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Metallothionein-inspired prototype of molecular pincer†

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Study of Zn and Pb release from complexes with natural and synthetic amidothiol motifs inspired the design of a "molecular pincer" that scavenges quantitatively metals from liquid environment and releases them, on-demand, under very mild oxidative conditions.

Understanding transport, effective scavenging and on-demand release of metals and metal ions has been an outstanding issue straddling various areas of chemistry, technology, medical and environmental sciences.[1–4] An excellent illustration, and one of several possible solutions to the related problems, can be found in the area of biological chemistry where researchers have long recognized the importance of metals and studied their function in biosystems.[1,5–7] While on the one hand, metals are beneficial for their various roles in diverse enzymatic cycles, on the other hand, metals may be detrimental to proper systemic functions due to their high toxicity.[8,9] Existence of a biochemical vehicle that would facilitate controlled transport of metals is therefore of immense importance.[10] Systems that deliver metals to target sites, where the "cargo" is unloaded selectively, frequently involve high cysteine containing peptides that escort metals, such as copper, zinc, *etc*., from one place to another.[11–15] A prominent example of this molecular platform is found in the chemistry of metallothioneins[16–21] In such scenario the ease of metal loading to the cysteine is facilitated primarily by the well-established affinity of soft metals to sulfur.[19,22] Concurrently, however, the strong metal/sulfur bonding also obstructs selective metal release, particularly under mild conditions. While, *in vitro*, the equilibrium can be shifted conveniently towards metal release by implementing vigorous chemical means (*i.e*., using strong acid, varying the concentration of metals),[23] which lead to metal liberation, those rather harsh conditions would likely be detrimental when applied *in vivo* environments.

Recent experiments on biological assays have revealed that oxidative pathways can facilitate more pertinent means of metal unloading.[24–27] In this fashion metallophilic thiolates have been proposed to transform oxidatively to non-methallophilic disulfides, forming a highly beneficial thermodynamic sink that closes effectively the process gate (*cf*. Fig. 1). The role of metal thiolates, including their proposed oxidative transformation,[24] may thus be of great importance in elucidating biological function mechanisms involving metallothioneins. Given these important observations on living systems, it comes as no surprise that related chemistries are of great scientific interest.[28]

The purpose of this work is twofold. First, we intend to supplement the circumstantial evidence of the oxidative release of metals from metal–thiolate complexes with direct measurements quantifying chemical liberation of selected metals (Pb, Zn) from metallocysteine moieties. Second, we employ robust synthetic model systems based on mercaptoamide/thioimide motifs featuring a reaction pattern that is inspired structurally by cysteine. We demonstrate the efficacy of metal release even under biologically benign conditions thanks to the advantageous conformation of the sulfur terminus.

In order to substantiate the concept of the bio-relevant oxidative metal unloading, we synthesized corresponding metallocysteine Pb (**1a**, **2a**) and Zn (**1b**, **2b**) based thiolates and subjected them to aerobic oxidative conditions. The top portion of Fig. 2 depicts the proposed chemical pathway facilitating oxidative release of metals from cysteine residues. Metals are liberated by the formation of disulfides and the concentration of the resultant disulfide thus represents a direct measure of the release efficacy. Two classes of compounds have been synthesized and tested; they contain either amide (**1a**, **1b**) or ester (**2a**, **2b**) functionalities. Interestingly, the cursory kinetic evaluation of the oxidative metal release indicated the superior reactivity of the amide **1a**, **1b** (native to cysteinyl peptides and hence the metallothioneins) over the ester **2a**, **2b** for both metals studied. The critical role of the cysteinyl amide can be rationalized by an intermittent

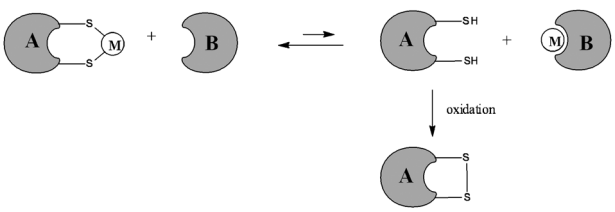

Fig. 1 Metallothionein function in cellular metal transport. Metals bound to the metallothionein complex (A) are delivered to the target (B), as suggested in ref. 24.

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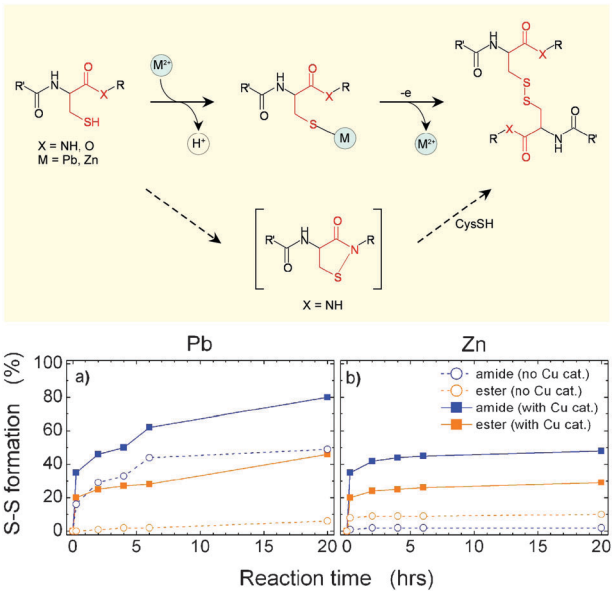

