# Intramolecular migration of $[Pt(dien)]^{2+}$ (dien = 1,5-diamino-3-azapentane) from sulfur to imidazole-$N^1$ in histidylmethionine (his-metH)

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**HPLC and NMR investigations of the kinetics of competitive binding by neighbouring side chains in his-metH demonstrate that at pH > 6 initial S coordination is followed by slow isomerization to an imidazole-$N^1$ bound complex through a dinuclear intermediate $[\{Pt(dien)\}_2$(his-metH-1κ$N^1$:2κ$S$)$]^{4+}$**

Pt binding by sulfur-containing bioligands such as L-methionine (Hmet) is believed to be responsible for the concentration-dependent nephrotoxicity of the widely used antitumor agent cisplatin, *cis*-$[PtCl_2(NH_3)_2]$.[1] Recent reports[2,3] of the replacement of a thioether S by guanine-$N^7$ in the square-planar coordination sphere of the model fragment $[Pt(dien)]^{2+}$ have also nourished the concept of a drug reservoir mechanism in which initial protein binding may provide a route to DNA platination.[4] Interestingly, whereas 5′-GMP selectively displaces Hmet in $[Pt(dien)(Hmet\text{-}\kappa S)]^{2+}$, no reaction is observed[3] between the likewise imidazole-containing amino acid L-histidine (Hhis) and this complex, even after 3 days. In contrast to this finding, we now report HPLC and NMR evidence for his-$N^1$ replacement of S-bound met, when both are neighbouring peptide residues.

Equimolar 0.8mM solutions of the three model dipeptides his-metH, met-hisH and *cyclo*-his-met with $[Pt(dien)(H_2O)]^{2+}$ in the range 3 < pH < 11 were incubated at 313 K for 14 days, after which the reaction products were separated by reversed-phase HPLC in the presence of 0.1% (v/v) pentafluoropropionic acid as an ion-pairing agent.[5,6] The distribution diagrams presented for the $[Pt(dien)(H_2O)]^{2+}$/his-metH system in Figs. 1–3 were calculated using peak areas of the separated species at the detection wavelength of 220nm and individual molar absorbance coefficients obtained from a least-squares fit for the chromatographic data collected over the full pH range. Products were characterized by FAB mass spectrometry and multinuclear ($^1$H,$^{195}$Pt) NMR spectroscopy.† Methionine κ$S$ coordination leads to a pronounced downfield shift for the thioether δ-$CH_3$ protons from $\delta$ 2.12 in the free dipeptide (pH* = 6.9) to $\delta$ 2.53 in $[Pt(dien)(his\text{-}metH\text{-}\kappa S)]^{2+}$ **1**, the dominant Pt complex in acid solution. At pH > 6.1, the $N^1$-bound complex, $[Pt(dien)(his\text{-}metH\text{-}\kappa N^1)]^{2+}$ **2**, provides the major species; **2** is characterized by its lack of a δ-$CH_3$ downfield shift, the equivalence of its $^3J(^1H\text{–}^{195}Pt)$ values (19 Hz) for the imidazole $H^2$ and $H^5$ resonances and its typical $^{195}$Pt NMR chemical shift (δ −2861) for a square-planar $N_4$ coordination sphere.[7] In contrast to Hhis itself with an $N^3/N^1$ binding ratio of 1.5 for $[Pt(dien)]^{2+}$ at pH* = 6.5,[8] the κ$N^3$ complex appears to play a minor role for his-metH in neutral aqueous solution, as is also the case for met-hisH and *cyclo*-his-met. These latter dipeptides exhibit respective crossover pH values of 5.9 and 3.8 for the change in their preferred binding mode from κ$S$ to κ$N^1$. Inspection of Fig. 1 indicates that a dinuclear complex $[\{Pt(dien)\}_2(his\text{-}metH\text{-}1\kappa N^1:2\kappa S)]^{4+}$ **3**, characterized by its FABMS base peak and two $^{195}$Pt resonances, reaches its maximum concentration at the crossover pH value and a similar state of affairs was established for met-hisH and *cyclo*-his-met.

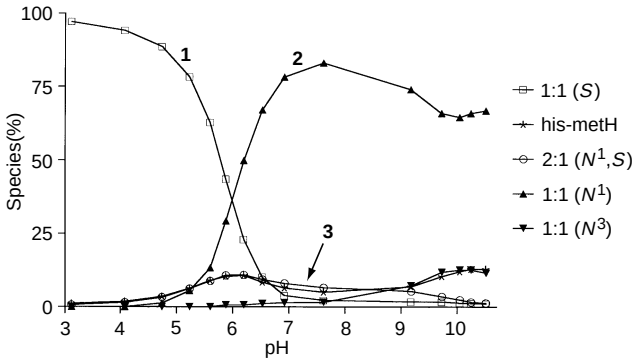



**Fig. 1** Species distribution for the 1 : 1 $[Pt(dien)(H_2O)]^{2+}$–his-metH reaction system as determined by HPLC for the range pH 3.0–10.5 ($T$ = 313 K, $t_{reaction}$ = 14 d). Two minor species are omitted for clarity.

