



Letter

A zinc complex that suppresses the expression of a reactive sulfur species-producing enzyme, cystathionine γ -lyase, in cultured vascular endothelial cells

Akane Takahashi^{1,*}, Musubu Takahashi^{1,*}, Tomoya Fujie^{2,*}, Takato Hara², Eiko Yoshida¹,
Chika Yamamoto² and Toshiyuki Kaji¹

¹Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda 278-8510, Japan

²Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi 274-8510, Japan

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ABSTRACT — Persulfide species present in mammalian cells play important toxicological roles against oxidative stress and methylmercury. Cystathionine γ -lyase (CSE) and cystathionine β -synthase (CBS) are enzymes that produce persulfide species using cysteine as a substrate in the presence of vitamin B₆. However, little is known about the regulatory mechanisms underlying the expression of these enzymes. In the present study, we searched for molecular probes from a library of 27 zinc complexes to analyze the mechanisms in vascular endothelial cells. We found dichloro(1,10-phenanthroline)zinc(II), termed Zn11, as a zinc complex that suppressed endothelial CSE expression without any change in CBS expression. This zinc complex can be used as molecular probes to analyze the regulation underlying endothelial CSE expression.

Key words: Zinc complex, Cystathionine γ -lyase, Cystathionine β -synthase, Reactive sulfur species, Vascular endothelial cell

INTRODUCTION

Bio-organometallics is a new strategy to study biological systems using organic-inorganic hybrid molecules such as organometallic and metal coordination compounds (Fujie *et al.*, 2016a). Organic-inorganic hybrid molecules comprise metal(s) and organic ligand(s), and exhibit unique biological activities.

We have suggested that the cytotoxicity of organic-inorganic hybrid molecules is dependent on intramolecular metal(s), organic structure, or interaction between the metal(s) and the structure (Kohri *et al.*, 2015; Murakami *et al.*, 2015; Hara *et al.*, 2016; Nakamura *et al.*, 2017). Thus, the biological activities, including cytotoxicity, of either the molecular structures or the metals that comprise hybrid molecules, cannot be used to predict the biological

properties of such hybrid molecules.

Reactive sulfur species have gained increasing attention as protective agents against oxidative stress (Ida *et al.*, 2014) and methylmercury (Yoshida *et al.*, 2011). Cystathionine γ -lyase (CSE) and cystathionine β -synthase (CBS) are enzymes that produce reactive sulfur species using cysteine as a substrate in the presence of vitamin B₆. However, little is known about the regulatory mechanism underlying the expression of these enzymes.

We hypothesized the presence of intracellular pathways that regulate the expression of CSE and CBS in vascular endothelial cells and that these pathways may be analyzed using organic-inorganic hybrid molecules as molecular probes. In the present study, we search for candidate molecular probes from a library of zinc complexes.

Correspondence: Toshiyuki Kaji (E-mail: t-kaji@rs.tus.ac.jp)

*These authors equally contributed to this work.

MATERIALS AND METHODS

Materials

Bovine aortic endothelial cells were purchased from Cell Applications (San Diego, CA, USA). Dulbecco's modified Eagle's medium (DMEM) and Ca^{2+} - and Mg^{2+} -free phosphate-buffered saline (CMF-PBS) were obtained from Nissui Pharmaceutical (Tokyo, Japan). Fetal bovine serum (FBS) was purchased from Biocera (Kansas, MO, USA). Tissue culture dishes and plates were supplied by AGC Techno Glass (Shizuoka, Japan). Immobilon-P polyvinylidene difluoride membranes (0.45 μm) was procured from Millipore (Billerica, MA, USA) and anti-glyceraldehydes-3-phosphate dehydrogenase monoclonal antibody (peroxidase conjugated) and Immunostar Basic from WAKO Pure Chemical Industries (Osaka, Japan). Anti-CSE rabbit polyclonal antibody was supplied by Professor Yoshito Kumagai of University of Tsukuba, Tsukuba, Japan, while anti-CBS mouse monoclonal antibody (M01) was obtained from Abnova Corporation (Taipei, Taiwan). Horseradish peroxidase-conjugated anti-rabbit IgG antibody (#7074) and horseradish peroxidase-conjugated anti-mouse IgG antibody (#7076) were provided by Cell Signaling (Beverly, MA, USA). Zinc complexes used in this study were supplied by Research Associate Takehiro Nakamura of Kindai University, Osaka, Japan. Other reagents were

procured from Nacalai Tesque (Kyoto, Japan).

Cell culture and treatments

Vascular endothelial cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C in DMEM supplemented with 10% FBS. Confluent cells were transferred to 35-mm dishes and further cultured until confluency. The medium was discarded, and the cells were washed twice with fresh serum-free DMEM; the cells were treated with zinc complexes at 5 and 10 μM concentrations for 24 hr in fresh serum-free DMEM.

