Contribution from the Departments of Chemistry and Pharmacology, The University of Vermont, Burlington, Vermont 05405, and The Vermont Regional Cancer Center, Burlington, Vermont 05405

Chemical Properties and Antitumor Activity of Complexes of Platinum Containing Substituted Sulfoxides [PtCl(R′R″SO)(diamine)]NO_3. Chirality and Leaving-Group Ability of Sulfoxide Affecting Biological Activity

Nicholas Farrell,*[†,§] Donna M. Kiley,[†] Wendy Schmidt,[‡] and Miles P. Hacker[‡,§]

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The preparation and antitumor activity in L1210 leukemia of a novel set of platinum complexes of formula [PtCl(R′R″SO)(diam)]NO_3 (diam = bidentate amine such as 1,2-diaminocyclohexane (dach) or 1,1-bis(aminomethyl)cyclohexane (damch) and R′R″SO = substituted sulfoxides such as dimethyl (Me_2SO), methyl phenyl (MePhSO), methyl benzyl (MeBzSO), diphenyl (Ph_2SO), and dibenzyl sulfoxide (Bz_2SO)) is reported. The complexes are the first well-defined antitumor platinum complexes containing sulfur as ligand. The antitumor activity is dependent on both the nature of the amine and, especially the nature of the sulfoxide. In the case of asymmetric sulfoxides, such as methyl *p*-tolyl sulfoxide (MeTolSO), preparation of the optically pure forms shows a distinct effect of chirality of the sulfoxide ligand on the biological activity. The possible mechanisms of antitumor action are discussed. Studies on displacement of sulfoxide by Cl^- and H_2O show the order of lability to be Ph_2SO > MePhSO > MeTolSO > Bz_2SO > MeBzSO > Me_2SO. The lability is also dependent on amine, with damch complexes being significantly more reactive than their dach analogues. The pseudo-first-order rate constants, which range over 2 orders of magnitude, preclude a simple loss of sulfoxide as a mechanism of antitumor activity. The complexes may act by binding to DNA with subsequent loss of sulfoxide ligand.

Introduction

This paper reports on the chemistry and antitumor activity of the series of complexes [PtCl(R′R″SO)(diamine)]NO_3 where the diamine is a bidentate amine such as 1,2-diaminocyclohexane (dach) or 1,1-bis(aminomethyl)cyclohexane (damch) and R′R″SO is a substituted sulfoxide. The complexes are of interest in that they are the first set of platinum complexes with sulfur donor ligands, as well as being one of the first series of well-defined cationic species with good antitumor activity, thus violating the empirical structure–activity relationships set down initially for platinum complexes. The activity of the complexes is dependent on the nature of both the amine and the sulfoxide, and where unsymmetrical sulfoxides are used, the activity also depends on the chirality of the sulfoxide. Neutral species such as *cis*-[$PtCl_2(NH_3)_2$], cisplatin, represent by far the most active class of platinum complexes, but there is increasing acceptance that antitumor activity is not strictly limited to complexes of this type. Development of structurally unique complexes with good antitumor activity is important for a rigorous statement of structure–activity relationships, mechanistic studies (new mechanisms and modes of DNA attack), and eventual clinical application (possible lack of cross-resistance with cisplatin and a new spectrum of activity).

The rationale for the use of sulfoxides (R′R″SO) as a leaving group in [$Pt(Me_2SO)_2$(diam)]$^{2+}$ has been previously explained.[1,2] Briefly, the mutual labilization of the two Me_2SO ligands results in initial loss of a sulfoxide ligand to give the monoaqua species [$Pt(Me_2SO)(H_2O)$(diam)]$^{2+}$.[3] Thus, despite the high trans-labilizing influence of Me_2SO, complexes can be prepared that preferentially lose dimethyl sulfoxide. This situation is in contrast to the deactivation that occurs upon dissolution of Pt–amine complexes in Me_2SO.[4,5] These original bis(sulfoxide) complexes were substantially inactive in vivo, due perhaps to the 2+ charge and lack of penetration into the cell. Further, the rate at which the second sulfoxide ligand is replaced, either giving the active diaqua species or reacting directly with DNA, may be too slow for useful biological activity. To overcome these problems, we examined the series [PtCl(R′R″SO)(diam)]NO_3 for the following reasons:

(1) The complexes should be water-soluble because of the charge.

(2) The aqua species [$Pt(R'R''SO)(H_2O)$(diam)]$^{2+}$ should also be produced upon initial chloride hydrolysis.

(3) Use of sterically hindered sulfoxides should give ligands that will hydrolyze faster than the parent Me_2SO ligand and allow correlation of sulfoxide lability with biological activity.

(4) Platinum complexes with dach or damch ligands are potential "second-generation" antitumor complexes because of their good activity in some primary screens and their lack of cross-resistance with cisplatin-resistant tumor lines. Problems including lack of stability, purity, and difficulties in formulation have prohibited any definitive clinical trials of these complexes. The use of bidentate amines also diminishes the trans labilization by the sulfoxide ligand, maintaining intact the Pt(diam) unit.

Experimental Section

Materials and Methods. IR spectra were obtained as KBr disks on Nicolet FT6000 series and Perkin-Elmer 1430 spectrophotometers. UV/visible spectra were run on a Perkin-Elmer Lambda 4B instrument. NMR spectra were run on Bruker 250- and 270-MHz spectrometers. Pt NMR spectra (250 MHz) were run in D_2O with reference to a 0.1 M Na_2PtCl_6 solution in D_2O as external reference. Samples were run by using a pulse width of 15 μs. Usually a sweep width of 30 KHz was used, and 5000–10000 scans were adequate. All shifts are positive to lower shielding. Circular dichroism spectra were obtained in MeOH on a Jobin-Yvon Autodichrograph Mark V instrument. Optical rotations were performed on a Perkin-Elmer 141MC polarimeter in MeOH.

Preparation of Complexes. Standard sulfoxides were purchased from Aldrich and used without further purification. The optically pure methyl *p*-tolyl sulfoxides were prepared by the literature procedure.[6] Repeated recrystallization gave ligands of >95% chemical purity and 85–90% optical purity as judged by CD and optical rotation.[7] The amine *trans-l*-1,2-diaminocyclohexane was purchased from Aldrich. All amine complexes [$PtCl_2$(diam)] were prepared from K_2PtCl_4 by standard procedures.