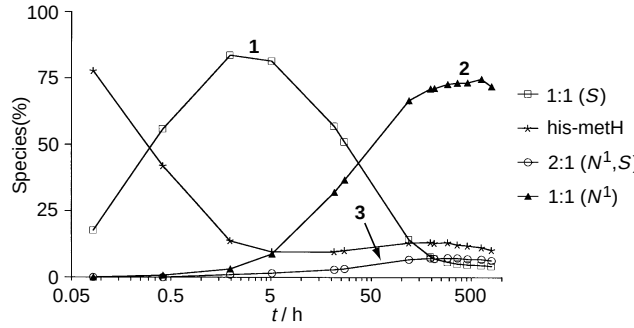



**Fig. 2** Time-course of the 1 : 1 reaction between $[Pt(dien)(H_2O)]^{2+}$ and his-metH at pH = 9.4 as monitored by reversed-phase HPLC ($T$ = 313 K). A minor species is omitted for clarity.

Time-dependent HPLC studies of the $[Pt(dien)(H_2O)]^{2+}$–his-metH reaction system at 313 K (Figs. 2 and 3) demonstrate that, as expected,[2–4,9] the kinetically favoured S-bound complex **1** is formed rapidly and reaches a maximum concentration within 2–3 h. At the pH values considered (6.5, 9.4), **1** then slowly isomerizes to the thermodynamically preferred κ$N^1$ complex **2** over a period of 500 h (Scheme 1). In Fig. 2, the dinuclear complex **3** appears in chromatograms together with **2** for the

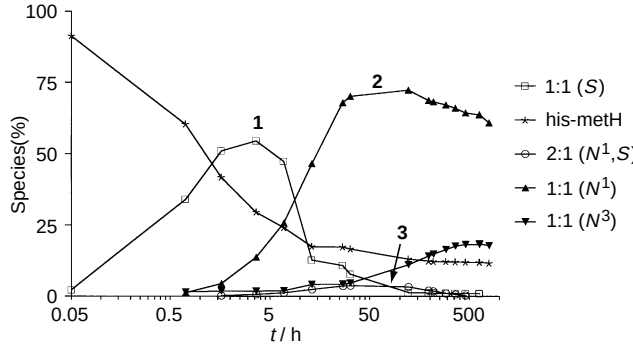
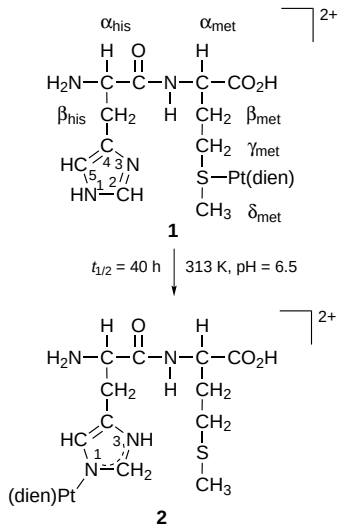



**Fig. 3** Time-course of the 1 : 1 reaction between $[Pt(dien)(H_2O)]^{2+}$ and his-metH at pH = 9.4 as monitored by reversed-phase HPLC ($T$ = 313 K). A minor species is omitted for clarity.




**Scheme 1** Isomerization **1** → **2**

first time after 2 h and exhibit a distribution curve with a time-dependence similar to that of the $N^1$-bound complex. Contrastingly, **2** reaches a maximum concentration after *ca*. 30 h at pH 9.4 (Fig. 3) and can no longer be detected after 500 h. In accordance with the findings of Sadler and coworkers,[3] we also confirmed[10] that the individual amino acid Hhis plays only a minor role in Pt binding when $[Pt(dien)(H_2O)]^{2+}$ is also allowed to react with an equimolar solution of Hmet and Hhis for 14 days at 313 K in the range $3 < pH < 11$. The S-bound complex $[Pt(dien)(Hmet\text{-}\kappa S)]^{2+}$ is present over the whole experimental range and exhibits a crossover pH (as dominant species) of 8.3 to a second N(amino)-bound Hmet complex. A two-step intramolecular route involving the dinuclear intermediate product **3**, which would contrast to a normal five-coordinate intermediate, can be discussed for the isomerization of **1** to **2**. Initial coordination of the kinetically favoured thioether S in **1** is the first step and blocks this binding site for further reactions. Subsequently a second $Pt(dien)^{2+}$ fragment is bound to the thermodynamically preferred imidazole-$N^1$ and the dinuclear complex **3** is formed. For an equimolar ratio of Pt to ligand, this complex dissociates to the mononuclear species **2** by cleavage of the less stable Pt–S bond (Fig. 3). This final step is favoured by the associated reduction in cation charge from +4 to +2. At the higher pH value (9.4), the required dinuclear intermediate **3** can no longer be detected after completion of the conversion **1** → **2**. In contrast, no intermediate could be established for the intramolecular migration of $Pt(dien)^{2+}$ from S to guanine-$N^7$ in S-guanosyl-L-homocysteine.[2,11] This isomerization is more rapid ($t_{\frac{1}{2}}$ = 10 h at 295 K, pH < 6.5) than for the conversion **1** → **2**, which exhibits a half-life ($t_{\frac{1}{2}}$ *ca.* 40 h at 313K, pH = 6.5) more comparable with that reported for the reaction of $[Pt(dien)(Hmet\text{-}\kappa S)]^{2+}$ with 5′-GMP ($t_{\frac{1}{2}}$ = 167 h at 300 K, pH* = 7.0).[3] Under pseudo-first-order conditions, the formation of the dinuclear complex **3** exhibits a reaction rate of $1 \times 10^{-4}$ $s^{-1}$, which is nearly an order of magnitude slower than that for **1** ($k = 9 \times 10^{-4}$ $s^{-1}$) but similar to the rate constant of **2** ($k = 8 \times 10^{-5}$ $s^{-1}$). Such values are, of course, not directly comparable with intramolecular competition conditions (1 : 1), because of pronounced changes in the final product distribution. The observed acceleration in formation of **2** at pH 9.4 (Fig. 3) could be due to faster coordination of a second $Pt(dien)^{2+}$ fragment to the now non-protonated imidazole ring. Temporary formation of **3** is then followed by disappearance of this dinuclear complex which is not thermodynamically stable under these conditions.

