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Disulfide Bond Cleavage Induced by a Platinum(II) Methionine Complex

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The cleavage of a disulfide bond and the redox equilibrium of thiol/disulfide are strongly related to the levels of glutathione (GSH)/oxidized glutathione (GSSG) or mixed disulfides in vivo. In this work, the cleavage of a disulfide bond in GSSG induced by a platinum(II) complex [Pt(Met)Cl_2] (where Met = methionine) was studied and the cleavage fragments or their platinated adducts were identified by means of electrospray mass spectrometry, high-performance liquid chromatography, and ultraviolet techniques. The second-order rate constant for the reaction between [Pt(Met)Cl_2] and GSSG was determined to be 0.4 M^{-1} s^{-1} at 310 K and pH 7.4, which is 100- and 12-fold faster than those of cisplatin and its monoaqua species, respectively. Different complexes were formed in the reaction of [Pt(Met)Cl_2] with GSSG, mainly mono- and dinuclear platinum complexes with the cleavage fragments of GSSG. This study demonstrated that [Pt(Met)Cl_2] can promote the cleavage of disulfide bonds. The mechanistic insight obtained from this study may provide a deeper understanding on the potential involvement of platinum complexes in the intracellular GSH/GSSG systems.

Introduction

Disulfide bonds play important roles in establishing and maintaining three-dimensional folding and structure of proteins.[1,2] They can act as switches for protein function or as regulators for secreted proteins through bond cleavages.[3] Disulfide redox systems control numerous important events in cellular processes such as the regulation of cell growth and proliferation[4] and cancer development.[5] Several mechanisms of disulfide bond cleavage, for example, dithiol−disulfide redox exchange,[6] simple alkaline hydrolysis,[3] and acid−base-assisted hydrolysis,[7] have been identified or suggested in secreted proteins. Reactions between a disulfide bond and transition-metal complexes have been reported recently.[8,9] These studies could provide new insights into the chemistry of transition-metal sulfur clusters with biological and industrial significance.[10] The reduction of disulfides to thiolates and the interconversion between them have been widely utilized in biological systems[11] and in the development of functional materials as a reversible redox process.[8,12]

Cisplatin and related compounds have been widely used in clinics as anticancer agents because Pt(II) is a "soft" metal ion showing a high affinity for sulfur-containing biomolecules such as methionine (Met), glutathione (GSH), and metallothionein.[13] Intracelluar thiols such as GSH may be

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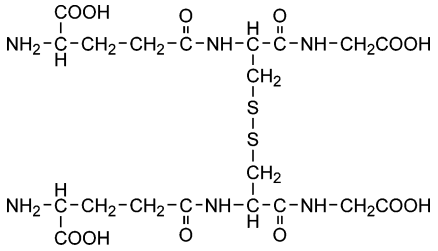
Chart 1. Molecular Structure of GSSG



GSSG

involved in the activation of Pt(IV) antitumor complexes, which leads to the formation of oxidized glutathione (GSSG).[14] The interactions between platinum(II) complexes and disulfide bonds are of great interest for the understanding of the mechanism of action of Pt(II) and Pt(IV) drugs. It is surprising that few studies have been reported on this aspect.[15,16] The cleavage of a disulfide bond and the formation of Pt−GS adducts were observed in the reaction of platinum(II) complexes with GSSG.[16] Such a cleavage in human serum albumin may result in a significant structural change of the protein.[15] However, the focus of these studies has been centered mainly on the kinetic behavior of the reactions, and the platinated adducts were not clarified.

In this study, platinum complex [Pt(Met)Cl_2] was chosen as a cisplatin model to simplify the reaction process because it is known that ammines in cisplatin could be released easily when reacting with sulfur-containing ligands.[17] The disulfide substrate GSSG (Chart 1) was employed, and the mechanism of the disulfide bond cleavage was suggested. The results could provide a deeper understanding on the potential involvement of platinum complexes in the intracellular GSH/GSSG systems.

