Mutagenic Effects of Rhodium(I) and Ruthenium(II) Organometallic Complexes in Bacteria*

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Abstract

Microorganisms are suitable models for studying the mechanisms of action of chemical compounds with therapeutic potential. Listed in order of increasing complexity, the following microbial systems can be considered: transforming DNA, bacteriophages and other viruses, bacteria, fungi, and mammalian cells in culture.

For more than 10 years, cisplatin (*cis*-dichlorodiammine-Pt(II)) has been known to exert a number of effects in bacteria, such as mutation induction, selective inhibition of DNA synthesis, degradation of DNA, induction of lambda prophage, filamentous growth, and higher toxicity for strains unable to repair damaged DNA. The *trans*-isomer does not exert many of these effects, even if it also reacts with bacterial DNA, it is only marginally mutagenic and its selective toxicity for bacterial strains with defects in the DNA repair systems is less pronounced. The response of cultured mammalian cells matches these findings in bacteria. As antitumor activity is restricted to cisplatin, one may suppose that the therapeutic effects and the many differences shown in microbial systems are consequences of the different way of interaction of the two isomers with cellular DNA.

In the search for less toxic congeners of cisplatin, a number of metalloorganic compounds have been synthesized as possible antitumor drugs: either platinum with other ligands, or compounds of rhodium, ruthenium and other metals. A preliminary inquiry into their effects in microbial systems may indicate which of them are the most promising.

Introduction

In a search for transition metal complexes which could mimic the effect of cisplatin (*cis*-dichlorodiammine-Pt(II) in bacteria, we found that different rhodium(I) complexes were able to produce filamentous growth of *Escherichia coli* and to induce lambda prophage in lysogenic bacteria. The more active compounds were derivatives of the type Rh–chelating(ligand–ligand), where the chelating moiety was 1,10-phenanthroline and the ligand was either 1,5-cyclooctadiene or 1,5-norbornadiene [1]. The octahedral ruthenium(II) derivative $RuCl_2$-(dimethylsulfoxide)$_4$ also showed a similar effect [1, 2].

These facts prompted us to test the mutagenic activity of these compounds on *Salmonella typhimurium* and *Escherichia coli* in view of the well-known correlation between mutagenic, oncogenic and antitumor activity of chemicals [3].

TABLE I. Bacterial Strains

Strain	Genetic markers	Reference
E. coli B		
WP2	trpE	4
WP2uvrA	trpEuvrA	4
WP2/TM1	WP2/pKM101	5
WP2/TM4	WP2uvrA/pKM101	5
S. typhimurium		
TA 92	hisG46/pKM101	6
TA 98	hisD3052, rfa, ΔuvrB/pKM101	6
TA 100	hisG46, rfa, ΔuvrB/pKM101	6

Experimental

Bacterial Strains

The bacterial strains used are listed in Table I.

Chemicals

Rhodium(I) complexes were prepared following described methods [7]. They include [Rh(phen)-COD]$^+Cl^-$, [Rh(phen)NBD]$^+Cl^-$, [Rh(4,7-dimethyl-phen)COD]$^+Cl^-$, [Rh(4,7-dimethyl-phen)NBD]$^+Cl^-$, [Rh(3,4,7,8-tetramethyl-phen)COD]$^+Cl^-$, and [Rh-(3,4,7,8-tetramethyl-phen)NBD]$^+Cl^-$ (phen = 1,10-phenanthroline, COD = *cis*,*cis*-1,5-cyclooctadiene, NBD = 1,5-norbornadiene). $RuCl_2$(dimethylsulf-

