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Comparative ^{113}Cd-n.m.r. studies on rabbit $^{113}Cd_7$-, (Zn_1,Cd_6)- and partially metal-depleted $^{113}Cd_6$-metallothionein-2a

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Rabbit $^{113}Cd_7$-metallothionein-2a (MT) contains two metal–thiolate clusters of three (cluster B) and four (cluster A) metal ions. The ^{113}Cd-n.m.r. spectrum of $^{113}Cd_6$-MT, isolated from $^{113}Cd_7$-MT upon treatment with EDTA, is similar to that of $^{113}Cd_7$-MT, but the cluster B resonances are lower in intensity, suggesting its co-operative metal depletion. $(Zn_1,^{113}Cd_6)$-MT, formed upon addition of the Zn(II) ions to $^{113}Cd_6$-MT, shows ^{113}Cd-n.m.r. features characteristic of cluster B populations containing both Cd(II) and Zn(II) ions. The overall intensity gain of the mixed cluster B resonances per Cd as to those in $^{113}Cd_6$- and $^{113}Cd_7$-MT suggests a stabilization effect of the bound Zn(II) ions upon the previously established intramolecular ^{113}Cd exchange within this cluster.

INTRODUCTION

In recent years a number of studies have been conducted on the elucidation of the structure of metal-binding sites in MT, a low-molecular-mass (6–7 kDa) cysteine- and metal-rich protein (Vašák & Kägi, 1983). Thus the ^{113}Cd-n.m.r. (Otvos & Armitage, 1979, 1980) and Co(II)-e.p.r. and magnetic-c.d. (Vašák, 1980; Vašák & Kägi, 1981) studies on the respective metalloform of this protein have established the existence of two metal–thiolate clusters, of three and four metal ions, possessing overall tetrahedral tetrathiolate metal co-ordination. Additional support for the two-cluster model came from the enzymic cleavage of this protein and subsequent isolation of the *C*-terminal cluster domain (cluster A) enfolding four metal ions (Winge & Miklossy, 1982). More recently, both the crystal structure (Furey *et al.*, 1986) and the solution n.m.r. structure (Frey *et al.*, 1985; Braun *et al.*, 1986) have been solved. While both structures agree on the presence of two metal–thiolate clusters, they differ in details of the sequence-specific metal–cysteine connectivities (Wagner *et al.*, 1987).

Since it is assumed that the physiological function of MT is in metal metabolism (Zn, Cu) and in heavy-metal detoxification (Hg, Cd) (Kägi & Nordberg, 1979), the differential reactivity of the metal ions bound and the properties of partially metal-depleted MT are of great interest. In order to probe for the differential reactivity the chelating agent EDTA has often been used. Early electronic absorption studies dealing with the effect of EDTA on Zn_7- and (Zn_3,Cd_4)-MT have shown that Zn(II) ions are removed relatively rapidly, in contrast with Cd(II) ions, whose removal is very slow (Li *et al.*, 1980). Armitage & Boulanger (1983) reported the disappearance of the ^{113}Cd-n.m.r. resonances of the Cd_3 cluster (cluster B) of rabbit liver $^{113}Cd_7$-MT-1 on treatment with EDTA. Nicholson *et al.* (1987) demonstrated, by using ^1H-n.m.r., electronic absorption and atomic absorption spectroscopy, that most Zn(II) ions in Zn_7-MT react rapidly with EDTA. However, in (Zn_2,Cd_5)-MT only one to two Zn(II) ions and none of the Cd(II) ions were labile. Furthermore, under the same conditions only one Cd(II) ion has been extracted from Cd_7-MT.

The aim of the present work was to characterize the metal-deficient Cd_6-MT form by using ^{113}Cd-n.m.r. spectroscopy and to explore further the binding properties of this form with respect to the Zn(II) ions.