The present investigation provides evidence for intramolecular Pt migration from a kinetically favoured met residue to a thermodynamically preferred his side chain in peptides. Although the conversion is relatively slow, it should be remembered that Pt has an *in vivo* half-life of several days after administration of cisplatin.[12] The presence of spatially neighbouring his residues could, therefore, influence the reactivity of met-bound Pt in DNA-binding proteins and should be considered in discussions on the mechanism of action of Pt anticancer drugs.

## Footnotes and References

† $^1H$ and $^{195}Pt$ NMR data ($D_2O$) with chemical shifts ($\delta$) relative to respectively sodium 3-(trimethylsilyl)tetradeuteriopropionate (TSP, $\delta$ = 0.00) or sat. $K_2[PtCl_4]$–1mol $dm^{-3}$ NaCl (external, $\delta$ = −1628) at 295K. pH* values were not corrected for deuterium isotope effects.

his-metH: $^1H$, $\delta$ 8.06 ($H^2$), 7.17 ($H^5$), 4.29 ($\alpha_{met}$), 4.16 ($\alpha_{his}$), 3.24 ($\beta_{his}$), 2.52 ($\gamma_{met}$), 2.12 ($\delta_{met}$), 2.12, 1.98 ($\beta_{met}$) (pH* = 6.9). $[Pt(dien)(his\text{-}metH\text{-}\kappa S)]^{2+}$ **1**: FABMS, *m/z* 584 $M^+$; $^1H$, $\delta$ 8.68 ($H^2$), 7.47 ($H^5$), 4.61 ($\alpha_{his}$), 4.46 ($\alpha_{met}$), 3.56 ($\beta_{his}$), 2.53 ($\delta_{met}$), 2.4, 2.22 ($\beta_{met}$), 2.85–3.3 ($\gamma_{met}$, dien-$CH_2$), $^{195}Pt$, $\delta$ −3378 (pH* = 3.2). $[Pt(dien)(his\text{-}metH\text{-}\kappa N^1)]^{2+}$ **2**: FABMS, *m/z* 584 $M^+$; $^1H$, $\delta$ 7.88 [$H^2$, $^3J(^1H\text{–}^{195}Pt)$ 19 Hz], 6.88 [$H^5$, $^3J(^1H\text{–}^{195}Pt)$ 19 Hz], 4.26 ($\alpha_{his}$), 3.75 ($\alpha_{met}$), 3.29 ($\beta_{his}$), 2.5 ($\gamma_{met}$), 2.13 ($\delta_{met}$), 2.07, 1.94 ($\beta_{met}$), 2.85–3.3 (dien-$CH_2$); $^{195}Pt$, $\delta$ −2861 (pH* = 8.2). $[\{Pt(dien)\}_2(his\text{-}metH\text{-}1\kappa N^1 : 2\kappa S)]^{4+}$ **3**: FABMS, *m/z* 879 $M^+$; $^1H$, $\delta$ 7.94 [$H^2$, $^3J(^1H\text{–}^{195}Pt)$ 19 Hz], 6.99 [$H^5$, $^3J(^1H\text{–}^{195}Pt)$ 19Hz], 4.68 ($\alpha_{met}$), 4.28 ($\alpha_{his}$), 3.3 ($\beta_{his}$), 2.52 ($\delta_{met}$), 2.38, 2.18 ($\beta_{met}$), 2.8–3.3 ($\gamma_{met}$, dien-$CH_2$); $^{195}Pt$, $\delta$ −2861, −3380 (pH* = 1.0).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