DALTON
FULL PAPER

Substitution reactions of $[Pt(dien)Cl]^+$, $[Pt(dien)(GSMe)]^{2+}$, *cis*-$[PtCl_2(NH_3)_2]$ and *cis*-$[Pt(NH_3)_2(GSMe)_2]^{2+}$ (GSMe = *S*-methylglutathione) with some sulfur-bonding chemoprotective agents †

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Sulfur-containing compounds are used as rescue agents to protect normal tissue from the toxic side effects of *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin) without affecting its anti-tumour activity. Reactions of the model complexes $[Pt(dien)Cl]^+$ (**1**) and $[Pt(dien)(GSMe)]^{2+}$ (**2**) (GSMe = *S*-methylglutathione) with the rescue agents glutathione (GSH), thiourea, thiosulfate, and diethyldithiocarbamate (DDTC) have been studied in a 1.0 mol dm^{-3} aqueous perchlorate medium at 37 °C and pH 7.30 using stopped-flow and conventional UV/VIS spectrophotometry. The reactions of **1** with 2-mercaptoethanesulfonate (mesna) and 2-mercaptoethylamine (cysteamine), which is the parent compound of 2-(3-aminopropylamino)ethylphosphorothioic acid (WR2721), and of *cis*-$[Pt(NH_3)_2(GSMe)_2]^{2+}$ with thiosulfate have also been studied. The reactions between cisplatin and DDTC, thiourea and thiosulfate were investigated in an unbuffered 0.10–0.05 mol dm^{-3} chloride medium at 37 °C. All reactions follow the rate law: $-d[complex]/dt = k_2[Pt(II)][Nu]$ where k_2 denotes a second-order rate constant and [Nu] is the total concentration of nucleophile. The rate law indicates that the reactions proceed *via* a direct nucleophilic substitution pathway. The rescue agents display a relatively narrow range of reactivity towards **1**, mesna < Hcysteamine < GSH < thiourea < thiosulfate < DDTC with rate constants k_2 of 0.156 ± 0.001, 0.22 ± 0.01, 0.237 ± 0.003, 1.17 ± 0.01, 5.57 ± 0.04, and 8.0 ± 0.1 mol^{-1} dm^3 s^{-1}, respectively. The sequence is consistent with the report that DDTC is the most effective rescue agent discovered so far when administered after cisplatin. The activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ have been determined and the products of the reaction of **1** with the rescue agents have been characterised.

Introduction

The biological activity of platinum anti-tumour agents is governed by their complex chemical reactions with a variety of biomolecules. The activity of cisplatin, *cis*-$[PtCl_2(NH_3)_2]$,[1,2] is ascribed to interactions between the complex and DNA.[3–6] Its clinical effectiveness is limited by its toxicity in several normal tissues, especially the kidneys; nephrotoxicity being a dose-limiting side effect.[7] It is hypothesised that part of the nephrotoxicity, gastrointestinal toxicity, and possible bone marrow suppression may involve reaction of platinum with sulfur-containing compounds with subsequent inactivation of essential enzymes and other proteins.[8–11] For instance, Gonias *et al.*[12] have demonstrated that cisplatin-induced deactivation of the plasma protein α_2-macroglobulin occurs by cross-linking of subunit sulfhydryl groups.

In addition to the search for new and structurally modified drugs of improved selectivity, chemoprotection represents another approach to inhibit normal tissue toxicity without compromising anti-tumour response. The affinity of thiols for platinum(II) complexes has led to the investigation of numerous sulfur-containing compounds as inhibitors of cisplatin nephrotoxicity,[13,14] such as 2-(3-aminopropylamino)-ethylphosphorothioic acid (WR2721; Amifostine),[13,15] sodium thiosulfate,[14,16] sodium diethyldithiocarbamate (DDTC),[17,18] and glutathione (GSH).[19,20]

Knowledge of the kinetic parameters and the reaction products for the reactions of platinum(II) complexes with these nucleophiles is important for the understanding of the mechanisms of chemoprotection and platinum metabolism. Reedijk *et al.*[21] have investigated the reactivity of $[Pt(dien)Cl]^+$ (**1**) and $[Pt(dien)(OH_2)]^{2+}$ toward GSH, *S*-methylglutathione (GSMe), and guanosine 5′-monophosphate (5′-GMP) under mildly acidic conditions using ^1H NMR. In the same laboratory, the reaction between platinum–sulfur compounds and sulfur-donor nucleophiles such as DDTC and thiourea has been studied.[22]

However, so far no quantitative kinetic study on substitution reactions of platinum(II) complexes with rescue agents seem to have appeared. This fact and the importance of sulfur-containing species in the biological activity of platinum complexes in general prompted us to undertake the present investigation. The main objective was to determine the kinetic parameters characterising the substitution reactions of the model platinum(II) complexes $[Pt(dien)Cl]^+$ (**1**) and $[Pt(dien)(GSMe)]^{2+}$ (**2**) with some sulfur-donor chemoprotective agents at the physiological conditions of pH 7.30 and 37 °C. Complex **1** is used to mimic the first binding step of cisplatin to biomolecules[23] and **2** serves as a model for cisplatin–methionine adducts in proteins.[24] The former has the advantage that the chelated dien ligand is not displaced in the presence of sulfur-donor ligands in the *trans* position, unlike the ammines in cisplatin.