Preparation of [PtCl(R′R″SO)(diam)]NO_3 from [$PtCl_2$(diam)]. All cationic complexes were prepared from the diamine precursors by the same procedure, and so one example only is given. An equimolar amount of methyl phenyl sulfoxide (MePhSO) (0.9151 g, 0.0065 mol) was added to a slurry of [$PtCl_2$(damch)] (2.652 g, 0.0065 mol) in HPLC grade MeOH (30 mL). To this was added 1 equiv of $AgNO_3$ dissolved in hot MeOH. The reaction mixture was stirred overnight in the dark. The insoluble AgCl precipitate was filtered off and the filtrate rotoevaporated until the volume of methanol was approximately 2 mL. The concentrated solution was diluted with ether until a white solid just began forming.

[†] Department of Chemistry, The University of Vermont.
[‡] Department of Pharmacology, The University of Vermont.
[§] The Vermont Regional Cancer Center.

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The flask was then placed in the freezer overnight, and the resultant white crystals were filtered and washed with ether. A second recrystallization may be performed in the same manner. The complex was dried in vacuum over P_2O_5.

Stability and Displacement Studies. High-pressure liquid chromatography (HPLC) separations were performed on a system consisting of Waters M-45 pumps (Waters, Inc., Milford, MA), a Waters 720 system controller, a Waters 730 data module, a Rheodyne injector, and a Spectra-Physics 770 spectrophotometric detector. The column was a 145 × 4.5 mm C18 column from Rainen (Woburn, MA). All water was double-distilled in glass and then passed through a Water I System (Gelman, Ann Arbor, MI) to a resistance greater than 12 MΩ. Prior to analysis, compounds were scanned over wavelengths from 300 to 220 nm on a Perkin-Elmer spectrophotometer to determine the wavelength of maximal absorption. For sulfoxides with phenyl groups, all complexes showed a band at 245 nm, which was used for all subsequent analyses. For Me_2SO complexes, this was changed to 220 nm. Initial scanning of the complexes for optimal organic solvent concentrations for elution within a reasonable time period was performed on a gradient fron 70% methanol in 25 mM KH_2PO_4 containing 0.01% triethylamine (TEA) to 5% methanol in 25 mM KH_2PO_4 containing 0.01% TEA over 40 min at a flow rate of 1.0 mL/min. Retention times for the peaks of interest were between 4 and 9 min with optimal % MeOH concentrations usually between 30% and 60%. All analytical scans were performed in the isocratic mode at a flow rate of 1.0 mL/min. Analysis of platinum content was performed by flameless atomic absorption on a Perkin-Elmer 560 AA spectrophotometer monitoring at 266.8 nm.

Each compound was dissolved to a concentration of 1.0 mg/mL in either HPLC grade water or the appropriate concentration of NaCl and maintained at a constant temperature of 37 °C. Immediately following dilution, the solution was injected onto the column. From the integration of the area under the curve (AUC), the ratio of the AUC of the peak of interest to the total AUC for all peaks was expressed as a percentage. The percentage area under the peak of interest was used to find the concentration of the parent compound. The disappearance of the peak corresponding to complex followed first-order kinetics, and rate constants were then calculated by using $-\ln (c_0/c_t) = kt$ where c_0 is the initial concentration of undissociated complex and c_t is the concentration at time t.

Biological Assays. The in vitro and in vivo biological activities in L1210 cell lines were assessed by using standard assays.[8] Briefly, for the in vivo activity, L1210 cells grown in RPMI-1640 medium supplemented with either 10% horse serum (L1210/0) or 10% fetal bovine serum (L1210/R or L1210/dach) were exposed to varying concentrations of complex dissolved in H_2O for 72 h. Final cell concentrations were measured by using a Coulter particle counter, and ID_{50} values (drug concentrations required to kill cell growth by 50%) were calculated for each complex. For in vivo studies, BDF_1 mice were inoculated ip with 10^6 L1210/0 cells (day 0) and treated ip with the dose of test complex on days 1, 5, and 9. Mean survival times (MST) of treated mice and control tumor-bearing mice were calculated and % T/C determined by % T/C = MST(treated)/MST(control). Long-term survivors (defined as alive on day 60) were not included in % T/C calculations.

Results and Discussion

Figure 1 shows the structures of the amines and sulfoxides used. The sulfoxides were chosen to study both steric and electronic effects by systematic substitution of the "parent" dimethyl sulfoxide with phenyl and benzyl groups. The principal diamines used were 1,2-diaminocyclohexane (dach) and 1,1-bis(aminomethyl)cyclohexane (damch). These were chosen because their platinum complexes are in general non-cross-resistant with *cis*-$[PtCl_2(NH_3)_2]$. Ethylenediamine (en, five-membered chelate) and 1,3-propanediamine (pn, six-membered chelate) complexes were used as simple analogues of the above amines. In vitro cytotoxicity studies indicated that en and pn complexes were significantly less active than those of dach and damch, and their in vivo activity was not pursued.

Two general methods are available for the preparation of the $[PtCl(R'R''SO)(diam)]^+$ cation:

$$[PtCl_2(R'R''SO)_2] + \text{diam} \rightarrow [PtCl(R'R''SO)(diam)]Cl \quad (1)$$

$$[PtCl_2(\text{diam})] + R'R''SO \rightarrow [PtCl(R'R''SO)(diam)]Cl \quad (2)$$

The nitrate salts may then be prepared by metathesis with $AgNO_3$,

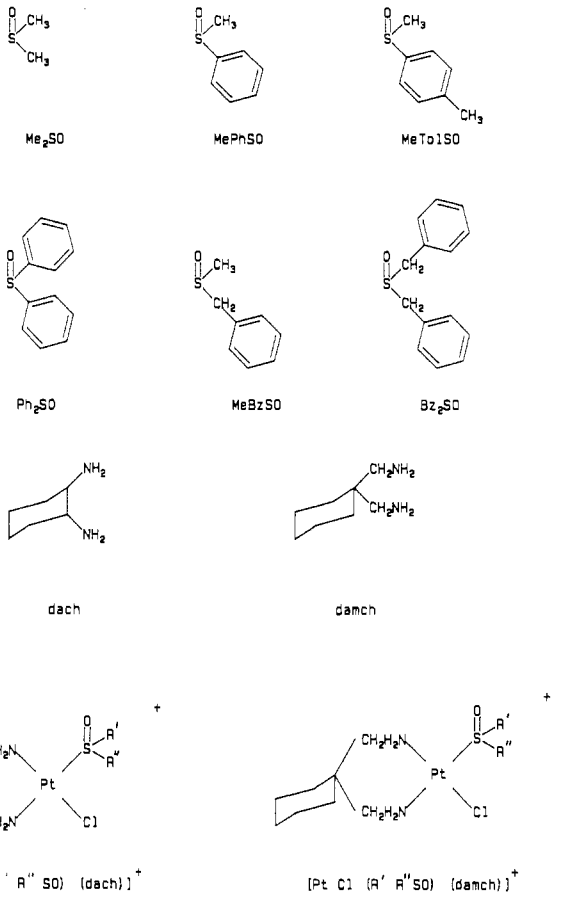

Figure 1. Structures of substituted sulfoxides and their diamine complexes of platinum studied for antitumor activity.

