Cleavage of the Pt–S bond of thiolated terpyridine–platinum(II) complexes by copper(II) and zinc(II) ions in phosphate buffer

Chien-Chung Cheng* and Yen-Lin Lu

Institute of Chemistry, Academia Sinica, Taipei, Taiwan 11529, Republic of China

Using the chelation of a triamine moiety and an S-bridged heterodinuclear unit, cleavage of the Pt–S bond in the thiolate–platinum complexes, $[Pt(terpy)(BAT)]^{4+}$ and $[Pt(terpy)(AET)]^{+}$ is induced by adding transition metals such as Cu^{2+} or Zn^{2+} in phosphate buffer (pH 7–8) at room temp.

Many efforts in the development of platinum complexes as anticancer drugs have been contemplated in order to reduce the cytotoxicity and to improve the platin drug-resistance.[1] Since sulfur-containing compounds such as glutathione and metallothioneins are rich in cells, the formation of the S–Pt bond is generally believed to be one of the major mechanisms in the resistance to *cis*-platin drugs in biological processes.[2] In contrast, recent NMR studies by Sadler and coworkers reveal that S-bound L-methionine on Pt^{II} acts as a reversible binding ligand and is capable of undergoing intermolecular replacement by 5′-GMP in phosphate buffer (pH 7.2).[3] In fact, the concentration of glutathione (GSH) in intracellular cells is commonly high (> 1 mM). Therefore, in the reaction with platin drugs, the formation of thiolate–platinum adducts is inevitable.[4] The thiolate ion is capable of providing a stronger binding affinity owing to its better σ-donating ability.[5] Such a Pt–S bond is considered relatively inert, which may cause the inhibition of the anticancer activity of platin drugs. Thus, the great challenge is to break the Pt–S bond in the thiolate–platinum adducts.

To approach this goal, thiolated derivatives of terpy–platinum(II) complexes are used as a source to study the Pt–S bond owing to their characteristic electronic absorption and NMR spectra.[6–8] Here, we report that transition metals such as Cu^{II} or Zn^{II} are capable of inducing cleavage of the Pt–S bond of thiolated terpyridine platinum(II) complexes, $[Pt(terpy)(AET)]^{+}$ (AET = 2-aminoethanethiol) and $[Pt(terpy)(BAT)]^{4+}$ [BAT = *N*,*N*′-bis(aminoethyl)aminoethanethiol], particularly in phosphate buffer as shown in Fig. 1. The introduction of a triamine moiety to the complex structure increases the dissociation rate and the selectivity of the Pt–S bond cleavage in thiolate–platinum complexes. To our knowledge, this is the first reported example of Pt–S bond cleavage among thiolate–platinum(II) complexes in phosphate buffer.

$[Pt(terpy)(AET)]^{+}$ was prepared by published methods.[11] The synthesis of a triamine derivative, $[Pt(terpy)(BAT)]^{4+}$, was achieved by the coupling reaction of $[Pt(terpy)Cl]^{+}$ with the modified multistep synthesis[12–15] of triaminothiol, **1**, in water as illustrated in Scheme 1. The NMR spectrum of $[Pt(terpy)(BAT)]^{4+}$ in D_2O showed a new downfield peak at δ 8.82 and the UV–VIS spectrum displayed the absorbances at 486 nm (ε = 698 dm^3 mol^{-1} cm^{-1}), 342 (12 959), 329 (12 397) and 311 (11 903) in phosphate buffer (pH 8.0), which agreed with the characteristic features for establishing the formation of thiolate–platinum bonds in the previous report.[11] In addition, the integration ratio of protons of terpyridine and the triamine moiety in NMR spectra (D_2O) was approximately 0.93, suggesting no S-bridged diplatinum complex was generated.[14]

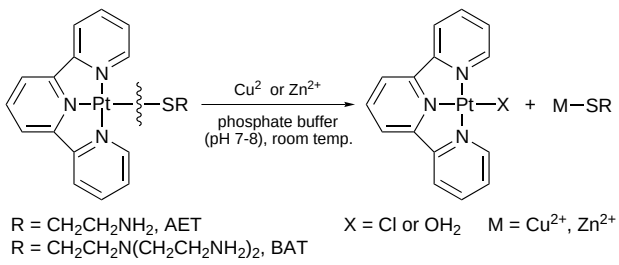
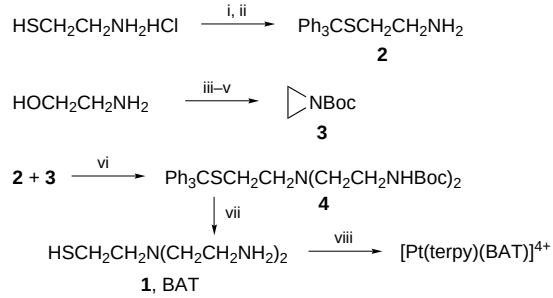


