Thiolate-bridged heterodinuclear platinum–zinc chelates as models for ternary platinum–DNA–protein complexes and zinc ejection from zinc fingers. Evidence from studies using ESI-mass spectrometry†

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Structures for model ternary platinum–DNA–protein complexes and zinc ejection from zinc fingers by platinum were deduced from the ESI-MS spectra of the interaction of model Zn and Pt complexes.

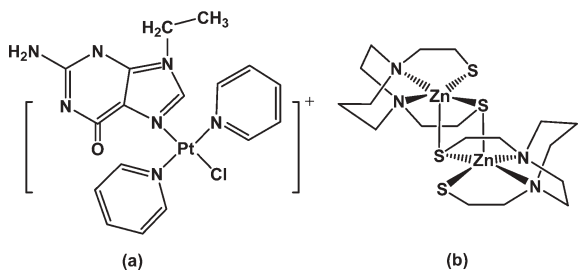
Zinc finger proteins are involved in a variety of DNA transcriptional and repair functions. With ever-increasing structural and biological information, zinc-fingers have also become important cellular targets for potential drugs against cancer[1] and HIV inactivation.[2,3] A dominant strategy is the design of chelators to effect zinc ejection, thus inhibiting protein function.[4] In parallel, the biological relevance of metal ion–zinc-finger or zinc-based protein interactions has come from a variety of sources. Studies on zinc replacement by Cd, Hg and Pb have suggested mechanisms whereby such metals may manifest their toxicity.[5,6] The anti-arthritic gold compound, aurothiomalate, inhibits the DNA binding of the zinc finger transcription factor OB2-1, thus downregulating OB2-1 dependent c-erbB-2 transcription.[7] The purported zinc site of OB2-1 was suggested as the target. A cobalt macrocycle based on the Schiff base salen system specifically attacks histidine residues with subsequent zinc displacement from a model zinc finger.[8] The platinum complex *[SP-4-2]*-[PtCl(9-EtGua)(NH_3)(quin)]$^+$ and analogues eject zinc from the C-terminal knuckle (residues Lys34 – Glu53) of the HIV nucleocapsid NCp7 protein.[9] The mechanism by which *cis*-DDP inhibits DNA polymerase from P-3A prostate tumor cells is also suggested to be through Zn-ejection.[10]

DNA–protein crosslinks are a minor component of the cellular adducts formed by anticancer drugs such as cisplatin and oxaliplatin.[11] The proportion of such adducts may vary depending on complex structure.[12] The structures of the DNA–protein adducts formed by platinum are poorly understood, especially in comparison to the extensive knowledge on bifunctional DNA–DNA adducts.[13] The mononuclear *trans*-DDP cross-links the zinc-finger nucleocapsid NCp7 protein to HIV-1 RNA.[14] Similarly, ternary adduct formation has been shown for the DNA adducts of dinuclear platinum compounds with Zn-finger containing transcription factors such as Sp1 and the UvrABC bacterial repair protein.[15,16] Incorporation of steric hindrance into an ethylenediamine chelate may also allow for ternary DNA–UvrA protein crosslinking due to retardation of the second DNA binding step and favorable competitive reaction with protein.[17] In all these cases, protein complexation is considered to occur through a monofunctional Pt–DNA intermediate. Similarly, the induction of Topoisomerase I–DNA complexes in human tumor cells by complexes of type *trans*-[$PtCl_2$(pyridine)$_2$] and *trans*-[$PtCl_2(NH_3)$(thiazole)$_2$] may involve ternary Pt–DNA–protein crosslinking mediated by a monofunctional Pt–DNA species.[12]

The mechanistic aspects of metal exchange on bioligands such as zinc-fingers therefore deserve examination from the basic chemical viewpoint. In this communication we show mass spectrometric evidence for formation of heterodinuclear (Pt,Zn) thiolate-bridged systems through the reaction of a model zinc chelate and cytotoxic platinum compounds followed by eventual formation of a platinum macrocycle through zinc ejection. The results also suggest reasonable chemical models for zinc ejection by platinum compounds as well as suggesting possible mechanisms and structures for ternary DNA–protein crosslinking mediated by platinum complexes, and repair of monofunctional Pt–DNA adducts through the initial formation of a heterodinuclear compound.