Table I. Elemental Analyses for $[PtCl(R'R''SO)(diam)]NO_3$

complex no.	diam	R'R''SO	anal. % C	% H	% N
I	*R,R*-dach	Me_2SO	19.8 (20.1)	4.1 (4.3)	8.7 (8.6)
II	*R,R*-dach	MePhSO	28.6 (28.5)	4.0 (3.9)	7.7 (7.6)
III	*R,R*-dach	MeBzSO	29.7 (30.0)	4.1 (4.3)	7.1 (7.5)
IV	*R,R*-dach	Ph_2SO	35.5 (35.7)	4.0 (4.1)	6.9 (6.7)
V	*R,R*-dach	Bz_2SO	37.7 (37.5)	4.4 (4.3)	6.6 (6.4)
VI	en	MePhSO	21.8 (21.9)	3.0 (3.3)	8.3 (8.5)
VII	damch	Me_2SO	23.4 (23.3)	4.7 (4.2)	8.2 (7.7)
VIII	damch	MePhSO	31.2 (31.3)	4.3 (4.5)	6.7 (6.6)
IX	damch	MeBzSO	32.6 (32.2)	4.7 (4.3)	7.3 (7.8)
X	damch	Ph_2SO	37.6 (37.6)	4.2 (4.4)	6.7 (6.6)
XI	damch	Bz_2SO	39.8 (40.2)	4.8 (4.4)	6.3 (6.1)
XII	pn	MePhSO	27.6 (27.2)	4.3 (4.0)	7.5 (7.7)
XIII	damch	(-)-MeTolSO	32.6 (32.4)	4.5 (4.6)	7.3 (7.0)
XIV	damch	(+)-MeTolSO	32.6 (31.8)	4.5 (4.5)	7.3 (7.1)
XV	*R,R*-dach	(-)-MeTolSO	28.6 (29.0)	4.0 (4.1)	7.5 (7.3)
XVI	*R,R*-dach	(+)-MeTolSO	28.6 (29.1)	4.0 (4.0)	7.5 (7.2)
XVII	*S,S*-dach	(-)-MeTolSO	28.6 (28.8)	4.0 (3.9)	7.5 (7.3)
XVIII	*S,S*-dach	(+)-MeTolSO	28.6 (28.6)	4.0 (4.0)	7.5 (7.3)

either in situ or independently. The second reaction was found to be the more convenient as the more labile sulfoxides tended to give large amounts of $[PtCl_2(diam)]$ as a byproduct of reaction 1. Further, for bulky sulfoxides, the product of the reaction between R'R''SO and the platinum starting material K_2PtCl_4 is highly dependent on the nature of the sulfoxide, giving mixtures of *cis*- and *trans*-$[PtCl_2(R'R''SO)_2]$ (R'R''SO = Bz_2SO) and $[PtCl_3(R'R''SO)]^-$ (R'R''SO = Ph_2SO).[9] Although all these species should afford $[PtCl(R'R''SO)(diam)]NO_3$ upon reaction with a chelating diamine, we have chosen reaction 2 for consistency.

Nature of the Sulfoxide Complexes. The characterizing data including elemental analyses and spectroscopic results are given

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Table II. Characterization Data for $[PtCl(R'R''SO)(diam)]NO_3$

complex no.	IR ν(SO),[a] cm^{-1}	^1H and ^{195}Pt NMR δ,[b] ppm δ(H) R'R''SO[c]	δ(H) diam[d]	δ(Pt)
I	1134	3.49, 3.47	2.65 (m), 2.05 (m) 1.63 (m), 1.1–1.4 (m)	−3297
II	1141	3.50, 3.52 7.5 (d), 8.1 (d)	*d*	−3304, −3311
III	1132, 1170	3.48, 3.47 7.6 (m)	*d*	−3309
IV	1100	7.5 (m)	*d*	−3317
V	1110, 1180	7.75 (m)	*d*	−3303
VI	1162	3.72 (21.5) 7.75 (d), 8.1 (d)	2.84 (42.0)	−3295
VII	1103	3.49 (23.0)	2.5–2.8 (m) 1.1–1.4 (m)	−3300
VIII	1145	3.73 7.75 (d), 8.1 (d)	*d*	−3310
IX	1125	3.55 7.60 (m)	*d*	−3305
X	1100	7.75 (m)	*d*	−3315
XI	1115	7.65 (m)	*d*	−3302
XII	1178	3.75 (27.0) 7.75 (d), 8.11 (d)	1.9 2.75 (41.0)	

[a] In KBr disks. [b] In D_2O. Singlets except where indicated; d = doublet, and m = multiplet. Numbers in parentheses in the ^1H NMR spectra refer to J(Pt–H) (Hz) and are given only when observed clearly. [c] Methylene resonances of III, V, IX, and XI obscured by D_2O. [d] All diamine peaks are multiplets centered at quoted values. Only one set is given for each example for clarity.

Table III. Chemical Characterization Data for $[PtCl(MeTolSO)(diam)]NO_3$ Complexes

complex no.	diam	MeTolSO	Chemical Data[a] IR ν(SO), cm^{-1}	NMR $\delta(SCH_3)$	NMR δ(Pt)	CD λ_{max}, nm ($\Delta\epsilon$)
XIII	damch	*S*,(−)	1110	3.67	−3300	244 (−5.38) 267 (+2.07)
XIV	damch	*R*,(+)	1110	3.67		244 (+3.62) 267 (−1.80)
XV	*R,R*-dach	*S*,(−)	1145	3.69	−3298	244 (−10.2) 271 (+2.57)
XVI	*R,R*-dach	*R*,(+)	1120	3.67		244 (+6.93) 268 (−3.66)
XVII	*S,S*-dach	*S*,(−)	1120	3.67	−3312	244 (−10.2) 271 (+2.69)
XVIII	*S,S*-dach	*R*,(+)	1145	3.69		244 (+6.35) 268 (−3.27)

[a] IR as KBr disks; ^1H NMR and ^{195}Pt NMR in D_2O. All complexes gave peaks at approximately δ 8.0 (d), 7.5 (d), and 2.5 (s) for the MeTolSO ligand. The damch protons gave multiplets at δ 2.5–2.8 and 1.1–1.4, while the dach protons occur at δ 2.65 (m), 2.05 (m), 1.63 (m), and 1.1–1.4 (m). Integration was as expected. The expected Pt satellites were barely seen at the field strength employed and so are not reported.

in Tables I–III. The difference in chemical shift from free sulfoxide ligands is typical of S-bonded sulfoxide,[2] as are the ^{195}Pt chemical shifts of the complexes.[10] The IR spectra of all complexes also show the expected increase in ν(SO) upon complexation via sulfur. Because of overlap and possible mixing, no attempt was made at this time to distinguish ν(Pt–S) and ν(Pt–Cl).

