INHIBITION OF BACTERIAL GROWTH AND NUCLEIC ACIDS SYNTHESIS BY PLANAR COMPLEXES OF RHODIUM (I)

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Received 19 April 1974

1. Introduction

Coordination compounds of some transition metals have been shown to possess antimicrobial properties. Since the early results on the activity of Fe(II) and Ru(II) complexes with 1,10-phenanthroline, reviewed by Shulman and Dwyer [1], the silver complexes of sulphadiazine (see ref. [2]) and uracil [3] have been reported to have antibacterial effects on *Pseudomonas aeruginosa*. More recently, the phenanthroline complexes of Cu(II), Cd(II), Zn(II), Mn(II), Fe(II), Co(II), Ni(II), Ru(II) have been reported to have lethal action on *Candida albicans*, and to induce the formation of miniature-colonies in *C. albicans* and *Staphylococcus aureus* [4]. For what concerns the noble metals, the antibacterial effects of Rh(III) [5] and Pt(IV) and (II) [6] have been described. In particular, *cis*-$Pt(II)Cl_2(NH_3)_2$ and related compounds appear to be of wide interest, since these planar molecules have been subsequently shown to possess a number of biological activities. Noteworthy are their powerful antitumour effects in animal models, and encouraging are the results obtained in preliminary clinical trials [7].

These properties of platinum drugs have led us to examine the biological activities of molecules with a similar planar structure, but containing rhodium instead of platinum. The compounds are:

$[Rh(I)bipyCOD]^+Cl^-$ and $[Rh(I)phenCOD]^+Cl^-$
bipy = 2,2′-bipyridine phen = 1,10-phenanthroline
COD = *cis-cis*-1,5-cyclooctadiene

In this communication we report on the effects of these complexes on growth and metabolism of bacteria. The antineoplastic activity of such complexes will be reported elsewhere.

2. Materials and methods

The effects on bacterial growth were determined by adding the substance to be tested to cultures of different strains of *E. coli* or of *Staph. aureus* at the beginning of the exponential growth. The inhibitory effects were measured spectrophotometrically at a time corresponding to the end of the exponential growth in control cultures.

The effects on macromolecular syntheses were determined by adding 5 μCi (2.5 μg) of ^{3}H-methylthymidine, 5 μCi (3 μg) of [^{3}H]uridine or 5 μCi (13 μg) of [^{3}H]leucine (Radiochemical Center, Amersham, England) per ml of acetively growing cultures in MM, pretreated for 10 min with the Rh(I) complex. The acid insoluble radioactivity was measured on 50 μl samples, which were pipetted on Whatman 3MM chromatographic paper disks, subsequently processed according to the methods of Bollum [8].

The water–*n*-octanol partition coefficients $p = C_{H_2O}/C_{oct}$ were determined essentially as described by Fujita et al. [9]. Typically, a sample giving an extinction of about 0.5 in water, was partitioned with an equal volume of *n*-octanol. The extinction of the two phases was determined spectrophotometrically; adherence to Beer's law was checked in both solvents.

Table 1
Effects of rhodium complexes and their free ligands on bacterial growth

Bacterial strain	*E. coli* W3110	*E. coli* pol A⁻	*E. coli* HfrH	*E. coli* K12 λ⁻	*E. coli* HfrH	*E. coli* K12 λ⁻	*S. aureus* Oxford	
Medium	MM	MM	MM	MM	BHI	BHI	BHI	log. *p*
Bipy	126.8	110.2	168.6	364.7	318.5	640	640	−1.168
Rh-bipy	13.1	11.5	18.3	2.5–7.5	248	> 248	36.4	−0.120
Phen	19.4	21.0	21.1	13.7	83.4	74.5	68.9	−1.034
Rh-phen	25.4	20.8	24.7	4.54	110.3	80.9	11.7	−0.0267

Each value is the DI_{50} (μM) calculated by the method of Spermann and Karber on the per cent inhibition figures obtained as described in the experimental section. Bacteria were grown as indicated in minimal medium (MM), supplemented for W3110 and polA⁻ strains with 0.38 mM thymine [19], or in brain heart infusion DIFCO (BHI).

3. Results and discussion

Data reported in table 1 show that the bipyridine complex has an inhibitory activity on bacterial growth in MM much greater than that of its free ligand, with a DI_{50} ranging from 2.5 to about 18 μM. The sensitivity of *Staph. aureus* in BHI to Rh-bipy allow to rule out the possibility that the reduced sensitivity of *E. coli* HfrH and K12 λ⁻ when tested in BHI is due to inactivation of the rhodium complex by medium components.

On the contrary, the phenanthroline complex has an inhibitory capacity comparable to that of the free phenanthroline, except on *Staph. aureus*, where the complex is about six times more active on a molar basis. These results could be explained by considering: i) the liposolubility of these substances, expressed as the log *p*, which is much greater for phenanthroline than for the related complex, and ii) the differences in surface structure between Gram-positive and Gram-negative microorganisms. Even more important is the fact that phenanthroline, other than being considerably liposoluble, has been reported to have bactericidal effects on *Bacillus stearothermophillus*, attributed to its iron chelating properties [10].

