Mutagenic Activity of some Platinum Complexes with Monodentate and Bidentate Amines

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Abstract

The effect of bridging with an ethylene chain the two aminic nitrogens *cis*-coordinated to the metal in a platinum–dichloro–diamine complex has been examined by studying forward mutagenesis in strains of *Salmonella typhimurium* carrying hisG46 and hisD3052 mutations and differing at the uvrB locus. While the complexes with the monodentate amines NH_3 and α-methylbenzylamine (mba) exhibit strong mutagenic activity, the corresponding species with chelating diamines, 1,2-diaminoethane (en) and *N,N'*-bis(α-methylbenzyl)1,2-diaminoethane (mben), show much weaker activity. Moreover, the latter complexes appear to be more active via 'frame shift' than 'base substitution' mechanisms and do not differentiate appreciably between cells differing in the rfa locus. Complexes with amines bearing alkyl substituents of opposite chirality (*R*-mba and *S*-mba, *R,R*-mben and *S,S*-mben) were tested separately and appeared to have comparable activity.

Introduction

After the introduction of *cis*-$[PtCl_2(NH_3)_2]$ (*cis*-DDP) in clinical trials, several efforts have been devoted to a better understanding of its mechanism of action [1, 2]. In order to gain more information on this subject, we have embarked on a program of research into the mutagenic activity of different classes of platinum–chloro–amine complexes towards strains of *Salmonella Typhimurium* differing in their genotypes and repair capacities [3–10]. In a previous work we examined compounds of the general formula *cis*-$[PtCl_2LA]$ and *cis*-$[PtCl_2A_2]$ (L = ligand with a strong *trans*-labilizing effect, A = monodentate amine) [11]. We have now investigated the effect of bridging with an ethylene chain the two aminic nitrogens *cis*-coordinated to platinum. In the case of amines with chiral substituents, the different stereo-isomers were tested separately.

Materials and Methods

Chemicals

Commercial reagent-grade products were used without further purification. The ligands *R,R*-mben and *S,S*-mben [mben = *N,N'*-bis(α-methylbenzyl)1,2-diaminoethane] were prepared by the method of Terent'ev *et al.* [12]. The complexes with monodentate amines were prepared as reported in ref. 11.

The ligand *R,S*-mben was prepared by heating an equimolar mixture of racemic α-methylbenzylamine and 1,2-dibromoethane until a yellow crystalline precipitate of the hydrobromide separated out. This was filtered off, washed with ether and dried. The solid was then dissolved in water, treated with an excess of potassium hydroxide. The amine, which separated out as a brown oily liquid, was driven off and treated with the stoicheiometric amount of hydrochloric acid forming a yellowish crystalline precipitate. The mixture of *R,R*-, *S,S*-, and *R,S*-mben·2HCl was treated with methanol. The *R,R* and *S,S* components, being more soluble in this solvent, dissolved leaving a solid residue of almost pure *R,S*-mben·2HCl. This was separated by filtration of the mother liquor, washed three times with methanol, then washed with ether and dried. The compound was characterized by elemental analysis (*Anal.* Calc. for $C_{18}H_{26}Cl_2N_2$: C, 63.3; H, 7.7; Cl, 20.8; N, 8.2. Found: C, 63.1; H, 7.8; Cl, 21.2; N, 8.2%. IR and NMR spectra were significantly different from those of the corresponding *R,R* and *S,S* ligands.

The complexes *cis*-$[PtCl_2A_2]$ (A_2 = *R,R*-mben, *S,S*-mben, and *R,S*-mben) were prepared by decom-

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position of the cationic species *cis*-$[PtCl(dmso)A_2]^+$ (dmso = dimethylsulphoxide) obtained by the method of Romeo *et al.* [13]. The salts $[PtCl(dmso)A_2]Cl$ (1 mmol) were dissolved in methanol (10 cm^3) and by warming (80 °C, 60 h, A_2 = *R,S*-mben; 40 °C, 2h, A_2 = *R,R*-mben and *S,S*-mben) and stirring yellow precipitates of the desired compounds separated out. The compounds were crystallized from dichloromethane/methanol. In all cases the yields were above 80%. *Anal.* Calc. for $C_{18}H_{24}Cl_2N_2Pt$: C, 40.4; H, 4.5; Cl, 13.3; N, 5.2. Found: C, 39.9; H, 4.7; Cl, 14.0; N, 5.1% (*R,R*-ligand). C, 40.0; H, 4.5; Cl, 13.8; N, 5.2% (*S,S*-ligand). C, 40.1, H, 4.6; Cl, 13.8; N, 5.1% (*R,S*-ligand).

