Zinc Transfer Kinetics of Metallothioneins and Their Fragments with Apo-carbonic Anhydrase

HUANG, Zhong-Xian*(黄仲贤) LIU, Fang(刘芳) ZHENG, Qi(郑起) YU, Wen-Hao(余文浩)
Laboratory of Chemical Biology, Department of Chemistry, Fudan University, Shanghai 200433, China

The zinc transfer reactions from Zn_7-MT-I, Zn_7-MT-II, Zn_4-α fragment (MT-I) and Zn_4-α fragment (MT-II) to apo-carbonic anhydrase have been studied. In each reaction, no more than one zinc ion per molecule is involved in metal transfer. Zn_7-MT-I and Zn_7-MT-II donate zinc to apo-carbonic anhydrase and *de novo* constitute it at a comparable efficiency, while Zn_7-MT-II exhibits a little faster rate. Surprisingly, Zinc is released from Zn_4-α fragment (MT-II) with a much faster rate than from Zn_4-α fragment (MT-I), whose rate is close to that of Zn_7-MT-I. The reason for the difference is still unknown. Introducing complex compounds into this system may give rise to an effect on the reaction. The transfer from Zn_7-MT-II in the presence of reduced glutathione shows little difference compare to the control, suggesting that the reduced glutathione is not involved in zinc transfer process. However, glutathione disulfide does accelerate this zinc transfer reaction remarkably, indicating that the oxidative factors contribute to zinc release from metallothioneins.

Keywords Metallothioneins, zinc transfer, carbonic anhydrase, glutathione

Metallothioneins (MTs) are a group of low-molecular weight, cysteine- and metal-rich metalloproteins. Although metallothioneins have been known for over 40 years, the exact functions of these ubiquitous proteins remain to be a topic of discussions.[1,2] It is reported that MTs be involved in heavy metal detoxification and essential metal homeostasis. Unlike other zinc proteins, native metallothionein is able to transfer its zinc to some zinc-requiring apo-enzymes to restore their activity under physiological conditions.

MT-I and MT-II are two isoforms of metallothioneins. In rat liver MT, they both have 61 amino acids, but differ in 13 residues.[3] In the two isoforms, seven divalent metal ions are bound in two separate metal-cysteine clusters: one with four metal ions and 11 cysteines (consisting of the α domain, residues 31—61), and the other with three metal ions and 9 cysteines (consisting of the β domain, residues 1—30). It is reported that there is no direct contacts between the two domains[4] and MT remains its activity even when 16 amino acid residues were inserted into the linker region (residue 30—32) *in vivo*.[5] It seems that the two domains are independent from each other and involved in different functions. The kinetically labile *N*-terminal domain may function in metal-exchange processes, whereas the kinetically stable *C*-terminal domain is important in detoxification.[6] Some mutagenesis studies on MT support this conclusion,[7,8] but recently, the argument on the roles played by different domains appears again in various research groups.[9,10] The details of the specific roles and relationship of these two domains in the zinc transfer from MT to apo-carbonic anhydrase are still unknown. The present paper focuses on these questions and the studies might be helpful in understanding the roles of different domains and the functions of metallothionein isoforms.

Materials and methods

Materials

Bovine carbonic anhydrase (CA) was purchased

* E-mail: zxhuang@fudan.edu.cn
Received July 28, 2000; revised January 6, 2001; accepted January 9, 2001.
Project supported by the National Natural Science Foundation of China (No. 29671008).

from Sigma; dithizone and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were from Sigma; *p*-nitrophenyl acetate, $Fe(EDTA)^-$, $Cr(en)_3^{3+}$ were of reagent grade purity and re-crystallized before use. Glutathione, the metal salts and other chemicals were of reagent grade. All glasswares were treated by EDTA solution before use, and all buffers were dealt with dithizone/CCl_4 to remove free metal ions, then extracted by CCl_4 to remove the remaining dithizone.

