immunoassays, competitive enzyme inhibition, and DNA intercalation assays (You et al., 2015). However, the use of synthetic receptors with competitive binding assays has gained popularity within the past two decades, and the so-called "indicator displacement assay" or IDA, has become a standard strategy for molecular sensing (Biedermann et al., 2012, 2013). IDA is based on the competition between an indicator and an analyte for the binding of a receptor (Ghale and Nau, 2014). In a typical IDA experiment, an indicator is bound to a receptor first, creating the sensing ensemble. An analyte is then introduced, and the indicator is displaced from the sensing ensemble. Generally, the free and bound indicators have different optical (colorimetric or fluorescent) properties, resulting in a signal change. In recent years, graphene has been increasingly explored as a new star of excellent quenchers for fluorescent probes based on fluorescence resonance energy transfer (FRET) between a fluorescent dye and graphene in fluorescent sensing fields (Mondal and Jana, 2012; Mao et al., 2012). At present, fluorescent IDA (F-IDA), which holds great promise for potential applications in constructing a "switch–off–on" graphene fluorescent sensing platform, has been broadly used in detection (Nguyen and Anslyn, 2006). In an F-IDA, a fluorescent indicator is firstly allowed to bind reversibly to a receptor. Then, a competitive analyte is introduced into the system causing the displacement of the fluorescent indicator, which in turn modulates a fluorescent signal (Nguyen and Anslyn, 2006; Mao et al., 2012). Compared with the traditional method that assemble a dye labeled aptamer on graphene, such F-IDA recognition, carried out as a model system to construct a "turn–off–on" graphene fluorescent sensing platform, does not require the indicator to be covalently labeled to the receptor, which decreased the difficulty of

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synthesizing and greatly expanded its application in fluorescent sensing fields (Praetorius et al., 2008; Mao et al., 2012; Biedermann and Nau, 2014; Li et al., 2015).

Calixarenes, recognised as the third class of macrocyclic host molecules after crown ethers and cyclodextrins, have become important receptors because they can form stable host–guest complexes with various organic, inorganic, and biological guest molecules, which show high supramolecular recognition and enrichment capability (Mutihac et al., 2011; Dsouza et al., 2011). Water-soluble calixarenes, particularly p-sulfonated derivatives, have been widely investigated to develop different fluorescent sensing platforms due to their biocompatibility and simplicity of synthesis (Zhou et al., 2013). Graphene is one of the most promising materials that holds great promise for potential applications in many technological fields because of its high surface area, low cost, and high conductivity (Allen et al., 2010). A major drawback of the graphene is the inevitable aggregation owing to the strong π–π stacking tendency between the nanosheets (Marcano et al., 2010). It has been reported that the composites of calixarenes and carbon materials (e.g. carbon nanotube, graphene) could be formed by π–π interactions and hydrogen interactions (Chen et al., 2012; Zhou et al., 2013; Eroglu et al., 2013; Chen et al., 2015). If graphene is modified with water-soluble calixarenes, it is possible to obtain new functionalized materials that simultaneously possess the unique properties of graphene (excellent quenching ability and a large surface area) and calixarenes (high supramolecular recognition and good enrichment capability) and also can improve the dispersibility of graphene.

