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六氯合铂酸钾与金属硫蛋白的体外反应

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本文报道了 K_2PtCl_6 与兔肝 Zn_7MT-Ⅱ和 apoMT-Ⅱ的反应包含一个氧化还原反应和一个取代反应。通过紫外可见光谱、园二向色谱、柱层析和 X-光电子能谱研究了该反应的性质、铂在反应产物中的键合位置和氧化态。金属硫蛋白(MT)被氧化成单体、双聚和多聚产物,其中含有分子间和分子内 CyS-SCy 二硫键。Pt(Ⅳ)被还原成 Pt(Ⅱ)然后键合于产物中。随着 K_2PtCl_6 与 MT 的反应摩尔比和反应时间的增加,键合于产物中的 Pt(Ⅱ)的计量数增加而蛋白中所含 Zn(Ⅱ)的量减少,当 Zn_7MT 与 4 和超过 10 摩尔的 K_2PtCl_6 反应时,分别得到了 Pt_4Zn_4MT 和 Pt_8MT。当 apoMT 与 7 及超过 25 倍的 K_2PtCl_6 在 pH 2 条件下反应时,分别得到了 Pt_7MT 和 Pt_{15}MT。动力学数据表明 K_2PtCl_6 与 apoMT 的反应比与 Zn_7MT 的反应快。

六氯合铂酸钾 MT

关键词: K_2PtCl_6 金属硫蛋白 氧化还原反应 机理

REACTIONS OF K_2PtCl_6 WITH METALLOTHIONEINS *IN VITRO*

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A redox reaction following a substitution reaction from K_2PtCl_6 with rabbit liver Zn_7MT-Ⅱ or apoMT-Ⅱ is presented. The reaction features, the metal binding stoichiometry, and the binding sites and the oxidation state of platinum in the products are studied with UV-visible, circular dichroism (CD) spectroscopy, chromatography, and X-ray photoelectron spectroscopy methods. MT is oxidized to monomeric, dimeric, and higher oligomeric products with intra and intermolecular CyS-SCy linkages. Pt(Ⅳ) is reduced to Pt(Ⅱ) which binds to the products. The binding stoichiometry of Pt(Ⅱ) to the protein increases and the amount of Zn(Ⅱ) decreases as the reaction molar ratio of K_2PtCl_6 to MT increases and the reaction time prolongs. Pt_4Zn_4MT and Pt_8MT are obtained when the Zn_7MT reacts with four and beyond ten molar ratios of K_2PtCl_6, respectively. When apoMT-Ⅱ reacts with K_2PtCl_6, Pt_7MT and Pt_{15}MT are found for seven and 25 molar ratios of K_2PtCl_6 to MT

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at pH 2, respectively. The reaction of K_2PtCl_6 with apoMT is faster than that with Zn_7MT.

Keywords: K_2PtCl_6 **metallothionein** **redox reaction** **mechanism**

0 Introduction

Metallothionein (MT) is a low molecular weight protein found in all eukaryotic cells and is capable of binding a variety of metal ions[1-2]. The binding involves thiolates of the 20 cysteine residues, the bound metal ions which are tetrahedrally coordinated to four cysteine thiolates are distributed as a M_3S_9 complex (β-cluster) formed by the N-terminal β-domain and a M_4S_{11} complex (α-cluster) enfolded by the C-terminal α-domain (M is Zn and/or Cd ion) in the native mammalian MT molecule[2-4]. It is considered that the reactivity and metal-binding property of the two clusters are different from each other[2,5]. One opinion thinks that the metal binding process is "domain-specific" or "cooperative". This model was used to interpret the results obtained when Cu(I) reacted with Zn_7MT or Cd^{2+} reacted with apoMT, the first six Cu(I) or three Cd^{2+} ions replaced Zn^{2+} ions which located in the β-domain[5-6]. The other opinion believes that a "distributed" or "incooperative" model is an explanation suitable for the results from the reaction of four molar radios of Cd^{2+} or Co^{2+} with Zn_7MT and statistically two Cd^{2+} or two Co^{2+} ions were bound in each domain[5,7]. MT is also a potential candidate for involvement in the binding of platinum. This leads to the reactions which are important in the platinum metabolism, antitumor action, and drug resistance or cisplatin-cross resistance of certain Pt complexes[8-15].