EXPERIMENTAL

Rabbit liver MT used in the present study was purified as described previously (Kägi *et al.*, 1974; Kimura *et al.*, 1979). All MT preparations were characterized by amino acid analysis (Durrum 500) and by metal analysis by the use of atomic absorption spectroscopy (Instrumentation Laboratory IL 157 instrument). The protein concentration was determined spectrophotometrically by measuring the absorbance of the apoprotein at 220 nm in 0.01 M-HCl (ϵ_{220} 47300 $M^{-1}\cdot cm^{-1}$; Bühler & Kägi, 1979). The fully ^{113}Cd(II)-saturated $^{113}Cd_7$-MT was prepared by reconstitution followed by gel filtration on Sephadex G-50 (Vašák *et al.*, 1985). The metal-deficient $^{113}Cd_6$-MT was isolated from the $^{113}Cd_7$-MT form by incubation (5 min) with 20-fold molar excess of EDTA in 20 mM-potassium phosphate buffer, pH 7.0 (Nicholson *et al.*, 1987). In a subsequent step EDTA and the Cd(II)–EDTA complex were removed from the reaction mixture by using a gel-filtration (Sephadex G-50) column equilibrated with 20 mM-KCl/20 mM-Tris/HCl buffer, pH 7.0. In order to prevent the oxidation of the cysteine thiolates all solutions used in the preparation of $^{113}Cd_6$-MT were continuously purged with argon and all of the sample handling was performed in an argon-purged glove-box. Throughout these studies the accessibility of all 20 cysteine residues was monitored by Ellman's reagent [5,5′-dithiobis-(2-nitrobenzoic acid)] at 412 nm in 20 mM-potassium phosphate buffer, pH 7.0, containing 2 M-guanidinium chloride and 20 mM-EDTA (ϵ_{412} 13600 $M^{-1}\cdot cm^{-1}$; McGilvray & Morris, 1971). For n.m.r. measurements $^{113}Cd_6$-MT was concentrated in an Aminco

Abbreviation used: MT, metallothionein (isoform 2a).
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ultrafiltration apparatus (YM-2 membrane) and the n.m.r. tubes were sealed under reduced argon atmosphere. The (Zn_1,$^{113}Cd_6$)-MT sample was prepared from the $^{113}Cd_6$-MT form to which 1 equiv. of Zn(II) at pH 7.0 (20 mM-KCl/20 mM-Tris/HCl buffer) was added, and the sample was subsequently passed through a Sephadex G-50 column equilibrated with the same buffer.

^{113}Cd-n.m.r. spectra were recorded at 80 MHz on a Bruker AM-360 spectrometer with the use of broad-band ^{1}H decoupling applied during data acquisition. MT samples (5–7 mM) containing > 95%-enriched ^{113}Cd isotope (A.E.R.E., Harwell, Berks., U.K.) were placed in 5 mm tubes in 20 mM-KCl/20 mM-Tris/HCl buffer, pH 7.0, containing 10% (v/v) ^2H_2O to provide the field-frequency lock. Typical acquisition parameters were 90° pulse, acquisition time 0.4 s with 2.5 s pulse delay between consecutive pulses and 20000–30000 transients at 25 °C. A line-broadening function of 20 Hz was applied before Fourier transformation. Chemical shifts are reported in p.p.m. downfield from the ^{113}Cd resonance of 0.1 M-$Cd(ClO_4)_2$ in ^2H_2O.

RESULTS AND DISCUSSION

The incubation of fully metal-occupied $^{113}Cd_7$-MT with a 20-fold molar excess of EDTA at neutral pH followed by gel filtration (Sephadex G-50) resulted in formation of the metal-deficient ^{113}Cd-MT form containing exactly 6 mol of Cd(II)/mol of apo-MT. This suggests the existence of one labile metal-binding site in this protein. In order to gain insight into the structural features of this metal-deficient MT form, comparative ^{113}Cd-n.m.r. studies with $^{113}Cd_7$-MT were conducted. Fig. 1(*a*) displays the ^{113}Cd-n.m.r. spectrum of $^{113}Cd_7$-MT. The ^{113}Cd resonances designated 1, 5, 6 and 7 originate from the four-metal cluster (cluster A) and resonances 2, 3 and 4 come from the three-metal cluster (cluster B) in this protein (Otvos & Armitage, 1980; Frey *et al.*, 1985; Otvos *et al.*, 1985). The spectral origin of the minor 673 p.p.m. ^{113}Cd signal in the latter spectrum (marked with arrow) is not known. Before the n.m.r. measurement the $^{113}Cd_7$-MT form was passed through a gel-filtration column (Sephadex G-50) to ensure size homogeneity and that all cysteine residues in MT were accessible to modification by 5,5′-dithiobis-(2-nitrobenzoic acid). Therefore the presence of polymeric species brought about by oxidation of the cysteine residues, which might explain the 673 p.p.m. signal, is unlikely.