Substitution reactions of the biologically relevant complexes *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin) and *cis*-$[Pt(NH_3)_2(GSMe)_2]^{2+}$ have

† Electronic supplementary information (ESI) available: observed pseudo-first-order rate constants k_{obs} for substitution reactions of $[Pt(dien)Cl]^+$ (**1**) with thiosulfate, thiourea, GSMe, GSH, mesna, and Hcysteamine and for reactions of **1**, **2**, and cisplatin with DDTC. Observed pseudo-first-order rate constants k_{obs} for reaction of **1** with thiosulfate at variable temperatures and $[Cl^-]$-dependence of the observed pseudo-first-order rate constants for the reaction of **1** with GSMe at pH 7.30 and 37 °C. See http://www.rsc.org/suppdata/dt/b1/b110138m/

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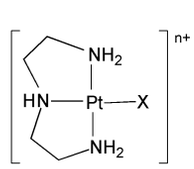
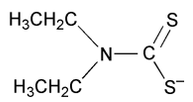
also been studied. In the case of reactions between these complexes and sulfur-donor ligands, we find that kinetic studies with excess nucleophile are complicated by consecutive substitution reactions with relatively similar rates involving the ammine ligands also. This complication was neglected in a previous study of reactions of cisplatin with biological thiols (*vide infra*).[25] Experiments with an excess of cisplatin would circumvent the problem of consecutive processes, but are not feasible due to the low solubility of the complex in water.

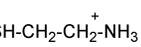
The structures of **1** and **2** and some of the nucleophiles used are depicted in Chart 1. HCysteamine is the parent compound for the most frequently studied pro-drug WR2721 which is activated *in vivo* through dephosphorylation to its metabolite 2-(3-aminopropylamino)ethanethiol (WR1065). It has been reported that the latter accumulates to a far greater degree in normal tissues than in tumours.[26]



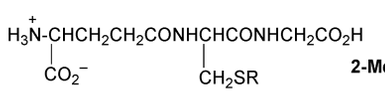
1 (X = Cl, n = 1)
2 (X = GSMe, n = 2)

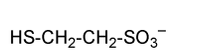


Diethyldithiocarbamate (DDTC)



2-Mercaptoethylamine (HCysteamine)





2-Mercaptoethanesulfonate (mesna)

Glutathione (GSH) (R = H)
***S*-Methylglutathione (GSMe) (R = CH_3)**

Chart 1

Experimental

Materials and solutions

The compounds $K_2[PtCl_4]$ (Johnson Matthey Chemicals Ltd.), *cis*-$[PtCl_2(NH_3)_2]$ (Sigma), sodium 2-mercaptoethanesulfonate (Sigma), *S*-methylglutathione (Sigma), thiourea (Merck), glutathione (Acros), sodium thiosulfate pentahydrate (Acros), 3-azapentane-1,5-diamine (dien) 99% (Aldrich), 2-mercaptoethylamine hydrochloride (Sigma), and sodium diethyldithiocarbamate trihydrate (Sigma) were used as received. The compound [Pt(dien)Cl]Cl was prepared according to the literature.[27] The complex $[Pt(dien)(GSMe)]^{2+}$ was prepared *in situ* by reacting $[Pt(dien)Cl]^+$ with one equivalent of GSMe in water,[28] and *cis*-$[Pt(NH_3)_2(GSMe)_2]^{2+}$ similarly by reaction between cisplatin and two equivalents of GSMe.[28] The UV/VIS and ^{195}Pt NMR spectra of these complexes agreed well with those reported.[27,28] All other chemicals used were of analytical grade. Except for reactions with *cis*-$[PtCl_2(NH_3)_2]$, a phosphate buffer (8.8×10^{-2} mol dm^{-3}) containing 0.10 mol dm^{-3} chloride was used to maintain a constant pH of 7.30. All solutions were prepared using deionised millipore water (MAXIMA).