A lot of investigations have been made on the reactions of Pt(II) complexes, especially cisplatin, with MTs *in vivo* and *in vitro*[9-14]. It appears that Pt_7MT or $Pt_{10}MT$ may be formed when Pt(II) complexes react with Zn_7MT, Cd_7MT or apoMT *in vitro*[11-16]. However, rare investigation has been made on the reaction with Pt(IV) complexes. We have reported that the reaction between native rabbit liver Cd_5Zn_2MT-II and K_2PtCl_6 comprises a redox and a substitution reaction[17]. The native Cd_5Zn_2MT is oxidized by K_2PtCl_6 to generate monomeric, dimeric platino-MTs and oligomeric products. The β-cluster of the protein is more reactive than the α-cluster which perhaps has a special stability when substoichiometric quantitative Pt(IV) (<0.5 K_2PtCl_6 per MT thiolate) reacts with the protein, and the α-cluster also takes part in the reaction until the molar ratio of Pt(IV) to MT is above stoichiometric quantities ($\geqslant 0.5$ K_2PtCl_6 per MT thiolate) for a long time ($\geqslant 4$ h).

In order to obtain more information on the reaction property of the α- and β-clusters in MT with Pt(IV) complexes, in this paper, the reactions of K_2PtCl_6 with rabbit liver Zn_7MT-II and apoMT-II have been reported. The reaction mechanism and the structure of the products are discussed upon the results obtained.

1 Experimental

1.1 Preparation and Characterization of MTs

Rabbit liver Zn_7MT- Ⅱ was isolated and purified by using the method reported in the literature[18], and was checked with measuring the contents of S and metal ions by inductively coupled plasma (ICP) spectrometric determination using emission line of S (181.978 nm), Pt(224.552 nm), and Zn(213.856 nm) performed on a JOBIN YVON JY38S ICP spectrometer[19]. ApoMT was prepared according to the literature[20] by gel-filtration chromatography (Sephadex G-25). An appreciate amount of lyophilized Zn_7MT was dissolved in 20 mmol · l^{-1} tris-HCl buffer solution at pH 8.60, containing equal amount of dithiothreitol, and was incubated for 1 h at room temperature. The pH was then adjusted to 1.0 by the rapid addition of 6 mol · l^{-1} HCl. The sample was centrifuged for 5 min at 10,000 g, then the supernatant was applied to a Sephadex G-25 column(1.6×30 cm) preequilibrated with 0.01 mol · l^{-1} HCl, and eluted with the same solution. After elution monitored by an absorption at 220 nm, the protein fractions of the major monomeric peak were collected. The sulfhydryl groups (SH) were measured with Ellmen' reagent[21] and the results showed that the apoMT contained 19±2 SH^-. The concentration of the protein was determined by the absorbance at 220 nm of apoMT at pH 2(ε=47,300 mol · l^{-1} · cm^{-1})[22].

1.2 Binding Kinetics

Rabbit liver MT- Ⅱ isoform and stock solutions of K_2PtCl_6 and K_2PtCl_4 in water were used for studies. All procedures were performed in a nitrogen atmosphere.

Deaerated Zn_7MT and apoMT solutions containing 250 molar ratios of K_2PtCl_6 were prepared to study the reaction kinetic properties by UV-visible spectral experiments. 36 μl of 0.208 mmol · l^{-1} Zn_7MT solution was added to 2.814 ml of 0.01 mol · l^{-1} potassium phosphate buffer solution, pH 7.40(final MT concentration was 2.5 μmol · l^{-1}). After degassed completely, this solution was transferred to a 1 cm cuvette and sealed with a parafilm, 1.50 ml of 1.25 mmol · l^{-1} K_2PtCl_6 degassed solution was added through a gastight syringe to the cuvette at time zero, and the changes of absorbance at 260 nm versus time were recorded on a Shimadzu 3100 spectrometer at 15℃. The reference solution contained all the regents except protein and K_2PtCl_6. Pseudo-first-order rate constant was obtained by plotting $\ln(A_0/A)$ against time(A_0 represents the absorbance at 260 nm of the reaction mixture at time zero). The pseudo-first-order rate constant of the reaction between apoMT and K_2PtCl_6 was obtained by using the same method.

