Electrochemical latent redox ratiometric probes for real-time tracking and quantification of endogenous hydrogen sulfide production in living cells

Kesavan Manibalana, Veerappan Mania, Pu-Chieh Changb, Chih-Hung Huanga,b, Sheng-Tung Huanga,b,⁎, Kasper Marchlewiczc,d, Suresh Neethirajanda

ARTICLE INFO

Keywords:
Hydrogen sulfide
Gasotransmitter
Real-time quantification
Electrochemical redox probes
Living cells
Whole blood analysis

ABSTRACT

Hydrogen sulfide (H2S) was discovered as a third gasotransmitter in biological systems and recent years have seen a growing interest to understand its physiological and pathological functions. However, one major limiting factor is the lack of robust sensors to quantitatively track its production in real-time. We described a facile electrochemical assay based on latent redox probe approach for highly specific and sensitive quantification in living cells. Two chemical probes, Azido Benzyl ferrocene carbamate (ABFC) and N-alkyl Azido Benzyl ferrocene carbamate (NABFC) composed of azide trigger group were designed. H2S molecules specifically triggered the release of reporters from probes and the current response was monitored using graphene oxide film modified electrode as transducer. The detection limits are 0.32 µM (ABFC) and 0.076 µM (NABFC) which are comparable to those of current sensitive methods. The probes are successful in the determination of H2S spiked in whole human blood, fetal bovine serum, and E. coli. The continuous monitoring and quantification of endogenous H2S production in E. coli were successfully accomplished. This work lays first step towards real-time electrochemical quantification of endogenous H2S in living cells, thus hold great promise in the analytical aspects of H2S.

1. Introduction

As a gasotransmitter, H2S plays significant roles in biological systems (Li et al., 2011). In fact, the focus of H2S research is now completely diverted to explore its biological roles and therapeutic potential, while its toxicology research has been virtually halted. Its substantial role in regulating cardiovascular, neuronal and immune systems was now uncovered (Yu et al., 2013), while its role as modulator in respiratory, cardiovascular, neuronal and immune systems were now uncovered (Yu et al., 2013). H2S is enzymatically produced in cells and tissues of mammals using enzymes such cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptoptyruvate sulfurtransferase (3MST). Studies revealed that bacterial genomes have orthologs of mammalian CBS, CSE, or 3MST and many similarities between mammalian gasotransmitter and that of bacterial. Bacteria produce H2S endogenously as a universal defense against antibiotics (Shatalin et al., 2011). On the other hand, its abnormal levels are related to several diseases such as, chronic kidney disease, liver cirrhosis, and Down’s syndrome (Hartle and Pluth, 2016). Despite the fact that H2S is linked to various physiological and pathological processes, a large part of the underlying molecular mechanisms remain still unknown (Hartle and Pluth, 2016; Zheng et al., 2016). The lack of reliable sensors that can track and quantify endogenous H2S in real-time is key limiting step in understanding its functions. As the amount of endogenous H2S is normally in low micromolar range (10–100 µM), developing a sensor with such low sensitivity is challenging (Peng et al., 2011). The presence of other sulfur species such as biothiols in biological medium are imposing interferences issues that has to be tackled. Therefore, the development of robust sensor with high selectivity and sensitivity is of current interest in analytical aspects of H2S.

The advantages and disadvantages of the previously developed H2S sensors have been summarized in Table S1. Most of the conventional methods such as, methylene blue test, spectrophotometry and chromatography are not viable for real-time quantification (Olson et al., 2014; Pandey et al., 2012). Over the past few years, fluorometric probes have garnered considerable attention in H2S monitoring (Lippert et al., 2011; Peng et al., 2011; Yu et al., 2014); however, Papapetropoulos

⁎ Corresponding author at: Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, Taipei 10608, Taiwan, ROC.
E-mail address: ws75624@ntut.edu.tw (S.-T. Huang).

http://dx.doi.org/10.1016/j.bios.2017.05.006
Received 22 March 2017; Received in revised form 2 May 2017; Accepted 3 May 2017
Available online 4 May 2017
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et al., had reported that a common problem of most fluorescent probes is their inability to measure endogenous H$_2$S in biological samples (Papapetropoulos et al., 2015). On the other hand, electroanalytical methods are simple, low-cost, portable, easy-to-use and requires minimal samples (Wang, 2006). The high sensitivity of electrochemical transducers together with their amenability to being miniaturized has made electrochemical sensors attractive in analytical science. Ion selective electrodes (ISEs, Ag/Ag$_2$S) are popular for H$_2$S detections; however, they require alkaline environment, encounter poor selectivity, exhibit narrow working range, and need every day electrode reconditioning and calibration and hence their use in biological samples is questionable (Olson et al., 2014; Xu et al., 2016).