Experimental Section

Complex [Pt(Met)Cl_2] was prepared according to the literature method,[18] and yellow blocky single crystals were obtained by slow evaporation of the reaction solution. GSSG and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma. Disodium 2-nitro-5-thiosulfobenzoate (NTSB) was synthesized from DTNB according to the reported method.[19] Samples for electrospray mass spectrometry (ESMS) determination were prepared using double-distilled water. Because of the limitation of the ESMS method, no buffer solution was employed in the preparation. The pH was adjusted by NaOH (1 M) or HAc (1 M) directly and measured after 24 h of reaction.

The ESMS spectra were recorded on an LCQ electron spray mass spectrometer (Finnigan) by loading 1.0 μL of a solution into the injection valve of the LCQ unit and then injecting that into the mobile phase solution (50% aqueous methanol), with the sample being carried through the electrospray interface into the mass analyzer at a rate of 200 μL min^{-1}. The employed voltage at the electrospray needles was 5 kV, and the capillary was heated to 200 °C. The maximum ion injection time was set to 200 ms with 10 scans. Zoom scanning was used in the experiments. The predicted isotope distribution patterns for each of the complexes were calculated using the Isopro 3.0 program.[20] The pH measurement was carried out using a pHS-3C pH meter equipped with a Phoenix Ag−AgCl reference electrode (Cole-Parmer Instrument Co.), calibrated with phosphate buffer solutions at pH 4 and 10. The chromatograms were recorded using an Agilent 1100 series chromatograph equipped with a DAD detector and a ZORBAX SB-C18 column (15 cm × 4.6 mm, 5 μm, Agilent Technology). High-performance liquid chromatography (HPLC) was performed using H_2O containing 0.1% trifluoroacetic acid (TFA; solvent A) and acetonitrile containing 0.1% TFA (solvent B) as the mobile phase with a flow rate of 0.5 mL min^{-1}, and 220 nm was selected as the detection wavelength. The ^{195}Pt NMR spectra were acquired on a 500-MHz Bruker DMX spectrometer at 107.3 MHz using standard pulse sequences. The ^{195}Pt chemical shifts were measured in D_2O at 310 K using Na_2PtC1_6 (0.1 M) as an external standard.

The kinetic study was carried out as follows. Solutions of complex [Pt(Met)Cl_2] (1.060−3.036 mM) were prepared in phosphate buffers of different pH values. GSSG was then added to different complex solutions, with the concentration of GSSG being adjusted to 100 μM. In all cases, [Pt(Met)Cl_2] was in excess. The S−S bond of GSSG was determined following the procedures described in the literature;[15a] namely, a 1.0-mL aliquot of the "NTSB assay solution" (0.2 M Tris + 0.1 M Na_2SO_3 + 3 mM EDTA, pH 9.5) was added to 1.0 mL of the reaction solution at the appropriate time, and the absorbance at 412 nm was monitored. The S−S bond was determined according to the amount of 2-nitro-5-thiobenzoic acid formed.[21]

Results

The reaction between [Pt(Met)Cl_2] and GSSG was monitored by ESMS at different pH values. Figure 1 shows the time-dependent ESMS spectra (negative ion mode) at pH 2.16. After 2 h of reaction (Figure 1a), apart from the signals for reactants GSSG and complex [Pt(Met)Cl_2] (**1**), peaks given by Pt−GS or Pt−GSSG complexes were also observed. The peaks with *m*/*z* values of 953.9 and 476.1 can be assigned to one negatively charged ion [Pt(GSSG)(Met)-3H]$^-$ (**2**) and its two negatively charged ion (**2′**), respectively. The peak with a *m*/*z* value of 647.0 can be attributed to one negatively charged ion [Pt(SG)(Met)-5H]$^-$ (**3**). Few changes were observed after 5 h except a new peak with a *m*/*z* value of 680.0 (Figure 1b), which can be assigned to one negatively charged ion [Pt(GSO_2)(Met)-3H]$^-$ (**6**). After 32 h of reaction, the intensity of the peaks corresponding to GSSG was still very strong (Figure 1c), whereas the peak for [Pt(Met)Cl_2]