Preparation of Zn_7-MT-**I**, Zn_7-MT-**II**, Zn_4-α-MT-**I** *and* Zn_4-α-MT-**II**

Zn_7-MT-**I** and Zn_7-MT-**II**, were prepared as described[11] and Zn_4-α domain of MT-**I** and MT-**II** were obtained by means of subtilisin digestion. All of them were characterized by ^1H NMR, and the protein concentrations were determined by DTNB as described in the previous paper.[12] The metal content in metallothionein was quantified with an atomic absorption spectrometer.

Preparation of apo-CA

Zinc ion was removed from carbonic anhydrase as described.[13] The concentration of CA was determined by ultraviolet absorption ($\varepsilon_{280} = 5.7 \times 10^4$ mol·L^{-1}·cm^{-1}). The activities of native CA and apo-CA were assayed with *p*-nitrophenyl acetate as the substrate in an adaptation of published methods.[13]

Reaction kinetics of metallothionein with apo-CA

Zinc transfer reactions from metallothioneins or its fragments to apo-CA were studied according to the literature method.[14] Briefly, Zn_7-MT or Zn_4-α domain was incubated with apo-CA in 0.01 mol·L^{-1} Tris-HCl buffer (pH 7.5) at 25℃. In a certain time interval aliquots (100 μL) were drawn from the reaction system to monitor the enzyme activity of CA. Since the hydrolytic rate of *p*-nitrophenyl acetate is proportional to the concentration of reconstituted enzyme, one of the enzymatic products can be monitored at 348 nm, it is convenient to trace the reconstituting process. To study the effect of small molecules on these reactions, stoichiometric complex compounds were incubated with Zn_7-MT-**II** in the buffer for 10 min before apo-CA was added.

Results

Zinc transfer from metallothioneins and its fragments to apo-CA

The activity restoration of CA at different incubation time is shown in Fig. 1. Zn_7-MT-**I** and Zn_7-MT-**II** present similar curves, while the Zn_4-α-MT-**I** shows relatively lower capacity of zinc donation. In the molar ratio of [MT]/[apo-CA] ≈ 1, the activity restorations of apo-CA activated by Zn_7-MT-**I**, Zn_7-MT-**II** or Zn_4-α-MT-**I** are 40—50%, 40% and 30%, respectively (Fig. 1a). Surprisingly, Zn_4-α-MT-**II** completed the zinc transfer to apo-CA within 20 s (Fig. 1b), but it took 30 min for Zn_4-α-MT-**I** to transfer an equal zinc ion to apo-CA (Fig. 1a). This situation is held even in different ratios of [Zn_4-α-MT]/[apo-CA].

