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A novel pyrene based fluorescent probe for selective detection of cysteine in presence of other bio-thiols in living cells

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ABSTRACT

This manuscript reports the synthesis of pyrene-based fluorescent probe (PA-1) containing α,β-unsaturated carbonyl moiety and its application towards the selective and sensitive detection of cysteine (Cys) over other bio-thiols. The probe, 3-(2-hydroxyphenyl)-1-pyrenyl-2-propenone (PA-1) was synthesized through Claisen-Schmidt condensation between acetyl pyrene and salicylaldehyde. The formed product was characterized by ^{1}H NMR, ^{13}C NMR and GC–MS techniques. The probe exhibited absorption maximum at 374 nm and emission maximum at 467 nm ($\lambda_{ex}=342$ nm). The emission intensity of PA-1 was greatly enhanced while adding 2.5 nM Cys. This can be attributed to the nucleophilic attack of Cys to the α,β-unsaturated ketone resulting in switching off, intramolecular charge transfer (ICT) from pyrene moiety to the phenolic nucleus. This was confirmed by DFT measurements. The PA-1 exhibited an excellent selectivity towards the determination of 40 nM cys in the presence of 250,000-fold higher concentration of common interferents. The emission intensity was linearly increased and the limit of detection was found to be 10 pM/L (S/N=3). Interestingly, the response of the PA-1 towards Cys is less than 1 min. The confocal laser scanning micrographs of HeLa cells confirmed the cell permeability of the PA-1 and its ability to selectively detect Cys in living cells. In addition, the proposed probe was successfully applied for the determination of Cys in blood serum samples.

1. Introduction

Low molecular weight thiols such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) are abundant in organism and play pivotal role in variety of biological processes including reversible redox homeostatsis, cellular functions (Chen et al., 2010; Kim et al., 2003; Homocysteine in Health and Disease; Carmel and Jacobsen, 2001). Cys in particular facilitates crosslinking of biomacromolecules through disulphide bonds which signifies the function and structure of proteins (Voet et al., 1995). It is also associated with growth and delay of senility of cells and tissues in living systems (Kim et al., 2009; Wright et al., 2006; Refsum et al., 2004; Keelan et al., 2001). Further, it stands as a precursor for glutathione, Co-Enzyme A, taurine and also acts as a source of sulphide in Fe-S clusters (Reddie et al., 2008; Weerapana et al., 2010; Lill et al., 2006). However, elevated levels of Cys are associated with several problems including cardiovascular complications (Refsum et al., 1998; van Meurs et al., 2004), neurotoxicity (Klingman et al., 1989), Parkinson's disease (Heafield et al., 1990), Alzheimer's disease (Heafield et al., 1990), adverse pregnancy outcomes (El-Khairy et al., 2003) and rheumatoid arthritis (Bradley et al., 1994). On the other hand, low levels of Cys are responsible for retarded growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, weakness, hematopoiesis reduction, leucocyte loss, psoriasis and skin lesion development (Shahrokhian., 2001; Wu et al., 2008). Therefore, selective and sensitive detection of Cys under physiological condition would be of significant interest in diagnostic applications. Several techniques have been employed to determine Cys such as HPLC (Ogasawara et al., 2007), electrochemical assay (Lima et al., 2008), capillary electrophoresis (Chen et al., 2004), UV–vis spectroscopy (Wang et al., 2004; Wang et al., 2005), FT-IR spectroscopy (Sato et al., 2005), Mass spectrometry (Rafii et al., 2007), Gas chromatography and Immunoassay (Refsum et al., 2004). However, most of these techniques have several drawbacks which include more time consumption, operational inconveniences, high cost besides poor sensitivity and selectivity. On the other hand, the fluorescence based detection (Yi et al., 2009; Jung et al., 2011; Shi et al., 2012; McMahon et al., 2012) is exceptional and stands out as a desirable method of choice because of its simplicity (Quang et al., 2007; de Silva et al., 1997), low cost, high sensitivity, selectivity and versatility (Yue et al., 2011).

Innate fluorescent probes for thiols based on variety of sensing

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mechanisms have been designed (Zhou 2012; Chen et al., 2012) including Michael addition (Wang et al., 2012, Zhou et al. 2012, Zhou and Yoon, 2012, Jung et al., 2012., Yang et al., 2013, Wang et al., 2014), cyclization with aldehydes (Zhang et al., 2009; Lin et al., 2008; Kim et al., 2008, Duan et al., 2008, Li et al., 2011, Das et al., 2012), cleavage reaction of thiols (Cao et al., 2011; Niu et al., 2013, Wei et al., 2013), and metal complex displacement co-ordination (Shao et al., 2006). However, distinguishing Cys from Hcy and GSH is seriously hampered owing to their structural similarity (Shao et al., 2011). GSH being the most abundant intracellular thiol, development of fluorescent probe for selective detection of Cys over GSH will be of prime significance. Thus, the present study aims to synthesize pyrene derivative as a fluorescence probe for the selective detection of Cys. The probe 3-(2-hydroxy-phenyl)-1-pyren-1yl-propenone (PA-1) was synthesized in a single step with high yield. The probe has an unsaturated ketone group, which upon Michael addition of the thiol group of Cys, displayed enhanced fluorescence. We speculate that the addition of thiols hampers the extended conjugation which results in turning off the ICT process, accompanied by an increase in the fluorescence intensity. Further, its intracellular Cys selectivity was studied by fluorescence imaging in living cells. In order to demonstrate the practical application of the proposed sensor, the assessment of Cys was carried out in human blood serum samples.