Developing and exploring selective electrochemical latent redox probes for biologically important analytes is an ongoing research interest of our research group (Mani et al., 2015; Manibalan et al., 2016). Such chemical probes equipped with unique triggering groups and masked electroactive reporters. These probes selectively interact with the analyte of interest via user-designated biochemical events to unmask their inherent reporter signals (Manibalan et al., 2015). Here, we have designed two electrochemical probes, Azido Benzyl ferrocene carbamate (ABFC) (3a) and N-alkyl Azido Benzyl ferrocene carbamate (NABFC) (3b) by incorporating the concept of latent redox probe’ into their molecular design and exploited them in real-time tracking and quantification of H$_2$S in living cells (Scheme 1). The probes were composed of azide trigger groups that attached to the latent redox probes bridged via self-immolative carbamate linkage. H$_2$S molecules triggered the selective reduction of azide moieties attached on these probes which initiated a self-immolative 1,6-elimination reaction, as a result the reporter was ejected out (Gronowitz et al., 1975). The as-released reporter displayed its signature azide trigger fragments, which are completely away from biological interference region. Most importantly, we confined an accurate quantification platform, which can be used to track and quantify the amount of H$_2$S in living bacteria cells. To the best of our knowledge, this is the first report towards real-time electrochemical quantification of endogenously produced H$_2$S in living cells.

2. Experimental

2.1. Materials and methods

All the chemicals, reagents, and solvents were purchased from Sigma and used without further purification. Human blood sample was collected from healthy human and bovine serum isolated from cows was used in real sample analysis. The synthetic details and spectra for chemical structure characterizations of synthetic intermediates and 3a and 3b are given in Supplementary material. Bacterial cell counting or enumeration was performed using a spectrophotometer equipped with a xenon lamp and 1.0 cm quartz cells. NaHS was dissolved in buffer to in-situ production of H$_2$S. All the electrochemical experiments were acquired by CHI 660D electrochemical workstation at ambient conditions. The three electrode cell comprising GO film modified glassy carbon electrode (GCE) as a working electrode (area=0.071 cm$^2$), platinum-wire as a counter electrode, and saturated Ag|AgCl (saturated KCl) as a reference electrode was adopted to carry out electrochemical experiments.

2.2. Preparation of modified electrode and stock solutions

GO aqueous solution (1 mg mL$^{-1}$) was prepared by following our previous report (Manibalan et al., 2015). Then, 5 μL GO dispersion was drop-cast on pre-cleaned GCE and dried to obtain GO/GCE. 100 mM phosphate buffer saline (PBS, pH 7.4) was prepared using monosodium phosphate, sodium phosphate, NaCl, and KCl. 10 mM stock solutions of ABFC and NABFC were prepared in DMSO. Maximum H$_2$S sensing performances were attained when 50/50% and 20/80% volume ratios of DMSO/PBS, pH 7.4 were used as supporting electrolytes for ABFC and NABFC, respectively (Fig. S1). All the measurements had been repeated minimum of three times and their average was used to prepare figures and plots.

2.3. Assay procedure

The H$_2$S assay system consists of three steps; (1) the detection mixture was prepared as 1 mL aliquots containing 50 μL of ABFC (10 mM), 0.45 mL of DMSO and NaHS in 0.5 mL PBS buffer (100 mM). For NABFC incorporated assays, the volume ratio of DMSO and PBS was maintained to be 20/80%. (2) Each detection mixture was incubated at 40 °C for 30 min (3) the mixture was transferred to an electrochemical cell and analyzed using GO/GCE, wherein the release of reporter (FA or NFA) was monitored. All assays were performed in triplicate, and the results reported are the average of at least three experiments.