The amine 1,2-diaminocyclohexane exists as both geometric (*cis*-dach) and optical (*trans-l-* or *R,R*,-dach and *trans-d-* or *S,S*-dach) isomers. A complex of either pure optical isomer with a racemic sulfoxide such as MePhSO or MeBzSO gives a diastereomeric pair. The *R,R*-dach complex was used throughout this study. Using *R,R*-dach the presence of diastereomers was confirmed by the appearance of two peaks of equal intensity for the S–CH_3 protons in the ^1H NMR spectrum for complexes II (3.50 and 3.52) and III (3.48 and 3.47). Only complex II gave two resolved peaks in the ^{195}Pt NMR spectrum. Repeated recrystallization gave different ratios of these peaks indicating some preferential separation. No enantiomeric selectivity is observed in reaction 2 itself—evaporation of the reaction mixture to dryness and washing of the product with acetone showed equal proportions of the diastereomers by NMR, further confirmed by the absence of a CD spectrum corresponding to the presence of the chiral sulfoxide (see also below). The 1:1 diastereomer mixture was used for the initial antitumor and displacement studies in the case of MePhSO and MeBzSO, since elemental analyses and spectroscopic data were repeatable for the experimental procedure.

Interestingly, the ^1H NMR spectrum of the cation $[PtCl(Me_2SO)(R,R\text{-dach})]^+$ also showed two peaks for the S–CH_3 resonances. This was confirmed for the *S,S*-dach complex, but the complexes with en, pn, *cis*-dach, and damch (no optical activity) gave only one peak. The most reasonable explanation is that the CH_3 groups are diastereotopic. The $-CH_2-$ protons of the benzyl group in MeBzSO show a complicated pattern (doublet of doublets centered at δ 4.15) due to the inequivalence of the methylene protons attached to the chiral sulfur. These peaks are also shifted upfield approximately 1 ppm upon complexation but are now mixed with the D_2O signal. The $-CH_2-$ resonances of Pt-bound Bz_2SO are also obscured for the same reason.

To examine the effect of the chiral sulfoxide further, we prepared complexes of damch, *R,R*-dach, and *S,S*-dach with the optically pure enantiomers of methyl *p*-tolyl sulfoxide (R' = CH_3, R'' = *p*-$CH_3C_6H_4$, MeTolSO), and the spectroscopic data are given separately in Table III. The absolute configuration of the optically active free sulfoxides have been assigned—the (+) form is *R*.[5] The optical rotation of complex XIII was −51.92° (derived from (+)-MeTolSO, optical rotation = +144.05°) and that of Complex XIV was +43.88° (derived from (−)-MeTolSO, optical rotation = −144.3°) respectively. Thus, the sign (and handedness) of the sulfoxide changes upon complexation.[11] The signs throughout this manuscript refer to the sign of the sulfoxide *in the complex*.

Antitumor Activity. The in vivo antitumor activity (Tables IV and V) of the complexes in L1210 leukemia were studied by standard procedures. The in vitro parameter, ID_{50} in μM, is the concentration required to inhibit cell growth by 50%. The legends L1210/0, L1210/R, and L1210/dach in Table V refer to L1210 cell lines sensitive to cisplatin and rendered resistant to cisplatin and resistant to $[Pt(SO_4)(R,R\text{-dach})]$, respectively.[12] The parameter for in vivo activity is % T/C, which relates weight of tumor in treated animals to that of nontreated control animals (significant activity >150%). In these calculations the standard time span used was 60 days and long-term survivors at this time were not included in the % T/C calculation.

The complexes in general meet minimal standards of activity, and the series represents a novel class of antitumor platinum agents. The order of in vitro efficacy (data not shown) for a more limited series of these complexes is damch > dach and Ph_2SO > MePhSO > Me_2SO. As with most complexes of dach and damch, the complexes are active in cisplatin-resistant cell lines in vitro. The in vivo activity in L1210 leukemia shows some interesting trends. Use of the damch ligand gives a Me_2SO complex with good activity, and in general, damch complexes show greater efficacy than their *R,R*-dach analogues. Previous results on *cis*-$[PtCl(Me_2SO)(am)_2]^+$ (am = monodentate alicyclic amine)[13] indicated this series to be inactive. The reasons for this difference may be due, in part, to the enhanced lability of the damch derivative and also to the fact that the trans influence of the sulfoxide may result in displacement of the monodentate amine and loss of antitumor activity. In our own studies, the complexes *cis*-$[PtCl(R'R''SO)(NH_3)_2]^+$ (R'R''SO = Me_2SO, MePhSO) derivatives were also inactive with % T/C < 120. Analysis of the HPLC profile of the NH_3 complexes with time in the presence and absence of Cl^- did not show a clean loss of sulfoxide, and multiple peaks, attributed to species resulting from loss of amine, appeared. For both diamines MePhSO complexes are significantly

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Table IV. Antitumor Activity of $[PtCl(R'R''SO)(diam)]NO_3$ in L1210 Leukemia in Vivo

R'R''SO	complex no.	dose, mg/kg of mouse	% T/C[a]	complex no.	dose, mg/kg of mouse	% T/C
Me_2SO	I	3 × 100	131	VII	3 × 100	152
MePhSO	II	3 × 50	211 (1/6)	VIII	3 × 50	227 (2/6)
		3 × 25	151		3 × 25	176
MeBzSO	III	3 × 100	231 (2/6)	IX	3 × 100	169
		3 × 50	165			
Ph_2SO	IV	3 × 50	toxic	X	1 × 100	toxic
		2 × 25	177[b]		3 × 25	183
					3 × 12.5	163 (1/6)
Bz_2SO	V	3 × 100	142	XI	3 × 100	153

[a] Numbers in parentheses refer to long-term survivors and are not included in % T/C calculation. See Experimental Section for full details. [b] Only two doses given because of toxicity.

