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Lead (II) Binding to Thionein

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Abstract: Lead (II)-binding properties of rabbit liver thionein 2 (apo-MT2) have been studied. Two kinds of complexes, Pb_7-MT and Pb_7-MT', are observed upon the binding of Pb^{2+} to apo-MT at different pH. From their characteristic peaks in UV and CD spectra, it is deduced that Pb^{2+} in Pb_7-MT is coordinated by four sulfhydryl groups in Td microsymmetry, while multiple coordination types including two-coordination maybe present in Pb_7-MT'.

Keywords: Thionein; lead (II); UV spectra; circular dichroism; multiple coordination.

Metallothioneins (MTs), a class of low molecular weight, metal- and cysteine-rich proteins, are postulated to play a central role in metal-related cell-biological processes. Although available evidences strongly suggest that MT play an important role in regulating the intracellular toxicity of lead[1, 2], information on the molecular aspects of lead-binding MT is currently lacking. In this paper, we examine the properties of the interactions between apo-MT and Pb (II) under different conditions, particularly the effects of pH on the Pb (II)-MT adducts.

Experimental

Rabbit liver Zn_7MT2 was purchased from Wei-Ming biological engineering company of Peking University. Apo-MT was prepared by removing metal ion of Zn_7MT on a Sephadex G-25 column equilibrated with 0.01 mol/L $HClO_4$ solution. The concentration of MT was evaluated from its absorbance in 0.01 mol/L $HClO_4$ at 220 nm (ε_{220} =48200 $mol^{-1}Lcm^{-1}$)[3]. The protein sulfhydryl content was measured by Ellman assay (ε_{412} = 13600 $mol^{-1}Lcm^{-1}$)[4]. All other chemicals were analytical grade or better, and twice distilled water was used.

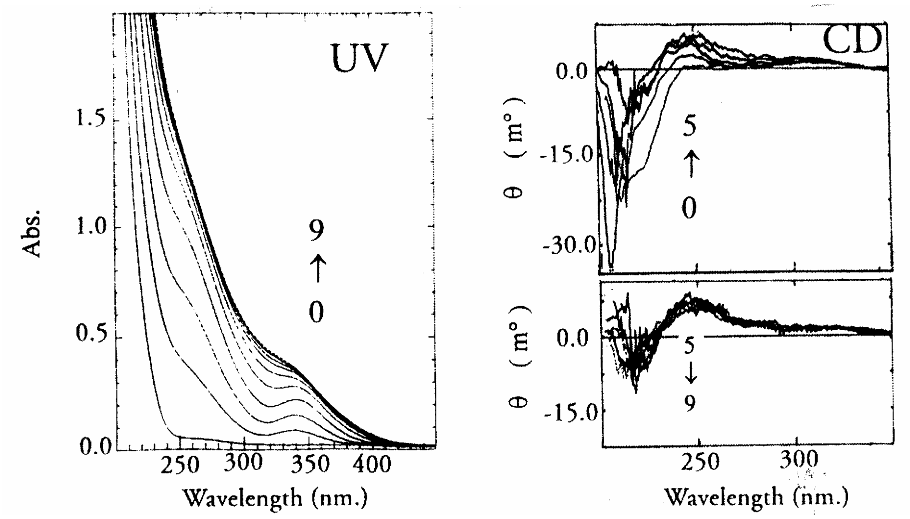
The titrations of apo-MT with Pb (II) as lead acetate were performed at pH 7.2 and 4.3 respectively. The solutions of apo-MT, lead acetate and Tris buffer were all deaerated by purging with high pure N_2 for 10 mins, then were quickly sealed and transferred into a N_2-purged glovebox. The pH values of apo-MT solutions were adjusted by additions of appropriate amount of Tris buffer in the glovebox. Titrations were carried out with a single solution of apo-MT: aliquots of Pb (II) were added by syringe to obtain the desired Pb (II)/MT molar ratios, then the electronic absorption (UV) and circular dichroism (CD) spectra were recorded until no further changes were observed. All the Pb (II)-binding studies were performed at least three times.

Spectrometric studies were done on a UV-240 spectrophotometer (Shimadzu, Japan) and a J-500C automatic recording spectropolarimeter (Japan Spectroscopic Co. LTD.)

Results and Discussion

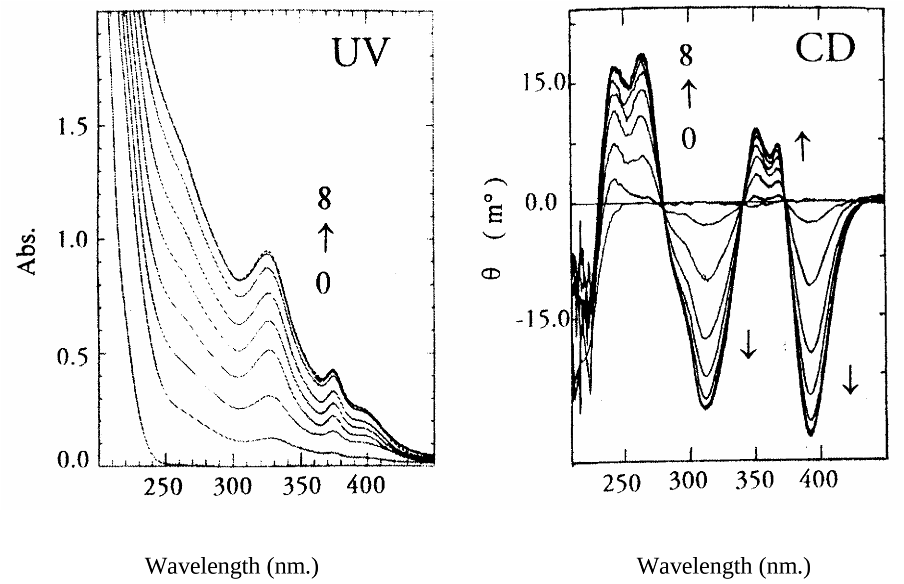
Stoichiometry of Pb (II) binding in MT: **Figure 1** and **2** show the UV and CD spectra recorded during the titration of apo-MT with Pb (II) at pH 7.2 and 4.3, respectively. The spectral changes are summarized in **Table 1**. Although the spectral envelops at pH 7.2 are quite different from those at pH 4.3, a clearly defined titration end-point at 7 Pb/MT is observed at both pH values. Therefore, both titration products contain 7 Pb (II) per MT molecule, namely Pb_7-MT (pH 7.2) and Pb_7-MT' (pH 4.3). The number of binding Pb (II) in Pb-MT adducts is consistent with the result of Nielson *et al.* (Pb/MT = 7)[5].