2.4. Procedures for bacteria cell culture and endogenous H$_2$S production

Escherichia coli strain MG1655 were grown overnight on a lysogenic broth (LB) agar plate at 80 °C. Subsequently, a single colony of grown E. coli was inoculated into 10 mL of LB medium and the

Scheme 1. represents the synthesis of ABFC and NABFC probes and H$_2$S-catalyzed breakdown of probes with subsequent release of reporter.
whole bacterial cell culture was incubated overnight at 37 °C with constant shaking. The cell counts were estimated by spectrophotometer analysis (OD600=0.65 at 37 °C) wherein the bacterial solution was diluted in fresh LB medium with 1:9 ratio. The amount of bacterial cells play crucial role in controlling H2S production. Microbial growth optical density value can be directly converted to bacterial cell count and the calculated number of bacterial count was ~ 2.1×10^9 cells/mL.

The assay procedure for the detection of endogenous H2S production form E. coli involved the following 4 steps: (1) cultivation of E. coli cells, (2) incubating the cells with either stimulant (1 mM cysteine) or inhibitor (10 mM aspartate) for 30 min at 37 °C (time varied in the case of tracking study), (3) addition of 500 µM NABFC and subsequent incubation for 30 min at 40 °C, and (4) performing DPV experiments and measuring the current response of NFA. All assays were performed in triplicate, and the results reported are the average of at least three experiments.

3. Results and discussions

3.1. Design and synthesis of ABFC and NABFC

The synthetic route of ABFC and NABFC is outlined in Scheme 1. The two known synthons, ferrocenyl azide (Goggins et al., 2015) and azidobenzyl alcohol (Udumula et al., 2016) were underwent a Curtius rearrangement to furnish ABFC with 80% yield. NABFC was obtained by direct N-alkylation of ABFC with 1-Bromo-2-(2-methoxyethoxy) ethane and the yield was 58%. The successful formations of the compounds were confirmed by NMR, and Mass (ESI). The only chemical structure difference between ABFC and NABFC is the additional ethylene glycol unit on NABFC in order to improve its aqueous solubility. The syntheses of both probes are facile and the probes are stable at ambient conditions.

3.2. H2S sensing via latent redox probe approach: Proof-of-concept

The electrochemical behavior of ABFC and its interaction with H2S was investigated by cyclic voltammetry (Fig. 1A) at GO/GCE. The optimized parameters for cyclic voltamogram (CV): potential range=–0.3 V to +0.5 V and scan rate=0.05 V s⁻¹. In the absence of H2S, the voltammogram of ABFC displayed a sharp redox couple at formal potential (E°) of +0.251 V (denoted as RCABFC) (a, Fig. 1A). The redox couple is related to one electron-one proton reversible process between ferrocene and ferrocenium cation. In presence of H2S, the peak currents of RCABFC were considerably decreased, but a new redox couple was appeared at E°' of +0.013 V (denoted as RCFA) (curve b, Fig. 1A). The E°' of newly appeared redox couple is consistent with that of pristine FA (inset to Fig. 1A). From these results, we inferred that H2S triggered the reduction of azide moiety attached on the ABFC, which initiated a self-immolative disassembly with concomitant release of FA, thus validated the proof-of-concept of our approach. The effect of scan rate on the electrochemical behavior of FA was studied (Fig. S2). Both the anodic (Ipa) and cathodic (Ipc) peak currents were increased as the scan rate increased. A plot between currents and square root of scan rates showed good linearity indicating diffusion controlled process of FA (inset to Fig. S2).

Furthermore, two more probes without the -N3 moiety, BFC (Sagi et al., 2006) and FCCD, (Manibalan et al., 2016) have been prepared and investigated. Different concentrations of NaHS were incubated with BFC (Fig. S3A) and FCCD (Fig. S3B) under optimized conditions and DPVs were performed. There were no signals generated at –0.06 V, thus the attachment of azide group on the probe is necessary in order to initiate its self-immolative disassembly. In addition, the oxidation current of FA was not altered when it interacted with different amounts of H2S (Fig. S3C). Thus, our method followed controlled pathway with highly specific trigger-analyte interaction chemistry.