The zinc chelate used was the dinuclear dithiolate complex formed from the tetradentate ligand [1,5-bis(mercaptoethyl)-1,5-diazacycloheptanato], [Zn(bme-dach)]$_2$, an [(N_2S_2)Zn]$_2$ complex such as that previously explored as a model of zinc sulfur methylation proteins.[18] The platinum compound was *trans*-[Pt(pyridine)$_2$(9-EtGua)Cl]$^+$, a model for a monofunctional adduct of platinum on DNA.[9] The structures of the compounds are shown in Scheme 1 and the mass spectra of the individual reactants are given in Figure S1 and Figure S2.

Fig. 1 shows the mass spectra of the reaction at early time points in the reaction. The major peak at 5 min (Fig. 1a) is due to the



Scheme 1 The structure of (a) *trans*-[Pt(pyr)$_2$(9-EtGua)Cl]$^+$ and (b) zinc finger model compound [Zn(bme-dach)]$_2$.

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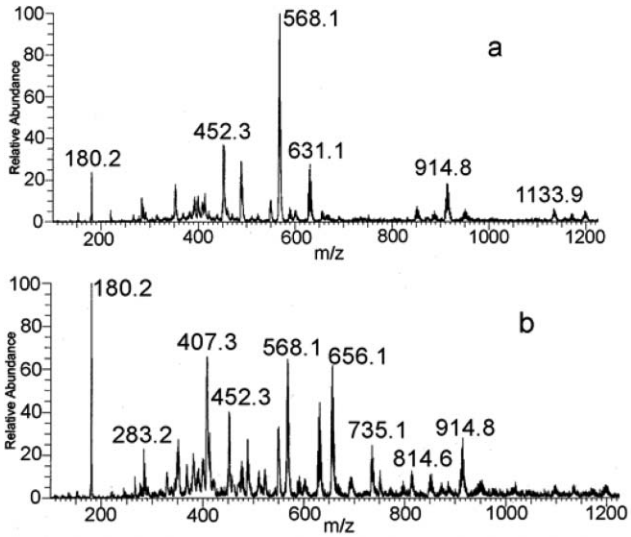


Fig. 1 The time dependent ESMS full scan (positive mode) of the reaction between *trans*-[Pt(pyr)$_2$(9-EtGua)Cl]NO$_3$ and [Zn(bme-dach)]$_2$ recorded after (a) 5 min and (b) 25 min at the ratio of 1 : 1, pH 7.6 and 310 K.

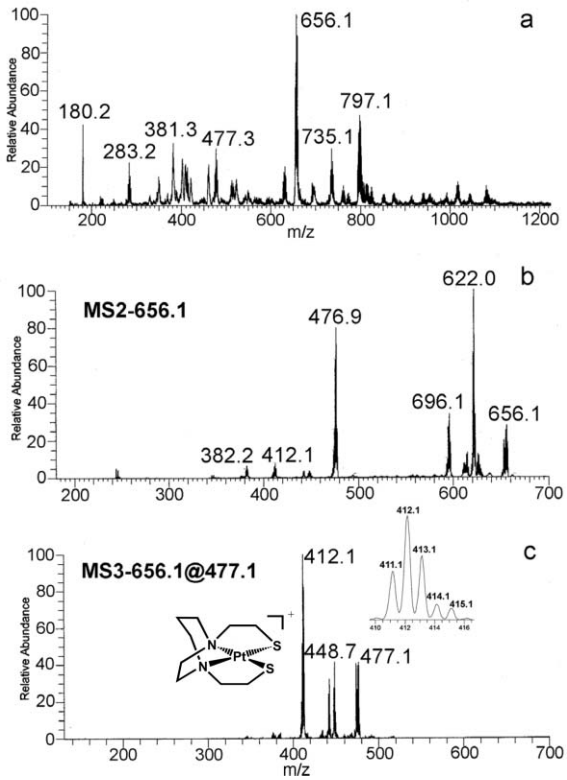
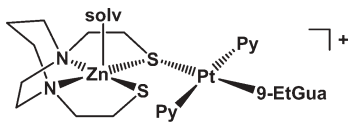


