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BODIPY-based azamacrocyclic ensemble for selective fluorescence detection and quantification of homocysteine in biological applications

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ABSTRACT

Considering the significant role of plasma homocysteine in physiological processes, two ensembles (F_{465}–Cu^{2+} and F_{508}–Cu^{2+}) were constructed based on a BODIPY (4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene) scaffold conjugated with an azamacrocyclic (1,4,7-triazacyclononane and 1,4,7,10-tetraazacyclododecane) Cu^{2+} complex. The results of this effort demonstrated that the F_{465}–Cu^{2+} ensemble could be employed to detect homocysteine in the presence of other biologically relevant species, including cysteine and glutathione, under physiological conditions with high selectivity and sensitivity in the turn-on fluorescence mode, while the F_{508}–Cu^{2+} ensemble showed no fluorescence responses toward biothiols. A possible mechanism for this homocysteine-specific specificity involving the formation of a homocysteine-induced six-membered ring sandwich structure was proposed and confirmed for the first time by time-dependent fluorescence spectra, ESI-MS and EPR. The detection limit of homocysteine in deproteinized human serum was calculated to be 241.4 nM with a linear range of 0–90.0 μM and the detection limit of F_{465} for Cu^{2+} is 74.7 nM with a linear range of 0–6.0 μM (F_{508}, 80.2 nM, 0–7.0 μM). We have demonstrated the application of the F_{465}–Cu^{2+} ensemble for detecting homocysteine in human serum and monitoring the activity of cystathionine β-synthase *in vitro*.

1. Introduction

Biological thiols (biothiols) such as glutathione (GSH), cysteine (Cys) and homocysteine (Hcy) present in biological systems play vital roles in physiological processes, including protein structure and function maintenance, redox homeostasis control and detoxification (Ivanov et al., 2000; Wood et al., 2003). Altered levels of biothiols are associated with various physiological diseases (Murakami et al., 1989; Ross et al., 1997; Heafield et al., 1990; Refsum and Ueland, 1998).

Hcy is a key intermediate amino acid generated by the demethylation of methionine in S-adenosylmethionine-dependent transmethylation reactions (Langman and Cole, 1999). Hcy is metabolized mainly through a transsulfuration pathway catalyzed by cystathionine β-synthase (Stead et al., 2004). Plasma Hcy exists in three major forms, with trace amounts (~1%) existing in the reduced form. Approximately 70% is bound to albumin, and the remaining 30% forms low molecular weight disulfides predominantly with Cys. The sum of all these Hcy species is termed total Hcy (tHcy) (Ueland, 1995). Normal levels of fasting plasma tHcy in healthy adults are considered to be in the range of 5–15 μM (Ueland et al., 1993). However, impaired Hcy metabolism generally caused by cystathionine β-synthase deficiency results in excessive concentrations of tHcy (> 15 μM) in plasma and urine, a condition that is clinically diagnosed as hyperhomocysteinemia. Moderate, intermediate, and severe hyperhomocysteinemia refers to concentrations of 16–30, 31–100, and > 100 μM, respectively (Kang et al., 1992). The mechanism by which Hcy exerts its effect has not yet been clearly defined, but it is generally accepted that the elevated Hcy in plasma can damage the endothelium and induce vascular injury (Ozkan et al., 2002). Clinical and epidemiological studies have shown a relationship between elevated tHcy levels and coronary artery disease (Ozkan et al., 2002), stroke (Coull et al., 1990), and osteoporotic fractures (van Meurs et al., 2004). tHcy has also been linked to an increased risk of dementia and Alzheimer's disease (Seshadri et al., 2002), metabolic syndrome (Yakub et al., 2014), neural tube defects (Steegers-Theunissen et al., 1994) and pregnancy complications (Vollset et al., 2000). Consequently, assessment of tHcy levels in blood plasma and urine is of great clinical diagnostic significance.

Over the past decades, effective strategies have been developed for Hcy detection, and the methods are based mainly on chromatographic separation and enzyme immunoassay. The chromatographic