Table V. Biological Activity of $[PtCl(MeTolSO)(diam)]NO_3$ Complexes in L1210 Leukemia

complex no.	diam	MeTolSO	in vitro ID_{50}, μM[a] L1210/0	L1210/R	L1210/dach	in vivo % T/C[b] L1210/0
XIII	damch	*S*,(−)	0.43	2.88 (7)	5.95 (14)	245 (1/6)
XIV	damch	*R*,(+)	0.20	0.82 (4)	3.91 (20)	229 (2/6)
XV	*R,R*-dach	*S*,(−)	4.80	17.80 (4)	30.3 (6)	244 (1/6)
XVI	*R,R*-dach	*R*,(+)	5.00	17.80 (4)	>35.0 (>7)	129 (0/6)
XVII	*S,S*-dach	*S*,(−)	2.86	11.1 (4)	21.4 (7.5)	251 (0/6)
XVIII	*S,S*-dach	*R*,(+)	2.86	6.40 (2)	32.1 (11)	139 (0/6)
	cis-$[PtCl_2(NH_3)_2]$		0.3	13 (43)	1 (3)	210[c]
	$[Pt(R,R\text{-dach})SO_4]$		0.6	2.5 (4)	21 (35)	*d*

[a] Figure in parentheses refer to resistance factors. [b] BDF_1 mice injected ip/ip at a 50 mg/kg of mouse × 3 dose schedule (1, 5, and 9 days). Further protocol in Experimental Section. % T/C calculated at 60 days. Long-term survivors in parentheses not included in % T/C calculation. [c] Dose of 3 × 5 mg/kg of mouse. [d] The complex $[PtCl_2(R,R\text{-dach})]$ gave a % T/C of 392 at 2 × 12.5 mg/kg of mouse.[37]

more active than the Me_2SO analogues. For the more labile sulfoxides such as Ph_2SO, toxicity appears to increase and much lower doses are required to obtain an antitumor effect free of toxicity.

Effect of Chirality of Sulfoxide on Biological Activity. A highly interesting set of results is obtained upon comparison of the various pairs of enantiomers prepared with optically pure sulfoxide ligand (Table V). The in vitro results show that all the complexes again meet minimal standards of activity, retaining the lack of cross-resistance with L1210/R displayed by the parent dichlorides. For comparison typical values for *cis*-$[PtCl_2(NH_3)_2]$ and $[Pt(R,R\text{-dach})SO_4]$ are included in Table V. A unique difference is noted for the *S*,(−) and *R*,(+) forms of the sulfoxide ligand in the cell line resistant to cisplatin. Further, if we examine the activity of the four dach complexes in the cell line that is 35-fold *resistant* to $[PtCl_2(R,R\text{-dach})]$ we find that although the complex is much less active than in L1210/0, the resistance factor is significantly lower than 35 and again depends to some extent on the chirality of the sulfoxide ligand. In the biology of platinum antitumor compounds, most patterns of cross-resistance are dictated by the amine ligand, and the results here are an unusual indication that the leaving groups can affect this pattern. A similar dependence on leaving group in Pt(dach)-resistant lines has also recently been found for [Pt(dach)(CBDCA)] (CBDCA = 1,1-cyclobutanedicarboxylate) in the Pt(dach)-resistant line.[14]

For comparison, only one set of in vivo results using the same dose (50 mg/kg of mouse on a 1, 5, and 9 day schedule) is shown. Complexes XIII–XV and XVII all give significant activity with long-term survivors for the first three complexes. Table V shows the dominant effect of the chirality of the sulfoxide. For the *R,R*-dach complex, *S*,(−)-MeTolSO is more potent than its corresponding *R*,(+)-MeTolSO stereoisomer (see complexes XV and XVI), and this is also the case for *S,S*-dach (see complexes XVII and XVIII). Both XV and XVII are also more potent than their enantiomers (XVIII and XVI, respectively). The dependence of biological activity on the optical isomer used in simple complexes of dach has been observed previously,[15] but the effect is much more marked in the presence of the chiral sulfoxide. The data for the nonchiral ligand damch (complexes XIII and XIV) confirms that the differential activity is due to the chirality of the sulfoxide but is not as marked as for the dach complexes. In the case of damch the *R*,(+) enantiomer is, if anything, most potent.

The high activity of the MePhSO derivatives confirms the results on the resolved methyl *p*-tolyl sulfoxide (MeTolSO) derivatives. We reemphasise that, in the case of complexes II and III, the biological data refer to the 1:1 diastereomeric mixture only. As stated earlier, repeated recrystallization gives differing proportions of diastereomers and irreproducible biological results. Particularly striking is the high activity of Complex III, [PtCl(MeBzSO)(*R,R*-dach)], which is significantly more potent than its Me_2SO analogue (I), despite the fact that the labilities of the two ligands are very similar. Further, this is the only case where the *R,R*-dach complex is *more* active than the damch analogue of the same sulfoxide ligand. Neither does $[PtCl(MeBzSO)(damch)]^+$ show any greatly increased activity over the Me_2SO derivative (complexes VII and IX). A rationalization for these results may be found if we consider that the *R,R*-dach complex contains three chiral centers and the chiral nature of the molecule is much more pronounced in comparison to its damch analog. This is particularly true for the MeBzSO ligand because of the close similarity of the $-CH_3$ and $-CH_2C_6H_5$ groups attached to sulfur. We are currently preparing optically pure MeBzSO complexes, to further examine the role of the asymmetric sulfoxide ligand. The purpose of the results here is to emphasise the clear "promotion" of the MeBzSO ligand over the symmetric Me_2SO and Bz_2SO derivatives and to point to the combinative effect of the chiral centers on both "sides" of the platinum complex.

Mechanism of Action of Platinum–Sulfoxide Complexes. The principal cytotoxic lesion of platinum complexes is considered to be the intrastrand linkage in DNA formed by platinum binding to two adjacent bases, especially guanine.[16] Monodentate lesions by a complex such as [PtCl(dien)]Cl are not cytotoxic.[17] Therefore, the limiting possibilities for the mode of action of the platinum-sulfoxide complexes within the presently accepted mechanism are (i) loss of sulfoxide in either (a) an extracellular reaction to give $[PtCl_2(diam)]$ or (b) an intracellular hydrolysis

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Table VI. Pseudo-First-Order Rate Constants for the Reaction $[PtCl(R'R''SO)(diam)]^+ + Cl^- \rightarrow [PtCl_2(diam)] + R'R''SO$