3.3. Determination of H2S via Differential pulse voltammetry (DPV)

DPV method was adopted in order to frame a sensitive assay. The optimized DPV parameters: amplitude =0.05 V, sampling width =0.0167 s and pulse period =0.5 s Fig. 1B displayed the DPV curves recorded using GO/GCE towards different concentrations of NaHS incubated with 50 µM ABFC. As the concentration of H2S increased, the Ipa of RCABFC was linearly decreased, while that of RCFA was linearly increased. In other words, as the concentration of H2S increases, the amount of ABFC gradually decreased, while that of FA increased in the reaction mixture. The concentration of H2S can be correlated to either RCFA or RCABFC. Thus, two different electrochemical channels, i.e OFF/ON (RCFA) and ON/OFF (RCABFC) were activated simultaneously in response to H2S. Since the Ipa of RCFA was observed at biological interference free potential region, we decided to study this channel. Two linear ranges, one at low concentration range (2–10 μM with sensitivity of 0.2098 μAμM cm⁻², Fig. S4) and another at high concentration range (10–100 μM with sensitivity of 0.1338 μA μM cm⁻², Fig. 1B inset) were obtained. The limit of detection (LOD) was calculated to be 0.32 µM (10.6 ppb). The sensitivity obtained at high concentration range was smaller than that at low concentration range, which might be due to the occurrence of substrate inhibition effect at higher concentration range (Lyon and Stevenson, 2006).

In order to investigate the role of GO on the sensing performance, DPVs of unmodified GCE and GO/GCE were performed in 50 µM
ABFC incubated with 200 μM NaHS under optimized conditions (Fig. S5A). In comparison with unmodified GCE, GO/GCE had shown two-fold enhancement in current response. In addition, the overpotential of the FA oxidation peak was considerably lowered at GO/GCE. Besides, the electrochemical active areas of unmodified GCE and GO/GCE were calculated to be 0.037 cm$^{-2}$ and 0.076 cm$^{-2}$. The results manifested to the enhanced electrocatalysis and fast electron transfer of FA at GO/GCE over bare GCE which must be attributed to the outstanding properties of GO (large surface area, presence of abundant oxygen functionalities, rich edge density and defects) (Dreyer et al., 2010). The SEM image of GO given in Supporting information displayed the characteristic wrinkled thin sheet like morphology of GO (Fig. S6).

3.4. NABFC vs. ABFC: comparative discussion

The H$_2$S sensing mechanistic pathway of NABFC was similar to that of ABFC and hence all the results are similar which were given in Supporting information (Fig. S5B, S7, and S8). Interestingly, NABFC based assay offered additional advantages over ABFC. The electrochemical parameters of both the probes and their corresponding reporters were given as Table S2. The attachment of non-ionic substituents with multiple polar groups, such as poly(ethylene glycol) is a powerful approach to enhance water solubility of the organic compounds (Sun et al., 2016). Here, NABFC was constructed via incorporating diethylene glycol group at carbamate NH-position of the ABFC; as a result, NABFC requires only 20% DMSO, whereas ABFC requires 50% DMSO (Fig. S1). Under optimized experimental condition, the oxidation potential of NFA was −0.10 V, while that of FA was −0.06 V. The overpotential required to oxidize NFA was 40 mV lowered than that of FA which indicating that NABFC based assay is advantageous in biological samples. Using NABFC, the linear ranges were 0.4–10 μM (with sensitivity of 0.2656 μAμM cm$^{-2}$) and 10–100 μM (with sensitivity of 0.0662 μAμM cm$^{-2}$) (Fig. S8). The LOD was calculated to be 0.076 μM (2.54 ppb). Remarkably, the LOD of NABFC was 4-fold higher than that of ABFC. Although, the original purpose of NABFC design was to enable aqueous dispersion, interestingly our results revealed that NABFC has significant impact on sensor performance as well.

3.5. Selectivity

In order to investigate selectivity of our assay, the likely interfering compounds, such as, H$_2$O$_2$, dopamine, ascorbic acid, cysteine, glutathione, homocysteine, uric acid, Na$_2$S$_2$O$_4$, Na$_2$S$_2$O$_3$, Na$_2$S$_2$O$_5$ and Na$_2$SO$_3$ were incubated with ABFC and their DPV responses were monitored (Fig. 2). Neither biological compounds nor anionic sulfur species had produced electrochemical signal at −0.06 V; only NaHS and Na$_2$S able to produce signal. The result clearly revealed that the signal generated by this approach is highly specific for in-situ produced H$_2$S (The conceptual idea is based on the special chemical affinity between azide and H$_2$S).