Physical measurements

Measurement of pH was performed using a Metrohm 744 digital pH meter equipped with a combination Metrohm glass electrode and calibrated with standard buffers of pH 4.0, 7.0, and 9.0 (Merck). UV/VIS spectra were recorded with a CARY 300 Bio UV/VIS spectrophotometer using 1.0 cm Quartz Suprasil cells. Proton and ^{195}Pt NMR spectra were recorded at room temperature with a Varian Unity 300 MHz spectrometer operating at a frequency of 299.779 MHz (protons) and 64.438 or 64.238 MHz (platinum) with D_2O used as a solvent and the residual signal as a reference. ^{195}Pt NMR spectra were referenced to H_2PtCl_6 (external reference, $\delta = 0$).

Kinetic measurements

All reactions were investigated in aqueous perchlorate and/or chloride medium at 37.0 °C. The activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ for the reactions of **1** with some of the rescue agents were determined from temperature-variation experiments. Faster reactions were studied by an Applied Photophysics SX-18MV Stopped-Flow ASVD instrument and slower ones by a Cary 300 UV/VIS spectrophotometer. Constant temperature was maintained by an external RM6 LAUDA circulating-water bath (± 0.1 °C) for the stopped-flow and a CARY Peltier thermostat (± 0.01 °C) coupled with a circulating-water temperature control unit for the Cary UV/VIS measurements. The reaction of DDTC with **1**, **2**, and cisplatin was studied using the complexes in excess in order to avoid formation of a precipitate assumed to be $[Pt(DDTC)_2]$.[22] Reaction between cisplatin and thiourea was also monitored with cisplatin in excess in order to avoid precipitates due to subsequent processes. All other reactions were studied under pseudo-first-order conditions with excess of GSH, thiourea, thiosulfate, mesna, Hcysteamine and GSMe. All reactions except those with cisplatin were studied in a perchlorate medium with total ionic strength of 1.0 mol dm^{-3} and pH 7.30 maintained by use of a phosphate buffer (8.8×10^{-2} mol dm^{-3}). Measurements using cisplatin were performed in a biologically relevant 0.10 mol dm^{-3} chloride medium (to suppress aquation) at pH *ca.* 6, since the complex reacts with phosphate buffer.[29]

The reactions were followed for at least 4 half-lives with 3 or more repetitive runs at wavelengths where the respective absorbance changes were the largest. Single-exponential kinetic traces were collected in all cases and the observed pseudo-first-order rate constants k_{obs} were evaluated by on-line non-linear least-squares fit of the equation $A_t = A_\infty + (A_o - A_\infty)\exp(-k_{obs}t)$, where A_o and A_∞ are the initial and final absorbances, respectively, to the absorbance–time data using the Applied Photophysics[30] and Cary WinUV Bio[31] software packages. Quantitative kinetic measurements for the reaction of **2** with Hcysteamine and mesna were not possible due to interference from subsequent processes (*vide infra*). Similar subsequent reactions were also observed for the reaction of **1** with GSH, mesna, and Hcysteamine with no or minimal interference. These interfering reactions were not observed at pH > 7.30.

Results and discussion

Reaction products

The reaction products of $[Pt(dien)Cl]^+$ (**1**) with the sulfur-donor ligands were identified by ^{195}Pt-NMR spectroscopy. Table 1 summarises the product complexes together with the observed ^{195}PtNMR chemical shifts. Assignment of the peaks was made by comparison with the chemical shifts of the complexes $[Pt(dien)(GS)]^+$ ($\delta = -3154$ ppm)[32] and $[\{Pt(dien)\}_2(GS)]^{3+}$ ($\delta = -3185$ ppm).[32] Mononuclear platinum(II) complexes were formed as expected, except for the reactions with mesna and Hcysteamine which yield dinuclear species. In a similar fashion, the dinuclear complex $[\{Pt(dien)\}_2(GS)]^{3+}$ has been reported to be the sole product of the reaction of **1** with GSH below pH 7. Above pH 7 and in the presence of free GSH, the dinuclear complex splits into $[Pt(dien)(GS)]^+$.[33]

At pH 7.30 the complex $[\{Pt(dien)\}_2(Hcysteamine)]^{4+}$ splits completely into $[Pt(dien)(Hcysteamine)]^{2+}$ when a 10-fold excess of the thiol is used while $[\{Pt(dien)\}_2(mesna)]^{2+}$ does so only to some extent to give the mononuclear [Pt(dien)(mesna)] under similar reaction conditions. Splitting of $[\{Pt(dien)\}_2(mesna)]^{2+}$ was complete when the pH was raised to 8.50. The substitution processes formulated for Hcysteamine are depicted

