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Development of multi-metal interaction model for *Daphnia magna*: Significance of metallothionein in cellular redistribution

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ABSTRACT

Despite the great progress made in metal-induced toxicity mechanisms, a critical knowledge gap still exists in predicting adverse effects of heavy metals on living organisms in the natural environment, particularly during exposure to multi-metals. In this study, a multi-metal interaction model of *Daphnia manga* was developed in an effort to provide reasonable explanations regarding the joint effects resulting from exposure to multi-metals. Metallothionein (MT), a widely used biomarker, was selected. In this model, MT was supposed to play the role of a crucial transfer protein rather than detoxifying protein. Therefore, competitive complexation of metals to MT could highly affect the cellular metal redistribution. Thus, competitive complexation of MT in *D. magna* with metals like Pb^{2+}, Cd^{2+} and Cu^{2+} was qualitatively studied. The results suggested that Cd^{2+} had the highest affinity towards MT, followed by Pb^{2+} and Cu^{2+}. On the other hand, the combination of MT with Cu^{2+} appeared to alter its structure which resulted in higher affinity towards Pb^{2+}. Overall, the predicted bioaccumulation of metals under multi-metal exposure was consisted with earlier reported studies. This model provided an alternative angle for joint effect through a combination of kinetic process and internal interactions, which could help to develop future models predicting toxicity to multi-metal exposure.

1. Introduction

Metal contamination has always been a concern, especially since the industrial revolution (Cai et al., 2015; Fan et al., 2014; Luo et al., 2011). Over the past years, great progress has been achieved in terms of reduction and management of metal-induced toxicity and carcinogenicity (Valko et al., 2005). At the cellular level, it is widely believed that metal-induced toxicity may result from the bindings to sulfhydryl groups in proteins, leading to inhibition of activity or stimulation of free radicals/reactive oxygen species (Hall, 2002; Valko et al., 2005). However, a critical knowledge gap still exists in predicting and quantifying the adverse effects of heavy metals on organisms in the natural environment.

Several models have been developed to predict possible biological effects resulting from metals in ambient environments. The biotic ligand model (BLM) states that mortality occurs when metal-biotic ligand reaches a critical concentration, making toxicity statistics through laboratory toxicity testing possible (Di Toro et al., 2001). In the recent years, another toxicokinetic-toxicodynamic (TK-TD) model has been developed for simulating and predicting toxicity over time (Jager et al., 2011; Tan and Wang, 2012). Compared to classic BLM, the TK- TD model presents more mechanistic and biological relevance due to its features on bioaccumulation processes of pollutants and corresponding time-course of hazard/ toxicity. However, few studies used the mentioned models or their deuterogenic models (Balistrieri et al., 2015; Santore and Ryan, 2015) to systematically evaluate relevant interactions among heavy metals. Besides, because natural waters are becoming frequently contaminated with metal mixtures due to human activities, it becomes important to comprehend and descript multi-metal interactions. Lately, a novel project called ''Metal Mixture Modeling Evaluation'' was proposed to look into the problem (Farley and Meyer, 2015; Farley et al., 2015; Van Genderen et al., 2015). However, unfortunately, the kinetics of uptake and signaling of biological feedback processes, which may affect the interaction process were still unconsidered.

According to existing knowledge system, the interaction among heavy metals under multi-metal exposure should follow two aspects: i) competitive uptake through the cell membrane (related to influx rate), and ii) subsequent interactions among heavy metals with detoxifying proteins in the cell (related to efflux rate), such as metallothionein

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(MT). This hypothesis is proposed based on that only metabolically available metals are related to toxicity (Rainbow, 2002, 2007). Thus, toxicity resulting from multi-metal exposure is induced by metabolically available metals, which are free from the detoxifying proteins. Recently, Gao et al. (Gao et al., 2016) used a refined TK-TD model combined with BLM to predict the accumulation and toxicity of metal mixtures in zebrafish larvae. However only external competition was considered. In a real environment, internal interactions *in vivo* should be taken into consideration due to the weak complexation competition at low level of multi-metal exposure. Overall, information regarding these processes is still lacking.

In this study, a multi-metal interaction model which includes external complexation competition and internal interaction was developed using kinetic description. This aimed to systematically investigate the behavior of metal bioaccumulation under multi-metal exposure. In the model, MT played the primary role in the internal interaction due to its high affinity with d10 electron configuration metals, including essential (Zn and Cu) and non-essential (Cd and Hg) metals (Isani and Carpene, 2014). The competitive complexations of metals like Cu^{2+}, Cd^{2+} and Pb^{2+} with MT in *Daphnia magna* were studied using electrochemistry analysis. *Daphnia magna* was selected as it is a widely used model organism in toxicity tests. The competitive properties of metals were used to modify the kinetic parameters and explain the observed phenomenon under multi-metal exposure.

This study was the first of its kind combining the kinetic processes and internal competitive complexations of metals with MT to mechanistically comprehend the behavior of bioaccumulation issued from multi-metals. The multi-metal interaction model provided a reasonable explanation for multi-metal bioaccumulation, which could help to develop models predicting toxicity prediction by multi-metal exposure.