Circular dichroism (CD) spectra were also used to study the reaction feature of Zn_7MT with K_2PtCl_6. The time course of the reaction between Zn_7MT and 25 molar ratios of K_2PtCl_6 were recorded on a Jasco J-500 circular dichroism spectrometer. The sample for CD spectral measurement was prepared as mentioned above (final MT concentration was 20 μmol · l^{-1}).

1.3 Binding Stoichiometry

The reaction mixtures of a series of the deaerated Zn_7MT- Ⅱ solutions containing 2, 4, 10 and 25 molar ratios of K_2PtCl_6 were prepared in 3.6 mmol · l^{-1} potassium phosphate solution, pH 7.40(final

MT concentration was 0.4 mmol · l^{-1}) and were left standing for 2, 24 and 72 h at 25 C. Each of these solutions was introduced into a Sephadex G-50 column(1.6×65 cm), eluted with 3.6 mmol · l^{-1} potassium phosphate solution, pH 7.40 at 8 C, and monitored at 254 nm. Fractions (5 ml/tube) were pooled and analyzed for S, Pt, and Zn by ICP spectrometry.

1.4 X-Ray Photoelectron Spectroscopic Measurement

The X-ray photoelectron spectroscopic (XPS) measurement was performed on an ESCALAB MK I electron spectrometer using Al-$K\alpha$ radiation (1,486.6 eV) as the X-ray excitation source. The samples for the measurement were prepared from the products of the reaction between Zn_7MT and 2, 4 or 7 molar ratios of K_2PtCl_4. As a comparison, the sample of the Pt-MT product in reaction of K_2PtCl_4 with Zn_7MT was prepared and measured also.

2 Results and Discussion

The UV-visible spectra versus time recorded for the reactions of Zn_7MT and apoMT with 250 molar ratios of K_2PtCl_6 at pH 7.40, 15 C were showed in Fig. 1. It was seen that the characteristic absorbance at 260 nm of the spectrum of K_2PtCl_6 decreased rapidly at the starting stage of the reaction, and the apparent absorption changed no more after 120 min and 40 min for Zn_7MT and apoMT, respectively. Two kinetic steps which obey pseudo-first-order can be resolved in plots of $\ln(A_0/A)$ versus time (A_0 was the initial absorbance at 260 nm). The pseudo-first-order rate constants for the first step were obtained in terms of the decrease of the absorbance at 260 nm, which were $1.96\pm0.25\times10^{-2}$ and $6.82\pm0.45\times10^{-2}$ min^{-1} for Zn_7MT and apoMT, respectively. These were bigger than the value for Zn_7MT with K_2PtCl_4, $8.80\pm0.65\times10^{-3}$ min^{-1}[18]. This means that the reaction of MT with K_2PtCl_6 is more rapid than that with K_2PtCl_4.

