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SECONDARY STRUCTURE OF RABBIT Cd₅Zn₂MT-II, Zn₇MT-II AND Pt₇MT-II BY FOURIER TRANSFORM INFRARED SPECTROSCOPY

Keywords: Secondary structure analysis; NMR analysis; FTIR spectroscopy and Metallotioneins (MTs).

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Abstract: The secondary structures of Cd₅Zn₂MT-II and Zn₇MT-II from rabbit liver and their derivative Pt₇MT-II in solid state were investigated by Fourier transform ir spectroscopy. The Cd₅Zn₂MT-II contains about 30% turns and half turns, 17% 3₁₀-helix, 4% unordered and 9% β -extended chain, which is similar to the content determined by the nmr analysis and is similar to the structures of the Pt₇MT-II and Zn₇MT-II. The results indicate that there is no evident conformation changes of MTs' secondary structure when the tetrahedral Zn and Cd ions of native MTs are replaced by square-planar Pt ion.

INTRODUCTION

Metallotioneins (MTs) are small, structurally unique, and functionally enigmatic protein which exist ubiquitously in nature¹. Their rich cysteine residues

involved in the binding of a large variety of divalent and trivalent metals *in vitro* and *in vivo*. It has been established that they have putative roles in cadmium detoxification, essential metal metabolism, radical scavenging, the stress response, and the pharmacology of metallodrugs and alkylating agents²⁻⁴.

Platinum(II) complexes including cisplatin and carboplatin are widely used in treating a number of human cancer². Many studies have demonstrated that the reactions of Pt(II) ions with native MTs *in vivo* and *in vitro* can produce platinum-metal-metallothioneins⁵⁻⁷. However, the geometry of coordinated platinum usually is square planar; while the case of the Cd and Zn are both tetrahedral. It is still unclear whether there is conformation change of MTs when the tetrahedral Zn and Cd ions of native MTs are replaced by square-planar Pt ion. In order to elucidate the influence of metal ions with different geometry on the conformation of bond MTs, we investigated the secondary structures of MTs by using FTIR spectroscopy.

EXPERIMENTAL

Materials

Rabbit liver Cd₅Zn₂MT-II and Zn₇MT-II were isolated and purified from adult male rabbit injected with CdCl₂ and Zn(NO₃)₂ solution, respectively^{8,9}. The Pt₇MT-II were prepared as described in the literature¹⁰. The purity of the metallothionein was checked by HPLC. Contents of S and metal ions were determined by inductively coupled argon plasma-atomic emission (ICP) spectrometric method¹¹ performed on a JOBIN YVON JY38S ICP spectrometer. During the experiments, all chemicals used were reagent grade or better, and deionized water was used.

Infrared Spectroscopy and Data Manipulation

Infrared spectra were measured with a Nicolet 170SX FTIR spectrophotometer at room temperature. The instrument was purged with dry air overnight prior to measurements. The solid MTs were measured as KBr pollets.

For each spectrum, a 1200-scan interferogram was collected at single beam mode with a 2 cm^{-1} resolution from 4000 to 1000 cm^{-1} . Difference spectra were generated by subtraction of the gaseous water following the procedure of Dong et al¹². Second-derivative spectra were obtained by taking the first derivative twice and were used to verify the peak assignments of the deconvolved spectra. Fourier self-deconvolution of the amide I absorbance bands was performed by using a lorentzian of 23 cm^{-1} half-bandwidth and a resolution enhancement factor (k value of 2.7), which generally give optimal resolution enhancement without producing evidence of excessive deconvolution, such as side lobe or other artifacts. The accurate absorption position and strength of each absorbance in amide I region was obtained by iterative curve fitting to the deconvolved spectra. The relative amounts of different secondary structure were assigned to the protein by integrating the areas under the curves assigned to a particular peptide chain configuration.

RESULTS AND DISCUSSION

The original ir spectra of $\text{Cd}_5\text{Zn}_2\text{MT}$, Zn_7MT and Pt_7MT in amide I region are showed in Figure 1. Little secondary structural information can be obtained directly from the original ir spectra showed in Figure 1. In order to get more information, we can take their secondary derivative spectra, from which the accurate number of components is obvious. Figure 2 shows the deconvoluted spectra and the fitted derivative spectra and their secondary derivative spectra. From Figure 2, it can be seen that the number and wavenumber of the peaks in the second-derivative spectra are consistent with the corresponding value in the deconvolved spectra, which indicate that our results are reliable. Curve fitting of the deconvolved spectra by multiple Gaussian profiles gives the individual component bands. For all three samples, nine components between about 1700 and 1620 cm^{-1} are considered to be due to the vibrations of the peptide bond. The two bands below 1620 cm^{-1} are probably from the side-chain vibrations of amino