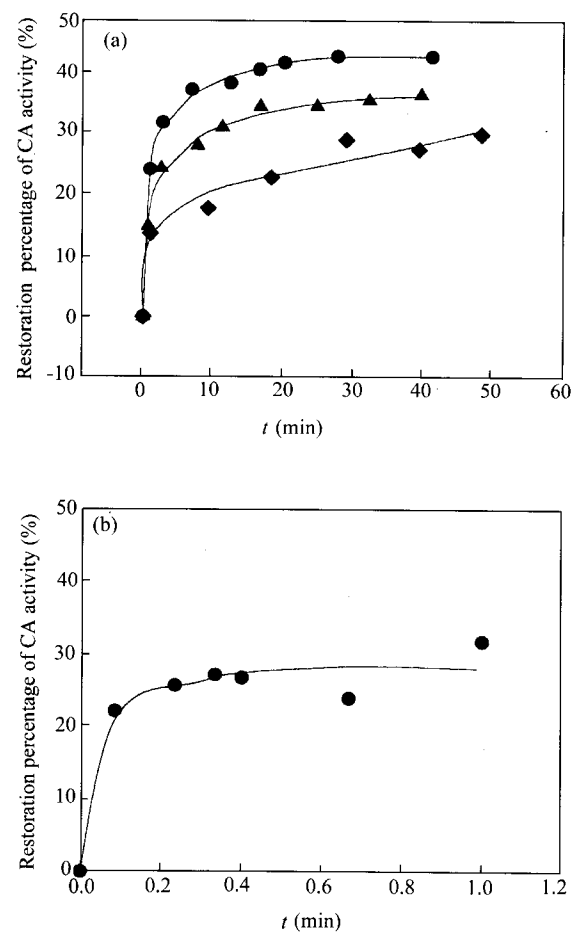

Fig. 1 Restoration of apo-carbonic anhydrase activity by various metallothioneins. (a) Zn_7-MT-**I** (●), Zn_7-MT-**II** (▲), Zn_4-α-MT-**I** (◆); (b) Zn_4-α-MT-**II**.

The reaction of MT activating apo-CA is likely a second-order reaction as follows:

$$Zn_7\text{-MT} + \text{apo-CA} \longrightarrow \text{Zn-CA} + \text{products}$$

It has the following kinetic formula:[15]

$$\ln([\text{MT}]/[\text{apo-CA}]) = ([\text{MT}]_0 - [\text{apo-CA}]_0)\,k_2 t - \ln([\text{MT}]_0/[\text{apo} - \text{CA}]_0)$$

The concentration of reconstituted CA can be detected from the reaction, then the concentrations of apo-CA and MT can be obtained according to the reaction formula. By plotting $\ln([\text{MT}]/[\text{apo-CA}])$ *vs*. time, a series of straight lines were obtained. The zinc transfer rate constants can be deduced from the slops and were listed in Table 1. Data of the reaction between Zn_7-MT-**I** and apo-CA were listed and the calculation was illustrated in Table 2. Note that the unusual constant of Zn_4-α-MT-**II** is at least 20 times faster than the others.

Table 1 Efficiency of zinc donation from the different metallothioneins

Zinc donor	Maximum CA-constituted percent (%) (at equal molar ratio)	Rate constant ($(\text{mol/L})^{-1}\cdot\text{min}^{-1}$)
Zn_7-MT-**I**	40—50	$(1.0 \pm 0.2) \times 10^3$
Zn7-MT-**II**	~40	$(2.8 \pm 0.6) \times 10^3$
Zn_4-α-MT-**I**	~30	$(1.1 \pm 0.5) \times 10^3$
Zn_4-α-MT-**II**	~30	$(1.2 \pm 0.5) \times 10^5$

Table 2 Data of the zinc transfer reaction between Zn_7-MT-**I** and apo-CA

Time (min)	Activity restoration of CA (%)	[apo-CA] (μmol/L)	[MT] (μmol/L)	ln([MT]/[apo-CA]) ($\times 10^4$)
0.8	24.44	8.674	8.664	-11.54
2.7	32.22	7.781	7.771	-12.86
6.8	37.78	7.143	7.133	-14.01
12.3	38.89	7.015	7.005	-14.26
16.5	41.11	6.760	6.750	-14.80
20.1	42.22	6.633	6.623	-15.09
27.6	43.33	6.506	6.496	-15.38
41.3	43.33	6.506	6.496	-15.38

$[Zn_7\text{-MT}]_0 = 11.47$ μmol/L, $[\text{apo-CA}]_0 = 11.48$ μmol/L.

Zinc transfer reactions in the presence of small complex molecules

As demonstrated by X-ray structure analysis on metallothionein, the metal-sulfur clusters are far from wrapped up completely by its peptide chains. Each domain has a cleft containing three cysteine residues which is exposed to the solvent.[4] NMR probing study indicated that the cleft in α domain is one of binding sites for $Fe(EDTA)^-$.[16] Thus, studying the effect of $Fe(EDTA)^-$ on the reaction may provide additional information about whether the cleft plays a role in the zinc transfer reaction (Fig. 2). Experimental results shows that the zinc transfer reaction from Zn_7-MT-**II** to apo-CA in the existence of $Fe(EDTA)^-$ was extended and the rate became slower, as the stoichiometric complex anion was added into the reaction system. On the contrary, the complex compounds with positive charge such as $Cr(en)_3^{3+}$, did not give rise to similar blocking effect (Fig. 3). This is consistent with our previous study that the positive charge ion, $Cr(en)_3^{3+}$, had little accessibility to metal-sulfur cluster.[16] This result strongly suggested that the cleft be involved in the zinc transfer reaction.