The complex *cis*-$[PtCl_2(Bu_2^ten)]$ (Bu_2^ten = *N,N'*-bis(terbutyl)1,2-diaminoethane) was prepared by

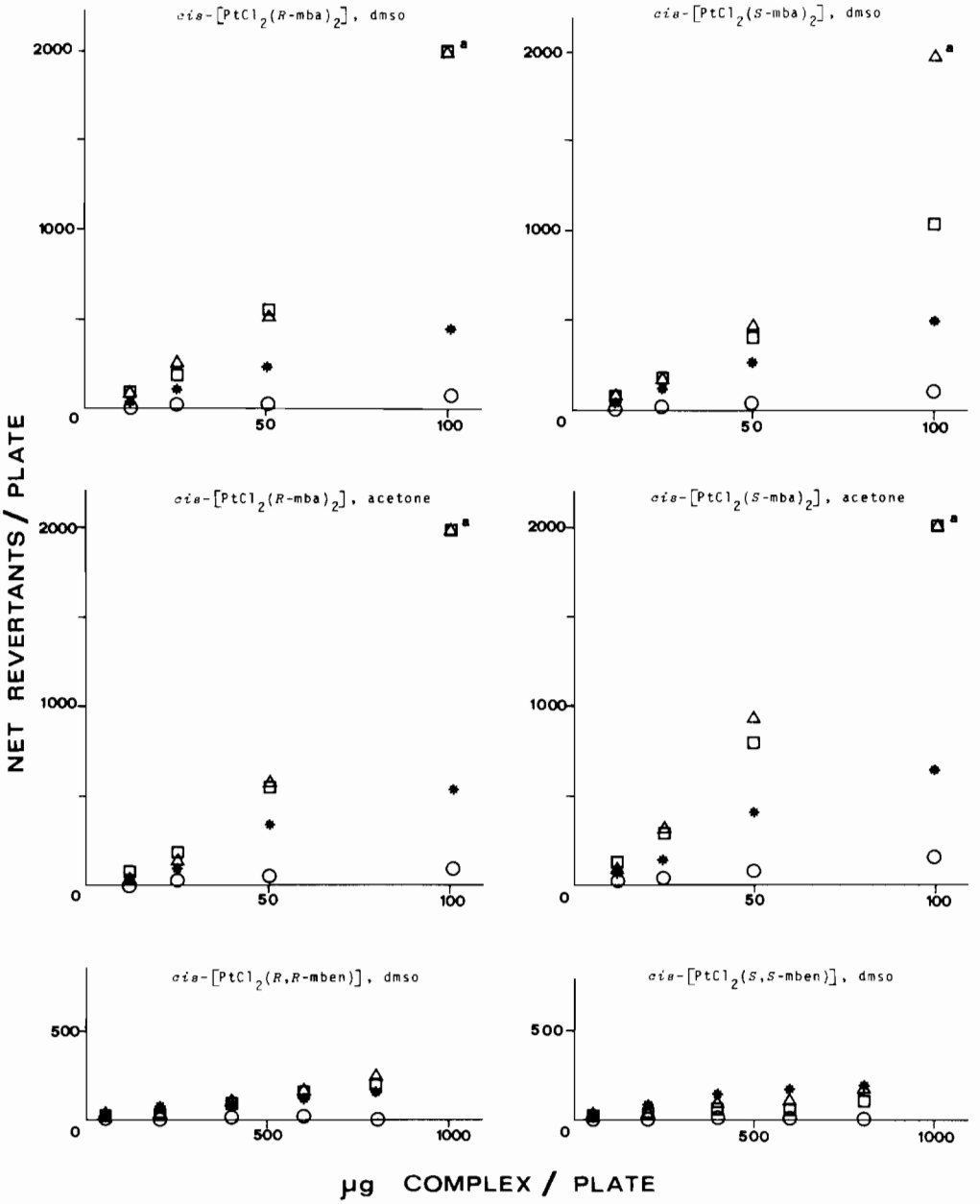

Fig. 1. Mutagenicities of *cis*-$[PtCl_2(R\text{-mba})_2]$, *cis*-$[PtCl_2(S\text{-mba})_2]$, *cis*-$[PtCl_2(R,R\text{-mben})]$, and *cis*-$[PtCl_2(S,S\text{-mben})]$ complexes towards *Salmonella typhimurium* strains TA92 (○), TA2410 (□), TA100 (△), and TA98 (*). The mutagens were added to the top agar in either dimethylsulphoxide (dmso) or acetone (see 'Materials and Methods'). [a]Revertant colonies per plate above 2000 are not countable.

slow addition of a solution of Bu_2^ten (0.172 g, 1 mmol) in methanol (10 cm^3) to a solution of $[PtCl_2(dmso)_2]$ (0.422 g, 1 mmol) in the same solvent (40 cm^3). After 65 h stirring at room temperature, the resulting yellow solution was concentrated to small volume (5 cm^3) under reduced pressure, filtered and cooled to 0 °C. Yellow crystals of $[PtCl_2(Bu_2^t en)]$ slowly separated out; these were filtered off, washed twice with methanol and dried, yield 80%. *Anal.* Calc. for $C_{10}H_{24}Cl_2N_2Pt$: C, 27.4; H, 5.5; Cl, 16.2; N, 6.4. Found: C, 27.5; H, 5.6; Cl, 16.4; N, 6.3%.

Mutagenesis Assay

The bacterial strains used are listed in Table I [14]. The mutagenic activity of the platinum compounds was measured according to the protocol of Ames *et al.