2. Multi-metal interaction model

2.1. Model concept

The detailed mechanisms of *in vivo* multi-metal interactions were not uncovered yet. Most of the studies performed to date used metal-metal competition at the biotic binding site to predict the toxicity of metal mixtures in living organisms. These include biotic ligand model (Iwasaki et al., 2015), bidentate biotic ligand (Balistrieri et al., 2015), and multi-metal multi-biotic ligand model (Santore and Ryan, 2015). However, only additive and antagonistic effects were observed if competition at the binding site was considered as the solely restricted perspective. Norwood et al.(Norwood et al., 2003) found that approximately 25% of interactions have synergistic effects. Apparently, metal competition for uptake sites could not provide a satisfactory explanation. Besides, several studies were focused on metals uptake in aquatic organisms under multi-metal exposure (Komjarova and Blust, 2008, 2009; Van Ginneken et al., 2015). Similarly, the competition model could not fully explain the enhancing effect in uptake rates of metals resulting from the metal-metal interaction. Therefore, a comprehensive consideration of incorporating uptake process with internal metal competition in the cell (detoxification processes) should be examined.

Based on the two-compartment TK-TD model for single metal proposed by Tan et al.(Tan and Wang, 2012), a multi-metal interaction model was developed (Fig. 1). This model suggested three places where metal-metal interactions might occur. Firstly, competitions of metals at binding sites in cell membranes were widely acknowledged. This would directly determine the influx rates of the metals. It should be noticed that metal ions can be transferred into the cell through two different ways: membrane transport proteins or ion channel (Alberts, 2009). Therefore, the inhibition effect of influx rates may be prevented even at high metal levels (Fig. 1). Secondly, the other place where metals may interact with each other should be the metabolic pool. Banci et al. (Banci et al., 2010) reported that copper chaperones and glutathione

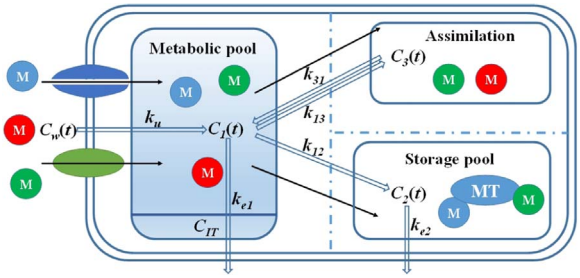

Fig. 1. Schematic diagram showing the multi-metal interaction concept.

(GSH) constituted an exchangeable cellular copper-binding pool, where affinity gradients drove the copper to cellular destinations (Cu, Zn-SOD1, and MT). They found that affinity gradients and protein-protein interactions may lead to the distribution of cellular metal ions.

Therefore, Fig. 1 proposed that the cellular metal could be separated into three portions: (i) metabolic pool where metal combined with low molecular substances like GSH, (ii) assimilated portion where metal was involved in the synthesis of the enzyme, and (iii) storage pool where metal was detoxified or combined with detoxifying protein like MT. In the metabolic pool, the multi-metal interaction may occur through the competitive occupation of capacity. Lastly, metals may also compete with detoxifying proteins, where MT would play a key role due to its high capacity and elevated complexation constant with common heavy metals, such as Cd^{2+}, Hg^{2+}, Cu^{+}, Ag^{+}, and Pb^{2+} (Ryvolova et al., 2011).

Until now, a number of studies reported that *in vivo* depuration of metals was highly related to their partition in MT or metallothionein-like protein (MTLP) (Ng and Wang, 2005; Serafim and Bebianno, 2007; Wang and Wang, 2014). This indicated that MT or MTLP may not act as a final sink. As for marine mollusks, it has been reported that MT combined with heavy metals could be transferred into lysosome, then degraded for subsequent excretion from the organisms (Isani et al., 2000). Wang et al. (Wang and Rainbow, 2010) reviewed the significance of MT in metal accumulation kinetics in marine animals and found that MT was more dynamic than metals and such dynamic changes might subsequently affect the metal distribution. Therefore, it would be reasonable to suggest that MT could play a dual role, as a transporter and a chelating protein, to regulate cellular metal distributions in the multi-metal interaction model.

In general, metal in solution should primarily enter the metabolic pool through the cell membrane, which subsequently is transferred to assimilation portion or storage pool depending on the metal properties. Meanwhile, metals can be excreted from the metabolic pool and storage pool. MT was supposed to play a significant role in regulating the cellular metal distribution by combining and transferring metals from the metabolic pool to storage pool. Moreover, metals distributed in the metabolic pool should critically affect toxicity if the concentration of metals in the metabolic pool exceeded the threshold concentration (C_{IT}) according to Rainbow and Luoma (Rainbow and Luoma, 2011).

A unified model for metal bioaccumulation process was also described in Fig. 1. However, this was not suitable to any metal. For essential metals, it was supposed that metals can be transferred from the metabolic pool to the other portions, while non-essential metals can only be transferred from metabolic pool to the storage pool in an irreversible manner.

2.2. Mathematical derivation

Using the multi-metal interaction model (Fig. 1), bioaccumulation processes of single metals can be described using Eqs. (1)–(5).

$$C_{in}(t) = C_1(t) + C_2(t) + C_3(t) \quad (1)$$