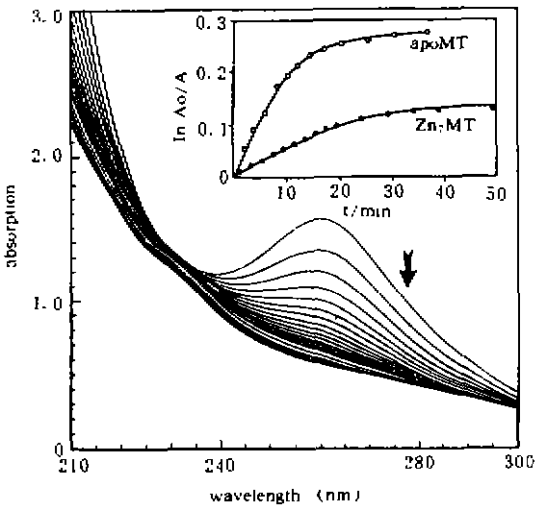

Fig. 1 Absorption spectral changes during the processes of reactions of rabbit liver Zn_7MT- Ⅰ and apoMT- Ⅱ with 250 molar ratios of K_2PtCl_6 in 0.01 mol · l^{-1} potassium phosphate solution, pH 7.40 at 25℃. Concentration of MT was 2.5 μmol · l^{-1}. The insert shows the time dependence of $\ln(A_0/A)$, A_0 and A were the absorbance at 260 nm at 0 and t min respectively.

The time course of the reaction of K_2PtCl_6 with Zn_7MT was also monitored by CD spectra. As shown in Fig. 2, the addition of Pt(Ⅳ) profoundly changed the simple CD spectrum of the Zn_7MT which exhibits the profile with maximum at 242 nm(+), and 228 nm(−), before the signal is dominated by the peptide chiralty below 220 nm[3,6]. All these bands diminished and four new bands with maximum at 270 nm(+), 264 nm(−), 256 nm(+), and 244 nm(−) appeared during the first 2 min after K_2PtCl_6 was added. The band at 244 nm(−) also disappeared in the process of the re-

action, but a positive reflection with a redshift from 244 to 249 nm appeared at 10 min. The change of bands between 280～250 nm was complicated and inordinate. There were a weak band with maximum at 250 nm(+) and a most intense band with maximum at 268 nm(−) in the spectra with no further changes after 120 min. These results were different from those observed for K_2PtCl_4 (Fig. 3) or CDDP with Zn_7MT which revealed the characteristic bands with maximum at 255 nm (+) and 235 nm(−)[12]. These results indicated that the reaction between Zn_7MT and K_2PtCl_6 was more complicated and was not a simple substitution reaction comparing with that Pt(Ⅱ) simply replaced Zn(Ⅱ) in Zn_7MT to form Pt-thiolate clusters in the reaction of Zn_7MT with K_2PtCl_4 or CDDP.

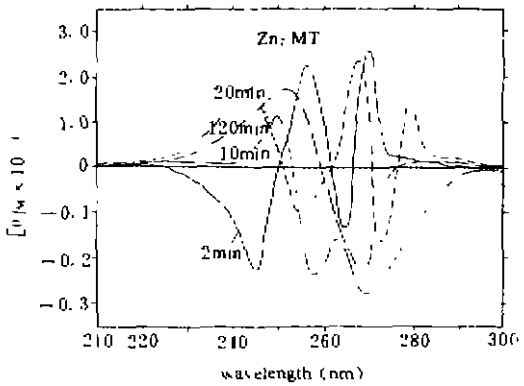

Fig. 2　CD spectra recorded for the reaction of rabbit Zn_7MT-Ⅰ with 25 molar ratios of K_2PtCl_6 versus time in 0.01 mol·l^{-1} potassium phosphate solution, pH 7.40, at 15℃ (Concentration of MT was 20 μmol·l^{-1}).

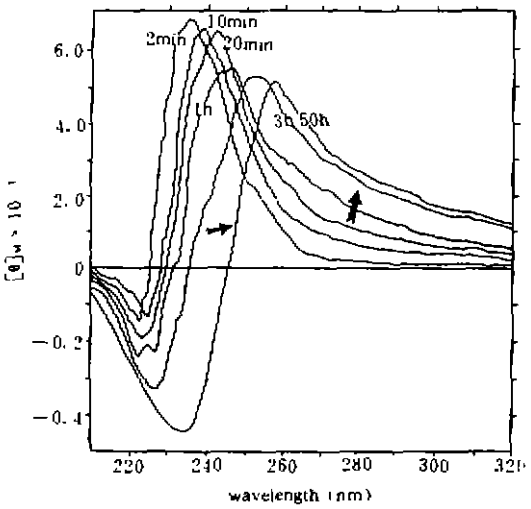

Fig. 3　CD spectra recorded for the reaction of rabbit Zn_7MT-Ⅰ with 25 molar ratios of K_2PtCl_4 versus time in 0.01 mol·l^{-1} potassium phosphate solution, pH 7.40, at 15℃ (Concentration of MT was 20 μmol·l^{-1}).