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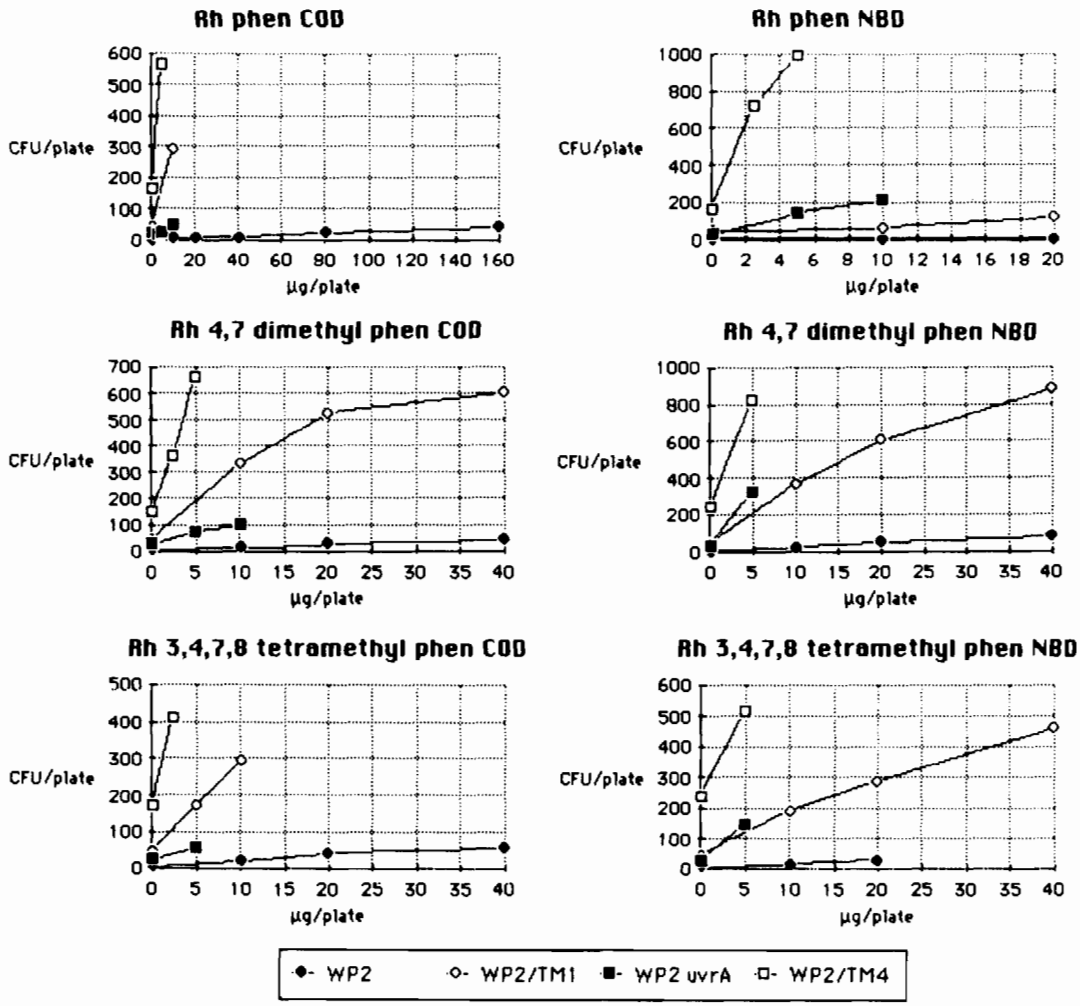

Fig. 1. Mutagenic activity of rhodium(I) complexes on *Escherichia coli* strains. Revertants are counted as colony forming units (CFU) per plate after a 48 h incubation in the presence of the indicated amounts of the compounds.

oxide)$_4$ was obtained according to Evans *et al.* [8]. Chemicals were dissolved in distilled water and filtered through a Millipore membrane (0.22 µm) immediately before use.

Mutation Assay

The mutation assay was carried out according to Ames [6]. Briefly, an appropriate amount of drug solution and 0.1 ml of an overnight culture of bacteria were added to 2 ml of molten top agar and poured onto Petri dishes containing 20 ml each of SEM agar (Davis–Mingioli minimal medium additioned with 1.25% Nutrient broth) for *E. coli* B, or of Vogel–Bonner agar [6] for *S. typhimurium*. Revertant colonies were counted after 48 h incubation at 37 °C.

Results and Discussion

Results of the mutation assay for the rhodium(I) and ruthenium(II) complexes are reported in Figs. 1 to 3. It is evident that all the compounds tested are able to raise the number of revertants over the spontaneous level, both when *S. typhimurium* or *E. coli* are used as tester organisms. It is also evident that the strains of *E. coli* carrying the pKM101 mutator plasmid are more prone to be mutated by the tested compounds, and that *E. coli* and *S. typhimurium* lacking the excision repair activity for damaged DNA show a higher number of revertants.

When the same bacterial strains were similarly exposed to equimolar concentrations of the chelating moiety of the complexes (*i.e.* phen, 4,7-dimethyl-phen, and 3,4,7,8-tetramethyl-phen) no induced revertant was produced in the mutation plates (data not shown).

The above results clearly show that the complexes of rhodium(I) and ruthenium(II) tested possess mutagenic activity for bacteria. It seems reasonable to conclude that these compounds are able to cross the bacterial membrane and interact inside the cell