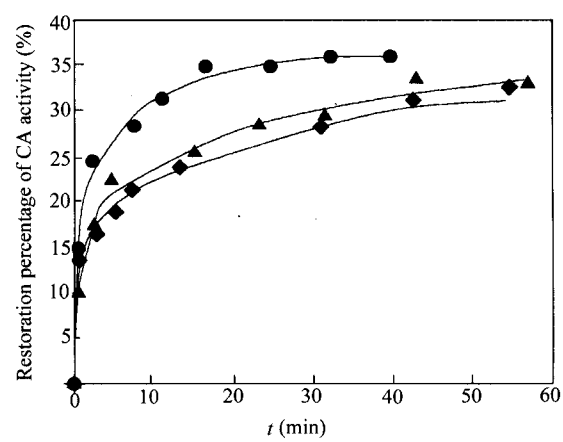

Fig. 2 Restoration of apo-carbonic anhydrase activity by Zn_7-MT in the presence of $Fe(EDTA)^-$. The ratio of $[Fe(EDTA)^-]/[Zn_7\text{-MT-II}]$: 0 (●), 2 (▲), 10 (◆). The rate constants of 0-fold, 2-fold and 10-fold $Fe(EDTA)^-$ were $2.22 \times 10^3 (\text{mol/L})^{-1}\cdot\text{min}^{-1}$, $7.90 \times 10^2 (\text{mol/L})^{-1}\cdot\text{min}^{-1}$ and $6.21 \times 10^2 (\text{mol/L})^{-1}\cdot\text{min}^{-1}$.

Glutathione, as an important metal chelator, usually maintains a high and stable level in cells (approximately 2—3 mmol/L). It has been reported that its existence may have some effects on metal transfer in

cells. For instance, an intracellular copper transfer between reduced glutathione (GSH) and Cu-MT was observed.[17] However, here in the zinc transfer reaction with 0 and 50 times excess of GSH solution, little difference was observed both in precession curves and second order rate constants, as shown in Fig. 4. On the contrary, glutathione disulfide (GSSG) could accelerate this zinc transfer reaction obviously. As shown in Fig. 5, the rate constants in the presence of GSSG was raised from 2.66×10^3 $(mol/L)^{-1} \cdot min^{-1}$ to 7.34×10^3 $(mol/L)^{-1} \cdot min^{-1}$ in the same system. In the latter system, 200-fold excess of GSSG was added, since the oxidation reaction is rather slow when the ratio of GSSG/MT is less than 100.

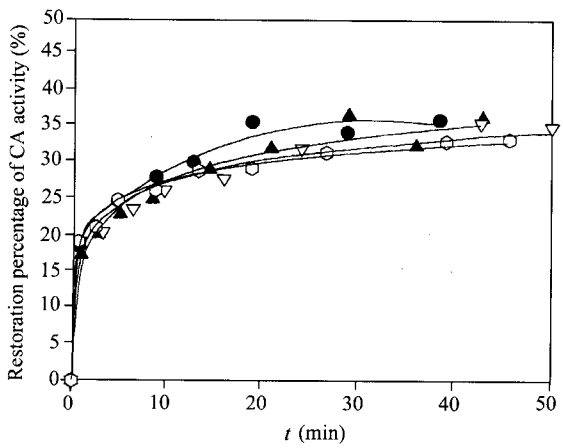

Fig. 3 Restoration of apo-carbonic anhydrase activity by Zn_7-MT in the presence of $Cr(en)_3^{3+}$. Neither acceleration nor inhibition was observed even when 20 times higher $Cr(en)_3^{3+}$ were incubated with protein. The ratio of [$Cr(en)_3^{3+}$]/[Zn_7-MT-II]: 0 (●), 2 (▲), 5 (▽), 20 (○).