Fig. 2 The ESMS full scan (positive mode) of the reaction between *trans*-[Pt(pyr)$_2$(9-EtGua)Cl]NO$_3$ and [Zn(bme-dach)]$_2$ (a) recorded after 20 h and (b) the MS2 of the peak 6561. (c) MS3 of peak 656.1@477.1.

presence of both reactants, with slight changes to peak intensities centered at *m*/*z* = 568.1. The observed isotopic distribution for the reactant mixtures is identical to that calculated from the coincidental superposition of the individual isotopic distributions of the Zn (*m*/*z* M$^+$ 569.1) and Pt (*m*/*z* M$^+$ 567.1) starting materials. After 25 minutes the relative intensities of the reactants has diminished and a number of new peaks arise, Fig. 1b. Noteworthy are the peaks at *m*/*z* 814.6 and 407.3 corresponding to the 1$^+$ and 2$^+$ ions of a Pt, Zn species [Zn(bme-dach){Pt(pyr)$_2$(9-EtGua)}]. Free 9-ethylguanine is also observed (*m*/*z* 180) along with a peak at 656.1. Over time (up to 20 h) this latter peak, along with the peak at 797.1 becomes the major species and no signals due to reactants are observed (Fig. 2a). Free 9-ethylguanine was also observed after 35 min by ^{1}H NMR spectroscopy, run under the same solvent conditions as the MS. Interestingly, the NMR spectrum at 15 min showed two peaks attributable to H8 of Pt-bound 9-ethylguanine at 8.3 and 8.4 ppm, possibly suggesting the presence of an intermediate Pt, Zn species. Table 1 gives the assignment of the major mass spectral peaks shown in Figs. 1 and 2.

The chemical structure of the [{Zn(bme-dach)}{Pt(pyr)$_2$(9-EtGua)}] species responsible for the peaks at 407.3 and 814.6 can be described structurally as a heterodinuclear {Pt,Zn} species (see Figure S3) with bridging thiolate (Scheme 2):

The propensity of thiolate in cysteine and glutathione adducts of platinum compounds to form bridged dinuclear structures



Scheme 2 Proposed structure of the intermediate species [Zn(bme-dach)-Pt(pyr)$_2$(9-EtGua)]$^+$.

supports this assignment.[19–22] This is, to our knowledge, the first heterodinuclear species using biologically relevant ligands. The intimate involvement of the zinc finger sites in DNA/RNA recognition in the HIV-1 NCp7, Sp1 and UvrABC cases suggests that the results and structures discussed here present reasonable models for the molecular structure of ternary DNA–protein complexes involving zinc finger proteins.

The chemical composition of the final product peaks centered at 656.1 and 797.1 are [{Zn(bme-dach)}{Pt(9-EtGua)}]$^+$ and

Table 1 Assignment of the major peaks in Figs. 1 and 2

Peak observed	Charge	Corresponding species	Calculated
451.3–455.3	+1	{[Pt(9-EtGua)(pyr)]$^{2+}$ − H$^+$}$^+$	452.0
281.1–288.1	+1	{[Zn(bme-dach)] + H$^+$}$^+$	284.6
654.1–661.1	+1	{[Zn(bme-dach)Pt(9-EtGua)]$^{2+}$ − H$^+$}$^+$	656.2
811.0–819.0	+1	{[Zn(bme-dach)Pt(9-EtGua)(pyr)$_2$]$^{2+}$ − H$^+$}$^+$	814.2
406.0–410.0	+2	[Zn(bme-dach)Pt(9-EtGua)(pyr)$_2$]$^{2+}$	407.6
473.0–481.5	+1	{[Zn(bme-dach)Pt]$^{2+}$ − H$^+$}$^+$	477.5
793.0–808.0	+1	{[Zn(bme-dach)]$_2$PtCl}$^+$	797.8
732.0–741.0	+1	{[Zn(bme-dach)Pt(9-EtGua)(pyr)]$^{2+}$ − H$^+$}$^+$	735.5