No significant differential sensitivity to these drugs is shown by *E. coli* polA⁻. This bacterial strain, lacking the DNA repairing capacity [11], has been shown to be much more sensitive than pol A⁺ to a large number of agents known to alter cellular DNA [12], and has been used in order to investigate the occurence of interactions between Rh(I) compounds and bacterial DNA.

As reported in fig. 1, at 1 μg/ml Rh-bipy causes a selective inhibition of nucleic acids synthesis, leaving substantially unaffected the protein synthesis.

It is interesting to compare these data with those already reported for platinum compounds. These substances have different effects on bacterial and tumour growth, depending on their structure. While charged molecules are bactericidal at concentrations similar to those reported above for rhodium complexes [6], they have little or no effects on tumours [13]. On the contrary, the neutral species, which at the same concentrations cause only an elongation of the bacterial cells [6], have powerful antineoplastic properties [7], and induce prophages in lysogenic strains [14].

The charged Rh(I) complexes are similarly bactericidal and do not induce phage production, as shown by the absence of a greater sensitivity of HfrH strain to these substances. A pulse treatment with Rh-bipy for 2, 5, or 10 min, followed by titration of the phages produced, also showed no effect.
A partial elongation (2–3x) in about 80% of the cells is observed only with Rh-bipy at 3 μhr/ml.

As for the possible mechanism of action, the selective inhibition of nucleic acids synthesis caused by Rh-bipy could be attributed to an interaction of the complex with the bacterial nucleic acids. Coordination with platinum has been reported to occur both with purified DNA [15] and DNA of mammalian cells cultivated in vitro in the presence of platinum drugs [16]. In the latter case however only DNA synthesis was selectively and irreversibly inhibited [17,18]. In view of these considerations it is conse-

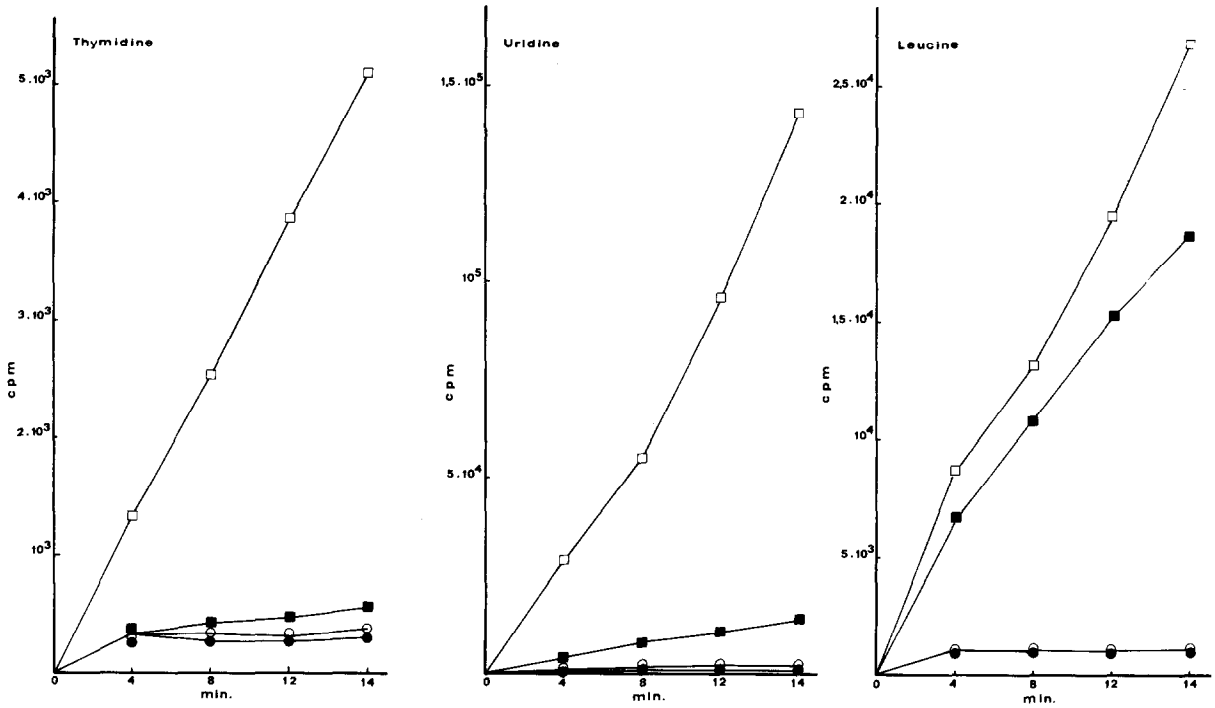

Fig. 1. Effects of rhodium-bipy on macromolecular syntheses in *E. coli* K12 λ^-. The labelled precursors were added 10 min after the addition of the rhodium complex to a final concentration of 0 (□), 1 (■), 3 (○), 10 (●) μg/ml, and the acid insoluble radioactivity determined when indicated.

quently difficult to understand the effects rhodium compounds on bacterial macromolecular metabolism, and the absence of a differential sensitivity between polA$^-$ and polA$^+$ strains, unless interactions more complex than coordination of bacterial DNA occur.

All these data and the preliminary results of screening for antineoplastic activity, seem to encourage a deeper examination of the biological activities of these and related compounds.

Acknowledgements

We wish to thank the C. and D. Callerio Foundation for the award of a fellowship to one of us (M.C.).

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