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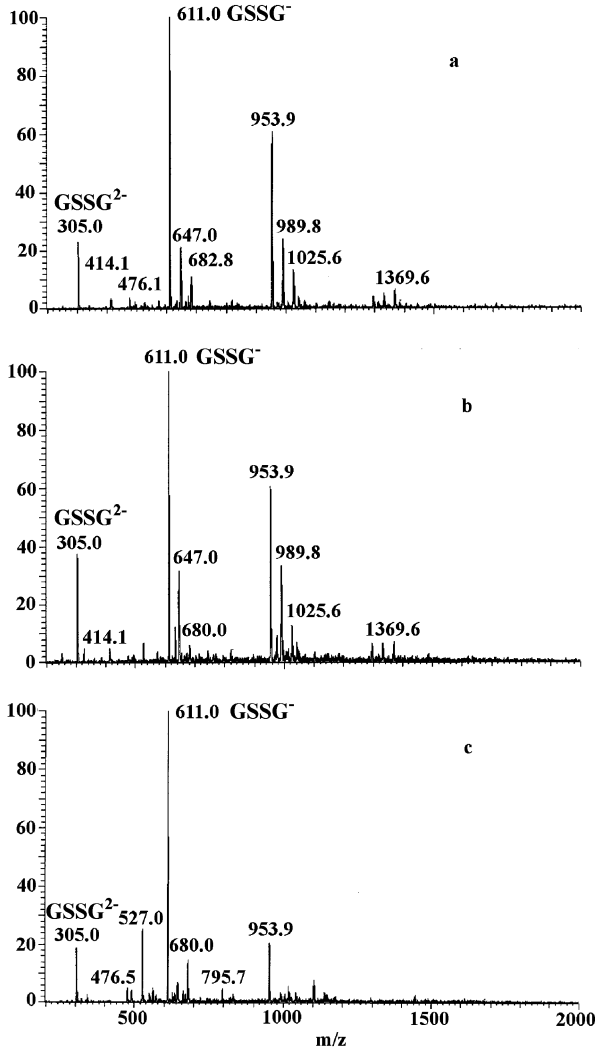

Figure 1. ESMS (negative ion mode) for the reaction of $[Pt(Met)Cl_2]$ with GSSG (molar ratio 2:1) recorded at pH 2.16 and 310 K after (a) 2 h, (b) 5 h, and (c) 32 h (see Table 1 for peak assignments).

disappeared completely even though the molar ratio of these two reactants is 2:1. The formation of Pt−GS complexes such as complexes **3**, **3′**, **4**−**6**, **7**, and **7′** in ESMS spectra (see Table 1) suggested the cleavage of the S−S bond in GSSG. Similar to the reaction of $[Pt(Met)Cl_2]$ with GSH,[13]

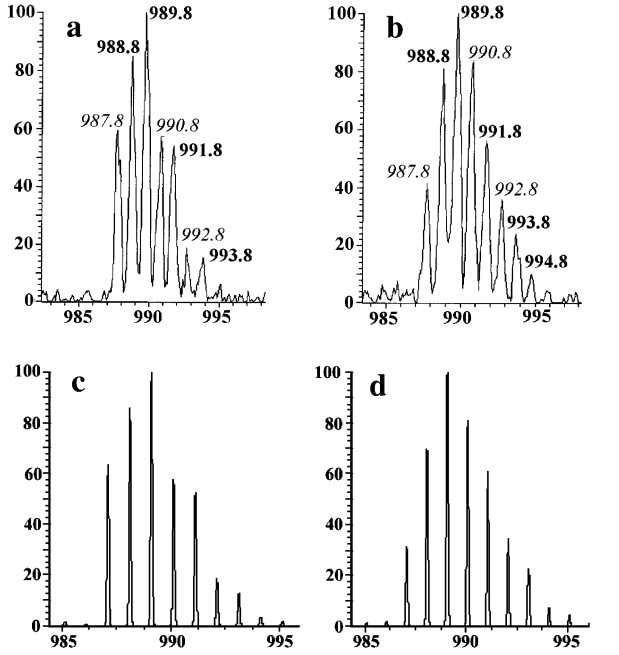

Figure 2. Determined isotopic distribution of the peak *m*/*z* 989.8 at 30 min (complex **2″**) (a) and at 2 h (complex **7**) (b). The italic numbers indicate the evident changes for the peaks. Simulated isotopic distributions for complexes **2″** and **7** are shown in parts c and d, respectively.