[Cl⁻], M	diam	R′R″SO	complex no.	10^5k, s^{-1}	$t_{1/2}$, h	diam	R′R″SO	complex no.	10^5k, s^{-1}	$t_{1/2}$, h
0	*R,R*-dach	Me_2SO	I	0.022 (6)	>200	damch	Me_2SO	VII	0.005 (1)	>200
0.154				0.091 (4)	213 (9)				0.263	73.4 (3.2)
0	*R,R*-dach	MePhSO	II	0.04	>200	damch	MePhSO	VIII	0.175 (6)	110 (4)
0.154				0.418 (47)	47.2 (5.1)				2.64 (19)	7.38 (0.59)
0	*R,R*-dach	MeBzSO	III	0.005 (3)	>200	damch	MeBzSO	IX	0.054 (10)	393 (74)
0.154				0.049 (7)	>200				0.196 (11)	98.9 (5.2)
0	*R,R*-dach	Ph_2SO	IV	0.237 (3)	81.5 (1)	damch	Ph_2SO	X	1.86 (18)	10.6 (1.1)
0.154				2.12 (59)	11.0 (3.5)				16.18 (2.12)	1.23 (0.15)
0	*R,R*-dach	Bz_2SO	V			damch	Bz_2SO	XI		
0.154									0.286 (15)	67.8 (3.8)
0	en	MePhSO	VI	0.078 (28)	>200	pn	MePhSO	XII	0.33 (2)	58.2 (3.0)
0.154				0.400 (13)	48.2 (1.5)				2.99 (5)	6.86 (1.2)
0	damch	*S*,(−)-MeTolSO	XIII			damch	*R*,(+)-MeTolSO	XIV		
0.154				1.32 (9)	14.8 (1.0)				1.31 (10)	14.8 (1.0)

to produce $[Pt(H_2O)_2(diam)]^{2+}$, whereupon these species could then react in their "normal" manner, or (ii) the formation of a monodentate linkage followed by closing of the intrastrand link by sulfoxide displacement on DNA:

$$[PtCl(R'R''SO)(diam)] \rightarrow [Pt(diam)(H_2O)_2]^{2+} \rightarrow [Pt(diam)DNA]$$

$$[PtCl(R'R''SO)(diam)] \rightarrow [Pt(R'R''SO)(diam)DNA] \rightarrow [Pt(diam)DNA]$$

Both limiting mechanisms will have the biological activity dependent on the structure of the sulfoxide. To study structure–activity relationships and the possible mechanism of action, we have studied the stability of the complexes in solution especially with respect to displacement of sulfoxide by chloride:

$$[PtCl(R'R''SO)(diam)]^+ + Cl^- \rightarrow [PtCl_2(diam)] + R'R''SO$$

Displacement Studies. The displacement of the sulfoxide ligand was followed by disappearance of the HPLC peak of the intact complex and concurrent appearance of the free sulfoxide peak. In the case of Cl^- displacement, the insoluble dichlorides precipitated from the reaction mixture and were identified by comparison of IR spectra with authentic samples. A concentration of 0.154 M Cl^- was used to approximate extracellular concentrations. The area of the initial HPLC peak corresponded within experimental error to the concentration of complex when compared with the UV/visible spectrum and the HPLC analysis showed at all times only two peaks corresponding to complex and free sulfoxide. Further, analysis by atomic absorption showed only the original peak to contain platinum.

As stated earlier, complexation of an optically active sulfoxide to Pt changes the sign in comparison to free ligand and when the substitution reaction by Cl^- is followed by circular dichroism the reappearance of the free sulfoxide with sign opposite to that in the complex is observed (Figure 2). Since Cl^- was used to excess in all reactions and the ion concentration remained essentially constant, in general no noncoordinating anions such as ClO_4^- were added. Analysis of loss of complex vs time gave first-order plots and plots of k_{obsd} vs Cl^- are straight lines passing through the origin within experimental error. The pseudo-first-order rate constants are collected in Table VI.

Effect of Sulfoxide. Examination of the kinetic data shows that the order of lability is Ph_2SO > MePhSO > MeTolSO > Bz_2SO > MeBzSO > Me_2SO. This trend is expected on steric grounds. There is no major difference between MeBzSO and Me_2SO, indicating that substitution by the Ph group does not produce any large steric effects when not directly bound to sulfur. Substitution in both CH_3 groups increases lability, but the effect is still not as marked as when the Ph group is bound to the sulfur atom. In this case there is an approximate order of magnitude increase in lability if we compare data for MePhSO vs MeBzSO, and this effect is further marked upon going to Ph_2SO. The lability of the sulfoxide may be changed 50- to 80-fold by systematic substitution. As expected, the kinetics of displacement by Cl^- of the resolved forms of (±)-MeTolSO were essentially the same for both enantiomers. There is a slight difference in comparison to MePhSO, with displacement of MeTolSO being slightly slower.

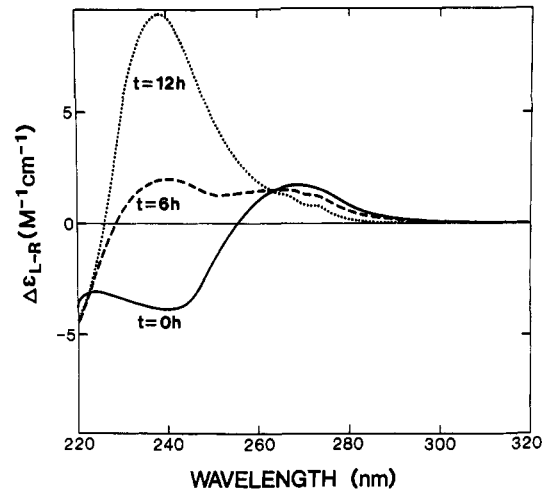

Figure 2. Circular dichroism spectra of complex XIII, [PtCl{*S*,(−)-MeTolSO}(damch)]NO_3, upon reaction with a large excess of chloride. The spectra show the appearance of free sulfoxide of opposite sign to that of the bound ligand.

Some kinetic data are available for similar Cl^- displacement of Me_2SO, but due to widely different experimental conditions and effects of different charges, no quantitative comparisons should be made. The pseudo-first-order rate constants for displacement of Me_2SO from $[Pt(dien)Me_2SO]^{2+}$ by 0.2 M Cl^- is 2.04×10^{-5} s^{-1}.[18] This is significantly faster than the rates reported here, but the different effects of ionic charge in the reactions and different conditions do not allow quantitative comparison, although our studies give a self-consistent idea of relative rates of displacement. We have also noted further an effect of amine on the rate of displacement (see below), and this will also help account for the apparent discrepancy.