* [15] without metabolic activation. Briefly, multiple 2.0 cm^3 aliquots of top agar containing 5×10^{-5} mol dm^{-3} biotin and 5×10^{-5} mol dm^{-3} histidine were prepared and kept at 45 °C. 0.1 cm^3 of a solution of the compound to be tested and 0.1 cm^3 of a fresh overnight bacterial culture were added rapidly to the top agar, mixed and poured onto the Vogel–Bonner minimal media plate [16]. After 2 days incubation at 37 °C, the number of colonies per plate (histidine revertants) were counted. The number of revertant colonies per plate, expressed as the mean value of three different experiments from which the spontaneous revertants have been subtracted TA92, 41 ± 7; TA2410, 140 ± 20; TA100, 116 ± 21; TA98, 18 ± 6), are reported in Figs. 1–3; some data relative to monodentate amines, previously reported in tabulated form, are also included for comparison [11].

TABLE I. Strains of *Salmonella typhimurium*

Strain	Relevant genotype	Reference
TA 92	hisG46, pKM101	14
TA 2410	hisG46, uvrB, pKM101	14
TA 100	hisG46, uvrB, rfa, PKM101	14
TA 98	hisD3052, uvrB, rfa, pKM101	14

Results

Figure 1 summarizes the behaviour of the complexes with monodentate and bidentate amines bearing the α-methylbenzyl substituent. Two solvents with different coordinating ability (dimethylsulphoxide and acetone) were used in order to check the stability of the substrates with monodentate amines under the experimental conditions used in this work. Complexes with alkyl substituents of opposite chirality were tested separately and their plots are placed one opposite to the other in order to better demonstrate any difference in their behaviour. The lower diagrams, which refer to the compounds with chelat-

