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# Sensitive detection and quantification of hydrogen sulfide as a gasotransmitter by combining monobromobimane-based derivatization to triple quadrupole LC/MS/MS

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## Overview

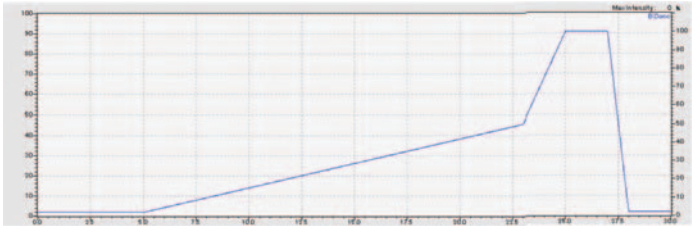
Hydrogen sulfide ($H_2S$) regulates various cellular functions as a gasotransmitter and reliable and accurate quantification of $H_2S$ concentration in biological samples is required. Here the quantitative analysis of endogenous $H_2S$ level in tissue samples was performed by coupling the monobromobimane-based assay to triple quadrupole LC/MS/MS. Furthermore, this method has an advantage of the measurement at neutral pH to quantify biologically free-gaseous $H_2S$ not acid-labile $H_2S$ and this approach is expected to be a useful method to quantify free $H_2S$ level in tissue samples.

## Introduction

Hydrogen sulfide ($H_2S$) is known to be a third gaseous mediator and also to have important roles on a vasodilation in both the peripheral and cerebral circulation. To elucidate various signal transduction pathways elicited by $H_2S$, reliable and accurate measurement of $H_2S$ concentration in biological samples becomes increasingly significant. Until now, head space gas analysis using gas chromatography and measurement by polarographic sensor have been reported to quantify $H_2S$ concentration in biological samples. However these approaches often yielded a higher value resulting from acid-labile $H_2S$ not from biologically free-gaseous $H_2S$. Here we developed sensitive quantitative analysis of $H_2S$ in biological samples, mouse brain tissues by combining a monobromobimane-based derivatization at a neutral pH to SRM (selected reaction monitoring) using triple quadrupole LC/MS/MS.

## Methods

Thiol-specific derivatization agent monobromobimane was used to quantify the endogenous $H_2S$ in brain tissues. Frozen brains from 12-d-old mice were placed in two volumes of ice-cold 5 mM monobromobimane (mBBr) in 10 mM Tris-HCl (pH 7.5) and then homogenized. Methanol was added to precipitate proteins. The mixture was vortexed and centrifuged. A supernatant was desalted by a solid-phase extraction and filtrated through a 5-kDa cutoff filter. An aliquot of 10 µL was analyzed to quantify sulfide-dibimane (SDB), monobromobimane-based derivatives of $H_2S$ by LC/MS/MS instrument, Nexera UHPLC system and LCMS-8030 triple quadrupole mass spectrometer. SDB was eluted from an ODS column (150 mmL. X 2.1 mmI.D. 1.7 µm particle size) with a gradient of acetonitrile, detected in negative mode ESI / SRM mode.



| | |
|---|---|
| Column | : commercially available ODS column (150 mmL. X 2.1 mmI.D. 1.7 mm particle size) |
| Flow rate | : 0.2 mL/min |
| Column temp. | : 40°C |
| Mobile phase | : A) water containing 0.1% formic acid |
| | : B) acetonitrile containing 0.1% formic acid |
| Time prog. | : 2%B (0 → 5min) → 50%B (23 min) → 100%B (25 → 27 min) → 2%B (28 → 30 min) |
| Ionization mode | : ESI negative |
| DL temp. | : 250°C |
| HB temp. | : 400°C |
| Nebulizing gas flow | : 3.0 L/min |
| Drying gas flow | : 15 L/min |
| Transition | : *m/z* 413.10 → *m/z* 191.15 (Q1 : 30 V, CE : 20 V, Q3 : 20 V) |

Fig. 1 Analytical condition of SRM to quantify endogenous hydrogen sulfide

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## Results

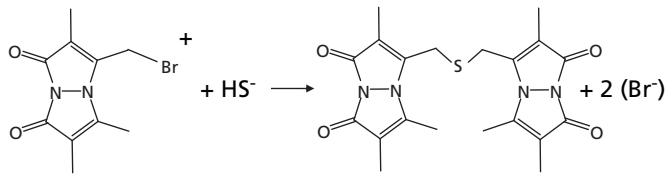


Fig. 2 Scheme of the derivatization of hydrogen sulfide with monobromobimane

Hydrogen sulfide is derivatized with monobromobimane reagent and forms sulfide-dibimane (SDB). Reacted SDB is stable at room temperature and is measured on SRM analysis. In this study, the transition of SDB was determined at *m/z* 413 → 191 at CE of 20 V in ESI/negative mode.

