Review

Toxicology of organic-inorganic hybrid molecules: bio-organometallics and its toxicology

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ABSTRACT — Bio-organometallics is a research strategy of biology that uses organic-inorganic hybrid molecules. The molecules are expected to exhibit useful bioactivities based on the unique structure formed by interaction between the organic structure and intramolecular metal(s). However, studies on both biology and toxicology of organic-inorganic hybrid molecules have been incompletely performed. There can be two types of toxicological studies of bio-organometallics; one is evaluation of organic-inorganic hybrid molecules and the other is analysis of biological systems from the viewpoint of toxicology using organic-inorganic hybrid molecules. Our recent studies indicate that cytotoxicity of hybrid molecules containing a metal that is nontoxic in inorganic forms can be more toxic than that of hybrid molecules containing a metal that is toxic in inorganic forms when the structure of the ligand is the same. Additionally, it was revealed that organic-inorganic hybrid molecules are useful for analysis of biological systems important for understanding the toxicity of chemical compounds including heavy metals.

Key words: Organic-inorganic hybrid molecule, Metallothionein, Endothelial cell, Bio-organometallics, Toxicity

OPENING REMARKS: WHAT IS BIO-ORGANOMETALLICS?

Bio-organometallics is a research strategy of biology that uses organic-inorganic hybrid molecules—organometallic compounds and metal coordination compounds—which is so-called “bio-element strategy.” Organic-inorganic hybrid molecules are composed of metal(s) and organic ligand(s) in a common feature and can have a characteristic of both organic and inorganic compounds of the component (Fig. 1). It is possible that intracellular interaction of the organic structure (ligand) and metal atom give a new characteristic to the hybrid molecules. Historically, organic-inorganic hybrid molecules were used for the first time in science as reagents of chemical reactions by pioneers such as Grignard and Wittig (Grignard, 1900; Wittig and Schöllkopf, 1954). After that, organic element chemistry has made rapid progress; today, most of elements can be available and necessary physical properties and functions as well as necessary structures can be obtained in this chemistry. However, the evaluation of organic-inorganic hybrid molecules has been that as synthetic chemical reagents. The achieve-

Fig. 1. Organic-inorganic hybrid molecules. There are two types of chemical compounds; one is organic compounds and the other is inorganic compounds. Organic-inorganic compounds have characteristics of both types of compounds. This suggests that organic-inorganic compounds may exhibit novel bioactivities by their unique structures.

ment of organic-inorganic hybrid molecules in life sciences is little and there has been no strategy of application of the molecules to the biological systems.

There are numerous studies on the toxicity of metals

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and metalloids. Such studies published these past three years in the *Journal of Toxicological Sciences* investigated the toxicity of many types of metals, including cadmium (Ohtani *et al.*, 2013; Miura *et al.*, 2013; Baba *et al.*, 2014; Du *et al.*, 2014; Park *et al.*, 2015; Lukkhananan *et al.*, 2015), mercury (Yoshida *et al.*, 2013; Kim *et al.*, 2014), methylmercury (Hwang *et al.*, 2013a, 2013b; Iwai-Shimada *et al.*, 2013; Chang *et al.*, 2013; Hirooka *et al.*, 2013; Kim *et al.*, 2013; Watanabe *et al.*, 2013; Shao *et al.*, 2015; Toyama *et al.*, 2015), aluminum (Zhang *et al.*, 2013; Jinzhu *et al.*, 2015), manganese (Fujishiro *et al.*, 2013), uranium (Lestaevl *et al.*, 2013), arsenic (Tokumoto *et al.*, 2013; Wang *et al.*, 2015), arsine (Kato *et al.*, 2014), organic bismuth (Kohri *et al.*, 2015), chromium (Kimura *et al.*, 2015), and tributyltin (Oyanagi *et al.*, 2015). Although methylmercury is one of the organic-inorganic hybrid molecules (the minimum unit!), the authors do not have such a concept. They think that methylmercury is only a metal compound in environmental pollutants.