$[\{Zn(bme\text{-}dach)\}_2PtCl]^+$ respectively. The isotopic distributions are identical with those calculated in both cases. While a number of possible structures may be drawn, tandem MS gives information on the structures of these species. An MS2 experiment on the 656.1 peak, Fig. 2b, gives two major peaks at 622.01 and 477.1 corresponding to loss of a thiol (656.1-33-1) and 9-ethylguanine (656.1-179) respectively. A further tandem MS experiment on the 477.1 peak gives as final product a species with a *m*/*z* of 412.1 corresponding to loss of zinc (477.1-65) and the simple platinum chelate [Pt(bme-dach)] as final product, Fig. 2c. Interestingly MS2 and MS3 experiments on the 797.1 peak also gave as final product the [Pt(bme-dach)] species *via* a major peak at 514.1 (see Figure S4). The sequence of decomposition for both species is therefore:

$$[\{Zn(bme\text{-}dach)\}\{Pt(9\text{-}EtGua)\}]^+ \rightarrow [\{Zn(bme\text{-}dach)Pt\}]^+ \rightarrow [Pt(bme\text{-}dach)]^+$$

and

$$[\{Zn(bme\text{-}dach)\}_2PtCl]^+ \rightarrow [\{Zn(bme\text{-}dach)\}PtCl]^+ \rightarrow [Pt(bme\text{-}dach)]^+$$

Regardless of the exact structures of these intermediates the tandem MS experiments provide strong evidence for zinc ejection and concomitant platinum incorporation into the macrocycle. The proposed ejection of zinc from its structural site therefore occurs upon coordination of the second metal in close proximity to the active site. The Pt(bme-dach) complex was prepared independently and isolated pure as a yellow solid with MS identical to that of the zinc exchange described above.

The bme-dach ligand and N_2S_2 analogs are very versatile metal-binding agents. Monometallic derivatives include square planar Ni, Pd and Cu. Dinuclear Fe, Co and Zn in square pyramidal coordination result from thiolate sulfur bridges in the formation of $[(N_2S_2)M]_2$.[18,23–25] The S-based reactivity of the Ni derivative has been exploited to produce cluster compounds incorporating Pd through thiolate bridges – the structurally characterised $[\{Ni(bme\text{-}dach)\}_2PdCl]^{+}$[25] is at least compositionally and likely structurally similar to the $[\{Zn(bme\text{-}dach)\}_2PtCl]^+$ species observed in this study by mass spectrometry. These compounds are formally analogous to the intermediate species described here, as the cluster (polynuclear) formation is based on an Zn(Ni)–S–Pt(Pd) bridging thiolate motif.

Amongst the reasons for study of zinc and other metal–thiolate systems is that they provide precedent for *S*-alkylation reactions in nature. The thiolate ligand binding sites of (bme-dach)Zn are sufficiently nucleophilic to be readily alkylated by alkylating agents.[18] As expected, on alkylation the thiolate becomes a more weakly binding thioether. The persistence of the thioether–metal linkage is dependent on the exact nature of the alkylating agent. We therefore suggest that a formal analogy can be made between platination and alkylation (and indeed protonation). The *E. coli* ADA repair protein repairs DNA by transfer of a methyl group from a methylphosphodiester or methylguanine to a thiolate sulfur.[26] By analogy with the alkylating agent then, platination (and in general metallation) is also feasible. Whether such chemical repair occurs for monofunctional or even labile bifunctional Pt and other metal–DNA adducts is worthy of exploration.