the peak at *m*/*z* 527.0 assignable to $\{[Pt(Met)_2\text{-}2H] + Cl^-\}^-$ (**8**) was also observed in the reaction.

It is interesting to note that the isotopic distribution pattern of the peak with a *m*/*z* value of 989.8 showed gradual changes with time. These changes may correspond to the conversion of the chloride adduct ion **2″** to complex **7** (Figure 2a,b). This hypothesis was confirmed by the calculated isotopic distribution pattern shown in Figure 2c,d.

The reaction was also monitored in an alkaline medium. Figure 3 shows the ESMS spectra recorded at pH 8.12 after 2, 5, and 32 h, respectively. Compared with the reaction at pH 2.16 within the same periods of time, the intensities of the peaks corresponding to GSSG are much weaker than those observed in Figure 1a. On the contrary, the intensities of the peaks corresponding to Pt−GSSG or Pt−GS adducts such as complexes **2** (or **2″**), **3**, and **6** are much stronger. The new peak with a *m*/*z* value of 1294.7 in Figure 3a,b can

Table 1. Assignments of the Peaks Observed in the ESMS Spectra for the Reaction of $[Pt(Met)Cl_2]$ and GSSG (See Figures 1 and 3)

species	formula	observed	calculated
$[GSSG\text{-}2H]^{2-}$	$C_{20}H_{30}N_6O_{12}S_2$	305.0	305.0
$[GSSG\text{-}H]^-$	$C_{20}H_{31}N_6O_{12}S_2$	611.0	611.0
$[Pt(Met)Cl_2\text{-}H]^-$ (**1**)	$C_5H_{11}NO_2SCl_2Pt$	414.1	414.1
$[Pt(Met)(GSSG)\text{-}3H]^-$ (**2**)	$C_{25}H_{40}N_7O_{14}S_3Pt$	951.9−955.9	953.3
$[Pt(Met)(GSSG)\text{-}4H]^{2-}$ (**2′**)	$C_{25}H_{39}N_7O_{14}S_3Pt$	475.2−477.6	476.1
$\{[Pt(Met)(GSSG)\text{-}2H] + Cl^-\}^-$ (**2″**)	$C_{25}H_{41}N_7O_{14}S_3ClPt$	987.7−995.8	989.7
$\{[Pt(Met)(GSSG)\text{-}3H]^- + Cl^-\}^{2-}$ (**2‴**)	$C_{25}H_{40}N_7O_{14}S_3ClPt$	493.2−496.6	494.4
$[Pt(Met)(GS)\text{-}5H]^-$ (**3**)	$C_{15}H_{23}N_4O_8S_2Pt$	644.9−648.9	646.5
$\{[Pt(Met)(GS)\text{-}4H]+Cl^-\}^-$(**3′**)	$C_{15}H_{24}N_4O_8S_2ClPt$	680.8−686.8	682.8
$[Pt_2(\mu\text{-}SG,Cl)(Met)_2\text{-}4H]^-$ (**4**)	$C_{20}H_{35}N_5O_{10}S_3ClPt_2$	1023.6−1029.6	1027.0
$[Pt_2(\mu\text{-}SG)_2(Met)_2(Cl)_2\text{-}3H]^-$ (**5**)	$C_{30}H_{53}N_8O_{16}S_4Cl_2Pt_2$	1369.6	1369.9
$[Pt(Met)(GSO_2)\text{-}3H]^-$ (**6**)	$C_{15}H_{25}N_4O_{10}S_2Pt$	678.9−682.9	680.5
$[Pt_2(\mu\text{-}SG)(Met)_2\text{-}5H]^-$ (**7**)	$C_{20}H_{34}N_5O_{10}S_3Pt_2$	989.7−995.8	990.3
$[Pt_2(\mu\text{-}SG)(Met)_2\text{-}6H]^{2-}$ (**7′**)	$C_{20}H_{33}N_5O_{10}S_3Pt_2$	494.2−496.6	494.6
$\{[Pt(Met)_2\text{-}2H]+Cl^-\}^-$ (**8**)	$C_{10}H_{20}N_2O_4S_2ClPt$	525.5−528.0	526.0
$[Pt_2(\mu\text{-}GSSG)(Met)_2\text{-}5H]^-$ (**9**)	$C_{30}H_{49}N_8O_{16}S_4Pt_2$	1292.8−1300.8	1295.6
$[Pt_2(\mu\text{-}GSSG)(Met)_2\text{-}6H]^{2-}$ (**9′**)	$C_{30}H_{48}N_8O_{16}S_4Pt_2$	645.9−649.7	647.3
GSO_2^-	$C_{10}H_{16}N_3O_8S$	338.0	338.0