Grignard reagent—organic magnesium halide expressed as R-Mg-X—has a property of nucleophile and reacts with carbonyl compounds, forming the corresponding alcohol with alkylation. However, the components of Grignard reagent—alkane, alkyl halide, and magnesium halide—cannot induce the Grignard reaction. Similarly, the toxicity of the components of methylmercury—methane and inorganic mercury—is completely

different from that of methylmercury. These phenomena led us to an idea that the biological activities and toxicity of organic-inorganic hybrid molecules are ones different from those of the components—the molecular structure and metal(s)—of the molecules (Fig. 2). Therefore, new toxicological studies, in other words, toxicology of bio-organometallics, should be required as two types of research. First, toxicity of organic-inorganic hybrid molecules should be evaluated from the viewpoint of bio-organometallics. Metals that are nontoxic in inorganic forms may be toxic when they become a component of hybrid molecules. Conversely, metals that are toxic in inorganic forms may be nontoxic when they become a component of hybrid molecules. Second, applications of organic-inorganic hybrid molecules instead of toxic compounds to studies on clarification of biological systems, for example biological defense system, may be effective because of the unique biological activities. Bio-organometallics provides new aspects to toxicology, and may contribute to the new development of toxicology.

STRATEGY ON APPLICATION OF ORGANIC-INORGANIC HYBRID MOLECULES TO BIOLOGICAL STUDIES

What are the differences between organic-inorganic hybrid molecules and organic/inorganic compounds? First, three-dimensional structure of organic structure

Fig. 2. [A] Grignard reaction. It was revealed that organic-inorganic hybrid molecules can induce novel chemical reactions that are impossible for organic and inorganic compounds. [B] Methylmercury (left), methane (middle), and inorganic mercury (right). Although significance of organic-inorganic compounds in biological systems has been unclear, methylmercury is a good model to understand it. Only methane is gaseous among these three compounds. It is well known that the toxicological properties of inorganic mercury and methylmercury are completely different. For example, the target of inorganic mercury and methylmercury is the kidney and the nervous system, respectively. This supports the hypothesis that organic-inorganic hybrid molecules may exhibit unique biological activities that are impossible for organic and inorganic compounds.

can be dramatically changed by intramolecular metal(s) (Muranaka *et al.*, 2009). Conversely, it is likely that the molecular structures influence to the properties of the intramolecular metal(s). Interaction between the molecular structure and intramolecular metal(s) may change the electron state in the hybrid molecules. It is possible that these changes of molecular properties induce new biological activities and toxicity of organic-inorganic hybrid molecules. In fact, we reported that organobismuth compounds with certain molecular structures exhibit cytotoxicity via an interaction between the molecular structure and the bismuth atom, and this cytotoxicity can be diminished by replacing the bismuth atom with an antimony atom, resulting in lower intracellular accumulation. (Kohri *et al.*, 2015). Second, although a whole structure may be important for the biological activities of organic-inorganic hybrid molecules, hybrid molecules with a certain molecular structure may serve as a donor of specific metal ion to some specific proteins. Bis(L-cysteinato)zincate(II) gives zinc ion to a transcriptional factor metal response element (MRE)-binding transcription factor-1 (MTF-1) and induces transcriptional induction of metallothionein without nonspecific cell response (Kimura *et al.*, 2012), supporting this hypothesis (Fig. 3).

These results suggest that organic-inorganic hybrid molecules can be used as seeds of drug development, tools of analysis of biological systems, including cellular defense system from the viewpoint of toxicology, and

tools of analysis of the interrelationship between biological activities and electron state of the compounds.

TOXICITY OF ORGANIC-INORGANIC HYBRID MOLECULES

We believe that there are two types of studies in toxicology of organic-inorganic hybrid molecules. One is studies on toxicity of organic-inorganic hybrid molecules. Organic element chemistry is rapidly developing; however, chemists are very interested in the usefulness but not toxicity of the hybrid molecules synthesized by themselves. Chemists choose metals whose toxicity is low in inorganic forms or without deeply thinking about the toxicity when they synthesize “useful” organic-inorganic hybrid molecules. We know that cytotoxicity of hybrid molecules containing a metal that is non-toxic in inorganic forms can be more toxic than that of hybrid molecules containing a metal that is toxic in inorganic forms when the structure of the ligand is the same. For example, tris(pentafluorophenyl)phosphane is more toxic than tris(pentafluorophenyl)arsane to cultured vascular endothelial cells (Murakami *et al.*, 2015). The other is studies on clarification of the molecular mechanism underlying toxicity or defense systems using organic-inorganic hybrid molecules as a tool. Although the defense system is in general regulated by biological molecules, hybrid molecules may reveal the alternative mechanisms

Fig. 3. Bis(L-cysteinato)zincate(II) as a zinc ion donor to a transcription factor metal response element (MRE)-binding transcription factor-1 (MTF-1). This zinc complex activates MTF-1 probably by providing zinc to the protein, resulting in transcriptional induction of metallothionein without activation of nonspecific cell response (Kimura *et al.*, 2012). In other words, the zinc complex serves as a donor of zinc ion to a specific protein. This is a new function of organic-inorganic hybrid molecules in biological systems.

that activate or suppress the system. In this review, we introduce our recent study on the mechanisms underlying metallothionein induction in vascular endothelial cells.