Discussion

Carbonic anhydrase is known as an efficient metalloenzyme and its structure was determined by previous workers.[18-21] In holo-enzyme, the catalytic zinc ion is located near the center of the enzyme molecule and closes to the bottom of a 15 nm-deep active site cavity. And no protein atoms displayed any obvious positional change in metal-free enzyme. In the zinc transfer process, a zinc ion entered the cavity and *de novo* constituted a holo-enzyme. The second-order kinetics suggested an association mechanism.[15] It means a direct zinc transfer took place within the intermediate complex, which was composed of metallothionein and apo-carbonic anhydrase.

$$Zn_7\text{-MT} + \text{apo-CA} \longrightarrow [Zn_7\text{-MT-apo-CA}]^* \longrightarrow \text{products}$$

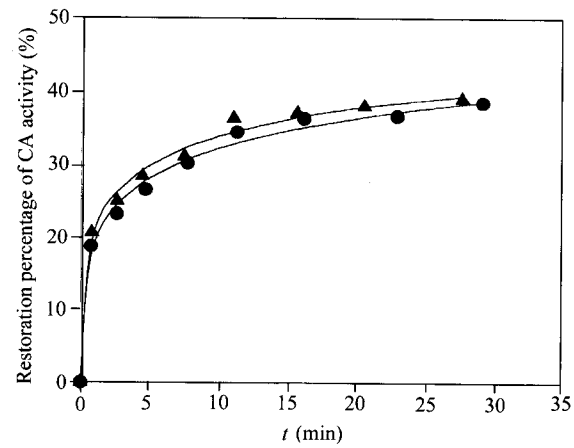

Fig. 4 Zinc transfer reaction between Zn_7-MT-II and apo-CA in the presence of GSH. The ratio of [GSH]/[Zn_7-MT-II]: 0 (●), 50 (▲).

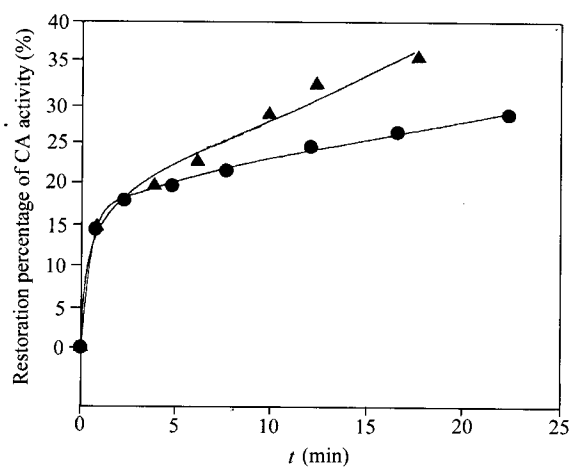

Fig. 5 Zinc transfer accelerated by glutathione disulfide. The concentration of Zn_7-MT-II and apo-CA was 4.06×10^{-6} mol/L and 6.42×10^{-6} mol/L, respectively. Glutathione disulfide was incubated with Zn_7-MT-II at 25℃ for 10 min before apo-CA was mixed. The ratio of [GSH]/[Zn_7-MT-II]: 0 (●), 200 (▲). The rate constant was accelerated from 2.66×10^3 $(mol/L)^{-1} \cdot min^{-1}$ to 7.34×10^3 $(mol/L)^{-1} \cdot min^{-1}$.