Figure 1. Lead (II) titration of apo-MT at pH 7.2 ($C_{apo\text{-}MT} = 1.68 \times 10^{-5}$ mol/L)
0-9 indicate the Pb/MT ratios



Coordination of Pb (II) in Pb-MT adducts: the UV and CD spectra of Pb_7-MT are similar to those reported previously[6], and the location of the first charge transfer (CT) transition coincides with that predicted for T_d-thiolate coordination of this metal using Jorgensen' s optical electronnegativity theory for charge-transfer excitations of metal ligand complexes[7], it appears very likely that Pb (II) is bound in T_d microsymmetry in Pb_7-MT, too. The presence of multiple peaks in the UV and CD spectra of Pb_7-MT' implies that the Pb (II) in Pb_7-MT' is bound in a quite different fashion and multiple coordination types maybe coexist in Pb_7-MT'. It has been reported that Pb (II) may exhibit multiple coordinations in long chain *phytochelatins* (PCs)[8], as indicated by the formations of two Pb (II)-binding species of $(\gamma\text{-Glu-Cys})_4$Gly ($n_4$): four-coordination type [Pb $(II)_1$-n_4] and two-coordination type [Pb $(II)_2$-n_4], when increasing Pb (II) equivalents were titrated into the peptide. The similarity of the CD spectrum of Pb $(II)_2$-n_4 and that of Pb_7-MT' except for the 395 (-) peak in the later, together with the absorption band at 325 nm observed in UV spectra of both species suggest that multiple Pb (II) coordination types including two-coordination maybe present in Pb_7-MT'.

Figure 2 Lead (II) titration of apo-MT at pH 4.3 ($C_{apo\text{-}MT} = 1.45 \times 10^{-5}$ mol/L)
0-8 indicate the Pb/MT ratios



pH effects on Pb-MT adducts: the formation of Pb_7-MT or Pb_7-MT' depends on pH. Pb_7-MT' is the only product under weak acid conditions (it is important to note that the whole spectral envelop exhibits the same dependence on the Pb/MT ratio), while Pb_7-MT is the single product under physiological conditions. It is probably that the amount of deprotoned sulfhydryl groups in MT plays an important role in the formation of Pb_7-MT or Pb_7-MT'. Pb_7-MT is formed only if sufficient deprotoned sulfhydryl groups are present in MT, otherwise, Pb_7-MT' is formed.

Table 1: Spectral changes during the titration of apo-MT with Pb (II)

titration conditions	changes in UV spectra	changes in CD spectra
pH 7.2	an absorption shoulder at 280 nm and a broad peak at 350 nm develop with the increasing amount of Pb^{2+}, and reach maxima at 7 Pb^{2+}	bands at 240 (+) and 330 nm (+) intensify to a maximum at 4 Pb^{2+}, no further changes at more than 5 Pb^{2+}
pH 4.3	two intense peaks at 325, 374 nm and a shoulder at 400 nm grow upon the addition of Pb^{2+}, and reach maxima at 7 Pb^{2+}	a strong CD envelop with maxima at 240 (+), 265 (+), 320 (-), 350 (+), 370 (+) and 395 (-) forms isodichroically (280, 340 and 375 nm), and reaches a maximum at 7 Pb^{2+}

A new MT species ------ Pb_7-MT’: Quite unlike other divalent metal ions (Zn^{2+}, Cd^{2+} *et al.*), two kinds of metal-MT adducts, Pb_7-MT and Pb_7-MT’, are formed during the Pb (II) titrations of apo-MT at different pH conditions, especially Pb_7-MT’, in which multiple Pb (II) coordination may coexist. This is a remarkable phenomenon, since it is believed that one kind of metal ion can only be bound in single coordination geometry in one MT molecule. The detection of Pb_7-MT’ implies that a novel structure motif of MT is adopted by this MT species.

Up to date, the two-cluster, two-domain model has been well established upon X-ray diffraction[9] and 2D-NMR[10] spectroscopic analysis of Cd_5Zn_2-MT and Cd_7-MT, that all the seven bivalent metal ions are tetrahedrally coordinated by four thiolate ligands, and two metal-sulfur clusters, M_4S_{11} and M_3S_9, are present in α-, β-domains respectively. But the detection of Ag_{18}-MT[11] and Hg_{18}-MT[12] indicates that MT can bind more metal ions than those expected under given conditions, and a new 3D structure may exist. The present investigation reveals that the Pb (II)-binding property of rabbit liver MT2 is influenced by pH, and a new species, Pb_7-MT’, is formed at low pH. It is not sure whether the formation of Pb_7-MT’ is unique to this isoform, and the structure of Pb_7-MT’ is unknown. Further studies are needed to resolve these problems.

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