In view of the existence of the cluster structure in MT, the removal of one ^{113}Cd from a specific site was expected to bring about marked changes in the ^{113}Cd-n.m.r. spectrum of the $^{113}Cd_6$-MT form. Thus the transformation of bridging to terminal thiolate ligands would generate low-field-shifted ^{113}Cd resonances, e.g. the chemical-shift position of ^{113}Cd nuclei bound to four cysteine residues in horse liver alcohol dehydrogenase occurred at 751 p.p.m. (Bobsein & Mayers, 1980). The ^{113}Cd-n.m.r. spectrum of $^{113}Cd_6$-MT between 850 and 540 p.p.m. reveals a set of resonances between 610 and 675 p.p.m. only (Fig. 1*b*). A comparison of the latter spectrum with that of $^{113}Cd_7$-MT shows their virtual identity (Figs. 1*a* and 1*b*), the only difference being the substantially lower intensity of the three-metal-cluster resonances 2, 3 and 4 in $^{113}Cd_6$-MT (Fig. 1*b*). The integration of each of the resonances 2, 3 and 4 shows only 48% intensity compared with the resonances 1, 5, 6 and 7. This effect indicates that a Cd(II) ion was removed from the cluster B. A chemical exchange of the remaining two Cd(II) ions among the three metal-binding sites, as a cause of the intensity decrease, is improbable, since chemical shifts differing from those found in $^{113}Cd_7$-MT would be expected. Thus the observed effect suggests the co-operative depletion of the three-metal cluster. From stoichiometric measurements of the Cd(II)/protein-fragment ratios obtained upon enzymic digestion with subtilisin (Nielson & Winge, 1983) and from the ^{113}Cd-n.m.r. studies in which the effect of increasing concentrations of EDTA on the ^{113}Cd resonances of the protein-inhomogeneous $^{113}Cd_7$-MT-1 form was followed (Armitage & Boulanger, 1983), a similar co-operative process in the cluster B domain has been inferred. In our case a direct observation of this effect on the characterized $^{113}Cd_6$-MT sample and in the absence of complexing agent (EDTA) was made. This result is in agreement with our recent ^{113}Cd-n.m.r. titration studies of apo-MT with Cd(II) ions, in which, at neutral pH, a sequential co-operative cluster formation of both metal–thiolate clusters, cluster A being formed first, has been clearly found (M. Good, R. Hollenstein, P. J. Sadler & M. Vašák, unpublished work). In view of the established co-operative metal binding and since in the native (Zn_2,Cd_5)-MT form the Zn(II) ions appear to be distributed between both clusters (Nettesheim *et al.*, 1985),

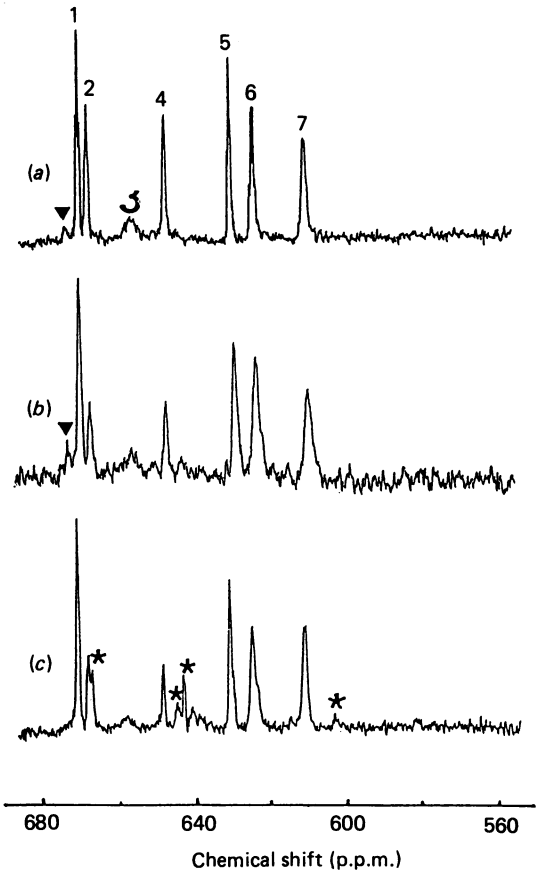

Fig. 1. ^{113}Cd-n.m.r. spectra of rabbit liver MT at pH 7.0

(*a*) $^{113}Cd_7$-MT; the numbers at each resonance refer to the position in the cluster (Otvos & Armitage, 1980; Frey *et al.*, 1985); (*b*) $^{113}Cd_6$-MT; (*c*) (Zn_1,$^{113}Cd_6$)-MT. For details see the text.