It was also proved by our ultrafiltration experiment. When Zn_7-MT was subjected to ultrafiltration with Amicon YM-5 membrane, the filtrate could not restore the apo-CA activity. As previously described, only 40% apo-CA was reconstituted when equal molar Zn_7-MT was

added to react with apo-CA, and Zn_4-α fragment under the same condition had 30% apo-carbonic anhydrase restored activity (Fig. 1 and Table 1). There would be two possible forms for this zinc transfer reaction:

a. Zn_7-MT + apo-CA ⟶ [Zn_7-MT-apo-CA]* ⟶ Zn_6-MT + Zn-CA

b. Zn_7-MT + apo-CA ⟶ [Zn_7-MT-apo-CA]* ⟶ 1/7 apo-MT + Zn-CA + 6/7 Zn_7-MT

If the second pathway occurred, since the free zinc ion can restore almost 100% (~97.5%) of the CA activity,[22] it would infer that only 1/7 amount of Zn_7-MT would be enough to restore the whole activity of apo-CA. But this is not the case and is inconsistent with our experiments. In most of our experiments the results show that when the ratios of Zn_7-MT to apo-CA are greater than 0.5 (in some experiments it even reaches 2) the restored activities of CA are no more than 75%. These facts support the pathway **a**. This means no more than one zinc ion per metalloprotein molecule is involved in the zinc transfer process.

Winge *et al.*[23] reported that two *iso*-metallothioneins isolated from rat liver exhibited variation in their ability to reconstitute apo-carbonic anhydrase. Our results show that MT-**I** restored apo-CA activity slightly higher than MT-**II** (Table 1), which is consistent with the result reported by Winge *et al.*[23] However, the present paper gives a result, which is not completely consistent with theirs, that is the isoform **II** exhibits a little faster reaction. The restored activity of apo-CA by isoform **II** usually reaches its maximum within 15 min, while the similar process with isoform **I** needs 30 min (see Fig 1. a, b and Table 1). Not only holo-protein isoforms display such variation, the two Zn_4-α fragments derived from the different isoforms also perform the zinc transfer reaction with enormous different rates. To the Zn_4-α-domain of MT-**II**, the rate is about 1.2×10^5 $(mol/L)^{-1} \cdot min^{-1}$, while to that of MT-**I**, 1.1×10^3 $(mol/L)^{-1} \cdot min^{-1}$ (see Table 1). The former is two orders magnitude faster than the latter. Since the knowledge about the structural difference between these isoforms remains poor, and almost all detailed structural data concerned are on MT-**II** due to its high homogeneity, a satisfactory explanation for the different zinc transfer rates of isoforms is not available yet.

Although crystal structure analysis indicates that two domains are independent of each other,[4,5,24] the interaction between domains in solution can not be ruled out.[9] Listed in Table 1, the second-order rate constant of Zn_4-α fragment is dozens of times faster than that of holo-protein. This constant is almost one tenth of that activated by free Zn^{2+} ion.[25] Absence of β domain does affect the zinc transfer process. A similar situation was occurred in the metals chelation of MTs by EDTA.[26,27] Recent study shows that zinc transfer potentials of α- and β-domain of MT are affected by domain interactions in the whole molecule.[9] It is also noted that such difference in MT-**II** fragments can not be observed between Zn_7-MT-**I** and Zn_4-α-MT-**I** when reacting with apo-carbonic anhydrase. Although the exact reason is unknown yet, it can be inferred reasonably to the different conformation of Zn_4-α-MT-**I** from that of Zn_4-α-MT-**II**, which leads to the variation of either the stability of construction or the binding of two proteins.

We have little knowledge about the structural difference between Zn_4-α domain in metallothioneins and the isolated Zn_4-α fragment. A reliable explanation needs further experiments. However, the dimerization of holo-metallothioneins (with an association constant of 3.0×10^4 mol/L) in the system should be one of the reasons. This dimerization reaction competes with the zinc transfer reaction. Particularly, it is considered that the β domain is inactive in the dimmer.[26] As pointed out, the dimer of metallothionein is constituted of monomers in the pattern of head-to-tail, *i.e.* α- to β-linked.[4] In the absence of β domains, it is difficult for the sole Zn_4-α fragments to form a dimer.