2. Experimental

2.1. Chemical reagents

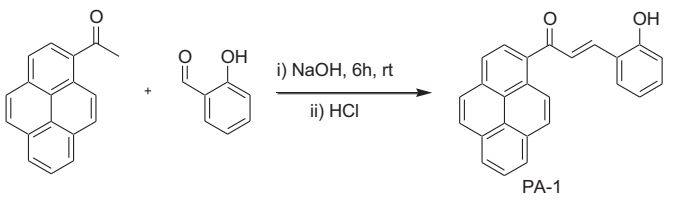
1-Acetyl pyrene, salicylaldehyde and amino acids were purchased from Sigma-Aldrich. All other chemicals used in this investigation were of analytical grade and used directly without further purification. Deionized water was used throughout the experiments.

2.2. Instrumentation

UV–visible spectra were measured by JASCO V-550 UV–visible spectrophotometer. Fluorescence spectral measurements were performed on JASCO-spectrofluorimeter FP-8500. The NMR spectra were recorded using Bruker 300 MHz spectrometer, with TMS as internal standard. The Mass spectrum was obtained on a GC–MS-QP2010 Plus, SHIMADZU-Electron Ionization instrument. Cell imaging was performed with a Ziess confocal fluorescence microscope.

2.3. Synthesis of compound PA-1

An ethanolic solution of acetyl pyrene (2 mmol) was added with salicylaldehyde (2 mmol). The mixture was stirred in the presence of catalytic amount of NaOH for 6 h at room temperature. After the completion of the reaction, the reaction mixture was neutralized with dilute HCl resulting in the formation of precipitate (Scheme 1). The precipitate was filtered off, washed with ethanol for several times and dried to give the desired product as orange solid (85% yield). ^{1}H NMR (300 MHz, $CDCl_3$) (Fig. S1): δ 10.7(1H, S), 9.108–9.058(1H, d, 15 Hz), 8.580–8.549(1H, d, 9.3 Hz), 8.427–8.400(1H, d, 8.1Hz), 8.309–.025(8H,m), 7.939–7.888(1H, d, 15 Hz), 7.562–7.553–7.510(1H, t, 2.7 Hz), 7.092–7.064(1H, d, 8.4 Hz), 7.014–6.988–6.963(1H, t, 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) (Fig. S2): δ193.4, 163.7, 142.0, 136.3, 133.2, 131.2, 130.6, 129.6, 129.0, 128.9, 128.3, 127.3, 126.4, 126.2, 126.0, 125.05, 124.5, 4.5, 124.2, 122.4, 121.8, 120.1, 118.9, 118. 6. MS (ESI) m/z (Fig. S3): 348.



Scheme 1. Synthetic route to probe PA-1.

2.4. Spectroscopy measurements

The stock solution of 10^{-4} M probe PA-1 was prepared in DMSO. From the stock solution 10^{-5} M solution was obtained by diluting the stock using phosphate buffered saline (PBS, pH 7.4). The amino acids cysteine (Cys), homocysteine (Hcy), cystine, tryptophan (Trp), asparagine (Asn), lysine (Lys), leucine (Leu), isoleucine (Ile), methionine (Met), threonine (Thr), tyrosine (Tyr), valine (Val), aspartic acid (Asp), alanine (Ala), serine (Ser), glutamine (Gln), glutamic acid (Glu), glycine (Gly), phenylalanine (Phe), Histidine (His), proline (Pro), arginine (Arg) and glutathione (GSH), stocks were prepared in deionized water with a concentration of 10^{-2} M. For fluorescence measurements, excitation wavelength was fixed at 342 nm with slit width at 2.5 nm and emission slit width at 2.5 nm. The fluorescence and UV-Visible titration were performed using 10 μM probe PA-1 in buffer solution (pH 7.4, PBS) with varying concentration of Cys in nanomolar concentrations.

2.5. DFT calculations

Geometry optimizations were performed using density functional theory method at B3LYP-6-311G (d, p) level of theory in Gaussian 09 program package.

2.6. Cell culture and confocal microscopy study with probe PA-1

HeLa cells were grown in modified Eagle's medium supplemented with 10% FBS (fetal bovine serum) at 37 °C. They were incubated with probe PA-1 (10 μM) containing PBS (pH=7.54) for 30 min. To remove the excess of the probe in the cells, it was washed thrice with PBS and then imaged through confocal fluorescence microscope. In another set of experiments, HeLa cells were further incubated with N-ethylmaleimide (NEM) containing media for 1 h and washed with PBS. The NEM treated cells were again incubated with PA-1 (10 μM) for 10 min. The fluorescence image and bright field images of the probe PA-1 and NEM treated cells were recorded. To study the cysteine selectivity in HeLa cells, the cells were cultured in the well plate. It was incubated with cysteine (100 μM) at 37 °C in PBS for 30 min. The Cys treated cells were again incubated with probe PA-1 for 10 min and were imaged through confocal microscopy.

3. Results and discussion

3.1. Spectral characterization of probe (PA-1)

The probe PA-1 was synthesized by Claisen-Schmidt condensation reaction of salicylaldehyde with 1-acetyl pyrene. It was characterized by different spectral techniques.

The absorption spectrum of PA-1 (10 μM, pH=7.4, PBS) shows bands at 288 nm, 374 nm, 401 nm and a shoulder band at 464 nm corresponding to π-π* transitions and fluorescence spectra with monomer bands at 380 and 396 nm and emission maximum at 467 nm while exciting at 342 nm. It exhibited weaker monomer and excimer emission than pyrene due to ICT process.