Dyes and Pigments xxx (xxxx) xxx

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: http://www.elsevier.com/locate/dyepig

Discriminative detection of cysteine/homocysteine and glutathione in HeLa cells by dual-channel fluorescent probe

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ARTICLE INFO

Keywords:
Fluorescent probe
Green-red signals
Biothiols
Cellular imaging

ABSTRACT

Biothiols such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) play important roles in various physiological and pathological processes. However, it is still a great challenge to selectively detect Cys/Hcy and GSH in biological samples due to the similarity of structures and re-activities of these biothiols. In this paper, a new dual-channel near infrared-7-nitro-1,2,3-benzoxadiazole (**NIR-NBD**) fluorescent probe has been developed for the discriminative detection of Cys/Hcy and GSH. The probe is composed of two functional parts: a near-infrared dye of dicyanoisophorone, as fluorophore precursor, and NBD as both fluorophore precursor and sensing group. It was observed that **NIR-NBD** probe displayed two different fluorescent signals in response to biothiols, green-red for Cys/Hcy and red for GSH. It also showed a near infrared emission (756 nm), low detection limit, fast response, high stability, selectivity and sensitivity. Furthermore, **NIR-NBD** was effectively applied for the discriminative detection of Cys/Hcy and GSH in HeLa cells.

1. Introduction

Cysteine (Cys), homocysteine (Hcy) and glutathione (GSH), the three common biothiols in biosystems, play important roles in various physiological and pathological processes [1,2]. Cys, a proteinogenic amino acid, is a precursor of GSH, and participates in many enzymatic reactions due to its highly reactive sulfhydryl group [3]. The abnormal concentration of Cys can cause many diseases such as retarded growth, skin lesions, liver damage, edema and Parkinson's diseases [4–6]. Hcy, a non-protein amino acid, is a precursor of Cys, and is biosynthesized by transsulfuration or methionine-conserving pathways. Therefore, over-concentration of Hcy has been associated with many diseases, such as cardiovascular disease, neuropsychiatric illness, Alzheimer's disease and cancer [7–9]. GSH, the most abundant intracellular thiols, is a vital endogenous antioxidant, which plays an important role in anti-oxidative stress, signal conduction and regulation of apoptosis [10,11]. Abnormal concentration of GSH has been associated with many diseases, such as leucocyte loss, liver damage, AIDS, Alzheimer's disease and cancer [12–14].

The discriminative detection of different biothiols is of great interest in terms of early diagnostics and pathological analysis of some diseases [15]. Fluorescent probes have attracted great attention due to its fast-response, simple operation, non-invasion, good selectivity and high sensitivity characteristics [16–22]. In the past decade, many fluorescent probes have been developed for the detection of biothiols in living cells [23,24]. However, it is still a great challenge to achieve selective detection of Cys/Hcy and GSH in biological samples due to the similarity of structures and reactivities of these biothiols [25]. Recently, Tu et al. developed a fluorescent probe for the discriminative detection of Cys/Hcy and GSH based on blue-green emission channels from combining NBD (7-nitro-1,2,3-benzoxadiazole) with coumarin dye [26]. However, a disadvantage of the coumarin dye is its relatively short emission wavelength (about 460 nm), which can be exposed to multi-factorial interference by other compounds in biological materials or samples. In order to achieve selective or discriminative detection of Cys/Hcy and GSH, it was of interest to find a near infrared dye with emission wavelength greater than 700 nm.

In this study, we designed and synthesized a new dual-channel, fluorescent **NIR-NBD** probe for the discriminative detection of Cys/Hcy and GSH in HeLa cells. The probe is composed of two functional parts, including near infrared dye of dicyanoisophorone as fluorophore precursor and NBD as both fluorophore precursor and sensing group

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https://doi.org/10.1016/j.dyepig.2020.109015
Received 11 August 2020; Received in revised form 12 November 2020; Accepted 17 November 2020
Available online 25 November 2020