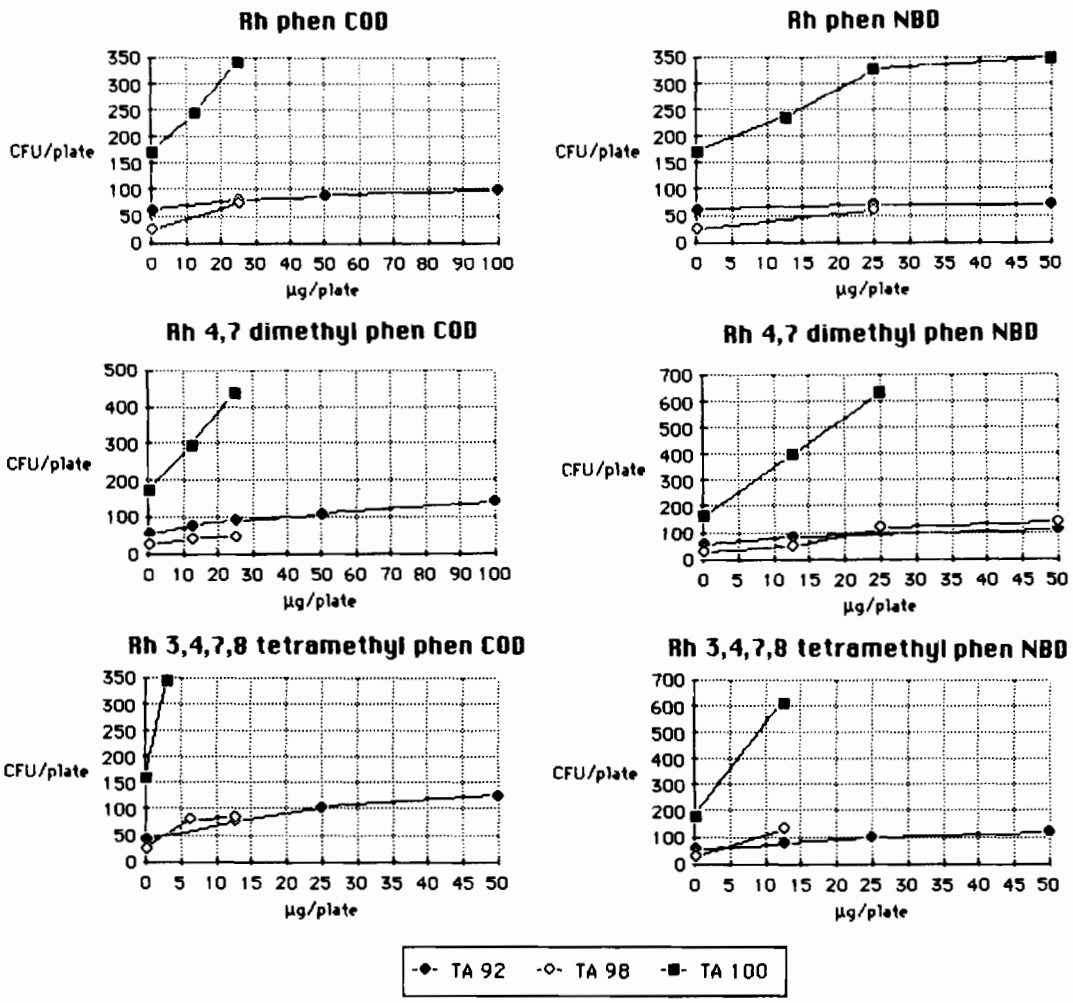

Fig. 2. Mutagenic activity of rhodium(I) complexes on *Salmonella typhimurium* TA 92, TA 98 and TA 100 strains.

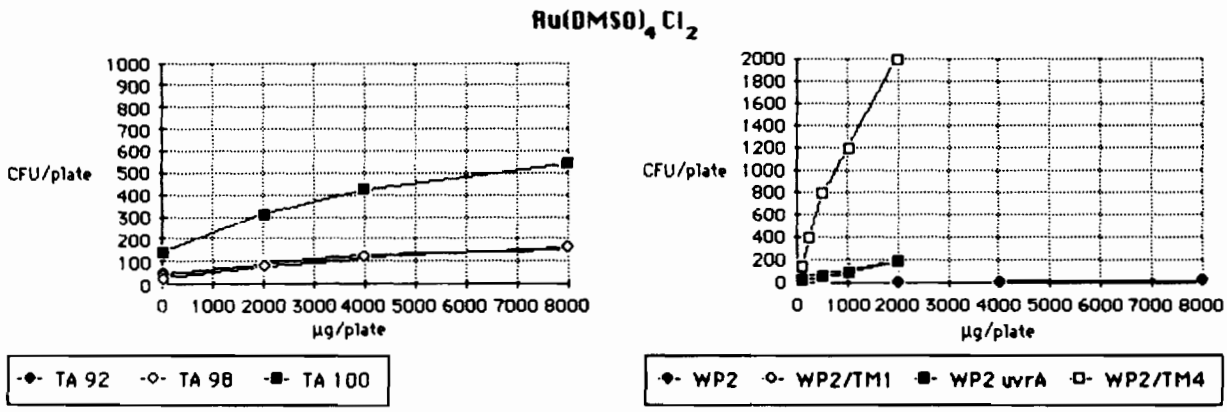

Fig. 3. Mutagenic response of *Salmonella typhimurium* and *Escherichia coli* strains to treatment with increasing concentrations of $RuCl_2(DMSO)_4$.

with bacterial DNA, leading to the production of adducts which activate the SOS repair mechanism. The activity of this DNA repair system ultimately fixes the DNA damage into mutations.

It is worth noting that, when tested as chemotherapeutic agents on different experimental tumors, some derivatives of Rh(I) and Ru(II) displayed antineoplastic activity [9–11].

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