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A sensitive and selective chemosensor for ascorbic acid based on a fluorescent nitroxide switch

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ABSTRACT

Ascorbic acid (AsA), also known as vitamin C, is a vital small-molecule antioxidant with multiple functions in vivo. It's the major natural antioxidant found in plants and is also an essential component of human nutrition. AsA plays a key role in many diseases-related biological metabolism. Therefore, sensitive and selective detection of AsA is greatly important in pharmaceutical, clinical and food industry. Here a sensitive and selective sensor for ascorbic acid detection based on the recovered fluorescence of NAPS-NO (*N*-propyl-triethoxysilane-4-(4-ylamino-1-oxy-2,2,6,6-tetramethylpiperdine)-naphthalimide) probe is described. The fluorescence of the naphthalimide moiety of NAPS-NO is inhibited by the nitroxide group, which is covalently linked to the fluorophore. Then, ascorbic acid reacts rapidly with the nitroxide moiety of NAPS-NO to form hydroxylamine, and the fluorescence properties of the naphthalimide moiety are recovered and the ESR signal decayed. Over a wide range from 80 nM to 50 μM, a good linear relationship between the fluorescence intensity and the concentration of ascorbic acid was found and the detection limit was estimated to be as low as 20 nM. To confirm the practical usefulness of the fluorophore–nitroxide probe, we demonstrated the use of NAPS-NO for the measurement of AsA in human blood serum and also successfully determined the concentration of AsA in HEK 293 cell lysate. Results from confocal laser scanning microscopy experiments demonstrated that this chemosensor is cell permeable and can be used as a fluorescent probe for monitoring ascorbic acid in living cells.

1. Introduction

Ascorbic acid (AsA), which cannot be synthesized in the human body, is used in large scale as an antioxidant in food, animal feed, beverages, pharmaceutical formulations and cosmetic applications [1]. AsA is the major natural antioxidant found in plants and is also an essential component of human nutrition [2,3]. It plays a key role in biological metabolism and has been commonly used for the prevention and treatment of common cold, mental illness, infertility, cancer and AIDS, etc [4]. Thus, the development of a simple and rapid method for the determination of AsA with high selectivity and sensitivity is desirable for diagnostic and food safe applications [5].

A wide variety of analytical methods is available for the determination of ascorbic acid, such as titrimetric [6,7], spectrophotometric [8,9] and chromatographic [10–12] methods. These methods rely on different phenomena and registration techniques suffering from several limitations. The spectrophotometric methods measure either the resultant color of products of certain reactions involving ascorbate [13] or absorbance of ascorbic acid itself [14]. However, the low selectivity of the reactions involved decreases the accuracy of the ascorbate analysis. Thereby, the reliability of these methods is limited by relatively low specificity. A number of specific assays for reductants including ascorbic acid, uric acid, tocopherol etc. have been developed based on high performance liquid chromatography (HPLC) [15,16]. These techniques require several complicated preliminary steps. In addition, HPLC separations would consume relatively long time, therefore, could not be considered as an express-method. Early methods utilizing nitroxide radicals for ascorbate quantifications were based on measurements of only ESR signal from nitroxide, therefore, suffered from general drawbacks of ESR spectroscopy. Although this technique permits investigations in high optical density substance, the ESR measurements are limited by high cost and complexity of equipments and difficulties in acquiring and processing experimental data. At present, electrochemical approaches become

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the major analytical methods for AsA determination because of their convenience and speediness. Nevertheless, the interference from other similar redox potential molecules can be serious, such as uric acid and dopamine [17]. What's more, the detection limit is always higher than that of fluorescence methods. Particularly, attempts have been made to detect ascorbic acid using nanoparticles. Wang et al. [17] developed a simple fluorescence sensor for sensitive turn-off detection of ascorbic acid by using protein-modified Au nanoclusters in aqueous media. Zhang et al. [18] fabricated an electrochemical sensor for selective detection of ascorbic acid in the presence of dopamine and uric acid by modifying the glassy carbon electrode with carbon-supported $NiCoO_2$($NiCoO_2$/C) nanoparticles. Wang et al. [19] prepared a palladium nanoparticle/graphene/chitosan/glassy carbon electrode which displayed excellent electrochemical catalytic activities towards ascorbic acid, dopamine and uric acid. But these methods have relatively high detection limit towards ascorbic acid. Fluorometric sensing is a preferable approach for detection of AsA due to its comparatively high selectivity, sensitivity, rapidity, operational simplicity, nondestructive methodology and direct visual perception [20].

In recent years, many fluorescent chemosensors were designed for ascorbic acid. Matsuoka et al. [21] synthesised several fluorescent nitroxide compounds based on naphthalene which were sensitive to ascorbic acid. They also demonstrated the use of Naph-DiPy nitroxide for the measurement of ascorbic acid in the plasma of osteogenic disorder Shionogi rats to confirm the practical usefulness of the fluorophore–nitroxide probe. Zheng et al. [22] demonstrated a fluorescent carbon dot probe, its fluorescence was quenched by Cr(VI) through inner filter effect, but Cr(VI) can be reduced to low valent chromium species easily by ascorbic acid, and then resulting in the elimination of the IFE and recovery of CD fluorescence. Therefore the CD – Cr(VI) mixture could behave as an off-on type fluorescent probe for ascorbic acid. Biqing et al. [23] developed a fluorescent turn-on detection for ascorbic acid based on hyperbranched conjugated polyelectrolyte. The fluorescence was superquenched by Cu^{2+} ions, upon addition of ascorbic acid, the paramagnetic Cu^{2+} ions can be transformed into diamagnetic Cu^{1+} ions, which inhibit the quenching and thus lead to fluorescence enhancement of the hyperbranched conjugate polymer. These fluorescent probes all have sensitive response to ascorbic acid, but none of them were used in intracellular environment.