It is assumed that the zinc transfer reaction takes place within the intermediate complex, [Zn_7-MT-apo-CA]*. This mechanism is further supported by the facts that some charged amino acids such as glutamine, asparagine and lysine have little effect on the zinc transfer reaction and nor ionic strength does (results not shown).

Despite several papers assumed metallothionein as a zinc donor,[14,15,28] direct evidence has not been obtained *in vivo* so far. In cells, a metal transfer process may be changed by various factors. For example, it may relate to some molecules other than metallothioneins, such as glutathione. Here we introduce glutathione into the zinc transfer system in order to explore the role of glutathione in zinc homeostasis. The GSH/GSSG redox couple has been clearly proven to regulate the mobility of zinc among metallothioneins and other zinc proteins. The re-

sults that addition of GSH did not show any acceleration of zinc release from Zn-MT means that GSH can not compete zinc against apo-CA. That is to say, no zinc transfer reaction from metallothioneins to GSH takes place. And the GSH-MT complex, even if it exists in this system, is similar to the MT itself as a zinc donor. A member of evidences indicated that oxidative stress contributes to zinc release from MT,[29,30] which is consistent with our results reported above that the oxidative glutathione accelerates the release of zinc (see Fig. 5). One possible reason is that the reaction between Zn-thiol cluster and GSSG shifts the reaction to the formation of RSSR in clusters, accelerating release of zinc. Considering that the ratio of GSSG : GSH differs significantly (from 1 : 300 to 1 : 1) in different cytoplasm and tissue fluids,[29] the ability of MT to donate zinc should be quite changeable by modulation of coupled reaction between GSH/GSSG and thionein/MT under a variety of physiological conditions.

From these *in vitro* experiments, we believe that Zn-MT may play a functional role on metal exchange and ligand substitution. In fact, evidence that such chemistry may also occur in cell has been reported.[31,32] Besides to be as a donor, metallothioneins exhibit a tendency of abstracting Zn^{2+} ion from less stable zinc proteins due to its high affinity for Zn^{2+}.[3] It was found that thionein could remove zinc ion from those zinc finger proteins such as Sp1 and TF IIIA, which consequently loses their ability to bind to its cognate DNA and to activate downstream gene transcription by RNA polymerase II.[33,34] Similar processes may be universal, for apo-metallothioneins are proven to be existed widespread in tumor cells.[35] Thus, the thionein/metallothionein couple may be considered as a collation/allocation system, regulating zinc balance among various zinc proteins.