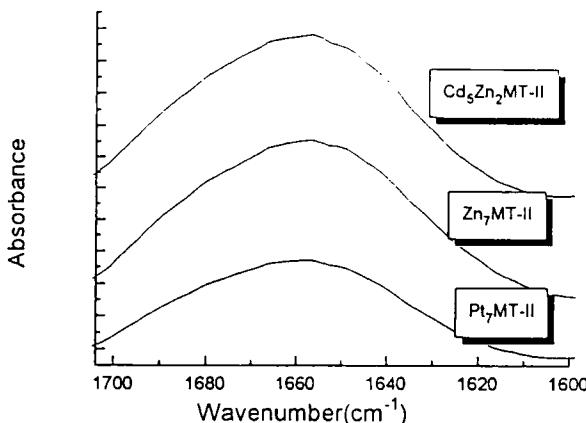


FIGURE 1 Original IR spectra (amide I region) of $\text{Cd}_5\text{Zn}_2\text{MT-II}$, $\text{Zn}_7\text{MT-II}$, $\text{Pt}_7\text{MT-II}$ in solid state at room temperature.

acides¹³. Assignment of amide I absorbance components gives the information of the secondary structures. The results are summarized in Table 1.

The amide I band frequency assignments for secondary structures available from previous studies are as follows¹⁴⁻¹⁷: α -helix ($1650 \pm 2 \text{ cm}^{-1}$), β -strand (1635 and 1620 cm^{-1}), unordered ($1640 \pm 2 \text{ cm}^{-1}$) and turns (1685 and 1665 cm^{-1}). The wavenumber of each type of above substructure is lower by approximately 5 cm^{-1} than the corresponding value in H_2O solution because each of the component bands of the amide I bands is shifted to lower frequencies in D_2O and solid state. According to these assignments, we can assign 1623 and 1631 cm^{-1} to the β -extended chain unambiguously.

At present there are no generally valid criteria for a reliable discrimination of the various bands between 1670 and 1695 cm^{-1} ¹⁸. Balyer et. al.¹⁹ assigned this region to turns, but Dong et. al.²⁰ assigned 1694 cm^{-1} in H_2O solution to β -extended chain. From our data, we can obtain four peaks 1699 , 1690 , 1678 cm^{-1}

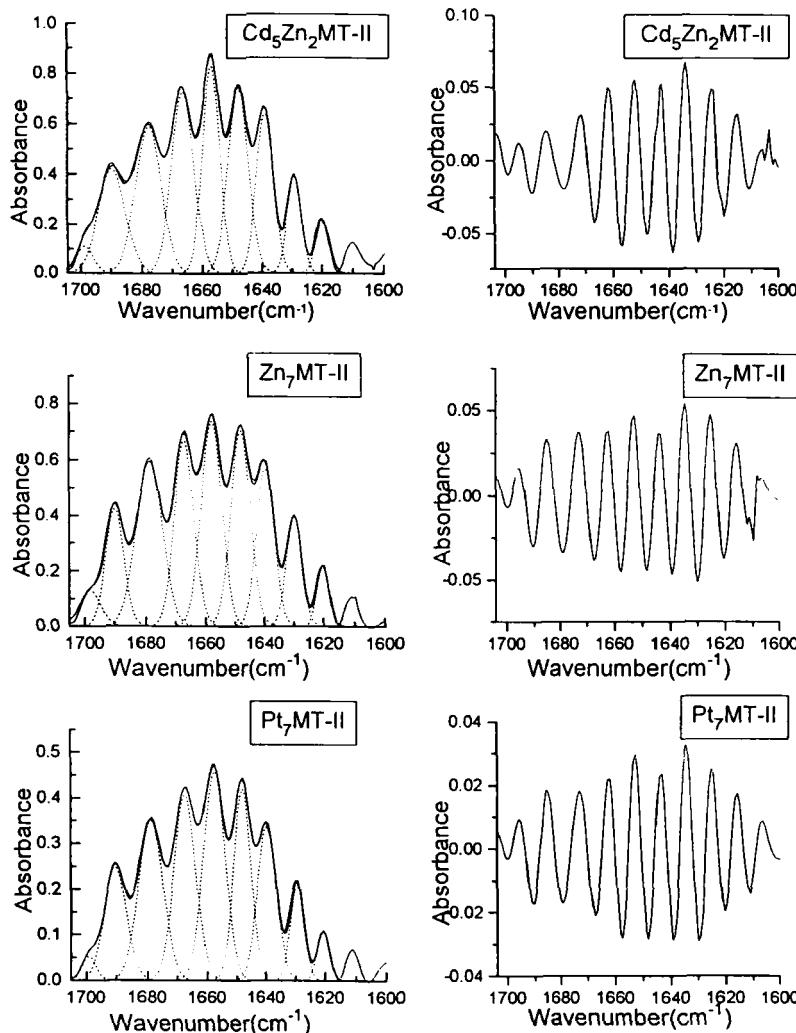


Figure 2 Deconvolved spectra and fitted amide I components for $\text{Cd}_5\text{Zn}_2\text{MT-II}$, $\text{Zn}_7\text{MT-II}$, $\text{Pt}_7\text{MT-II}$ and their second derivative spectra. In the upper curves, solid lines represent the experimental deconvolved spectrum; the dotted lines give the resolved components and the dash lines show the calculated sum of the resolved components (because the deviation between the calculated curve and the experimental data is so small, the former is virtually superimposed on the experimental spectrum).