the question arises as to the effect of Zn(II) ions on the ^{113}Cd-n.m.r. spectrum of $^{113}Cd_6$-MT when the full metal saturation of this form, i.e. (Zn_1,$^{113}Cd_6$)-MT, is restored. Since the Zn(II) ions show substantially lower affinity towards the metal-binding sites in MT compared with the Cd(II) ions (approx. 10^4-fold) (Vašák & Kägi, 1983) and provided that a complete co-operativity process exists, the expected formation of the Zn_3 cluster upon the addition of 1 equiv. of Zn(II) should leave the $^{113}Cd_6$-MT spectrum virtually unaffected Somewhat surprisingly, the ^{113}Cd-n.m.r. spectrum of (Zn_1,$^{113}Cd_6$)-MT (Fig. 1*c*) reveals, besides the Cd_3-cluster resonances 2, 3 and 4, a number of additional ^{113}Cd signals (marked with asterisks). It should also be noted that no apparent Zn(II) effect on the Cd_4-cluster resonances 1, 5, 6 and 7 is observed. The latter is in agreement with the established larger kinetic (Otvos *et al.*, 1987) as well as thermodynamic stability (Nielson & Winge, 1983; Armitage & Boulanger, 1983; Vašák & Kägi, 1983) of cluster A. Thus it is concluded that the additional ^{113}Cd-n.m.r. resonances unobserved in the $^{113}Cd_6$-MT spectrum (Fig. 1*b*) arise from the three-metal cluster populations in which both Cd(II) and Zn(II) ions are concomitantly present. The ^{113}Cd-n.m.r. studies by Nettesheim *et al.* (1985), in which the product of metal-exchange reactions of Zn(II) in Zn_7-MT by Cd(II) was monitored, resulted in non-selective Zn(II) replacements in both clusters. The apparent binding of Zn(II) ions to cluster B only is unique in our studies. Hence it would appear that under appropriate conditions both clusters can be filled independently. Indeed, in a preliminary note (Vašák & Good, 1987) the successful preparation of [$Co(II)_3$,$^{113}Cd_4$]-MT has been reported. As shown by the use of ^{113}Cd-n.m.r., electronic absorption and magnetic-c.d. data, the Co(II) ions selectively occupy cluster B and the Cd(II) ions occupy cluster A.

A comparison of the overall integral intensity of the three-metal-cluster resonances of the $^{113}Cd_7$-, $^{113}Cd_6$- and (Zn_1,$^{113}Cd_6$)-MT forms per Cd with the virtually unaffected resonances of the four-metal cluster reveals widely differing values (see below). At this point it may be noted that the ^{113}Cd signals of the three-metal cluster in this work (Fig. 1*a*) and in all ^{113}Cd-n.m.r. studies performed so far on the fully metal-occupied mammalian $^{113}Cd_7$-MTs have always been found to be lower in intensity (by approx. 20%) than the four-metal cluster resonances (Otvos & Armitage, 1980; Vašák *et al.*, 1985; Frey *et al.*, 1985). This observation prompted the detailed ^{113}Cd-n.m.r. studies of rabbit liver $^{113}Cd_7$-MT leading to the suggestion of conformational flexibility, thereby allowing dynamic processes within the cluster structure (Vašák *et al.*, 1985). Direct support for the dynamic process within cluster B was provided by ^{113}Cd-n.m.r. saturation transfer experiments performed on $^{113}Cd_7$-MT, which indicated an intramolecular metal exchange (Otvos *et al.*, 1987). Not much is known so far about the mechanism governing this process; however, on the basis of temperature-dependence studies of the cluster B resonances rapid dissociation and reassociation processes of ^{113}Cd ions have been excluded (Nettesheim *et al.*, 1985).

Under our conditions the ^{113}Cd resonances of the three-metal cluster amount to 2.4 Cd in $^{113}Cd_7$-MT, to 1.45 Cd in $^{113}Cd_6$-MT and to 1.85 Cd in (Zn_1,$^{113}Cd_6$)-MT. The last two values can be explained by a certain stabilization effect of the Zn(II) ions on the established dynamic process(es) within the three-metal-cluster domain.