In the reverse reaction, first-order rate constants for displacement of Cl^- by Me_2SO of 4.36×10^{-5} s^{-1} (in *cis*-$[PtCl_2(NH_3)_2]$ by Me_2SO as solvent at 23 °C)[4] and 12.28×10^{-5} s^{-1} (in K_2PtCl_4 by 1 M Me_2SO in CH_3OH/H_2O at 25 °C)[19] have been reported. The order of lability can be compared qualitatively with data for this latter reaction:

$$K_2PtCl_4 + R'R''SO \rightarrow K[PtCl_3(R'R''SO)] + KCl$$

In this case the first-order rate constants at equivalent concentrations for some sulfoxides used in this study followed the sequence Me_2SO > Me(*p*-Tol)SO > MePhSO, and the proportional differences are somewhat the same.

Effect of Amine. An interesting observation of relevance to the interpretation of the biological activity is that the amine affects the rate of displacement of the sulfoxide, and for damch, the displacement is 5–10 times faster than that of the corresponding dach complex. This is highly unlikely to be due to any electronic

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effect of the amine ligands. To examine this effect, we prepared MePhSO complexes with the diamines ethylenediamine (five-membered ring equivalent to dach) and 1,3-propanediamine (six-membered ring equivalent to damch) as analogues of the cyclic amines. Kinetic studies confirmed the steric effect of the amine with the rate for the propanediamine complex being nearly 6 times as fast as that of ethylenediamine and remarkably similar to that of damch.

Analysis of the Cl^- displacement in K_2PtCl_4 by R′R″SO mentioned above led to the conclusion that the rate-limiting step is in the transition (bond-making) step.[18] This must be valid for the reverse reaction discussed here. We are at present examining the structural features of these complexes to explain the greater reactivity of the damch ligands and the differences in lability of the various sulfoxides. Five-membered chelate rings have a significantly smaller bite (82–84°) in square-planar Pt compounds than six-membered rings (92–94°), and this difference may be more important in dictating leaving ability in a more crowded five-coordinate intermediate than in the four-coordinate starting complex. Further papers in this series will address this point.

Hydrolysis of the Sulfoxide. In the absence of Cl^-, the displacement of R′R″SO by H_2O can be studied. In this case, there will be further reactions caused by concurrent loss of chloride and deprotonation of the aqua ligand:

$$[PtCl(R'R''SO)(diam)]^+ \rightarrow [Pt(diam)(R'R''SO)H_2O]^{2+} \rightarrow [Pt(diam)(R'R''SO)(OH)]^+$$

A rigorous description of this reaction would require full allowance for all these factors, but plots of disappearance of complex vs time also followed first-order kinetics and allowed us to calculate pseudo-first-order rate constants and half-lives for the hydrolysis. These are also collected in Table VI.

The displacements of Me_2SO and MeBzSO were extremely slow, and half-lives in excess of days may be calculated for both dach and damch complexes. Although calculations were made over long periods of time, for the purposes of this discussion we assign half-lives of >200 h for the complexes with Me_2SO and MeBzSO, confirmed by studies of the initial rate of hydrolysis. Half-lives in water for the other sulfoxides range from 10 to 50 h, depending on amine ligand, with rates for damch complexes again significantly faster than those for the analogous dach species. For comparison, the relative rate (damch/dach) is 7.63 for displacement of Ph_2SO by Cl^- and 7.88 for displacement by H_2O. One of our initial postulates in studying steric effects of sulfoxides was that a leaving rate equivalent to chloride could be achieved by suitable substitution on the sulfur atom. We note that the hydrolysis of the Ph_2SO ligand from $[PtCl(Ph_2SO)(damch)]^+$ at a rate of 1.86×10^{-5} s^{-1} is on the order of the rate of Cl^- hydrolysis in *cis*-$[PtCl_2(NH_3)_2]$ ($k = 2.5 \times 10^{-5}$ s^{-1} at 20 °C).[20]

Summary and Conclusions. The series $[PtCl(R'R''SO)(diam)]^+$ represents a class of cationic Pt complexes with high antitumor activity. The activity is dependent upon the lability and, especially, the chirality of the sulfoxide ligand. The kinetic reactivity with respect to sulfoxide displacement spans almost 2 orders of magnitude and a unifying mechanism of action may be difficult to enunciate. Some interesting trends do emerge, however.

In the case of Ph_2SO, displacement of sulfoxide and production of the "active" diaqua species may account for the antitumor activity—this would also explain the high toxicity of complex X in comparison to the rest of the complexes. The fact that, in this complex, the lability of Ph_2SO is on the order of that of Cl^- supports this interpretation.

The more interesting results are with the intermediately and slowly displaced ligands. The inertness of Me_2SO, MeBzSO, and MePhSO with respect to displacement by both chloride and water precludes the possibility of these complexes acting by simple loss of sulfoxide ligand. Indeed, of these ligands, the shortest half-life in 0.154 M Cl^- is 7.38 h for $[PtCl(MePhSO)(damch)]^+$. The fact that the rates of chloride displacement and hydrolysis of the (±)-MeTolSO complexes are identical, despite their differing biological activity, also argues against a mechanism involving simple sulfoxide displacement.

The overall results most strongly point to mechanism ii as the mechanism of action and indicate that a formally nontoxic monodentate complex may be transformed into a toxic bidentate species upon reaction with DNA. Initial DNA-binding studies on the complexes, by assaying inhibition of restriction enzyme activity on DNA[21] and by using fluorescence techniques on calf-thymus DNA in the presence of ethidium bromide,[22] confirm that the sulfoxide complexes react at different rates, and thus both chiral recognition and differential displacement of sulfoxide by a target molecule such as DNA may contribute to the difference in antitumor activity observed. This interpretation is reminiscent of the argument for activation of carboplatin ($[Pt(NH_3)_2$(CBDCA)], CBDCA = cyclobutane-1,1-dicarboxylate). Despite the inert nature of the complex,[23] binding of the complex to DNA by displacement of one carboxylate is followed by a rapid closure of the intrastrand link.[24] With respect to displacement by DNA, effects of both asymmetry and lability will be important. Thus, the steric effect of the amine in lability could be of importance for Me_2SO where we note that damch > *R,R*-dach. Conversely, the high activity of complex III in comparison to complex IX may indicate an effect of chirality.