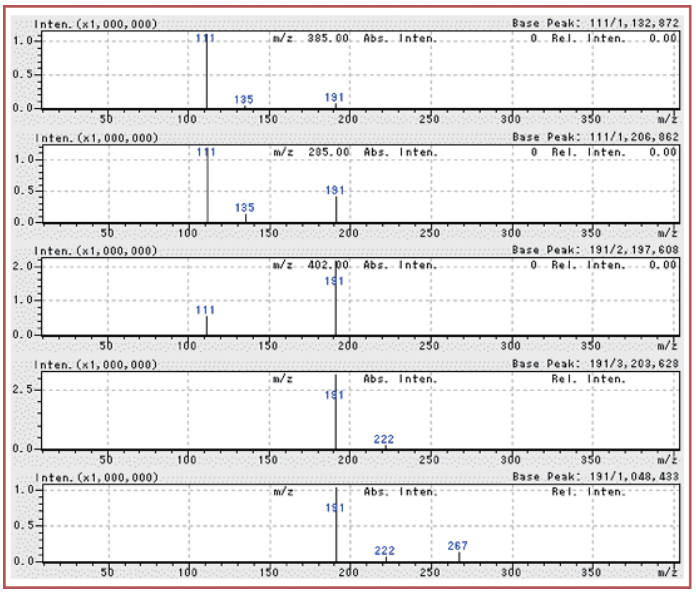

Fig. 3 MS/MS spectra of SDB standard on various collision energy (CE)

(A) A calibration curve of SDB standard.
A calibration curve was plotted by dilution series of SDB standard and were linear at a range of 1-5000 nM ($R^2$>0.99).

(B) A calibration curve of NaHS solution forming $H_2S$
A calibration curve was also plotted by using the derivatization with mBBr to $H_2S$ resulting from NaHS solution. Various concentrations of NaHS were reacted with excess monobromobimane (1 mM) for 10 min on ice.

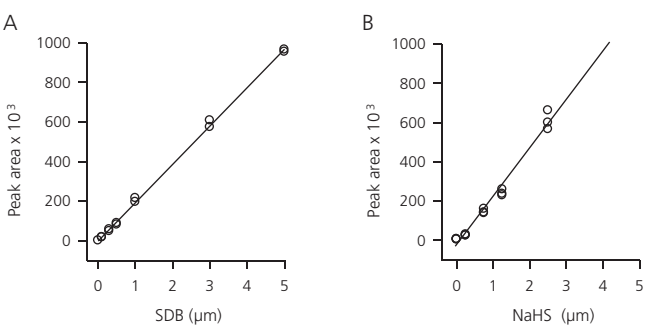


Fig. 4 Calibration curves of SDB and NaHS standard

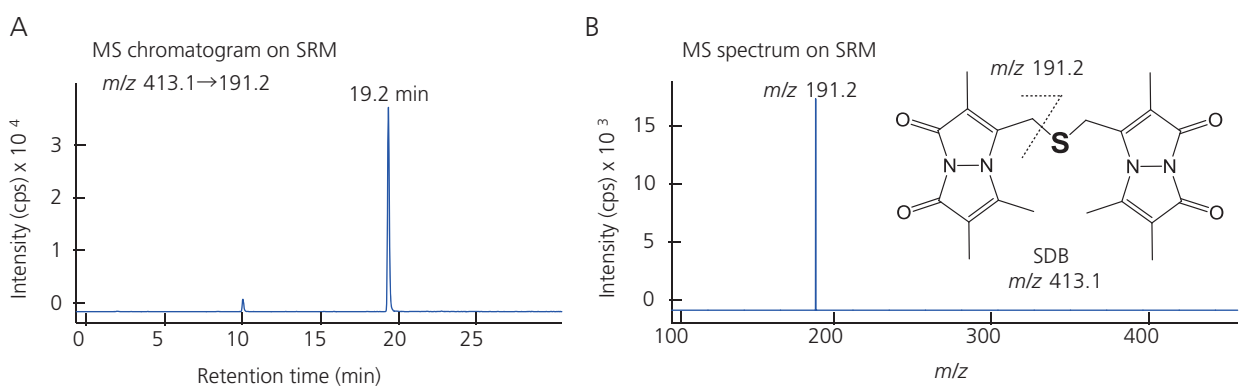


Fig. 5 MS chromatogram and MS spectrum on SRM of SDB derived from $H_2S$ in murine brain tissues