The occurrence of additional rather intense ^{113}Cd resonances in the (Zn_1,$^{113}Cd_6$)-MT spectrum, brought about by the presence of both metals in the cluster B, can be rationalized by incomplete co-operative metal binding in the $^{113}Cd_6$-MT form, increased kinetic stability and the preferential formation of the mixed $(Zn,Cd)_3$ cluster(s) as opposed to the Cd_3 cluster. Incomplete co-operativity is evident from the differences in intensity between the detectable ^{113}Cd resonances originating from cluster B in $^{113}Cd_7$-MT and $^{113}Cd_6$-MT, which amount to 2.4 and 1.45 Cd respectively. In the case of complete co-operativity approx. 10% higher intensity values per Cd in the $^{113}Cd_6$-MT form would be expected. The increased kinetic stability of the mixed $(Zn,Cd)_3$ cluster(s) is revealed by the observed overall rise in intensity of the cluster B resonances in the ^{113}Cd-n.m.r. spectrum of (Zn_1,$^{113}Cd_6$)-MT (1.85 Cd) when compared with that of $^{113}Cd_6$-MT (1.45 Cd). This implies that the presence of Zn(II) ions in the mixed $(Zn,Cd)_3$ cluster substantially decreases the intramolecular ^{113}Cd exchange and thus renders the ^{113}Cd resonances more accessible to observation on the ^{113}Cd-n.m.r. time scale. The evidence that the ^{113}Cd-n.m.r. profile of (Zn_1,$^{113}Cd_6$)-MT represents the thermodynamically as well as the kinetically most stable species is provided by the virtual identity of this spectrum (Fig. 1*c*) with that of (Zn_1,$^{113}Cd_6$)-MT prepared *in situ* by reconstitution of apo-MT with 6 equiv. of Cd(II) followed by the addition of 1 equiv. of Zn(II) at pH 7.5 (results not shown). Independent evidence for the greater kinetic stability of the Zn(II)–tetrathiolate complexes than the Cd(II) ones has been shown in the ^{1}H-n.m.r. studies of the adamantane-type Zn(II)–thiolate and Cd(II)–thiolate cluster models (Hagen *et al.*, 1982). Furthermore, it should be noted that the Cd_3-cluster resonances present in the (Zn_1,$^{113}Cd_6$)-MT form amount to approx. 0.9 Cd only. This value is almost 40% lower when compared with $^{113}Cd_6$-MT and may thus suggest the preferential formation of the mixed $(Zn,Cd)_3$ cluster(s). A similar conclusion has been reached in previous ^{113}Cd-n.m.r. studies from monitoring of the stepwise displacement of Zn(II) in Zn_7-MT by ^{113}Cd(II), though no selective occupancy of one cluster was observed (Nettesheim *et al.*, 1985). It may be noted that the biosynthetically enriched rat (Zn_2,$^{113}Cd_5$)-MT form, a species used in crystallographic studies (Furey *et al.*, 1986), revealed in solution at least 15 ^{113}Cd resonances, indicating the coexistence of multiple forms of proteins with different distribution of Zn(II) and Cd(II) ions in the individual sites of the metal–thiolate clusters (Vašák *et al.*, 1987). Altogether the results presented suggest that the mixed $(Zn,Cd)_3$ cluster forms in MT are thermodynamically and kinetically very stable entities. In this context the occurrence of two additional more intense ^{113}Cd resonances of comparable magnitude in the spectrum of (Zn_1,$^{113}Cd_6$)-MT, shifted downfield from the signals 2 and 4 (Fig. 1), at 667 and 643 p.p.m. is striking. These resonances could be attributed to increased population of two mixed $(Zn,Cd)_3$ clusters containing either two Zn(II) ions (singlets) or one Zn(II) ion in two different sites (doublets) with one of the ^{113}Cd signals being broadened. Another possibility is that a protein population exists in which one specific low-affinity metal-binding site is occupied by a single Zn(II) ion. A similar conclusion, as to the presence of the low-affinity metal-

binding site in Cd_7-MT, was reached in the differential modification studies of cysteine residues when the stepwise cluster formation was monitored (Bernhard *et al.*, 1986) and based on the removal of Cd(II) ions by EDTA from this protein (Nielson & Winge, 1983; Nicholson *et al.*, 1987). The existence of such a site has also been concluded on the basis of the increased Zn–S distances in the crystal structure of (Zn_2,Cd_5)-MT from rat liver (Collett & Stout, 1987). In the absence of ^{113}Cd–^{113}Cd connectivities in our studies such a conclusion would be tentative at most. However, the documented increased kinetic stability of the mixed $(Zn,Cd)_3$ cluster structure(s) may account for the failure to crystallize the metal-homogeneous $^{113}Cd_7$-MT form (Furey *et al.*, 1986).

