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Metal accumulation and toxicity: The critical accumulated concentration of metabolically available zinc in an oyster model

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ABSTRACT

Invertebrates typically carry out detoxification of accumulated metals. There is, therefore, no threshold total body concentration of accumulated metal initiating toxicity, the onset of toxic effects rather being related to a critical concentration of metabolically available (MA) accumulated metal. The challenge remains as to whether any particular combination of subcellular fractions of accumulated metal can be identified to represent this theoretical MA component. One candidate combined fraction is the so-termed metal sensitive fraction (MSF), consisting of metal bound to organelles and non-detoxificatory soluble proteins. In this study, we used laboratory zinc accumulation and toxicity data for four populations of the oyster *Crassostrea hongkongensis* with different histories of zinc exposure in the field to address the challenge. We conclude that in a 'control' population of the oyster, the MSF does approximate to the theoretical metabolically available zinc concentration. In populations with a history of field exposure to raised zinc bioavailabilities, however, the MSF would include more zinc detoxified in the lysosome component of organelle-bound metal, and the MSF in such populations would deviate more from the theoretical MA metal concentration.

1. Introduction

Invertebrates typically carry out at least some detoxification of accumulated metals, the percentage of accumulated body metal stored in detoxified form varying greatly with the metal and the invertebrate (Luoma and Rainbow, 2008; Rainbow and Luoma, 2011a,b). Thus for all such invertebrates there is no threshold total body concentration of accumulated metal that represents a threshold concentration initiating toxic effects (Adams et al., 2010; Casado-Martinez et al., 2010; Liu et al., 2013). Rather, the onset of toxicity effects is related to a critical concentration of metabolically available (MA) metal (Fig. 1A) (Rainbow and Luoma, 2011a). Thus models of the relationship between bioaccumulation and toxicity typically now take into account this metabolically available compartment of accumulated metal (Rainbow and Luoma, 2011a; Tan and Wang, 2012). However, there is currently no direct method to measure the MA metal concentration, which is, as yet, still a theoretical concept. Thus, its physical correspondence with any particular cellular/biochemical pool of accumulated metal is unknown.

A pragmatic approach to the subdivision of different categories of accumulated trace metals is the process of subcellular fractionation. Subcellular fractionation arbitrarily separates accumulated metals into five operational units defined by the subcellular component to which the metal is bound, using differential centrifugation and heating methods (Wallace and Luoma, 2003; Wallace et al., 2003). With this method, there are two soluble protein-bound fractions – metallothioneins (MT) [strictly metallothionein-like proteins (MTLP) which are characteristically relatively heat-resistant], and all other (heat-sensitive) proteins (HSP) which consist mostly of enzymes. The remaining three subcellular fractions are insoluble – organelles (Org), metal-rich granules (MRG) and cellular debris (CD) (Fig. 1B). The metals bound to MRG and MT are considered detoxified, while the remaining metal (bound to enzymes and other non-detoxificatory proteins, organelles and cell debris) is said to be non-detoxified. Part of this non-detoxified component (organelle and enzyme (HSP) fractions) has been termed the metal sensitive fraction (MSF). The changes in this particular combination of subcellular fractions showed the best correlation with observed toxicity effects in the original specific case of two bivalves exposed to zinc and cadmium by Wallace et al. (2003), as well as in marine diatoms (Wang and Wang, 2008a,b).

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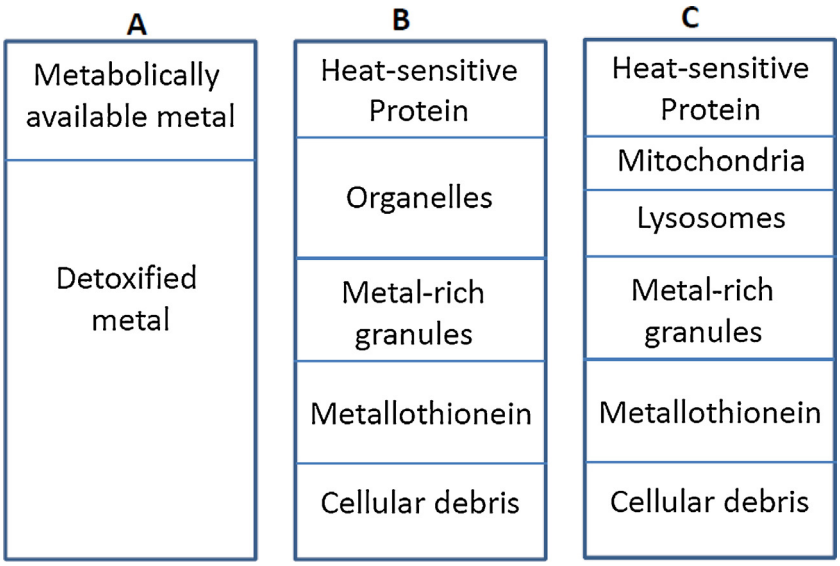

Fig. 1. Theoretical and operational schemes to subdivide an accumulated trace metal into different pools of ecotoxicological significance. (A) Theoretical division into metabolically available and detoxified metal (after Luoma and Rainbow, 2008); (B) Operational subcellular fractionation into 5 fractions (after Wallace and Luoma, 2003; Wallace et al., 2003); (C) Operational subcellular fractionation with further separation of organelle-bound metal into metal bound to mitochondria and metal bound to lysosomes (after Rosabal et al., 2012).