Fig. 2 (top) Proposed mechanism of metal capture and release by cysteines. (bottom) Metal release as indicated by the formation of S–S bond for Pb (**1a**, **2a**, left) and Zn (**1b**, **2b**, right) for cysteines bearing ester and amide functionalities. All attempts to isolate the S–N intermediate have been futile. Cu catalyst was used to speed up liberation of the metal. Compound **1**: X = NH, R′ = CH_3, R = naphthalene-2-yl; compound **2**: X = O, R′ = 4-CH_3-C_6H_4, R = CH_3.

formation of a putative S–N bond in the intermediate (*cf.* Fig. 2), which has been postulated to possess a likely influence on the reactivity of the cysteinyl residue.[29,30] Perhaps not surprisingly the data reveal that the capture/release efficiency depends on the metal. While the capture of both Pb and Zn is nearly instantaneous, the kinetics of Pb release is faster than that of Zn.[31]

Motivated by the prospect of unraveling the mechanism of metal release, model mercaptobenzamide compounds were synthesized that bore a molecular pattern analogous to cysteinyl amide but were stabilized by the structurally advantageous arrangement of the functional groups, *i.e.*, amide and thiol, that govern the capture/release of metals (*cf.* Fig. 3). These mercaptoamide/thioimide compounds act effectively as "molecular pincers".[32] Molecules comprising different combinations of X were synthesized bearing $–NO_2$ or –NH– groups representing electron withdrawing or donating elements, respectively (compounds **3** and **4**). The corresponding mercaptoamides were exposed to the same conditions as used in the aforementioned experiments with cysteinyl amides. In agreement with the above cysteinyl amide experiments, the resulting metallothiolates formed instantaneously under ambient conditions during which metal ions were attached to the sulfur residue as depicted in the scheme presented in the top portion of Fig. 3. The same scheme also indicates the proposed mechanism of metal release from the thioimide moiety. Mild aerobic oxidation was selected to satisfy simultaneously the criteria for metal unloading reactions and systemic biocompatibility. Specifically, all metal release experiments were carried in DMSO at room temperature utilizing air as oxidant;[33] the reaction rate was enhanced by adding a catalytic amount of $Cu^{(II)}$ acetate (for details see the Experimental section, ESI†).

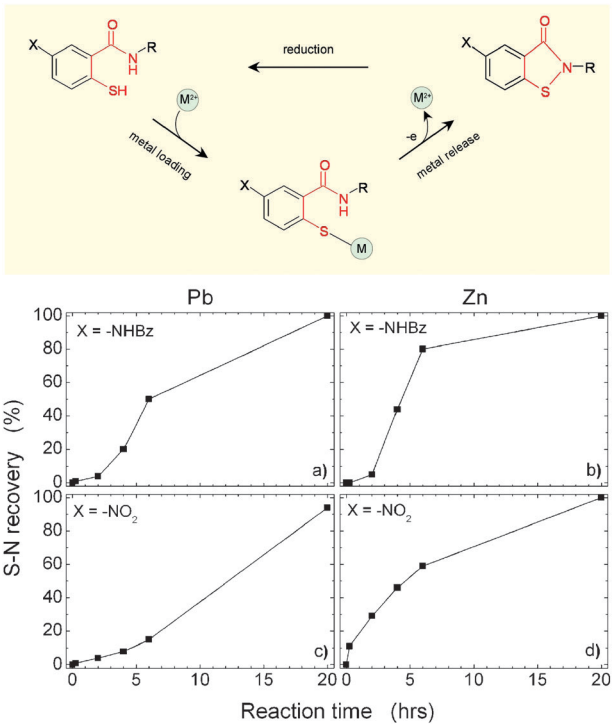

Fig. 3 (top) Proposed mechanism of metal capture and release by thioimides. (bottom) Metal release as indicated by the formation of S–N bond for Pb (left) and Zn (right) for thioimides with X = NH (a, b) or NO_2 (c, d). Compound **3**: X = NO_2, R = $CH(CH_3)_2$; compound **4**: X = 4-$CH_3C_6H_4CONH$, R = $CH(CH_3)_2$.

Successful metal release is associated with the formation of the S–N bond that closes the five-membered ring in the thioimide form of the pincer. By monitoring the population of the S–N bond in solution one can gain a quantitative understanding of metal liberation from the complex. In Fig. 3 we plot the time dependence of the concentration of the S–N bonds for the thioimides studied. From the data it is apparent that the release of both Pb and Zn is almost quantitative in 20 h regardless of X. Comparing these results with those obtained from the cysteine series it is evident that molecular pincers are more effective in liberating metals from the carrier metalloorganics. While the ability of the molecule to form stable five-membered rings is the likely explanation for the observed behavior, based on the current data it is impossible to specify the exact role of the chemical environment (*i.e.*, X and R) on the rate and completion of the metals release. In order to explain the function of X and R on metals release one has to carry out additional experiments with other metals and other combinations or X and R.[31]

In summary, metallothionein-inspired "molecular pincer" systems for loading and release of metals was developed and discussed. The devised molecule scavenges quantitatively selected metals from liquid environment and releases them, on-demand, under very mild oxidative conditions. Importantly, the conversion of the closed form of the "pincer" (thioimide) back to the open form (mercaptoamide) is possible; it can be accomplished by mild reducing agents, *e.g.* ascorbic acid. Fundamental understanding of interaction of metals with thiols may impact many practical areas, including biotechnology

and environmental sciences, that demand metal scavenging and metal delivery.[1–4,8,12] One can envision that the thioimide/mercaptoamide dyads would represent outstanding candidates for such functions, given their excellent synthetic accessibility complemented by their inherent structural flexibility, which allows for convenient chemical modification and incorporation into various environments.

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