Table 1 ^{195}Pt NMR chemical shifts δ of the product Pt(II) complexes for the reaction of $[Pt(dien)Cl]^+$ (**1**) with sulfur-donor nucleophiles at pH 3 (adjusted with HCl) (GSMe), *ca.* 6 (thiourea, thiosulfate), and 7.3 (mesna, Hcysteamine)

Nucleophile	Product	δ/ppm	Ref.
GSH	$[Pt(dien)(GS)]^+$	−3154	32
	$[\{Pt(dien)\}_2(GS)]^{3+}$	−3185	32
GSMe	$[Pt(dien)(GSMe)]^{2+}$	−3368 (−3364)	This work, 33
mesna	$[\{Pt(dien)\}_2(mesna)]^{2+}$	−3188	This work
	[Pt(dien)(mesna)]	−3153	This work
Thiourea	$[Pt(dien)(thiourea)]^{2+}$	−3235 (−3239)	This work, 22
Thiosulfate	[Pt(dien)(thiosulfate)]	−3132	This work
DDTC	$[Pt(dien)(DDTC)]^+$	−3355	22
HCysteamine	$[\{Pt(dien)\}_2(Hcysteamine)]^{4+}$	−3186	This work
	$[Pt(dien)(Hcysteamine)]^{2+}$	−3155	This work

by eqn. (1)–(4). An account of the formation and splitting of dinuclear platinum(II) complexes is given in the literature.[33]

$$[Pt(dien)Cl]^+ + HSCH_2CH_2\overset{+}{N}H_3 \xrightarrow{-HCl} [Pt(dien)(SCH_2CH_2NH_3)]^{2+} \quad (1)$$

$$[Pt(dien)(SCH_2CH_2NH_3)]^{2+} + [Pt(dien)Cl]^+ \xrightarrow{-Cl^-} [\{Pt(dien)\}_2(SCH_2CH_2NH_3)]^{4+} \quad (2)$$

$$2[Pt(dien)(SCH_2CH_2NH_3)]^{2+} \longrightarrow [\{Pt(dien)\}_2(SCH_2CH_2NH_3)]^{4+} + {}^-SCH_2CH_2\overset{+}{N}H_3 \quad (3)$$

$$[\{Pt(dien)\}_2(SCH_2CH_2NH_3)]^{4+} + HSCH_2CH_2\overset{+}{N}H_3 \text{ (excess)} \xrightarrow{-H^+} 2[Pt(dien)(SCH_2CH_2NH_3)]^{2+} \quad (4)$$

The case of DDTC is special in that the dien ligand of $[Pt(dien)(DDTC)]^+$ is ring-opened and co-ordinated as a bidentate ligand with DDTC also co-ordinated as a bidentate sulfur-donor ligand.[22] Similarly, we assume that this complex is formed in a rapid ring-opening process following the rate-controlling substitutions of chloride and GSMe by DDTC in **1** and **2**, respectively. Reactions of DDTC, GSH, thiosulfate, and thiourea with **2** are assumed to give the same products as with **1**.

Kinetics

The kinetic traces for reactions of DDTC with excess of **1**, **2**, and cisplatin, for reaction of thiourea with excess cisplatin, and for reaction of **1** and **2** with excess of the rest of the rescue agents are described by single exponentials, suggesting that the substitution reactions are first-order in the nucleophiles and in the platinum(II) complexes, respectively. Linear plots of the observed pseudo-first-order rate constants k_{obs} *versus* the total concentration of the nucleophiles (for complex **1**) pass through the origin within the limits of experimental error (Fig. 1). Plots of k_{obs} *versus* [Pt(II)] for the reactions of **1**, **2**, and cisplatin with DDTC are also linear, passing through the origin (Fig. 2). Reaction between an excess of cisplatin and thiourea (0.05 mmol dm^{-3}) at 37 °C in a 0.05 M aqueous NaCl medium gave the following values [cisplatin]/mmol dm^{-3}, 10^3k_{obs}: 0.50, 7.1 ± 0.1; 1.00, 12.2 ± 0.2; and 2.00, 28 ± 2 s^{-1}. All other observed pseudo-first-order rate constants are available as supplementary information Tables S1 and S2.† Based on these experiments the following rate law can be formulated:

$$-d[complex]/dt = k_2[complex][Nu] \quad (5)$$

where k_2 denotes a second-order rate constant and [Nu] is the total concentration of nucleophile. The second-order rate constants k_2, obtained from linear least-squares analysis of the kinetic data, are summarised in Table 2.