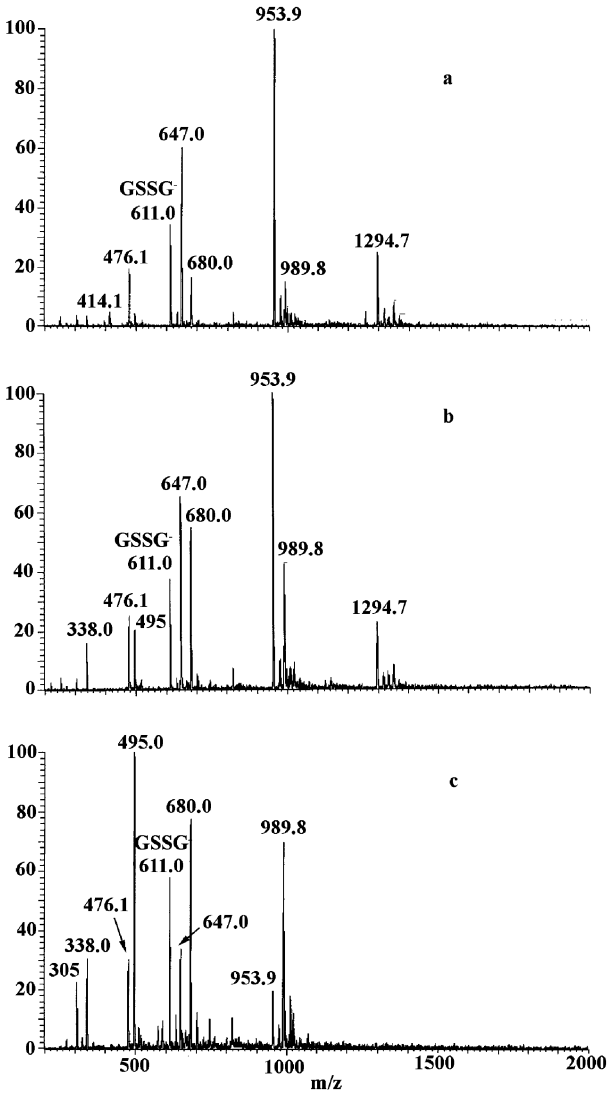

Figure 3. ESMS full scan (negative ion mode) for the reaction of [Pt(Met)Cl_2] with GSSG (molar ratio 2:1) recorded at pH 8.12 and 310 K after (a) 2 h, (b) 5 h, and (c) 32 h (see Table 1 for peak assignments).

be tentatively assigned to complex [Pt_2(μ-GSSG)$(Met)_2$-5H]$^-$ (**9**), where GSSG bridged two platinum atoms. After 5 h (Figure 3b), a peak with a *m*/*z* value of 338.0 was observed. This peak can be assigned to one negatively charged ion of GSO_2H, a hydrolyzed product of GSSG, which was also detected in the reaction between cisplatin and GSSG (data not shown). In the following time, the peaks corresponding to complexes **2** (*m*/*z* 953.9) and **3** (*m*/*z* 647.0) decreased in intensity while the peaks corresponding to complex **6** (*m*/*z* 680.0) and **7** (*m*/*z* 989.8) increased in intensity. After 32 h, the peaks corresponding to GSSG were still observable, but the dominant adducts were complexes **6** and **7** (or **7′**). The detailed assignments for peaks in Figures 1 and 3 are listed in Table 1.