VASCULAR ENDOTHELIAL CELLS AND METALLOTHIONEIN

Vascular endothelial cells are a cell type that covers the luminal surface of blood vessels in a monolayer. The cells directly contact with blood and regulate the blood coagulation-fibrinolytic system by synthesizing and secreting tissue factor (Maynard *et al.*, 1977), prostacyclin (Weksler *et al.*, 1977), thrombomodulin (Esmon and Owen, 1981), anticoagulant heparan/dermatan sulfate proteoglycans (Yamamoto *et al.*, 2005), plasminogen activators (Levin and Loskutoff, 1982), and plasminogen activator inhibitor type-1 (van Mourik *et al.*, 1984). Metallothionein is a low-molecular-weight and cysteine-rich protein containing heavy metals such as cadmium, zinc, and copper (Margoshes and Vallee, 1957). These heavy metals induce metallothionein and are bound to the protein, suggesting that metallothionein detoxifies heavy metal cytotoxicity by sequestering the heavy metals. We have studied the toxic effects of heavy metals on vascular endothelial cell functions. It was found that cadmium destroys the monolayer (Kaji *et al.*, 1992a). This cytotoxicity is protected by zinc through a metallothionein-independent mechanism (Kaji *et al.*, 1992b). In addition, cadmium lowers fibrinolytic activity by inducing the synthesis of plasminogen activator inhibitor type-1 (Yamamoto *et al.*, 1993; Yamamoto and Kaji, 2002). Lead also reduces the fibrinolytic activity but the mechanism is to inhibit the synthesis of tissue-type plasminogen activator (Kaji *et al.*, 1992c). In addition, lead inhibits the proliferation of vascular endothelial cells (Kaji *et al.*, 1995) by inhibition of a heparan sulfate proteoglycan, perlecan, which promotes the binding of fibroblast growth factor-2 to its receptor (Fujiwara and Kaji, 1999a, 1999b). These works suggest that heavy metals can be a risk factor of vascular disorders such as atherosclerosis.

Although the relationship between metallothionein induction and vascular disease has not been made clear, it is likely that the protein may protect vascular endothelial cells by sequestering heavy metals and reacting with oxidative stress (Sato and Bremner, 1993). In the process of these studies, however, we observed that zinc, a typical inducer of metallothionein, did not induce the protein in vascular endothelial cells in a Sephadex G-75 profile of the cytosol fraction, although cadmium can induce metallothionein (Kaji *et al.*, 1992b). Recently, we have confirmed that inorganic zinc does not induce met-

allothionein in vascular endothelial cells (Fujie *et al.*, 2016a); zinc does not activate MTF-1 that is activated by zinc ion (Redtke *et al.*, 1993; Zhang *et al.*, 2001) and initiates the induction of metallothionein (Heuchel *et al.*, 1994). Thus, inorganic zinc is in general a typical inducer of metallothionein but cannot be a tool to analyze mechanisms underlying metallothionein induction in vascular endothelial cells. Previously, we found that bis(L-cysteinato)zincate(II) activates MTF-1 and induces transcriptional induction of metallothionein in fibroblastic cells (Kimura *et al.*, 2012) but this zinc complex cannot induce metallothionein in vascular endothelial cells (Fujie *et al.*, 2016a).