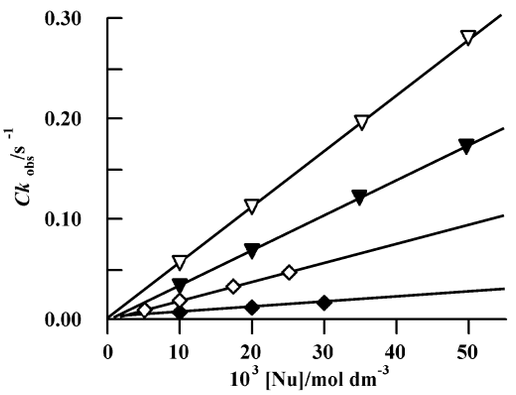


Fig. 1 Plots of the normalised observed pseudo-first-order rate constants Ck_{obs} *versus* total concentrations of thiosulfate (▽, C = 1), thiourea (▼, C = 3), GSH (◇, C = 8), and GSMe (◆, C = 5) for reactions with $[Pt(dien)Cl]^+$ (**1**) at pH 7.30 and 37 °C.

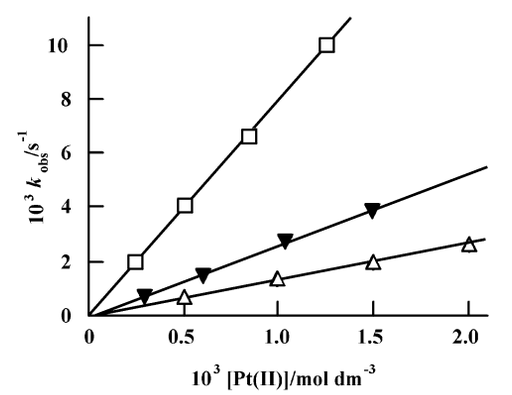


Fig. 2 Plots of the observed pseudo-first-order rate constants k_{obs} for the reactions of **1**, **2** and cisplatin with DDTC *versus* total concentrations of $[Pt(dien)Cl]^+$ (**1**) (□), $[Pt(dien)(GSMe)]^{2+}$ (**2**) (▼) and cisplatin (Δ) at pH 7.30 (**1**, **2**) and 5.5 (cisplatin); all at 37 °C.

Observed pseudo-first-order rate constants k_{obs} for the reaction between **1** and thiosulfate at variable temperatures are given in supplementary information Table S3.† Plots of these rate constants *versus* $[S_2O_3^{2-}]_{tot}$ are linear and pass through the origin (Fig. 3). For mesna, thiourea and DDTC single second-order rate constants k_2 were determined at temperatures other than 37 °C, assuming a similar kinetic behaviour to thiosulfate. The variable temperature measurements and the activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ are presented in Table 3. Excellent Eyring plots were obtained in all cases over the temperature ranges studied.

The reaction between *cis*-$[Pt(NH_3)_2(GSMe)_2]^{2+}$ and thiosulfate is complex. A plot of k_{obs} *vs.* $[S_2O_3^{2-}]_{tot}$ is linear with an intercept (Fig. 4), and neither the slope (63 ± 3 mol^{-1} dm^3 s^{-1}) nor the intercept (0.46 ± 0.03 s^{-1}) is affected by added free

Table 2 Second-order rate constants k_2 for substitution reactions of platinum(II) complexes with sulfur-donor nucleophiles from this work together with some relevant values from the literature.[21,38] Experimental conditions are 37 °C, pH 7.30 and ionic strength I = 1.0 mol dm^{-3} if not otherwise stated

	k_2/mol^{-1} dm^3 s^{-1}		
Nu	[Pt(dien)Cl]$^+$ (**1**)	[Pt(dien)(GSMe)]$^{2+}$ (**2**)	*cis*-[PtCl$_2$(NH$_3$)$_2$]
GSMe	0.117 ± 0.003 0.033[a]		
mesna	0.156 ± 0.001	See below[b]	(7.90 ± 0.01) × 10^{-2}[c]
GSH	0.237 ± 0.003 0.006[a]	(9.1 ± 0.7) × 10^{-2}	
HCysteamine	0.22 ± 0.01	See below[b]	
Thiourea	1.17 ± 0.01	(2.35 ± 0.04) × 10^{-2}	0.22 ± 0.02[d]
Thiosulfate	5.57 ± 0.04	1.260 ± 0.004	(1.9 ± 0.1) × 10^{-2}[d]
DDTC	8.0 ± 0.1 8.24 ± 0.01[e]	2.65 ± 0.04	1.34 ± 0.02[d]

[a] 22 °C, pH 5 (0.10 mol dm^{-3} phosphate buffer), from ref. 21. [b] Measurement was not possible due to interfering subsequent processes (*cf.* text). [c] 37 °C, pH 7.4 in 0.15 mol dm^{-3} chloride medium, from ref. 38. [d] Measured in a 0.10 mol dm^{-3} chloride medium at 37 °C. See also note 37. [e] Measured in an unbuffered 0.10 mol dm^{-3} chloride medium with I_{tot} = 1.0 mol dm^{-3}.