Fluorophore-linked nitroxides represent examples of such fluorescent sensors. The standard redox potential of nitroxides is high enough (E° = 0.54 V vs SHE for piperidine) to oxidize ascorbic acid which have been the only significant reducing agent studied and used in earlier applications of nitroxide radicals to blood and food product [24]. The fluorescence is intramolecular quenched by the linked nitroxide through electron exchange interactions [25–27], but can be recovered when the nitroxide moiety reacts with certain types of radicals, such as methyl radicals and some reducing agents, such as ascorbic acid [28]. Because of this, fluorophore-linked nitroxides have been used for the determination of reducing agents as well as radicals [29–32]. Nitroxide can shuttle between the nitroxyl radical form, the reduced hydroxylamine, and the oxidized oxoammonium cation form with one- and two-electron transfer reactions [21,26]. When the hydroxyl radical or superoxide reacts with the nitroxyl radical, the oxoammonium cation is produced. Most nitroxides are reduced to the corresponding hydroxylamine by reacting with ascorbic acid [24,33–35]. At the same time, the quenching effect of nitroxides disappeared, thus creating a decay of the ESR signal and enhancement of the fluorescence. This indicates that fluorophore–nitroxide coupled systems can act as promising reagents for the rapid and convenient fluorometric detection of ascorbic acid.

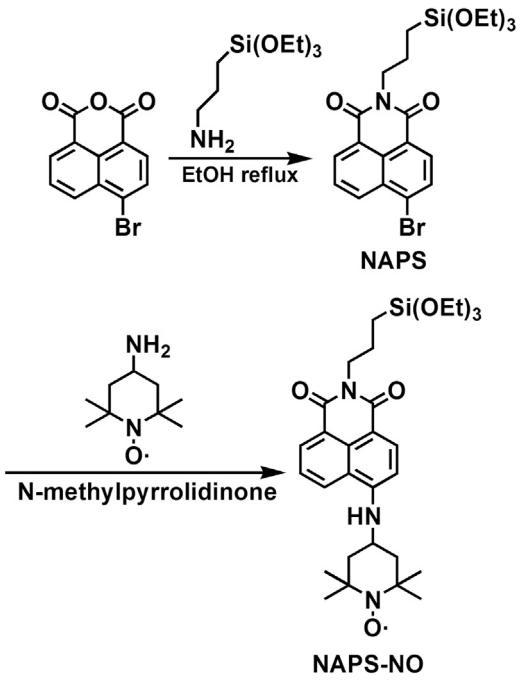
Because of the strong yellow–green fluorescence and good photostability of 1,8-naphthalimide derivatives, they have found wide application in spectral area [36–38]. Here, we describe a new naphthalimide-linked nitroxide probe (NAPS-NO) with high sensitivity and selectivity for the detection of ascorbic acid. According to the previous reports [39,40], the introduction of 3-aminopropyltriethoxysilane can increase the hydrophilicity of the probe, thus resulting in favorable biocompatibility. At the same time, the silane structure can provide a suitable binding site for the further construction of inorganic–organic complex probes, which have the obvious advantage in the efficient separation and analysis of the targets.

A modified previously reported procedure [41,42] was employed for the synthesis of NAPS-NO. It was synthesized from the reaction of 4-Br-1,8-naphthalimide and APTES, followed by reaction with 4-amino-tempo (Scheme 1). The structure of NAPS-NO was characterized by ESR, ESI-MS, FT-IR, and fluorophotometry. The fluorescent intensity of NAPS could be inhibited by the linked nitroxide through electron exchange interactions. When AsA reacted with NAPS-NO, the fluorescent intensity could be recovered. The as-prepared chemosensor exhibited a linear response to AsA concentrations ranging from 80 nM to 50 μM with a detection limit of about 20 nM. The concentration of AsA in human blood serum and HEK 293 cell lysate was determined respectively by the internal standard addition method. Moreover, results of confocal laser scanning microscopy experiments demonstrated that NAPS-NO could be used for monitoring ascorbic acid in living cells.

2. Experimental section

2.1. Apparatus

ESR determination was performed with a Bruker A300 ESR Spectrometer. Fluorescence spectra were measured on a Hitachi F-4500 spectrophotometer equipped with a 1 cm quartz cell. Mass spectra were obtained with MAT-261 spectrometer. FT-IR was recorded on a Thermo Scientific Nicolet 6700 FT-IR spectrometer (Sugar Land, TX, USA). Confocal fluorescence imaging was taken using a Leica TCS SP5 laser scanning confocal microscope (excitation wavelengths 468 nm).



Scheme 1. Synthetic routes to NAPS and NAPS-NO.