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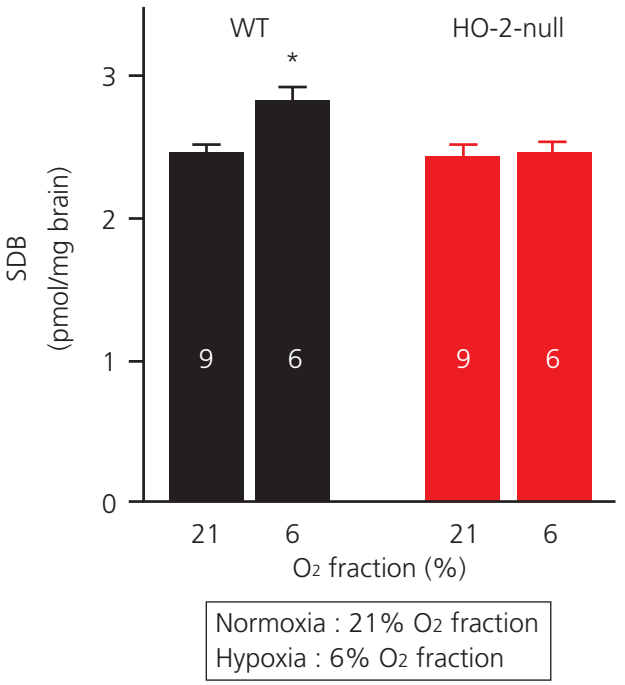


Endogenous $H_2S$ concentration in neonatal mouse brain was determined by SRM analysis using a monobromobimane-based assay. To quantify $H_2S$ level in brain tissues, a calibration curves was plotted by a SDB standard and applied as a external calibration. Fig. 6 shows $H_2S$ concentration in WT mouse increased under 6% $O_2$ fraction (hypoxia). On the contrary, the alteration of $H_2S$ concentration from heme oxygenase (HO)-2-null mouse brains could not be found between 21% and 6% fraction. Under normoxia, $H_2S$ levels were similar in WT and HO-2-null mice, presumably due to compensation from other sources of $H_2S$ in the transsulfuration pathway.

Error bar show a standard error (n=9 or 6).
*P<0.05 compared with WT at 21% $O_2$.

Fig. 6 Endogenous $H_2S$ concentration in neonatal brain of both WT and HO-2-null under normoxia/hypoxia

# Conclusions

Hydrogen sulfide is known to be as the gasotransmitter to control multiple biological functions. Therefore reliable and accurate quantitative analysis of $H_2S$ level in tissues is required. In this study we performed the quantitative analysis of hydrogen sulfide in mouse brain tissue by coupling the monobromobimane-based assay to triple quadrupole LC/MS/MS.

First analytical condition of SDB was determined on SRM by a SDB standard and then the linearity of calibration curves was validated by using a SDB standard and NaHS reagent forming $H_2S$. Correlation coefficient of these calibration curve was confirmed to be >0.99 and

The endogenous $H_2S$ level in mouse brain tissues was quantified as the derivatized SDB by triple quadrupole LC/MS/MS. This result shows the of the endogenous $H_2S$ is at the level of pmol/mg tissue. And the increase of the $H_2S$ level in WT mouse was confirmed under the hypoxia. On the other hand, the change of $H_2S$ level could not be found in the HO-2-null mouse. These results suggests this quantitative analysis by using triple quadrupole LC/MS/MS coupled with monobromobimane-based assay is helpful to quantify the endogenous $H_2S$ level in tissue samples.

# References

(1) Morikawa T, et al. (2012) Hypoxic regulation of the cerebral microcirculation is mediated by a carbon monoxide-sensitive hydrogen sulfide pathway. *Proc. Natl. Acad. Sci. U.S.A.* 109:1293-1298

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