Generally, H$_2$S assay encounters interferences from biothiols because their concentrations in biological samples are much higher than H$_2$S. Impressively, the interferences of biothiols can be completely circumvented in our method. In contrary, ISEs were unable to discriminate cysteine and H$_2$S in biological samples (Olson et al., 2014). H$_2$S in solution exists in the equilibrium: H$_2$S ⇌ HS$^-$, pKa$1=6.9$; SH$^−$ ⇌ S$^2$− pKa$2 > 12$. ISEs measure S$^2$− form of H$_2$S that requires an alkaline environment (i.e., pKa$2 > 12$) to favor the S$^2$− equilibrium (Kolluru et al., 2013). However, at alkaline environment cysteine in protein, including albumin and biothiols did liberate S$^2$− that will also be counted as H$_2$S by ISEs causing false positive results (Olson et al., 2014).

3.6. Real sample analysis in human blood, fetal bovine serum, LB medium and bacteria

Practical utility of our method was verified in whole blood, fetal bovine serum (FBS), LB medium and E. coli. Known amounts of NaHS were spiked and the release of H$_2$S was detected. The found and recoveries were calculated by standard addition method and the obtained results are in the satisfactory range, 97–103% (Table 1) and hence the method can be applicable in the determination of biological H$_2$S. Especially, our method was able to quantify the amount of spiked H$_2$S in whole human blood sample without any pre-treatments.

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added/μM</th>
<th>Found/μM</th>
<th>Recovery/%</th>
<th>RSD/%</th>
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<td>9.87</td>
<td>97.5</td>
<td>3.42</td>
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<tr>
<td></td>
<td>20</td>
<td>19.69</td>
<td>97.2</td>
<td>3.43</td>
</tr>
<tr>
<td>LB medium</td>
<td>10</td>
<td>10.13</td>
<td>103.3</td>
<td>2.83</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.12</td>
<td>97.6</td>
<td>3.18</td>
</tr>
<tr>
<td>FBS</td>
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<td>5.16</td>
<td>103.8</td>
<td>3.13</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.13</td>
<td>103.3</td>
<td>2.83</td>
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<tr>
<td>Bacteria</td>
<td>10</td>
<td>10.18</td>
<td>104.8</td>
<td>2.59</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.12</td>
<td>97.6</td>
<td>3.18</td>
</tr>
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</table>

* RSD= Relative standard deviation of three individual measurements
release from E. coli 500 µM NABFC. 7.4, at 37 °C. DPVs were obtained at GO/GCE in 20/80 DMSO/PBS, pH 7.4 containing presence of 10 mM aspartate (b) and stimulated without cysteine (c) in LB Medium, pH 7.4. (A) Bar graph: (a) E. coli +500 µM of NABFC, (b) E. coli +1 mM cysteine +500 µM NABFC, (c) E. coli +1 mM cysteine +500 µM NABFC +10 mM aspartate and (d) 1 mM cysteine +500 µM NABFC in LB Medium, pH 7.4, at 37 °C for 30 min. Inset shows the corresponding DPVs obtained at GO/GCE in supporting electrolyte 20/80 DMSO/PBS buffer pH 7.4. (B) Normalized current responses vs. [Endogenous H2S] vs. time; H2S release from E. coli stimulated by 1 mM cysteine (a), stimulated by 1 mM cysteine in presence of 10 mM aspartate (b) and stimulated without cysteine (c) in LB Medium, pH 7.4, at 37 °C. DPVs were obtained at GO/GCE in 20/80 DMSO/PBS, pH 7.4 containing 500 µM NABFC.

3.7. Real-time monitoring of endogenously produced H2S in bacterial cells

In bacteria, H2S can be produced endogenously by cysteine (stimulant) via 3-mercaptoacrylic acid sulfur transferase (3-MST) catalyzed chemical reaction (Madden et al., 2012). At the same time, H2S production can be inhibited by aspartate, a well-known 3-MST inhibitor (Eng et al., 1991). Exploiting these concepts into our method, we have demonstrated a real-time platform to track the endogenously produced H2S by E. coli using NABFC.

The current responses of endogenously released H2S from E. coli are shown in Fig. 3A. In the absence of cysteine, the DPV of E. coli did not show any signal (curve a); however, it displayed sharp signal at ~0.10 V (i.e. NFA) in presence of cysteine (b). As the cysteine molecules stimulated the production of H2S, the bacterial cells started to release H2S and that was detected as DPV signal. The DPV curve of E. coli incubated with mixture of 1 mM cysteine and 10 mM aspartate displayed decreased peak current indicated that the presence of aspartate suppressed the H2S production (curve c).