Western blot analysis

CSE and CBS proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on a 12% polyacrylamide gel and transferred onto an Immobilon-P membrane at 2 mA/cm² for 1 hr. Membranes were blocked for 1 hr with 5% skim milk in 20 mM Tris-HCl buffer solution (pH 7.5) containing 150 mM sodium chloride and 0.1% Tween 20 (TTBS) or 2% bovine serum albumin-TTBS solution and incubated overnight with a primary antibody at 4°C. The membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 hr at room temperature. Immunoreactive bands were visualized using the Immunostar Basic enhanced chemiluminescence western blot detection reagent and scanned using a LAS 3000 Imager

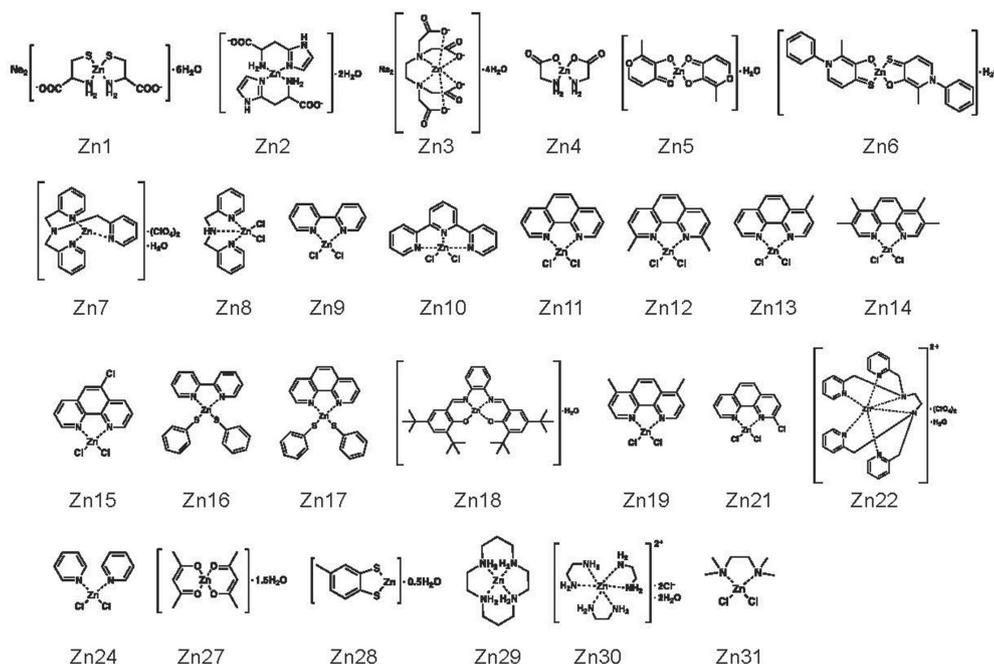


Fig. 1. Structures of the organic-inorganic hybrid molecules used in this study.