X-ray photoelectron spectroscopy (XPS) was used to determine the oxidation state of platinum by comparing the binding energies in the products. Fig. 4 shows the XPS spectra of the products from Zn_7MT with K_2PtCl_6 and K_2PtCl_4. After calibrating the spectra by using the C(1s) line from oil contamination (binding energy 285 eV) as an internal standard, the binding energies for $4f(7/2)$ and $4f(5/2)$ levels of platinum in the products from Zn_7MT with 4, 7, and 10 molar ratios of K_2PtCl_6 were 72.6, 75.9; 73.1, 76.6; and 72.4, 75.9 eV, respectively (Table 1), which just resembled those in K_2PtCl_4 (73.2 and 76.4 eV) and those in Pt-MT from Zn_7MT with K_2PtCl_4 (73.0, 76.4 eV), but were 2～3 eV less than those in K_2PtCl_6 (75.6 and 79.0 eV) for $4f(7/2)$ and $4f(5/2)$ levels respectively[23]. It can be concluded that the oxidation state of platinum in the MT products from Zn_7MT with K_2PtCl_6 was +2 and the reaction between Zn_7MT and K_2PtCl_6 involved a redox reaction. These were confirmed by the results obtained in out previous experiments when native rabbit liver Cd_5Zn_2MT reacted with K_2PtCl_6[17].

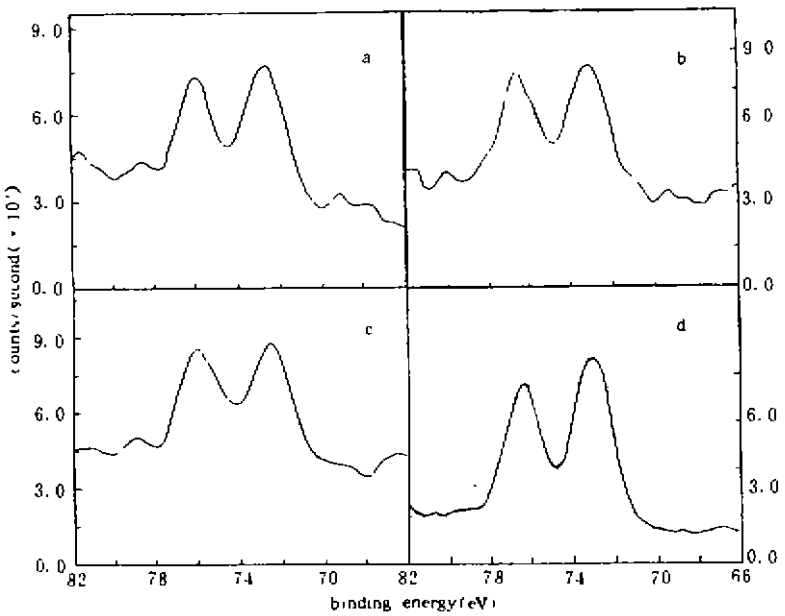

Fig. 4 X-ray photoelectron spectra for 4f levels of platinum in products from K_2PtCl_6 and Zn_7MT-Ⅱ with reaction molar ratios of (a)4 : 1, (b)7 : 1, and (c)10 : 1, and from K_2PtCl_4 with Zn_7MT-Ⅱ with molar ratio of (d)7 : 1. The C(1s) line from oil contamination (binding energy 285 eV) was used as an internal standard. The charge effect correction factors were 3.9, 4.2, 4.5, and 3.8 eV, respectively.