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REFERENCES

Armitage, I. M. & Boulanger, Y. (1983) in N.m.r. of Newly Accessible Nuclei (Laszlo, P., ed.), vol. 1, pp. 337–365, Academic Press, New York

Bernhard, W. R., Vašák, M. & Kägi, J. H. R. (1986) Biochemistry **25**, 1975–1980

Bobsein, B. R. & Myers, R. J. (1980) J. Am. Chem. Soc. **102**, 2454–2455

Braun, W., Wagner, G., Wörgötter, E., Vašák, M., Kägi, J. H. R. & Wüthrich, K. (1986) J. Mol. Biol. **187**, 125–129

Bühler, R. H. O. & Kägi, J. H. R. (1979) in Metallothionein (Kägi, J. H. R. & Nordberg, M., eds.), pp. 211–220, Birkhäuser Verlag, Basel

Collett, S. A. & Stout, C. D. (1987) Recl. Trav. Chim. Pays-Bas **106**, 182

Frey, M. H., Wagner, G., Vašák, M., Sorensen, O. W., Neuhaus, D., Wörgötter, E., Kägi, J. H. R., Ernst, R. E. & Wüthrich, K. (1985) J. Am. Chem. Soc. **107**, 6847–6851

Furey, W. F., Robbins, A. H., Clancy, L. L., Winge, D. R., Wang, B. C. & Stout, C. D. (1986) Science **231**, 704–710

Hagen, K. S., Stephan, D. W. & Holm, R. H. (1982) Inorg. Chem. **21**, 3928–3936

Kägi, J. H. R. & Nordberg, M. (eds.) (1979) Metallothionein, Birkhäuser Verlag, Basel

Kägi, J. H. R., Himmelhoch, S. R., Whanger, P. D., Bethune, J. L. & Vallee, B. L. (1974) J. Biol. Chem. **249**, 3537–3542

Kimura, M., Otaki, N. & Imano, M. (1979) in Metallothionein (Kägi, J. H. R. & Nordberg, M., eds.), pp. 163–168, Birkhäuser Verlag, Basel

Li, T.-Y., Kraker, A. J., Shaw, C. F., III & Petering, D. H. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 6334–6338

McGilvray, D. & Morris, J. G. (1971) Methods Enzymol. **17**, 585–589

Nettesheim, D. G., Engeseth, H. R. & Otvos, J. D. (1985) Biochemistry **24**, 6744–6751

Nicholson, J. K., Sadler, P. J. & Vašák, M. (1987) Proc. Int. Meet. Metallothionein 2nd (Kägi, J. H. R. & Kojima, Y., eds.), pp. 191–201, Birkhäuser Verlag, Basel

Nielson, K. B. & Winge, D. R. (1983) J. Biol. Chem. **258**, 13063–13069

Otvos, J. D. & Armitage, I. M. (1979) J. Am. Chem. Soc. **101**, 7734–7736

Otvos, J. D. & Armitage, I. M. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 7094–7098

Otvos, J. D., Engeseth, H. R. & Wehrli, S. (1985) Biochemistry **24**, 6735–6740

Otvos, J. D., Engeseth, H. R., Nettesheim, D. G. & Hilt, C. R. (1987) Proc. Int. Meet. Metallothionein 2nd (Kägi, J. H. R. & Kojima, Y., eds.), pp. 171–178, Birkhäuser Verlag, Basel

Vašák, M. (1980) J. Am. Chem. Soc. **102**, 3953–3955

Vašák, M. & Good, M. (1987) Recl. Trav. Chim. Pays-Bas **106**, 184

Vašák, M. & Kägi, J. H. R. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 6709–6713

Vašák, M. & Kägi, J. H. R. (1983) Met. Ions Biol. Syst. **15**, 213–273

Vašák, M., Nicholson, J. K., Hawkes, G. E. & Sadler, P. J. (1985) Biochemistry **24**, 740–747

Vašák, M., Wörgötter, E., Wagner, G., Kägi, J. H. R. & Wüthrich, K. (1987) J. Mol. Biol. **196**, 711–719

Wagner, G., Frey, M. H., Neuhaus, D., Wörgötter, E., Braun, W., Vašák, M., Kägi, J. H. R. & Wüthrich, K. (1987) Proc. Int. Meet. Metallothionein 2nd (Kägi, J. H. R. & Kojima, Y., eds.), pp. 149–157, Birkhäuser Verlag, Basel

Winge, D. R. & Miklossy, K. A. (1982) J. Biol. Chem. **257**, 3471–3476

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