Fig. 1 General equation representing the cleavage of the Pt–S bond

In the presence of $CuCl_2 \cdot 6H_2O$ (42 μM), the solution of $[Pt(terpy)(BAT)]^{4+}$ (40 μM) exhibited a decreasing UV–VIS absorption at 342 nm accompanied by an increasing absorption at 329 nm in phosphate buffer (10 mM, pH 8.0). An isosbestic point was observed at 337 nm in the spectrum, suggesting no intermediate had been generated in the reaction with metal ions. Also, no significant alteration was observed in the absence of Cu^{II}. The cleavage of the Pt–S bond of $[Pt(terpy)(BAT)]^{4+}$ (40 μM) in the titration with Cu^{II} (10–84 μM) was found to require 1 equiv. of Cu^{II} to complete the reaction in 120 min. This dissociation of the Pt–S bond of $[Pt(terpy)(BAT)]^{4+}$ was revealed to be a pH dependent process in the range pH 4.8–10.0. The reactivity of the Pt–S bond cleavage was reduced with a decreasing pH and ceased at pH < 6, implying that the participation of the amino group plays a vital role in the cleavage. In fact, when a hydroxylthiol derivative of $[Pt(terpy)(SCH_2CH_2OH)]^{+}$, was used instead of an amino derivative, under the same conditions, no cleavage of the Pt–S bond was observed.

The isolation of the cleavage product was performed in MeOH in order to simplify the product analysis. After the addition of $CuCl_2 \cdot 6H_2O$, a red solution of $[Pt(terpy)(BAT)]^{4+}$ turned into a blue-green suspension containing orange solid. This orange solid was isolated in a yield of 75–80% with electronic absorptions at 382, 332 and 320 nm in phosphate buffer (pH 8) and NMR peaks in D_2O at δ 7.8 (m), 7.5 (m) and 7.2 (t), suggesting it lacked the Pt–S bond linkage. Furthermore, FAB mass and isotope abundance simulation studies disclosed that the molecular ion at 464 contained a chloride ion, suggesting that $[Pt(terpy)Cl]^{+}$ was one of the cleavage products.



Scheme 1 *Reagents and conditions*: i, triphenylmethanol, neat TFA, 25 °C, 30 min, 89%; ii, NaOH(aq), 92%; iii, conc. H_2SO_4; vacuum (0.1 mmHg) at 80 °C, overnight, 62%; iv, KOH(aq), distillation; v, $(Boc)_2O$, dioxane–H_2O, 0 °C, warming to room temp. overnight; vi, MeCN, reflux, 3 days, 75–80%; vii, TFA, triethylsilane 84%; viii, deionized H_2O, $[Pt(terpy)Cl]Cl$, N_2, 25 °C, 5 h; recrystallization with MeOH–MeCN, 40%.

The chloride ion in the isolated product may be acquired from the use of metal chloride salt as the starting material. In addition, the residual Cu complex in the solution was determined to be a symmetric dimer of copper–triaminothiol complex by NMR and FAB MS analysis. The dimer adduct may be obtained by the coupling reaction with monomer of copper–triaminothiol once released from the platinum–thiolate complexes.

Furthermore, a comparison of the reactivity using different metals Ni^{II}, Cu^{II} and Zn^{II} in the Pt–S bond dissociation in $[Pt(terpy)(BAT)]^{4+}$ in phosphate buffer is also examined. Interestingly, the result shows that the dissociation induced by transition metals varied in a descending order: $Zn^{II} > Cu^{II} > Ni^{II}$, as illustrated in Fig. 2. The rate constant has been estimated under the first-order condition[15] to give a value of 7.9×10^{-3} s^{-1} for Zn^{II}, 5.3×10^{-4} s^{-1} for Cu^{II} and 1.9×10^{-4} s^{-1} for Ni^{II}. Moreover, the same cleavage product, $[Pt(terpy)Cl]^+$, also can be obtained from $[Pt(terpy)(BAT)]^{4+}$ using $ZnCl_2$ in phosphate buffer. Zn^{II} had the strongest preference for promoting Pt–S bond cleavage, and was about 15 times faster than $CuCl_2$. Besides, Zn^{II} also exhibited the highest selectivity in the cleavage of the Pt–S bond of the triaminothiol species, $[Pt(terpy)(BAT)]^{4+}$, but produced no reaction with the aminothiol species, $[Pt(terpy)(AET)]^+$.