Table 3 Rate constants and activation parameters for substitution reactions of [Pt(dien)Cl]$^+$ (**1**) with the rescue agents thiosulfate, mesna, thiourea, and DDTC at pH 7.30 and ionic strength I = 1.0 mol dm^{-3}

Rescue agent	T/°C	k_2/mol^{-1} dm^3 s^{-1}	$\Delta H^{\neq}$/kJ mol^{-1}	$\Delta S^{\neq}$/J K^{-1} mol^{-1}
Thiosulfate	20.2	1.82 ± 0.04	50.8 ± 1.8	−67 ± 6
	25.2	2.50 ± 0.05		
	30	3.82 ± 0.02		
	35	5.0 ± 0.2		
	40	7.2 ± 0.1		
mesna	22	(3.49 ± 0.04) × 10^{-2}	73 ± 1	−25 ± 3
	27	(6.1 ± 0.1) × 10^{-2}		
	32	(9.8 ± 0.2) × 10^{-2}		
	37	0.156 ± 0.001		
	42	(2.52 ± 0.01) × 10^{-1}		
Thiourea	22	(4.33 ± 0.02) × 10^{-1}	47.2 ± 0.4	−92 ± 1
	27	(6.08 ± 0.03) × 10^{-1}		
	32	(8.4 ± 0.3) × 10^{-1}		
	37	1.17 ± 0.01		
	42	1.56 ± 0.01		
DDTC	17	2.20 ± 0.01	50.7 ± 0.7	−63.4 ± 2.5
	22	3.17 ± 0.01		
	27	4.64 ± 0.01		
	32	6.46 ± 0.02		

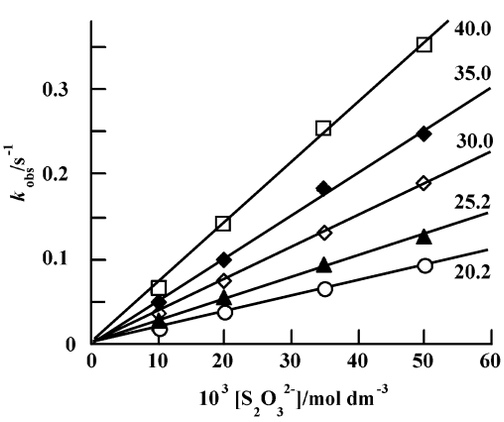


Fig. 3 Plots of observed pseudo-first-order rate constants k_{obs} *versus* $[S_2O_3^{2-}]_{tot}$ for the reaction between [Pt(dien)Cl]$^+$ (**1**) and thiosulfate at variable temperatures (°C).

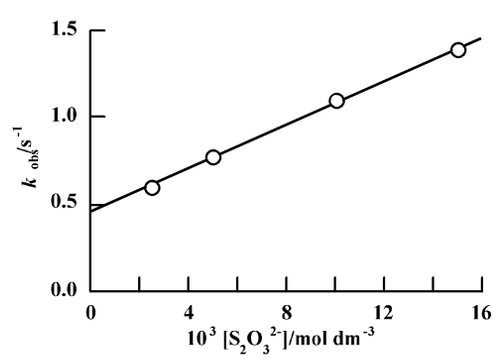


Fig. 4 Plot of the observed pseudo-first-order rate constants k_{obs} *versus* $[S_2O_3^{2-}]_{tot}$ for the reaction of *cis*-[Pt(NH$_3$)$_2$(GSMe)$_2$]$^{2+}$ with thiosulfate at pH 7.30 and 37 °C.

chloride. The ^{1}H NMR spectrum of a reaction mixture at pH *ca.* 8 with a 10-fold excess of thiosulfate showed the presence of a co-ordinated GSMe (δ = 2.575) and the ^{195}Pt{^{1}H} NMR spectrum displayed at least four peaks at δ −3551, −3575, −3690, and −3712 indicating the presence of several platinum(II) complexes. As expected, products are formed by substitution of the ammine ligands which are labilized due to the *trans*-effect of co-ordinated sulfur-donor ligands.[28] A single product assigned to be [Pt(S$_2$O$_3$)$_4$]$^{6-}$ (δ = −3708) was observed for the reaction of [PtCl$_4$]$^{2-}$ with a 10-fold excess of thiosulfate, the spectrum of which coincides with one of the products observed in the *cis*-[Pt(NH$_3$)$_2$(GSMe)$_2$]$^{2+}$-thiosulfate reaction. We conclude that complex consecutive substitution processes take place and unfortunately it is not obvious which particular substitution reaction(s) was followed in the kinetic measurement. On the other hand, the reaction of **2** with thiosulfate goes to completion as observed both from the kinetics and the ^{1}H NMR spectrum of the product, [Pt(dien)(S$_2$O$_3$)]. In **2** the chelating dien ligand is not substituted by thiosulfate.