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Notes and references

1 A. Klug, *FEBS Lett.*, 2005, **579**, 892–894.
2 R. A. Musah, *Curr. Top. Med. Chem.*, 2004, **4**, 1605–1622.
3 A. Rein, L. E. Henderson and J. G. Levin, *Trends Biochem. Sci.*, 1998, **23**, 297–301.
4 W. Beerheide, H. U. Bernard, Y. J. Tan, A. Ganesan, W. G. Rice and A. E. Ting, *J. Natl. Cancer Inst.*, 1999, **91**, 1211–1220.
5 H. A. Godwin, J. C. Payne and M. A. ter Horst, *J. Am. Chem. Soc.*, 1999, **121**, 6850–6855.
6 P. F. Predki and B. Sarkar, *Environ. Health Perspect.*, 1994, **102**, 195–198.
7 M. L. Handel, A. de Fazio, C. K. Watts, R. O. Day and R. L. Sutherland, *Mol. Pharmacol.*, 1991, **40**, 613–618.
8 A. Y. Louie and T. J. Meade, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 6663–6668.
9 D. A. Sartori, B. Miller, U. Biebach and N. Farrell, *J. Biol. Inorg. Chem.*, 2000, **5**, 575–583.
10 T. J. Kelley, S. Moghaddas, R. Bose and S. Basu, *Cancer Biochem. Biophys.*, 1993, **13**, 135–146.
11 L. A. Zwelling, T. Anterson and K. W. Kohn, *Cancer Res.*, 1979, **39**, 365–369.
12 N. Farrell, L. F. Povirk, Y. Ange, M. S. Gupta, G. Kohlhagen, Y. Pommier and D. Gewirtz, *Biochem. Pharmacol.*, 2004, **68**, 857–866.
13 K. R. Barnes and S. J. Lippard, *Met. Ions Biol. Syst.*, 2004, **42**, 143–177.
14 J. L. Darlix, C. Gabus, M. T. Nugeyre, F. Clavel and F. Barresinoussi, *J. Mol. Biol.*, 1990, **216**, 689–699.
15 V. Houten, B. S. Illenye, Y. Qu and N. Farrell, *Biochemistry*, 1993, **32**, 11794–11801.
16 M. Kloster, H. Kostrhunova, R. Zaludova, V. Brabec and N. Farrell, *Biochemistry*, 2004, **43**, 7776–7786.
17 B. Lambert, J. L. Jestin, P. Brehin, C. Oleyowski, A. Yeung, P. Mailliet, P. C. Pretot, J. B. Pecq, A. Jacquemin-Sablon and J. C. Chottard, *J. Biol. Chem.*, 1995, **270**, 21251–21257.
18 C. A. Grapperhaus, T. Tuntulani, J. H. Reibenspies and M. Y. Darensbourg, *Inorg. Chem.*, 1998, **37**, 4052–4058; M. L. Golden, Ph.D. Dissertation, Texas A&M University, 2004.
19 E. L. M. Lempers and J. Reedijk, *Adv. Inorg. Chem.*, 1991, **37**, 175–190.
20 K. A. Mitchell and C. M. Jensen, *Inorg. Chem.*, 1995, **34**, 4441–4446.
21 M. E. Oehlsen, A. Hegmans, Y. Qu and N. Farrell, *Inorg. Chem.*, 2005, **44**, 3004–3006.
22 P. del S. Murdoch, N. A. Kratochwil, J. A. Parkinson, M. Patriarca and P. J. Sadler, *Angew. Chem., Int. Ed.*, 1999, **38**, 2949–2951.
23 M. L. Golden, J. H. Reibenspies and M. Y. Darensbourg, *Inorg. Chem.*, 2004, **43**, 5798–5800.
24 M. L. Miller, S. A. Ibrahim, M. L. Golden and M. Y. Darensbourg, *Inorg. Chem.*, 2003, **42**, 2999–3007.
25 M. L. Golden, S. Jeffery, J. H. Reibenspies and M. Y. Darensbourg, *Eur. J. Inorg. Chem.*, 2004, 231–236.
26 L. C. Myers, M. P. Terranova, A. E. Ferentz, G. Wagner and G. L. Verdine, *Science*, 1993, **261**, 1164–1167.