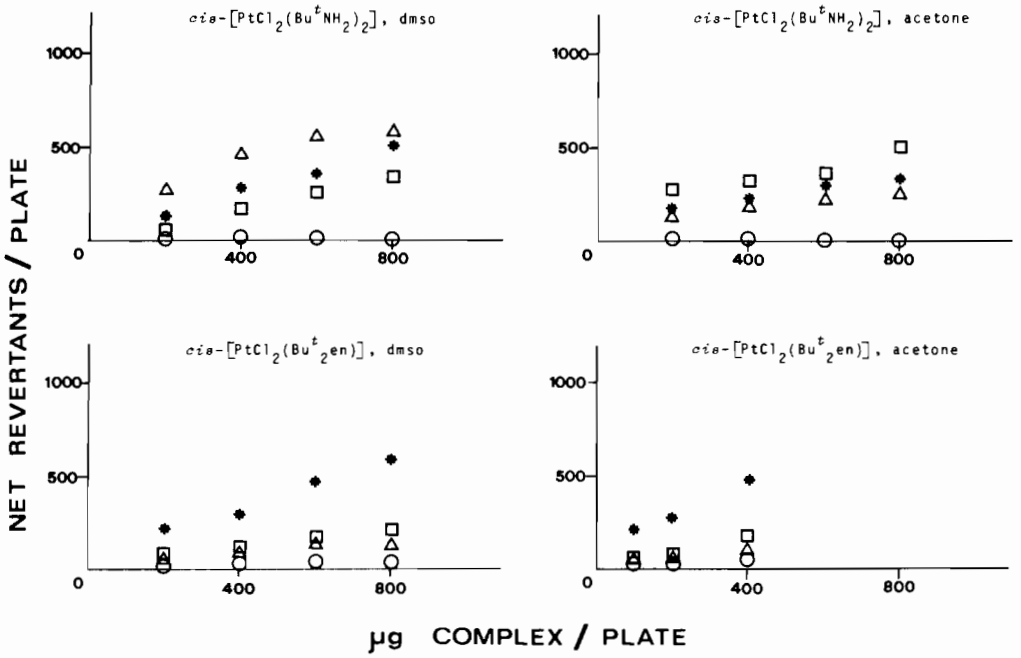

Fig. 2. Mutagenicities of *cis*-$[PtCl_2(Bu^tNH_2)_2]$ and *cis*-$[PtCl_2(Bu_2^t en)]$ complexes towards *Salmonella typhimurium* strains TA92 (○), TA2410 (□), TA100 (△), and TA98 (*). The mutagens were added to the top agar in either dimethylsulphoxide (dmso) or acetone (see 'Materials and Methods').