In order to eliminate the possibility of the aggregation of polyaromatic compounds,[10] which may enhance the cleavage by neighboring complexes, the dissociation process was also examined at a low concentration (10–12 μM) of $[Pt(terpy)(BAT)]^{4+}$. The result showed no significant difference in the dissociation rate of the Pt–S bond at either a low or high concentration of polyaromatic compounds. Moreover, the preliminary cleavage mechanism has been studied using Cu^{II} in NMR. In phosphate buffer, one of the terpyridine peaks and all of the aliphatic protons are shifted from δ 8.88 and 2.2–2.7 to δ 9.06 and 2.7–3.1 respectively, indicating the S-bridged heterodinuclear moiety is generated while Cu^{II} is coordinating to the triamine moiety. As a result, it will weaken the Pt–S bond, resulting in Pt–S bond dissociation by the other nucleophiles such as Cl^-. Thus, the different coordination of triaminothiol to tetrahedral Zn^{II}, square-planar Cu^{II} and octahedral Ni^{II} in the transition state may be attributed to the different reactivity and selectivity of different metal ions in the Pt–S bond cleavage. The detailed mechanism is under current investigation.

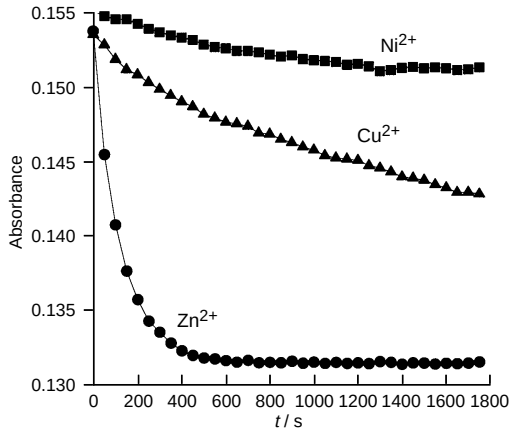


Fig. 2 Comparison of the reactivity in the cleavage of the Pt–S bond by Zn^{2+}, Cu^{2+} and Ni^{2+} ions. These data were collected at a fixed absorption wavelength of 342 nm at intervals of 50 s per cycle at a concentration of $[Pt(terpy)(BAT)]^{4+}$ (40 μM) and metal ion (40–42 μM) in 10 mM phosphate buffer (pH 8.0) at room temp. (●) Zn^{2+}, (▲) Cu^{2+}, (■) Ni^{2+}.

In summary, we have demonstrated that the cleavage of the Pt–S bond of $[Pt(terpy)(AET)]^+$ and $[Pt(terpy)(BAT)]^{4+}$ can be achieved by the addition of transition metal ions such as Zn^{II} or Cu^{II} in either phosphate buffer or MeOH. Importantly $[Pt(terpy)Cl]^+$ is isolated and identified as one of the major products in the dissociation of the Pt–S bond of $[Pt(terpy)(BAT)]^{4+}$ in the presence of $ZnCl_2$ or $CuCl_2$. In fact, the Cl ligand is very labile and readily replaced by other nucleophiles such as H_2O or imidazole.[6] Namely, in the biological system, the replacement of the labile Cl ligand may be achieved by surrounding guanine residues[2] resulting in the recovery of anticancer activity of platin drugs. Interestingly, the dissociation of the Pt–S bond and the formation of a labile chloro species can also be detected using $[Pt(dien)(BAT)]^{4+}$, (dien = diethylenetriamine) in phosphate buffer in the presence of Zn^{II} or Cu^{II} ions. Therefore, these results may provide an alternative pathway in the regeneration of the active Pt complexes from the platinum–thiolate adduct, which may relate to the restoration of the anticancer activity of platin drugs in biological systems.

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Footnote and References

* E-mail: ccheng@chem.sinica.edu.tw

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