One can see from Table 2 that the chemoprotective agents studied display a relatively narrow range of reactivity towards **1**, with DDTC being *ca.* 50 times more reactive than mesna: mesna (k_2 = 0.16 mol^{-1} dm^3 s^{-1}) < Hcysteamine (0.22) < GSH (0.24) < thiourea (1.17) < thiosulfate (5.57) < DDTC (8.0). In general, the substitution of GSMe by GSH, thiourea, thiosulfate, and DDTC was found to be slower than that of chloride by these nucleophiles under the same reaction conditions, as expected since chloride is a better leaving group than GSMe.

In the reactions with **2**, thiosulfate is favoured due to its doubly negative charge, DDTC and thiosulfate having the same reactivity within a factor of 2. Thiourea reacts more slowly, as observed also by Reedijk *et al.* based on ^{1}H NMR experiments at pH 7 and 21 °C.[34] Thus, DDTC is two orders of magnitude more reactive than thiourea towards **2** but only by a factor of 6–7 towards cisplatin and **1**. This is due to the decreased reactivity of thiourea with **2** while DDTC is a very strong nucleophile and displays little discrimination between the platinum(II) complexes **1**, **2**, and cisplatin (a factor of about 6 only).

In the case of thiosulfate, the reactivity with the three platinum complexes in Table 2 varies by a factor of almost 300, mainly due to changes in electrostatic interactions between the differently charged platinum complexes and $S_2O_3^{2-}$. It is noteworthy that the reaction of cisplatin with $S_2O_3^{2-}$ is *ca.* 70 times slower than that with DDTC.

Dedon and Borch[8] have reported a second-order rate constant for the cisplatin–DDTC reaction of (614 ± 26) × 10^{-4} mol^{-1} dm^3 s^{-1} at 37 °C and pH 7.4 based on HPLC experiments, which is more than 20 times smaller than the one we find here (Table 2). Their rate constant probably refers to a subsequent slow process, since the reaction was monitored by a slow analysis method following the appearance of [Pt(DDTC)$_2$], the final product in a series of stepwise processes. Similarly, their rate constant for the cisplatin–thiosulfate reaction (570 ± 27) × 10^{-4} mol^{-1} dm^3 s^{-1} deviates from our value in Table 2. Both these sets of experiments involved phosphate buffers, which are known to interact with cisplatin.[29] The experimental method and conditions used in the present study has allowed us to determine the rate constants without disturbance from such subsequent reactions and side reactions.

Perez-Benito and co-workers have published a study of the reactions of cisplatin with cysteine, penicillamine and glutathione based on spectrophotometric initial-rate measurements in aqueous solutions buffered with phosphate.[25] Their experimental method is not appropriate since accurate analysis of product build-up is not possible when consecutive reactions with relatively similar rates are involved and since phosphate buffers are known to interfere with reactions of cisplatin, especially so if hydrolysed species are present.[29] Moreover the protolysis constants of the thiols used were mixed up in that the pK_a of an –SH group was taken to be lower than that of the –NH$_4^+$ moiety; the converse is true for the thiols used.

Mechanism

No solvent path was observed in the present systems, suggesting that direct nucleophilic substitution is the major reaction pathway in all cases, as expected, when strongly nucleophilic sulfur-bonding ligands are involved. Similarly, the reaction of cisplatin with cysteine and glutathione has been reported to occur *via* a direct nucleophilic substitution.[35,36] This is in contrast to the reactions of nucleobases where hydrolysis of chloride is the rate-controlling step.[21] Added free chloride[37] does not influence the reaction rate (Table S4), † and the time-resolved spectra of **1** and cisplatin with DDTC exhibit at least one isosbestic point (Fig. 5). Similarly, reaction between 1 mmol dm^{-3} cisplatin and 0.1 mmol dm^{-3} thiourea results in a sharp isosbestic point at 246 nm. The latter observations suggest that there is no intermediate formed in significant concentrations during the course of the substitution reactions. The rate law in eqn. (5), the