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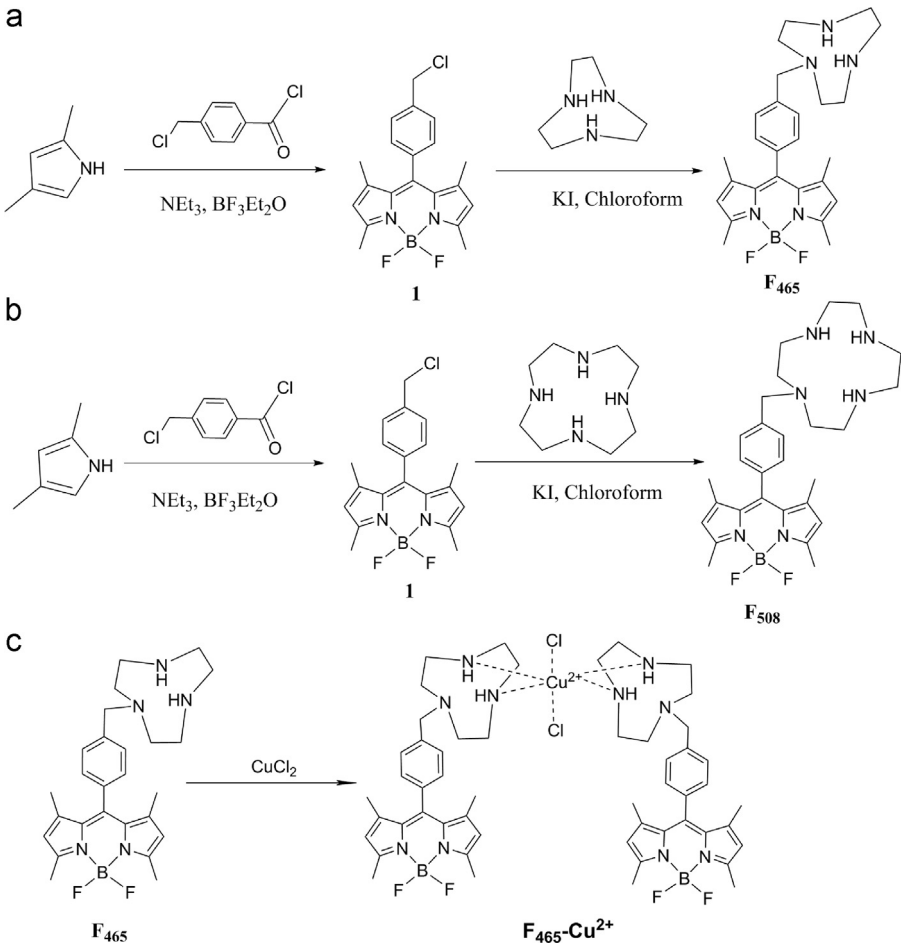
separation technique (Nekrassova et al., 2003; McMenamin et al., 2009) involves the derivatization of the sulfhydryl group, requiring tedious separation techniques such as gas chromatography with mass spectrometry (GC/-MS) and high performance liquid chromatography (HPLC), thus limiting the ability of Hcy to function as an important biomarker. Enzyme immunoassay (Shipchandler and Moore, 1995; Pernet et al., 2000; Newton et al., 2010) requires the use of the commercially available kits, using enzymes, antibodies and biomolecules that are expensive and intrinsically labile, generally using multi-step addition and washing procedures with specialized storage conditions. The instruments are typically sophisticated to operate. Therefore, there is an urgent need to develop simple and inexpensive methods for Hcy detection.

Fluorescence probing serves as the most attractive approach to monitor biologically relevant species because of its sensitivity and simplicity. In recent years, numerous fluorescent probes have been developed to distinguish biothiols from other amino acids (Guy et al., 2007; Yi et al., 2009; Lim et al., 2011; Lee et al., 2012; Zhang et al., 2011; Shao et al., 2012). However, due to the high degree of similarity of both structures and the reactivity among the biothiols, the reports on the selective detection of Hcy over Cys/GSH were addressed relatively seldom (Barve et al., 2014; Lee et al., 2014; Peng et al., 2014).

The Cu^{2+}-based ensemble is comprised of a Cu^{2+} center and a specific fluorescent molecule. Because of the strong quenching ability of paramagnetic Cu^{2+} for fluorophores, the ensemble is generally non-fluorescent after incorporating Cu^{2+} (Liu et al., 2013). As far as we know, the Chang group first reported the ensemble composed of fluorescein-dipicolylamine and Cu^{2+} for selective fluorescence responds of the sulfide anion (Choi et al., 2009). To date, the Cu^{2+}-based ensembles have been developed rapidly for sensing varied analytes such as sulfide (Sasakura et al., 2011; Fu et al., 2014), cyanide (Guliyev et al., 2009; Lou et al., 2012), and histidine (You et al., 2014; Reddy et al., 2014). However, these chemosensors all relied on the high affinity between Cu^{2+} and the analyte, and biothiols easily interfered with the detection of the analyte. Considering the critical role of biothiols in maintaining the redox status of biological systems, there are to date few cases of ensembles for probing biothiols utilizing a redox reaction. To our best knowledge, the Yang group reported a spiropyran-based ensemble for Hcy/Cys (Shao et al., 2006). No other Cu^{2+}-based ensemble is reported for the selective sensing of biothiols, especially Hcy. Therefore, to exploit a novel Hcy chemosensor based on a Cu^{2+} ensemble has become a current research challenge.

We designed and synthesized two ensembles (F_{465}–Cu^{2+} and F_{508}-Cu^{2+}) based on a BODIPY scaffold conjugated with an aza-macrocyclic Cu^{2+} complex, employing 1,4,7-triazacyclononane (TACN) and 1,4,7,10-tetraazacyclododecane (Cyclen), (Schemes 1a, b). F_{465}–Cu^{2+} can be employed to detect Hcy in the presence of Cys/GSH under physiological conditions with high sensitivity and selectivity in the turn-on fluorescence mode. We have then demonstrated an application of F_{465}–Cu^{2+} for Hcy detection in a real human plasma sample, which offers a potential application in the clinical diagnosis of coronary disease. We have also utilized the F_{465}–Cu^{2+} ensemble to monitor cystathionine β-synthase activity *in vitro*, thus establishing a methodology to evaluate cystathionine β-synthase activity and delivering a potential tool to regulate Hcy



Scheme 1. Synthetic procedure for F_{465}, F_{508} and F_{465}–Cu^{2+}.