Both selective uptake and selective attack on a biological substrate such as DNA may explain the different biological activity of enantiomers or stereoisomers. Chiral metal complexes such as the phenanthroline chelates recognize DNA differently, and their use as conformational probes is currently a very active and interesting area.[25] Differential DNA binding and different rates of sulfoxide displacement may not be the only explanation of the effect of chirality on antitumor activity. Early studies by Dwyer on the optical isomers of $[Os(phen)_3]^{2+}$ (phen = 1,10-phenanthroline) did show some differences in both uptake and elimination.[26] Uptake studies[27] showed an approximate 1.4-fold increase in uptake of complex XV over complex XVI in cultured L1210 cells, but this is unlikely to explain such large differences in in vivo antitumor activity.[28]

Cationic triamines $[PtCl(NH_3)_2(am')]^+$ (am′ = substituted pyridine) have antitumor activity,[29] and the complexes reported here represent a further well-defined cationic series that does not obey the standard structure–activity relationships for platinum complexes. They also represent the first example of a sulfur ligand incorporated into antitumor complexes with good activity. Platinum–sulfur complexes are of interest not only because of tissue binding and metabolism of platinum complexes[30] but also because of the nephroprotective effect of sulfur nucleophiles such as DDTC (diethyldithiocarbamate),[31] thiosulfate,[32] and WR2721,[33] the possible role of endogenous thiols such as glutathione in modulation of antitumor activity of platinum complexes,[34] and the use of thiourea in removal of Pt–DNA cross-links[35] and

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isolation of Pt-DNA monoadducts.[36] The incorporation of a Pt-S bond in a well-defined complex prior to any administration may have considerable effects on this biology and the fact that Pt-S complexes are antitumor active may be relevant to the mechanisms of some of the above-mentioned effects. An interesting point to note here also is that albumin, the predominant agent responsible for tissue binding of Pt complexes, has been used (as protein BSA) as a template for the preparation of chiral sulfoxides.[37] It is therefore axiomatic that chiral sulfoxide complexes will react differently with this enzyme. The chemical aspects of the biodistribution, tissue binding, and metabolism of platinum antitumor complexes are not as well understood as those of DNA binding, but it is clear that these properties may vary quite widely between, e.g., a neutral dichloride complex and a cationic sulfoxide species.

In conclusion, independent of the detailed mechanism of action of these complexes, we report on a new class of active platinum antitumor complexes based on sulfoxide ligands, with a unique effect of the chirality of the sulfoxide ligand upon the antitumor activity.

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Registry No. I, 124442-33-7; II (isomer 1), 124460-83-9; II (isomer 2), 124578-26-3; III (isomer 1), 124460-85-1; III (isomer 2), 124578-28-5; IV, 124442-35-9; V, 124442-37-1; VI, 124442-39-3; VII, 124442-41-7; VIII, 124442-43-9; IX, 124442-45-1; X, 124442-47-3; XI, 124442-53-1; XII, 124442-49-5; XIII, 124442-51-9; XIV, 124510-92-5; XV, 124460-87-3; XVI, 124578-30-9; XVII, 124578-32-1; XVIII, 124578-34-3; (*R*,*R*)-dach, 20439-47-8; en, 107-15-3; (*S*,*S*)-dach, 21436-03-3; damch, 4441-55-8; pn, 109-76-2.

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Contribution from the Department of Chemistry, The University of Wisconsin—Milwaukee, P.O. Box 413, Milwaukee, Wisconsin 53201

Biphasic Kinetics of Aurothionein Formation from Gold Sodium Thiomalate: A Novel Metallochromic Technique To Probe Zn^{2+} and Cd^{2+} Displacement from Metallothionein[1]

C. Frank Shaw III,* James E. Laib, M. Meral Savas, and David H. Petering

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A method has been developed to exploit the metallochromic dyes pyridylazoresorcinol and Zincon as monitors of the rate of zinc and cadmium displacement from Zn-, Zn,Cd-, Zn,Cd,Cu- and Cd-thioneins. In this report, the antiarthritic drug gold sodium thiomalate (AuSTm) is the displacing agent. In 5 mM Tris-HCl/100 mM $NaClO_4$ buffer, pH 7.4, at 25 °C, the reactions are biphasic. The fast and slow components are first order and independent of the choice of dye and its concentration. The reaction kinetics for each of the thionein preparations (except one Zn,Cd,Cu-thionein preparation) were also independent of the gold concentration. Thus, the rate law for aurothionein formation from AuSTm is rate = k_f[MT] + k_s[MT]. The averaged rate constants of the fast and slow steps obtained by using PAR with six different protein preparations were $k_f = (2.7 \pm 1.2) \times 10^{-2}$ s^{-1} and $k_s = (6.9 \pm 0.9) \times 10^{-4}$ s^{-1} and by using Zincon, were $k_f = 2.4 \pm 0.6 \times 10^{-2}$ s^{-1} and $k_s = 9.6 \pm 1.7 \times 10^{-4}$ s^{-1}. The same rate laws for metal displacement describe the reactions generating Au,Cd-Th or Au,Zn,Cd-Th (for which gold is the limiting reagent) and those forming $(TmSAu)_{20}$-Th with complete loss of protein-bound zinc and cadmium (for which the protein is the limiting reagent). Differences in the kinetics due to the source of the metallothioneins and their metal contents were all within the experimental error. The biological implications of the kinetics for aurothionein formation during chrysotherapy are discussed.

Introduction

Metallothionein is a curious, cysteine-rich, metal-binding protein found in mammalian tissues. Twenty of its 61 amino acid residues are cysteines. As isolated, it may contain zinc, copper, or environmentally accumulated cadmium in ratios dependent on the tissue, the species, and the age and history of the organism from which it is isolated. Cadmium and zinc are bound in two clusters with exclusive thiolate coordination: M_3S_9 and M_4S_{11}, localized in the N and C terminal ends of the peptide chain, respectively.[2-4]

Gold(I) thiolates provide successful treatments for rheumatoid arthritis.[5] In animal models the gold binds to metallothioneins in vivo, generating aurothioneins.[6-10] Aurothionein formation can be modeled by in vitro reactions of gold complexes with Zn_7-Th, Zn,Cd-Th and Cd_7-Th.[10-12] Gold sodium thiomalate (AuSTm)[13] displaces Zn^{2+} completely and Cd^{2+} in an equilibrium competition, forming Au,Zn,Cd-Th, Au,Cd-Th, and $(TmSAu)_{20}$-Th at progressively higher Au to protein ratios.[10,11] In the first two forms, gold is coordinated to two MT cysteines with loss of the thiomalate carrier ligand. In the latter, one AuSTm moiety

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(13) Abbreviations: apo-CA, apo-carbonic anhydrase; AuSTm, gold sodium thiomalate; DTNB, dithionitrobenzoic acid; EDTA, ethylenediamminetetraacetic acid; hrs, horse; kid, kidney; liv, liver; MT, metallothionein; PAR, pyridylazoresorcinol; rbt, rabbit; rds, rate-determining step; Th, thionein; ZI, Zincon.

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