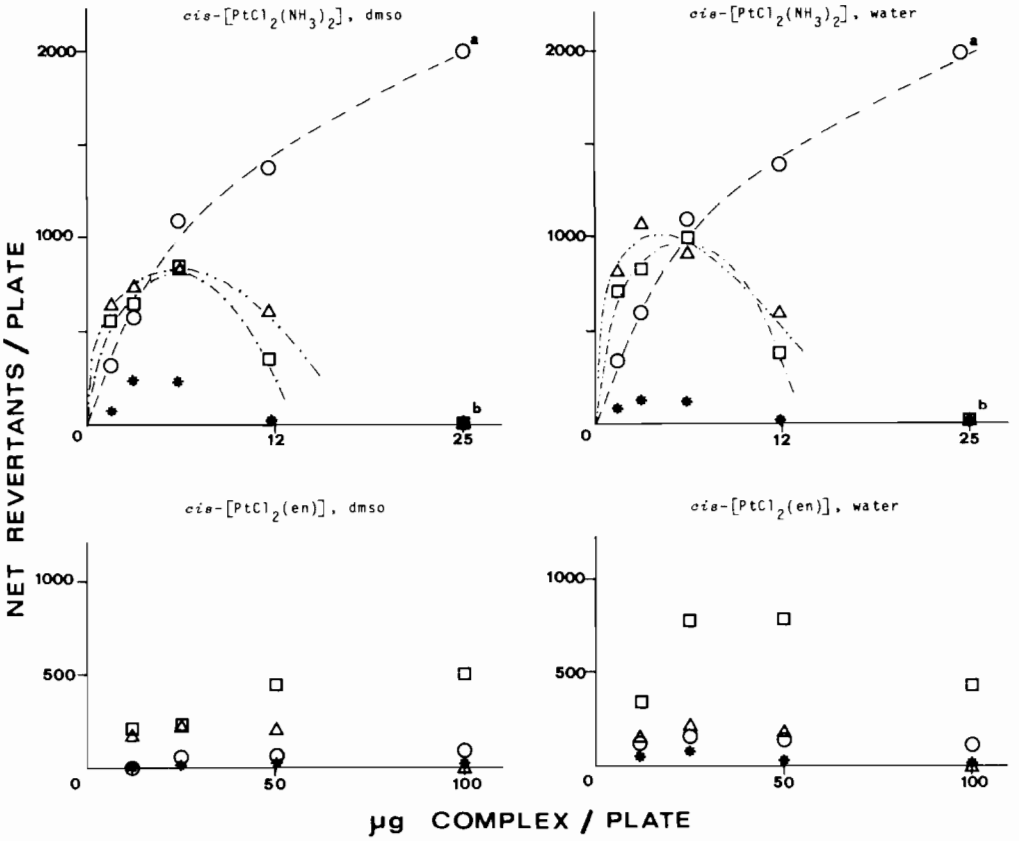

Fig. 3. Mutagenicities of *cis*-$[PtCl_2(NH_3)_2]$ and *cis*-$[PtCl_2(en)]$ complexes towards *Salmonella Typhimurium* strains TA92 (○), TA2410 (□), TA100 (△), and TA98 (*). The mutagens were added to the top agar in either dimethylsulphoxide (dmso) or water (see 'Materials and Methods'). [a]Revertant colonies per plate above 2000 are not countable. [b]Inhibition of background growth.

ing diamine, show how doses of complex ten times larger are required in order to have detectable mutagenic activity. The complex with *R,S*-mben, in contrast to those with *R,R*-mben and *S,S*-mben ligands, has negligible mutagenic activity and therefore is not reported in the diagram.

Figure 2 plots the results relative to the complexes with ter-butyl substituted monodentate and bidentate amines. An appreciable solvent effect, not detected in the previous case, occurs in *cis*-$[PtCl_2(Bu^t\text{-}NH_2)_2]$. Moreover, the mutagenic activity is comparable in the complexes with monodentate and chelating amine, but differs with respect to the different strains of bacteria.

Finally, Fig. 3 summarizes the behaviour of the complexes having ammonia and 1,2-diaminoethane as ligands. In this case, the overall proportionality between mutagenic activity and dose of complex is not retained due to an effect of toxicity exerted by the complexes at the higher concentrations [17].

Discussion

The first conclusion that can be drawn from the present investigation is that complexes with alkyl substituents at the nitrogen atoms of opposite chirality behave in a similar way; this applies not only to monodentate amines (*R*-mba and *S*-mba) but also to bidentate ligands (*R,R*-mben and *S,S*-mben). This result is not completely unexpected, since a nitrogen substituent which can freely rotate about the C–N bond will exert a sterical effect which is independent of its chirality. The situation could be completely different in cases in which the complexes have different chirality, either at the coordinated nitrogens themselves or at a carbon atom which is restricted in its motions, such as those of the organic chain bridging the two nitrogens of a diamine [18]. Both possibilities are presently being investigated in our laboratories. It is also to be noted that the complex with *R,S*-mben has negligible mutagenic activity; in this respect, it is different from the compounds with *R,R*- and *S,S*-mben.

A second observation is that the complexes with bidentate amines generally show a smaller activity than the corresponding species with monodentate amines [19]. The decreasing activity does not appear to depend upon a reduced ability to cross the cell membrane, since strains with different membrane permeability (TA2410 and TA100) behave in a similar way; it might be related to some other structural feature, such as a reduced number of protons on the aminic nitrogens or a less flexible molecular geometry of the complex.

Finally, it appears that complexes with bidentate amines are more active via 'frame shift' than 'base substitution' mechanisms (they are relatively more mutagenic towards hisD3052 than hisG46 strains). This effect is more evident in the complexes with ter-butyl substituted amines. In this case, when going from the monodentate to the bidentate species, the activity towards TA92, TA2410, and TA100 strains (all of hisG46 genotype) decreases sensibly, while that towards TA98 (hisD3052 genotype) remains unchanged or slightly increases. If 'frame shift' mutation can be put in relation to the formation of intercalation compounds, we should conclude that complexes, with chelating diamines, having a metallocyclic structure, have a higher tendency to give such a type of interaction [20].

Acknowledgements

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