To obtain quantitative kinetic information of the system, the solution containing an excess of [Pt(Met)Cl_2] was incubated at 310 K and pH 7.4. The pseudo-first-order rate constant, k_{obs}, was obtained by plotting $\ln(A_t - A_0)$ vs time (Figure S1 in the Supporting Information). On the basis of the reaction mechanism described in eq 1, the second-order

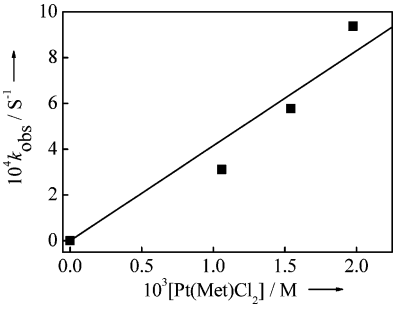

Figure 4. Relationship between the pseudo-first-order rate constants (k_{obs}) and [Pt(Met)Cl_2].

rate constant, k_1, can be derived from eqs 2 and 3.

$$\underset{\mathbf{A}}{\text{GSSG}} + \underset{\mathbf{B}}{\text{[Pt(Met-S,N)Cl}_2\text{]}} \xrightarrow{k_1} \text{product} \qquad (1)$$

According to the rate law

$$-\frac{\mathrm{d}[\mathbf{A}]}{\mathrm{d}t} = k_1[\mathbf{A}][\mathbf{B}] = k_{obs}[\mathbf{A}] \qquad (2)$$

$$k_{obs} = k_1[\mathbf{B}] \qquad (3)$$

A second-order rate constant (k_1) of 0.4 M^{-1} s^{-1} was obtained from the slope of the curve in Figure 4.

To investigate the pH influence on the reaction rate, GSSG was also incubated with an excess of [Pt(Met)Cl_2] at different pH values. k_{obs} was obtained in the range of pH 2.9−9.18 by the method described in the last part of the Experimental Section. The reactions at pH 3.0−6.0 were extremely fast, and the rate constants could not be determined accurately. We hence compared the residual concentration of GSSG at pH 3.6, 4.4, and 5.6, respectively. The remaining GSSG was 19.44 μM at pH 4.4 and 48.71 μM at pH 5.6, but no GSSG was detected at pH 3.6, which indicated that the S−S bond cleavage rate was much faster at pH 3.0−4.0 than at higher pH. This observation is consistent with the ESMS results.

The reaction between [Pt(Met)Cl_2] and GSSG was also monitored by HPLC. A typical HPLC chromatogram of the reaction after 24 h is shown in Figure S3 of the Supporting Information. Four peaks with retention times of 1.12, 1.35, 1.56, and 2.56 min, respectively, were observed. Except for the GSSG peak with a retention time of 1.35 min, all three other peaks could be attributed to the new adducts formed during the reaction.

Discussion

Disulfide bonds represent a significant structural motif in many peptides and proteins.[22] The reaction of a metal ion with a disulfide bond usually results in S−S bond cleavage and a conformational change of the biomolecules.[9,15] In this study, we investigated the S−S bond cleavage of GSSG affected by [Pt(Met)Cl_2].