References

1 Kagi, J. H. R.; Schaffer, A. *Biochemistry* **1998**, *27*, 8509.
2 Vasák M.; Hasler, D. W. *Curr. Opin. Chem. Biol.* **2000**, *4*, 177.
3 Kagi, J. H. R. In *Metallothionein III*, Eds.: Suzuki, K. T.; Imura, N.; Kimura, M., Birkkhauser Verlag Basel, Switzerland, **1993**, p.29.
4 Robbins, A. H.; McRee, D. E.; Williamson, M.; Collett, S. A.; Xuong, N. H.; Furey, W. F.; Wang, B. C.; Stout, C. D. *J. Mol. Biol.* **1991**, *221*, 1269.
5 Rhee, I.-K.; Lee, K. S.; Huang, P. C. *Protein Eng.* **1990**, *3*, 205.
6 Brouwer, M.; Hoexum-Brouwer, T.; Cashon, R. E. *Biochem. J.* **1993**, *294*, 219.
7 Cismowski, M. J.; Huang, P. C. *Biochemistry* **1991**, *30*, 6626.
8 Cismowski, M. J.; Narula, S. S.; Armitage, I. M.; Chernaik, M. L.; Huang, P. C. *J. Biol. Chem.* **1991**, *36*, 24390.
9 Jiang, L.-J.; Vasak, M.; Vallee, B. L.; Maret, W. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2503.
10 Munoz, A.; Petering, D. H.; Shaw III, C. F. *Inorg. Chem.* **1999**, *38*, 5655.
11 Zelazowski, A. J.; Stillman, M. J. *Inorg. Chem.* **1992**, *31*, 3363.
12 Good, M.; Hollenstein, R.; Vasak, M. *Eur. J. Biochem.* **1991**, *197*, 655.
13 Huang, Z.-X.; Hu, H.-Y. *Inorg. Chem.* **1992**, *8*, 240 (in Chinese).
14 Huang, Z.-X.; Hu, H.-Y.; Gu, W.-Q.; Sun, J.-Q. *Chin. J. Chem.* **1993**, *11*, 246.
15 Li, T.-Y.; Kraker, A. J.; Shaw III, C. F.; Petering, D. H. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 6335.
16 Huang, Z.-X.; Gu, W.-Q.; Zheng, Q.; Liu, F.; Sun, Y.-J. *Chin. J. Chem.* **1997**, *15*, 385.
17 Freedman, J. H.; Ciriolo, M. R.; Peisach, J. *J. Biol. Chem.* **1989**, *264*, 5598.
18 Liljas, A.; Kannan, K. K.; Bergsten, P.-C.; Waara, I.; Fridborg, K.; Strandberg, B.; Carlbom, U.; Jarup, L.; Lovgren, S.; Petef, M. *Nature, New Biology* **1972**, *235*, 131.
19 Henderson, L. E.; Henriksson, D.; Nyman, P. O. *J. Biol. Chem.* **1976**, *251*, 5457.
20 Sciaky, M.; Limozin, N.; Filippi-Foveau, D.; Gulian, J. M.; Laurent-Tabusse, G. *Biochimie* **1976**, *58*, 1071.
21 Hakansson, K.; Carlsson, M.; Svensson, L. A.; Liljas, A. *J. Mol. Biol.* **1992**, *227*, 1192.
22 Huang, Z.-X.; Hu, H.-Y.; Gu, W.-Q.; Wu, G. *J. Inorg. Biochem.* **1994**, *54*, 147.
23 Winge, D. R.; Miklossy, K.-A. *Arch. Biochem. Biophys.* **1982**, *214*, 80.
24 Schultze, P.; Worgotter, E.; Braun, W.; Wagner, G.; Vasak, M.; Kagi, J. H. R.; Wuthrich, K. *J. Mol. Biol.* **1988**, *203*, 251.
25 Henkens, R. W.; Sturtevant, J. M. *J. Am. Chem. Soc.* **1968**, *90*, 2669.
26 Gan, T.; Munoz, A.; Shaw III, C. F.; Petering, D. H. *J. Biol. Chem.* **1995**, *270*, 5339.
27 Winge, D. R.; Miklossy, K.-A. *J. Biol. Chem.* **1982**, *257*, 3471.
28 Udom, A. O.; Brady, F. O. *Biochem. J.* **1980**, *187*, 329.
29 Maret, W. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 237.
30 Maret, W. *Neurochem. Int.* **1995**, *27*, 111.
31 Meister, A.; Anderson, M. E. *Annu. Rev. Biochem.* **1983**, *52*, 711.
32 Hidalgo, J.; Garvey, J. S.; Armaric, A. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 554.
33 Zeng, J.; Heuchel, R.; Schaffner, W.; Kagi, J. H. R. *FEBS Lett.* **1991**, *279*, 310.
34 Zeng, J.; Vallee, B. L.; Kagi, J. H. R. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 9984.
35 Pattanaik, A.; Shaw III, C. F.; Petering, D. H.; Garvey, J.; Kraker, A. J. *J. Inorg. Biochem.* **1994**, *54*, 91.

(E200007146 SONG, J.P.; LING, J.)