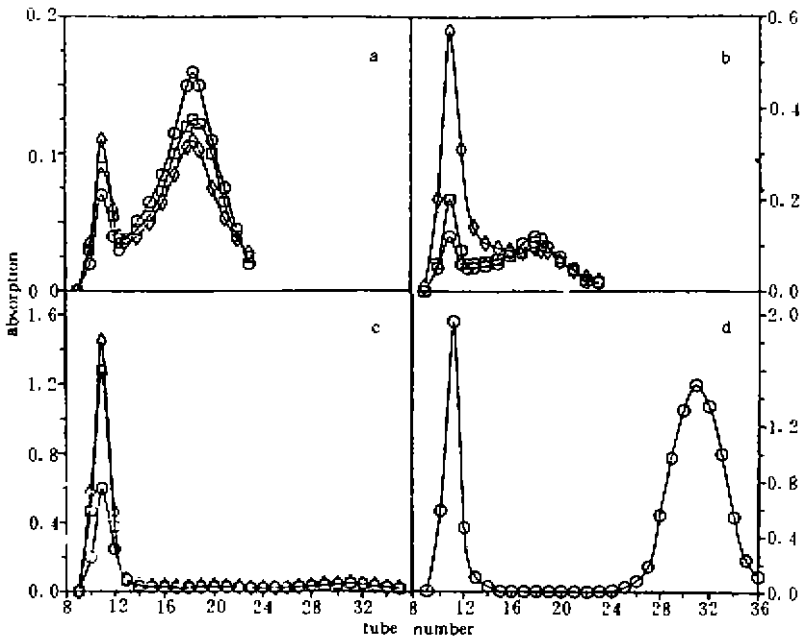

Fig. 5 Gel-filtration elution profile monitored at 254 nm of reaction mixtures of rabbit Zn_7MT-Ⅱ (0.40 mmol · l^{-1}) with K_2PtCl_6 in 3.6 mmol · l^{-1} potassium phosphate solution, pH 7.40, after standing for 2, 24, 72 h at 25℃. Sephadex G-50 column (1.6 × 65 cm), eluted with 3.6 mmol · l^{-1} potassium phosphate solution, pH 7.40. The reaction molar ratio of K_2PtCl_6 : MT was (a)2 : 1, (b)4 : 1, (c)10 : 1, and (d) 25 : 1. (○) 2 h, (□) 24 h, and (◇) 72 h.

Table 1　Binding Energies (eV) for the 4*f* Levels of Platinum[a]

reaction agents	binding energies of 4*f* 4*f*(7/2)	levels of Pt in products 4*f*(5/2)
K_2PtCl_4	73.2	76.4
K_2PtCl_6	75.7	79.0
$Zn_7MT+4K_2PtCl_6$	72.6	75.9
$Zn_7MT+7K_2PtCl_6$	73.1	76.6
$Zn_7MT+10K_2PtCl_6$	72.4	75.9
$Zn_7MT+7K_2PtCl_4$	73.0	76.4

a. Binding energies are accurate to ±0.2 eV.

The products from a set of reactions of Zn_7MT with 2, 4, 10 and 25 molar ratios of K_2PtCl_6 in 3.6 mmol · l^{-1} potassium phosphate buffer solution, pH 7.40, for 2, 24, and 72 h at 25℃ were fractionated on a Sephadex G-50 column. There was some precipitate which may be poorly dissolved platinum-containing oligomeric products in each case and it was removed by centrifugalization before fractionation. A typical elution profile monitored at 254 nm is shown in Fig. 5a, in which there were two peaks corresponding to high-molecule-weight component from 9 to 12 fractions and low-molecule-weight component between 12 and 23 fractions in the experiment with molar ratio of K_2PtCl_6 to Zn_7MT being 2 : 1 for 2 h. After pre-calibration with standard proteins, haemoglobin (MW 68,000 Da), bovine superoxide dismutase (33,000 Da), Cyt *c* (12,400 Da), and rabbit liver Zn_7MT (6,800 Da), the high-molecule-weight component was determined to be dimeric form of MT and the low-molecule-weight component was monomeric MT. The ICP measurement showed that the monomeric MT contained 0.53±0.19 Pt ions and 6.19±0.58 Zn ions, and the dimeric form contained 2.02 ±0.19 Pt ions and 6.48±0.61 Zn ions in one monomeric unit (Table 2).