In the reaction of [Pt(Met)Cl_2] with GSSG, platinated adducts such as Pt−GSSG, Pt−GS^-, and Pt−GSO_2^- were

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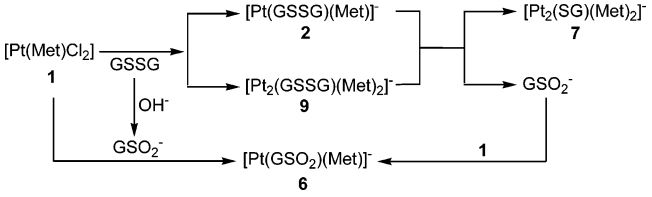
Scheme 1. Proposed Reaction Pathways between $[Pt(Met)Cl_2]$ and GSSG



observed. The identity of complex **3** (see Table 1) is uncertain, although the molecular mass and the isotopic distribution of this species (Figure S2 of the Supporting Information) suggested that the Pt center could be a Pt(IV) species. However, the corresponding Pt(IV) signal was not observed in the ^{195}Pt NMR spectra (Figure S4 of the Supporting Information), which may be due to the low abundance of the species. The limited solubility of the starting complex $[Pt(Met)Cl_2]$ in D_2O precludes the reaction being conducted at higher concentration. Complex **7** should be a species that contains one GS^- bridging two Pt atoms, which was similar to that reported previously.[16] Complex **9** should be $[Pt_2(\mu\text{-GSSG})(Met)_2\text{-}5H]^-$ as deduced by ESMS and its isotopic distribution, but its structure is unknown. There are 12 potential coordination sites for platinum(II) in GSSG, but it is difficult to clarify which one is more favorable. In an alkaline medium, the peaks for GSO_2^- and $[Pt(GSO_2)(Met)\text{-}3H]^-$ were observed, indicating that hydrolysis of GSSG occurred. A similar procedure was reported previously.[23,24] On the basis of the ESMS results, the pathway of S−S bond cleavage in GSSG is described in Scheme 1.

The HPLC chromatogram of the reaction between $[Pt(Met)Cl_2]$ and GSSG showed that at least four species appeared within 24 h, but the peaks are barely identifiable except that of GSSG (retention time of 1.35 min). Although the formation of new products is certain, the poor separation efficiency prevented HPLC from providing more valuable information for this study.

The kinetic study showed that the S−S bond cleavage followed a pseudo-first-order reaction in the case of $[Pt(Met)Cl_2]$ present in an excess amount over GSSG. The second-order rate constant for the reaction at pH 7.4 and 310 K is 0.4 M^{-1} s^{-1}, which is 100 and 12 times faster than those of cisplatin and its monoaqua species at 310 K and pH 7.4, respectively.[15] Therefore, the S−S bond cleavage of GSSG induced by $[Pt(Met)Cl_2]$ is much faster than those induced by cisplatin and its monoaqua species. The strong trans effect of the S-bound methionine may contribute to the fast formation of aqua species and eventually to the enhanced rate of cleavage of the disulfide. The pH-dependent reactions showed that the rate constant decreased with an increase of the pH, with the fastest reaction rate in the range of pH 3.0−4.0. This pH dependence is in agreement with those reported for cisplatin and its aqua complex.[15]

Conclusion

The thiol group of GSH is usually very reactive toward platinum complexes and thus prevents them from binding to DNA.[25,26] In this study, it is demonstrated that GSSG is also subject to the attack of platinum complexes. The kinetics for the reaction of GSSG with $[Pt(Met)Cl_2]$ revealed that this reaction is much faster than those with cisplatin and its monoaqua species. The main platinum-bound cleavage products of GSSG have been identified by ESMS and can only be partially separated by HPLC. These results suggest that GSSG can also potentially reduce the amount of intracellular platinum complexes available for interaction with DNA. Such potentiality may have important physiological implications in cellular defense and metabolism such as detoxification of platinum drugs and alteration of the thiol−disulfide status of cellular proteins.

Acknowledgment. We are thankful for financial support from the National Natural Science Foundation of China (Grants 20231010, 20228102, and 30370351).

Supporting Information Available: Figures of k_{obs} plots at different $[Pt(Met)Cl_2]$ concentrations, the isotopic distribution for complex **3**, the HPLC chromatogram, and the ^{195}Pt NMR spectrum of the reaction between $[Pt(Met)Cl_2]$ and GSSG. This material is available free of charge via the Internet at http://pubs.acs.org.

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