Next, the NABFC incorporated assay was employed to track the endogenous H2S level with respect to stimulation time. 1 mM cysteine was added to E. coli and the mixture was separated into aliquots and each aliquot was incubated for different time intervals at 37 °C. Subsequently, 500 µM NABFC was added and incubated (30 min at 40 °C) and then DPVs were performed. The peak current of NFA was monitored and plotted against the stimulation time (Fig. 3B, curve a). As shown in the plot, the response current was significantly increased from 0 to 30 min indicating linear increase in H2S production during this time period. Afterwards, the current followed decreased trend from 30 to 180 min and fell up to baseline at 180 min suggesting that the as-produced H2S were drained and no longer available to produce signal. No obvious current changes were observed from 180 to 240 min. The amount of H2S was directly quantified by matching the H2S tracking profile (Fig. 3B) with the calibration plot derived from the direct H2S detection done in E. coli via spiking method (Fig. S9). For instance, the quantified amounts of as-produced endogenous H2S were of 31.82 µM, 54.79 µM, and 51.41 µM corresponding to the stimulation time of 15, 30 and 60 min. Clearly, the experimental evidences revealed that our assay had accomplished real-time quantification of endogenously produced H2S in living cells. Notably, H2S production was maximum at 30 min and this result is consistent with recently reported fluorogenic assay performed mice cell (Zhang et al., 2017). After 240 min, H2S production was stimulated again by spiking 1 mM cysteine in reaction mixture followed by incubation. Interestingly, the current was steadily increased again for next 30 min (240–270 min) and dropped after 60 min. The amount of current observed during second stimulation was comparatively lower than that of first. The possible explanations for this result are currently under investigation.

The inhibition of H2S production was performed by adding mixture of cysteine (1 mM) and aspartate (10 mM) to E. coli. Then, the mixture was incubated and studied by DPV. The current responses were plotted against the corresponding inhibition time and concentration of H2S (Fig. 3B, curve b). The H2S release trend is similar to the stimulation plot; nevertheless, the current values obtained from inhibition plot (curve b) were lower (~ 30%) than that obtained for stimulation without inhibition (curve a). In addition to H2S quantification, the assay can be applicable to study the inhibitory effect of aspartate towards 3-MST activity.

Our work improves many of the limitations of current diagnostic tools for sensing H2S without compromising the sensitivity and selectivity (Table S1). Many fluorescent methods suffer the limitation of sensing the analytes in cloudy and colored samples; thus, pretreatment or serial dilutions of the sample to minimize interferences are required, and this compromises the sensitivity of the detection methods. The other disadvantages of fluorescence methods are their incompatibility in turbid samples (particularly in biological samples), low quantum yield, pre-sampling steps and auto-fluorescence. More recently, Zhang et al. (2017) reported near-infrared fluorescence probe that can visualize the H2S level in colorectal cancer cells in mice; however, the probe synthesis is complex. Although, researchers reported the endogenous H2S detection by fluorescence methods, they are unable to achieve the real-time quantification in living cells (Papapetropoulos et al., 2015). Moreover, the microscopic instruments are expensive, bulky and laborious. However, the current work described a simple method based on easy to operate electrochemical device and incorporated with small molecules synthesized by straightforward method. The approach is sensitive and selective to detect H2S under physiological condition, which many of the current methods cannot accomplish. The signal response is in the region of minimum interferences and the analysis is accurate in non-transparent solutions (i.e., whole blood) without any pre-treatment. Besides, the method has advantages over ISE, i.e., our methods works at physiological pH and did not interferences by biothiols.

4. Conclusions

We set up a novel electrochemical assay platform by employing the robust latent redox probes NABFC and ABFC to continuously track the H2S released by living cells (E. coli) in real-time. The probe synthesis is straightforward. The probe NABFC offered additional advantages over ABFC such as improved hydrophilicity, reporter with less overpoten-
tial, enhanced signal sensitivity and lower detection limit. Our method eliminates the interferences from biothiols and other sulfur species. The method can be applied to the real-time detection of H$_2$S in whole blood, fetal bovine serum and bacteria without any pre-sampling procedures. Importantly, we established a signal vs. time vs. concentration plot, which can be used to quantify the amount of H$_2$S release at any given time. The future work will be focused on the fabrication of stretchable electrochemical sensor devices for H$_2$S in mammalian cells.

Acknowledgements

This work was supported by the Ministry of Science and Technology, Taiwan (MOST 105–2113-M-027-003; MOST 105–2622-M-027-001-CC3).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2017.05.006.

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