Table 2　Binding Stiochiometry of Metal Ions to MTs[a]

reaction condition		monomeric products		dimeric products	
reaction molar ratio (MT : Pt^{II})	reaction time (hour)	Pt (g atoms metal/mole protein)	Zn	Pt (g atoms metal/monomeric unit)	Zn
Native Zn_7MT		— — —	6.73±0.12	— — —	— — —
1 : 2	2	0.53±0.19	6.19±0.58	2.02±0.19	6.48±0.51
1 : 2	24	1.37±0.11	6.41±0.48	2.87±0.49	6.66±0.62
1 : 2	72	1.11±0.10	5.95±0.58	2.14±0.21	5.91±0.26
1 : 4	2	3.68±0.30	3.32±0.41	4.23±0.33	3.90±0.16
1 : 4	24	3.71±0.25	4.40±0.63	4.05±0.12	3.73±0.31
1 : 4	72	3.96±0.32	3.61±0.35	4.11±0.22	3.63±0.34
1 : 10	2	— — —	— — —	8.61±0.74	0.53±0.13
1 : 10	24	— — —	— — —	7.92±0.55	0.72±0.25
1 : 10	72	— — —	— — —	8.20±0.68	0.50±0.16
1 : 25	2	— — —	— — —	8.81±0.92	0.40±0.17
1 : 7[b]	72	— — —	— — —	7.11±0.21	0.16±0.10
1 : 25[b]	72	— — —	— — —	14.83±1.02	0.11±0.12

[a] average±standard from three experiments

[b] reaction from apoMT with K_2PtCl_6 at pH2

When the reaction time prolonged up to 24 and 72 h, the intensities of the peaks for the high-molecule-weight components increased, but the intensities of the peaks for the low-molecule-weight components decreased. There were approximately one Pt and six Zn ions in the monomers while two Pt ions and six Zn ions in the dimers. These meant that, under a definite reaction molar ratio of Pt(Ⅳ) to MT, the degree of reaction increased but the metal binding stoichiometry was static when the reaction time prolonged. When the reaction molar ratio of K_2PtCl_6 to Zn_7MT was 4 : 1, also there were two peaks corresponding to high- and low-molecule-weight components in the absorption elution profile (Fig. 5b). It was also seen that the peak intensity of dimeric product increased along with the peak intensity of monomeric product decreased when the reaction time prolonged from 2 h to 24 h and to 72 h. About four Pt ions and four Zn ions bound to monomer and dimer of MT in all cases (Table 2).

As is known, K_2PtCl_6 is a strong oxidant (the value of reducing potential for $PtCl_6^{2-} \rightleftharpoons PtCl_4^{2-}$ is 0.73 V[24]) and MT may undergo thiolate-disulfide or other redox reactions under certain conditions[25]. In the experiments of Zn_7MT with two and four molar ratios of K_2PtCl_6, the XPS measurement showed that the oxidation state of platinum in the products was +2, and the chromatography revealed that the monomeric and dimeric MTs, and oligomeric products generated. Thus, the Pt(Ⅳ) was reduced to Pt(Ⅱ), the Zn_7MT was oxidized, and intra- and inter-molecular CyS-SCy linkages formed. On one hand, it can be assumed that the β-cluster reacted first. 1～2 Zn ions which located in the β-cluster were replaced when Zn_7MT reacted with two molar ratios of Pt(Ⅳ), also all of the Zn ions located in the β-cluster were replaced first, when Zn_7MT reacted with four molar ratios of Pt(Ⅳ), and Pt_4Zn_4MT was formed in which four Pt(Ⅱ) ions may be distributed as three of them, perhaps, with a some distortional square-planar geometry, locating in the β-domain and the fourth binding to the thioether side-chain of methionine in the β-domain[16,17]. The binding of Pt(Ⅱ) was "domain-specific" or "cooperative"[1,6]. On the other hand, considering the apparent stability constant of Zn-MT is 1.8×10^{11} per Zn which is about 10^{-3} less than that of Cd-MT, and the ratio of stability constants per Cd for each cluster (K_α/K_β) are larger than the ratio of stability constants per Zn[26], the "distributed" or "incooperative" model for the metal binding process was also a possible explanation for the results[6,7]. The hypothesis was that one of two or two of four Pt(Ⅰ) ions bound to each domain in the platino-MTs for the reaction of two or four molar ratios Pt(Ⅳ) with Zn_7MT, respectively. After all, the mechanism of the substitution process is unclear and needs a further study.