Table 1

	Frequency (cm ⁻¹)	Assignment	content (%)
Cd ₅ Zn ₂ MT-II	1699,1690,1678	β-turns and half turns	30 %
	1666	3 ₁₀ -helix	17 %
	1657,1648,1639	Unordered	44 %
	1629,1620	β-extended chain	9 %
Zn ₇ MT-II	1698,1690,1679	β-turns and half turns	28%
	1667	3 ₁₀ -helix	18 %
	1657,1648,1639	Unordered	44%
	1630,1621	β-extended chain	10 %
Pt ₇ MT-II	1699,1690,1679	β-turns and half turns	28%
	1667	3 ₁₀ -helix	17 %
	1658,1649,1639	Unordered	46 %
	1630,1621	β-extended chain	9 %

in this region and we assign them to β-turns and half turn. Because if we assign 1699 and 1690 cm⁻¹ to β-extended chain, the percentage of β-extended chain become too high (about 22 %). This is impracticable since the content of β-extended chain differ from the value estimated from the nmr analysis²¹, which shows that there are little β-extended chain. In human Cd₇MT-II solution, which secondary structure is very similar to the solid rabbit Cd₅Zn₂MT-II²¹, there are six half turns (Pro3-Cys5, Cys5-Cys7, Thr14-ala16, Ser32-Cys34, Cys34-Cys36 and Cys48-Cys50) and one turn (Val39-cys41), the total content of these turns are about 31 %. Because it is hard to identify the turns structure and half turns structure in FTIR, so we assign 1699, 1690, 1678 cm⁻¹ bands to turns and half

turns and the content of this structure (30 %) is similar to the content in nmr analysis²¹.

As we known, there are two 3_{10} -helixs in the structure of Cd₅Zn₂MT-II, which are located at residues 42-47 and 58-61, respectively²¹. Because 3_{10} -helixs are less common, they have been less well characterized by infrared spectroscopy²². However, synthetic α -aminobutyric acid-containing peptides, which are known to form 3_{10} -helixs, show strong amide I bands at 1662-1663 cm^{-1} . Other experimental and theoretical studies also indicate that distorted helical structures such as 3_{10} -helixs exhibit amide I frequencies which are higher than that observed for α -helices due to weaker hydrogen bonding²³. Therefore, we can reasonably assign the 1666 cm^{-1} band to 3_{10} -helixs structure, and the content of 3_{10} -helixs structure (17%) is similar to the structure of MT determined by nmr analysis.

In the most cases of reported proteins, the peak around 1657 cm^{-1} is empirically assigned to the α -helix. However, in the case of Cd₅Zn₂MT-II, since the content of the α -helix is very low, we cannot assign the 1657 cm^{-1} band to the α -helix as discussed previously¹⁴. Dong et al.²⁰ assigned 1659 cm^{-1} band to the unordered structure. Hence, the 1657 cm^{-1} band is considered to be due to the unordered structure mainly. As to 1648 cm^{-1} band and 1639 cm^{-1} band, we assigned them to the unordered structure.

In this way, the assignments of amide I bands of Zn₇MT-II and Pt₇MT-II were obtained.

Curve fit to the original spectra was also carried out as reported for the acetylcholine receptor by Méhot et al.²⁴, and similar results were obtained with those of curve fitting to the deconvolved spectra as described above. A typical example of the curve-fitted original spectrum of Pt₇MT-II is shown in Figure 3. The content of β -turns and half turns, 3_{10} -helix, β -extended chain and unordered

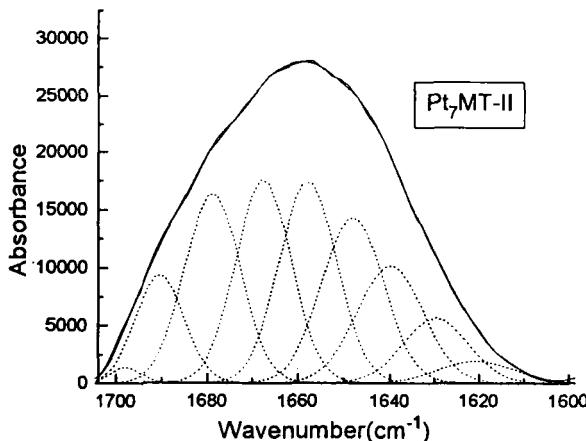


FIGURE 3 The curve-fitted original spectrum of Pt₇MT-II in amide I region.

structures estimated from the curve-fitted original spectrum are 28%, 17%, 9% and 46%, respectively, which are the same as those from the deconvolved spectrum (Figure 2). The number and position of peaks are almost the same as those in the deconvolved spectrum. These results indicate that the estimated secondary structures are reliable.

From Table 1, we obtained that the contents of the structures of the MTs binding with different metal ions are very similar. The results indicate that there is no evident conformation changes of MTs' secondary structure when the tetrahedral Zn and Cd ions of native MTs are replaced by square-planar Pt ion.

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