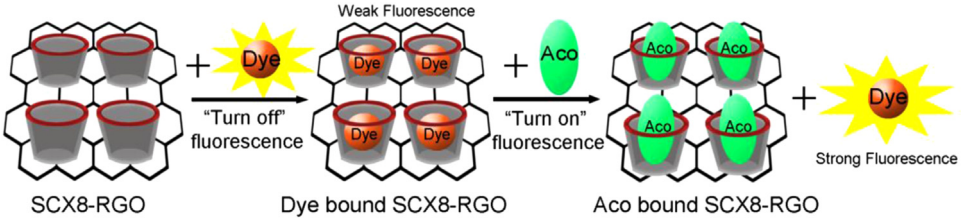
Aconitine is a diester-diterpene alkaloid derived from some Chinese medicinal herbs of genus *Aconitum* in the family of Ranunculaceae such as *Aconitum carmichaeli* Debx. and *Aconitum Kusnezoffii* Reichb. These medicinal herbs are widely used in clinics in China and other East Asian countries because of their effects against rheumatosis, rheumatoid arthritis and some other inflammations (Li and Cai, 2013). *Aconitum* is included in up to 600 formulations from both the historical literature and modern clinical reports (Singhuber et al., 2009). The annual consumption of Aconitum species in China exceeds 2,000,000 kg (Li et al., 2002). The most commonly used Aconitum-containing herbal products in the market are “Fuzi Lizhong Borus,” “Xiao Huoluo Dan,” “Jingui Shenqi Borus,” and “Shenfu Injection” (Wang and Lu, 1999). For instance, the Shenfu injection is a modern Chinese medicine preparation derived from a traditional formulation, Shenfu decoction. It is prepared from the extracts of red ginseng (steamed roots of *Panax ginseng*) and aconite (processed lateral roots of *Aconitum carmichaeli*) using multistage countercurrent extraction and macroporous resin adsorption technology (Song et al., 2015). Compared with the decoction, the Shenfu injection is more convenient for clinical use (Yang et al., 2014). However, the toxicity resulting from these alkaloids in these plants, such as aconitine, talatisamine, and Yunaconitine cannot be neglected. The tubers and roots of aconites, which have been proved to be neurotoxic and cardiotoxic, are applied only after cautious processing (usually boiling) in order to reduce their toxicity. Nevertheless, unexpected poisoning incidents caused by these toxic alkaloids remained in the herbal medicines have occurred from time to time because of improper processing (Yang et al., 2010; Zhang and Shi, 2012). There were 878 cases of accidental aconite ingestion in China From 1977 to 1985 (Zhu, 1988), whereas from 1989 to 1995, over 35 cases of aconite poisoning were reported in Hong Kong (But et al., 1994). Some suicidal and homicidal cases involving the use of aconite as poison have also been reported (Sun et al., 2009). The lethal dose 50% (LD_{50}), absolute lethal dose and arrhythmia dose of aconitine are 0.270 ± 0.002 mg/kg (mice), 0.102 ± 0.008 mg/kg (rats) and 0.034 ± 0.004 mg/kg (rats), respectively (Zhou et al., 1984). Oral dose of 0.2 mg aconitine was reported to be toxic and 3–5 mg to be fatal in humans (Zhou et al., 1984). Thus, the development of a valid and sensitive method to determine the *Aconitum* alkaloids is of great importance. So far, several methods have been reported for the determination of *Aconitum* alkaloids, including HPLC (Xie et al., 2005; Wang et al., 2006), capillary electrophoresis (Feng and Li, 2002), GC-MS (Wada et al., 2006), LC-MS (Kaneko et al., 2006; Beyer et al., 2007), etc. However, most of these methods need complex sample pretreatments, such as liquid-phase extraction, solid phase extraction and pre-column derivation, which are time-consuming, tedious, or need large amounts of toxic organic solvents (Yang et al., 2010). Therefore, designing and developing an effective, rapid, and simple analytical method for the determination of *Aconitum* alkaloids is highly desirable.

Herein, a novel fluorescent IDA-based approach for aconitine sensing based on a competitive host-guest recognition between p-sulfonated calix[8]arene (SCX8) and signal probe/target molecules was developed by using SCX8 functionalized reduced graphene oxide (SCX8-RGO) as a receptor. Three dyes (ST, RhB, BRB) and aconitine were selected as the probe and target molecules, respectively. As illustrated in Scheme 1, when the dye enters into the SCX8 host its fluorescence is quenched by RGO. However, upon the presence of aconitine to the performed SCX8-RGO · dye complex, the dye molecules are displaced by aconitine, resulting in reversion of the fluorescence of the dye, accompanied with a “turn on” fluorescence signal.

2. Materials and methods

2.1. Chemicals and materials

Graphite oxide was purchased from Nanjing XFNANO Materials Tech Co., Ltd. (Nanjing, China). Safranine T (ST), rhodamine B (RhB), and butyl rhodamine B (BRB) were obtained from Aladdin Chemical Reagent Lo., Ltd. (Shanghai, China). Aconitine (Fig. 1A) was extracted and separated from the roots of aconites (details in Supplementary Information). 4-Sulfocalix[8]arene hydrate (SCX8, Fig. 1B) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Preparation of the inclusion complex of aconitine/SCX8 and method for detection of aconitine in serum were provided in Supplementary Information. All aqueous solutions were prepared with deionized water (DW, 18 MΩ cm). All other reagents were of analytical grade.



Scheme 1. Indicator displacement assay for aconitine (Aco) using SCX8-RGO against fluorescent dye indicator.