When the reaction molar ratio of Pt(Ⅳ) to MT was up to 10 : 1 and 25 : 1, there exist only dimeric MT but no monomeric MT components (Figs. 5c,d). The intensities of the peaks for MT components were increased when the reaction molar ratio increased and the reaction time prolonged, too. The absence of monomeric MT indicated that the extent of the redox reaction was high and the amount of inter-molecular CyS-SCy bonds was large. The formation of large amounts of inter-molecular CyS-SCy bonds in the products may be the reason why the CD spectrum of the reaction between Zn_7MT and 25-fold molar excess K_2PtCl_6 appeared as no profile with maximum at 255 nm(+) and 235 nm(−) resulting from the formation of Pt-S clusters in Pt_7MT[11]. Almost all of Zn ions were replaced and about eight Pt(Ⅰ) ions were bound in the products from Zn_7MT with 10 and 25 molar ratios of

Pt(Ⅳ)(Table 2). These results indicated that both the α-cluster and the β-cluster participated in the reaction. These results were different from those in the reaction between native Cd_5Zn_2MT and K_2PtCl_6, there were still approximately four Cd ions left when Cd_5Zn_2MT reacted with 10 and 25 molar ratios of K_2PtCl_6 for 2 h, respectively, and one Cd ion left when the reaction molar ratio of Pt(Ⅳ) was ten for a long time. This may be due to the higher stability of Cd-S bond comparing with Zn-S bond in MT[26]. In Fig. 5c,d, the peak between 24 and 36 fractions corresponded to small-molecule component including excessive Pt(Ⅳ) and other inorganic ions.

When apoMT reacted with seven and 25 ratios of K_2PtCl_6 at pH 2, only dimeric MTs were obtained in the gel-filtration elution profiles (not shown in the article), and Pt_7MT and $Pt_{15}MT$ were obtained, respectively. It can also be assumed that a redox reaction occurred, the protein was oxidized while Pt(Ⅳ) was reduced because the free sulfhydryl groups are oxidized more easy than metal-S bond. The high level of bound Pt ions is also reported at low pH value and much excess reaction molar ratio of Pt in the reaction between MT and K_2PtCl_4[16].

From the UV-visible and CD spectra, chromatography, and XPS measurements, it can be found that the products contained monomeric and dimeric forms of MT and oligomeric products with intra and intermolecular CyS-SCy linkages, with the oxidation state of platinum in the products was +2 when Zn_7MT or apoMT reacted with K_2PtCl_6; that the Zn ions were replaced by Pt(Ⅱ) step by step, Pt_4Zn_4MT and Pt_8MT were formed when the reaction molar ratios of Pt(Ⅳ)-to-Zn_7MT were four and beyond ten, and Pt_7MT and $Pt_{15}MT$ were obtained in the reaction of apoMT with seven and 25 molar ratios of Pt(Ⅳ) at pH 2, respectively. From these results it can be concluded that the reaction of Zn_7MT or apoMT with K_2PtCl_6 comprises a redox reaction and a substitution reaction.

The mechanism of the reaction between K_2PtCl_6 and Zn_7MT or apoMT including a redox reaction indicates that the Pt(Ⅳ) complexes drugs can be reduced to Pt(Ⅱ) compounds by sulfhydryl-containing proteins (such as MT) or other reducing agents (such as cysteine and GSH) in normal or tumor tissues or other cells, then bind to MT (such as Zn-MT or apoMT) which may play important roles in metabolism, reducing cytotoxicity of Pt-containing drugs, and in developing drug resistance or cross-resistance.

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