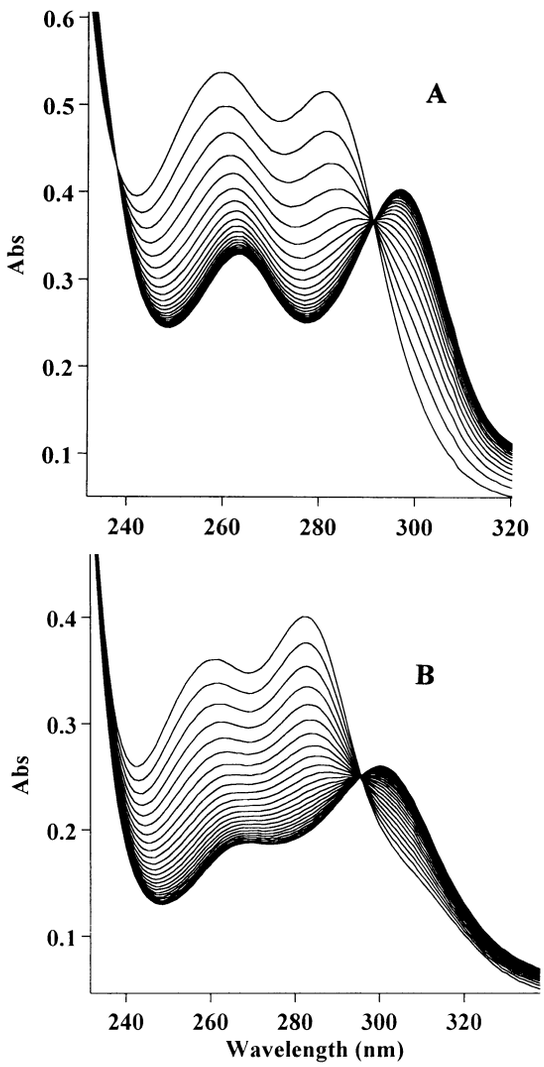


Fig. 5 Repetitive scan spectra for the reactions of [Pt(dien)Cl]$^+$ (**1**) (5 × 10^{-4} mol dm^{-3}) (A) and cisplatin (1 × 10^{-3} mol dm^{-3}) (B) with DDTC. Reaction conditions: [DDTC] = 4 × 10^{-5} (A), 2.5 × 10^{-5} mol dm^{-3} (B); [Cl$^-$] = 1.0 × 10^{-1} (A), 5.0 × 10^{-2} mol dm^{-3} (B); spectra were collected over 15 (A) and 30 min (B); T = 310 K (A, B).

sensitivity of the rates to the nature of the incoming ligands (*cf.* Table 2), and the negative entropies of activation are all indicative of the usual associative mode of activation.

Implication to mechanism of chemoprotection

A chemoprotective agent may inhibit reaction of cisplatin with proteins either by (1) a competitive reaction with the intact drug to form non-reactive complexes such as stable rings or (2) by a nucleophilic displacement of protein groups from their platinum(II) adducts. The results of this study are consistent with the report that DDTC, the most reactive of the nucleophiles studied, is the only chemoprotector effective when administered 1–4 h after cisplatin.[17,18] Glutathione could play a role as a rescue agent given its relatively high concentration (5.0 × 10^{-4} to 1.0 × 10^{-3} mol dm^{-3}) inside the cell and its ability to react with platinum–thioether compounds. Although thiosulfate could react with complex **2** with comparable ease to DDTC, it has been shown that protein-bound cisplatin cannot be released by thiosulfate.[34] *In vivo*, thiosulfate might be less effective in breaking such bonds due to its doubly negative charge hindering its approach to the target adducts. Hence, protection against renal damage by thiosulfate has been hypothesised to be due to inactivation of aquated cisplatin in the kidney nephron.[38] mesna is also proposed to have a similar mechanism of chemoprotection to thiosulfate,[38] which confirms

our observation that it is slightly less reactive than thiosulfate and thus is expected to involve a rather similar mechanism of kidney protection to thiosulfate. The compound WR1065 has been suggested to exhibit kidney protection through direct interaction with cisplatin.[39]

In summary, this study demonstrates that the reactivities of the rescue agents towards **1** vary by a factor of *ca.* 50. They can be expected to have a similar mechanism of protection against cisplatin toxicity (*cf.* Table 2). It is obvious that DDTC is the most reactive of the nucleophiles studied, consistent with its observed efficiency as a rescuing agent.[17,18] Differences in transport and tissue distribution properties of the rescue agents will also influence the mechanism of chemoprotection, however. The reactivity of thiosulfate is influenced very much by its doubly negative charge. For instance, the half-lives for the reactions of cisplatin and **2** with thiosulfate (50- and 35-fold excess, respectively) at 37 °C were measured to be 66 min and 15 s, respectively. Since the pH of a tumour cell is slightly lower than the pH of a normal cell[40,41] rescue agents containing the sulfhydryl group are expected to be more effective in normal cells than in cancer cells.

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