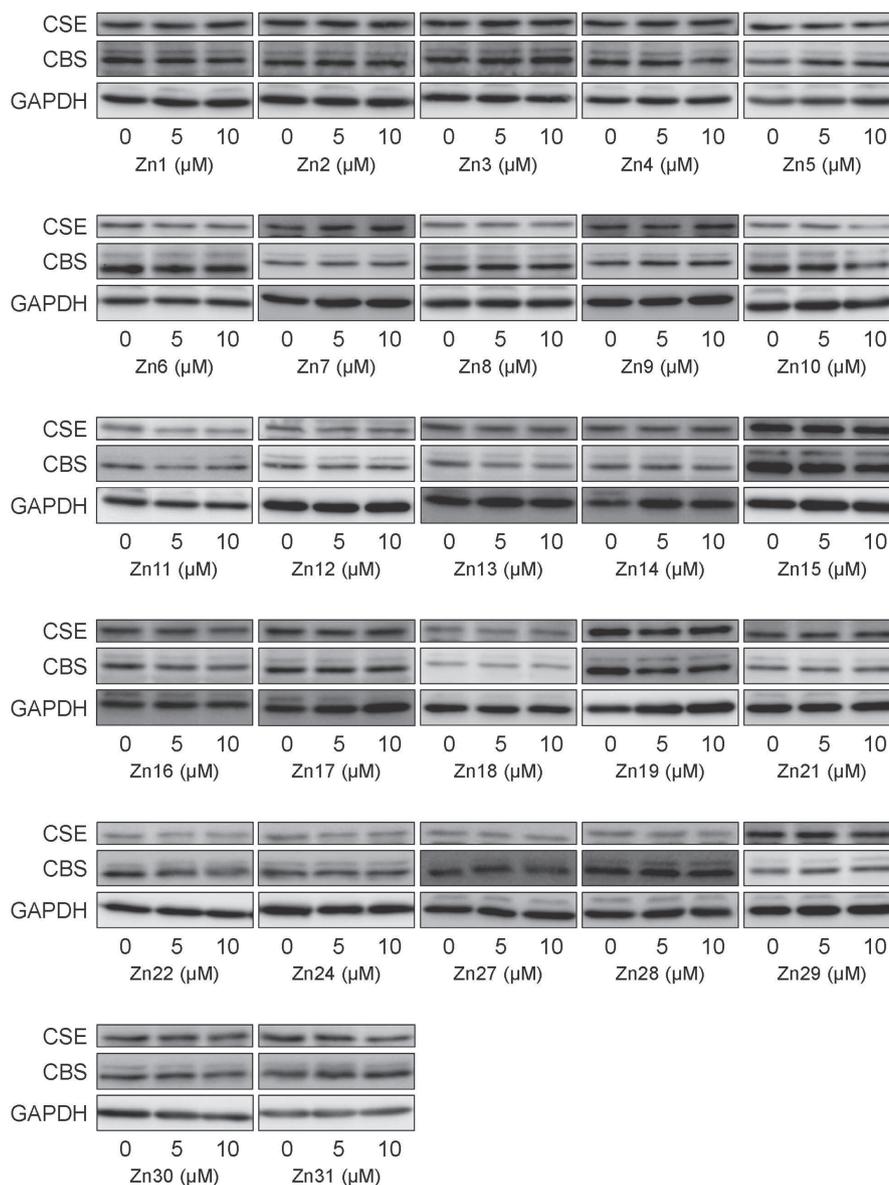
Zinc complexes and cystathionine γ -lyase

Fig. 2. The expression of CSE and CBS proteins in vascular endothelial cells after treatment with zinc complexes. Bovine aortic endothelial cells were treated with Zn1, Zn2, Zn3, Zn4, Zn5, Zn6, Zn7, Zn8, Zn9, Zn10, Zn11, Zn12, Zn13, Zn14, Zn15, Zn16, Zn17, Zn18, Zn19, Zn21, Zn22, Zn24, Zn27, Zn28, Zn29, Zn30, and Zn31 at 5 and 10 μ M concentrations for 24 hr. CSE and CBS protein expression was detected by western blot analysis.

(Fujifilm, Tokyo, Japan). Representative blots are shown from two or three independent experiments.

RESULTS AND DISCUSSION

The zinc complexes used in this study are shown in Fig. 1. Among the tested zinc complexes, Zn10

[dichloro(2,2':6',2''-terpyridine)zinc(II)] at 10 μ M decreased in the expression of both CSE and CBS, whereas Zn11 [dichloro(1,10-phenanthroline)zinc(II)] at 5 and 10 μ M markedly suppressed only the expression of CSE in vascular endothelial cells (Fig. 2). The expression of CSE and CBS was unchanged after treatment of vascular endothelial cells with the other zinc complexes tested.

In particular, Zn11 is a specific suppressor of endothelial CSE expression, indicative of its potential application as molecular probes for the analysis of the intracellular signaling pathways that selectively mediate endothelial CSE expression.

We used organic-inorganic hybrid molecules as molecular probes to study biological systems. The intracellular signaling pathways were different between MT-1 and MT-2, the isoforms of metallothionein, as observed in the presence of copper(II) bis(diethyldithiocarbamate) (Fujie *et al.*, 2016b) or tris(pentafluorophenyl)stibane (Fujie *et al.*, 2016c) in vascular endothelial cells. Furthermore, we analyzed the intracellular signaling involved in the expression of a specific type of proteoglycans in the cells (Hara *et al.*, 2017; Hara *et al.*, 2018). Organic-inorganic hybrid molecules used in these reports exhibited selective biological activities. For example, copper(II) bis(diethyldithiocarbamate), which activates the transcription factor NF-E2-related factor 2 (Nrf2) (Fujie *et al.*, 2016d) and modulates the fibrinolytic activity (Fujie *et al.*, 2017) in vascular endothelial cells, induces the expression of only syndecan-4 among the several types of endothelial proteoglycans (Hara *et al.*, 2018). The selective suppression of endothelial CSE expression by Zn11 suggests the suitability of this zinc complex as a molecular probe for the analysis of the mechanisms underlying endothelial CSE expression.

The present study revealed for the first time the possibility that the expression of CSE may be modulated by certain zinc complexes and suggests the potential application of zinc complexes as molecular probes for the analysis of CSE expression in vascular endothelial cells.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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