We found out copper(II) bis(diethyldithiocarbamate), termed Cu10, is an activator of NF-E2 related factor 2 (Nrf2) (Fujie *et al.*, 2016b), a transcription factor, regarded as a regulator of phase 2 detoxification and antioxidant enzymes (Itoh *et al.*, 1997). Since the promoter region of metallothionein genes has the antioxidant response element (ARE) (Ohtsui *et al.*, 2008) that binds Nrf2 (Motohashi *et al.*, 2002), we hypothesize that Cu10 can be a tool to analyze the role of Nrf2 in vascular endothelial metallothionein induction. The experiments (Fujie *et al.*, 2016c) revealed that Cu10 induces metallothionein in vascular endothelial cells at protein level; Cu10 increased the expression of mRNAs for metallothionein subisoforms *MT-1A*, *MT-1E*, and *MT-2A*; Cu10 activates both the MTF-1–MRE and the Nrf2–ARE pathways; the induction of metallothionein isoforms requires the activation of the MTF-1–MRE pathway; and Cu10-induced metallothionein-1 expression is downregulated by Nrf2 knockdown but metallothionein-2 expression is not affected by Nrf2 knockdown. Since Nrf2 is a transcriptional factor that induces the expression of antioxidant proteins such as hemoxygenase-1 (Alam *et al.*, 1999) and glutamate-cysteine ligase modifier subunit (Erickson *et al.*, 2002), the original role of metallothionein-1 may be cytoprotection against oxidative damage by injury or inflammation. On the other hand, metallothionein-2 may be an isoform that regulate intracellular zinc metabolism because the induction of this isoform required only the MTF-1–MRE pathway that is activated by zinc ion. We revealed that tris(pentafluorophenyl)stibane, an organoantimony compound, also induces gene expression of *MT-1A* and *MT-2A*; *MT-1A* is induced by activation of both the MTF-1–MRE and Nrf2–ARE pathways, whereas *MT-2A* expression requires only activation of the MTF-1–MRE pathway (Fujie *et al.*, 2016d), supporting the hypothesis that the original role of metallothionein isoforms is different (Fig. 4).

Toxicology of organic-inorganic hybrid molecules

Fig. 4. A possible mechanism of metallothionein isoform induction in vascular endothelial cells by copper(II) bis(diethyldithiocarbamate) and tris(pentafluoro)stibane. These two organic-inorganic hybrid molecules induce metallothionein isoforms—MT-1 and MT-2—in bovine aortic endothelial cells. At that time, activation of the MTF-1–MRE pathway is required for induction of both metallothionein isoforms whereas activation of the NF-E2 related factor 2 (Nrf2)—antioxidant response element (ARE) pathway is required for the induction of MT-1 but not MT-2 (Fujie *et al.*, 2016b, 2016d). This study showed for the first time that the signaling pathways for induction are different between metallothionein isoforms. Like this, organic-inorganic hybrid molecules can be an excellent tool to analyze biological systems.

CLOSING REMARKS

As stated above, vascular endothelial cells are involved in the regulation of blood coagulation-fibrinolytic system. Proteoglycans are macromolecules that consist of a core protein and one or more glycosaminoglycan side chains (Ruoslahti, 1988). The cells synthesize heparan sulfate and dermatan sulfate chains as glycosaminoglycan chains (Yamamoto *et al.*, 2005). Heparan sulfate and dermatan sulfate chains exhibit an anticoagulant activity by activating antithrombin III (Mertens *et al.*, 1992) and heparin cofactor II (Tollefsen *et al.*, 1983). Syndecan-4 is a trans-membrane heparan sulfate proteoglycan expressed in the cell surface of vascular endothelial cells (Kojima *et al.*,

1992). We found that the TGF- β signaling regulates the expression of syndecan-4 in vascular endothelial cells (Hara *et al.*, 2016a, 2016b). Recently, we found that there is an alternative pathway that induces endothelial syndecan-4 expression using 1,10-phenanthroline with or without zinc or rhodium (Hara *et al.*, 2017). Specifically, the syndecan-4 expression can be induced by activation of the hypoxia-inducible factor-1 α/β pathway via inhibition of prolyl hydroxylase-domain-containing protein 2. Our studies on endothelial metallothionein and syndecan-4 clearly indicate that organic-inorganic hybrid molecules are good tools to analyze biological systems (Fig. 5). Needless to say, evaluation of the toxicity of organic-inorganic hybrid molecules and clarification of the mecha-

Fig. 5. Induction of syndecan-4 expression by 1,10-phenanthroline (*o*-Phen) with or without zinc (Zn-Phen) or rhodium (Rh-Phen) in vascular endothelial cells. The induction activity is in the order of *o*-Phen > Zn-Phen > Rh-Phen, suggesting that endothelial syndecan-4 induction depends on the chelating activity of the compounds. In fact, *o*-Phen and Zn-Phen significantly inhibit the activity of prolyl hydroxylase-domain-containing protein 2 (PHD2) that contains Fe(II) in its active site. The hypoxia-inducible factor-1 α / β pathway is activated by this PHD2 inhibition, resulting in endothelial syndecan-4 induction (Hara *et al.*, 2017). Like this, we can find an alternative pathway that regulates biological response by comparison of the biological activities of organic-inorganic hybrid molecules and their common ligand.

nisms should be performed. Although bio-organometallics studies are not widespread, it is certain that organic-inorganic hybrid molecules should be evaluated as either toxic compounds or molecular probes for analyzing biological systems.

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

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