The ecotoxicological significance of metal bound to cellular debris remains much less well defined and understood.

In the literature, there has never been a direct data-led comparison between the theoretical MA component of an accumulated metal and the amount of metal accumulated in the metal sensitive fraction; the latter being derived from the measured concentrations of the metal bound to the organelles and heat-sensitive protein subcellular fractions. The challenge remains as to whether this particular combination of subcellular fractions has a wider general significance as a metal sensitive fraction, and indeed whether at all toxicity can be linked to the exceedance of a threshold concentration of accumulated metal binding to specific identifiable subcellular components. Therefore, in this study we use the data of Liu et al. (2013) to address the specific question – is it possible to identify any combination of subcellular fractions of accumulated Zn in the oyster *Crassostrea hongkongensis* that might physically represent the theoretical metabolically available fraction (Luoma and Rainbow, 2008; Rainbow and Luoma, 2011a), with particular reference to the MSF combination proposed by Wallace et al. (2003)?

2. Methods

Details of the experimental design of laboratory exposures of oysters to toxic dissolved Zn concentrations can be found in Liu et al. (2013). Briefly, we collected populations of oysters *C. hongkongensis* from four different locations in Southern China estuaries, with contrasting histories of Zn exposure. Three (Jiuzhen, LauFau and Shantou) represented a steep gradient of field Zn bioavailabilities from control to very high, while the fourth (Biajiao) was a multiple metal-contaminated site with particularly high bioavailability of copper as well as zinc (Liu et al., 2013). Jiuzhen is an oyster farm in the Jiuzhen estuary in Fujian province, China; LauFau is an oyster farm in Deep Bay Hong Kong, China; Shantou is an oyster farm in the Niutianyang estuary in Guangdong province, China; and Biaojao is in the Jiulong estuary, near Xiamen, Fujian Province (see Liu et al. (2013) for further details). In the laboratory, we then exposed these populations of *C. hongkongensis* from four sites for up to 60 days to an increasing laboratory series of dissolved Zn exposures (104, 484, 893, 4492 and 9705 μg Zn/L) that were potentially lethally toxic, although within the range of dissolved zinc concentrations in field-contaminated coastal waters (e.g., 100–57,000 μg Zn/L – Luoma and Rainbow, 2008; Wang et al., 2011).

Each day, the oysters were exposed to the dissolved Zn concentration for 20 h and fed with clean food for the other 4 h in clean seawater. Mortality was checked 4–6 times per day during the first two weeks and 2–3 times per day during the remaining exposure period. The LT50 was calculated as the exposure time at which Zn caused 50% mortality of the exposed oyster population. Oysters were collected at day 0 and on various days up to day 60, including the day of LT50 (or as soon after as possible), and subjected to metal tissue accumulation and subcellular fractionation measurements. Numbers of replicate oysters analysed are given in Tables 1 and 2. The five operationally defined subcellular fractions included cellular debris, organelles, metal rich granules, metallothionein-like proteins, and heat-sensitive proteins. The metal-sensitive fraction, as defined by Wallace et al. (2003) consisted of the combined organelle and heat-sensitive protein fractions.

This experiment (Liu et al., 2013) provided comparative data on the toxicity of Zn, the accumulation rates of Zn at different Zn exposure concentrations, and the subcellular fractionation (Wallace and Luoma, 2003; Wallace et al., 2003) of Zn in the four different oyster populations. The data presented in Tables 1 and 2 in this study are derived from the full data set of Liu et al. (2013), as opposed to the summarized data presented in that publication.

3. Results and discussion

3.1. Modelling of metabolically available (MA) Zn

We now attempt to model Zn bioaccumulation and its toxicity in the oyster *C. hongkongensis*.

Rainbow and Luoma (2011a) produced a biodynamic model relating Zn bioaccumulation and toxicity in three crustaceans with very differing degrees of storage accumulation of detoxified Zn, culminating in the case of a barnacle which stores extremely high concentrations of Zn in the form of metabolically inert zinc pyrophosphate granules (Luoma and Rainbow, 2008). The oyster is an analogue of the barnacle with similar, very pronounced