Effects of Monofunctional Platinum Agents on Bacterial Growth: A Retrospective Study

Timothy C. Johnstone, Sarah M. Alexander, Wei Lin, and Stephen J. Lippard*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States

Ⓢ *Supporting Information*

ABSTRACT: The effect of the novel and potent monofunctional platinum(II) agent phenanthriplatin on *Escherichia coli* and bacteriophage λ lysogens is reported. *E. coli* filamentation was observed by light microscopy when cells were grown in the presence of phenanthriplatin, *cis*-$[Pt(NH_3)_2(Am)Cl]^+$ where Am is phenanthridine. Treatment of lysogenic bacteria with this compound resulted in lysis and the production of viral particles, as indicated by plaque formation in a bacterial lawn. The results obtained with phenanthriplatin are contextualized by comparison with those obtained using cisplatin as well as other, less active, monofunctional compounds such as $[Pt(NH_3)_3Cl]^+$ and *cis*-$[Pt(NH_3)_2(py)Cl]^+$, where py is pyridine. The ability of phenanthriplatin to induce bacterial filamentation and initiate lysis in lysogenic bacteria corroborates the hypothesis that the biological activity of this complex is mediated by its interaction with DNA.

Platinum agents are among the most widely prescribed anticancer chemotherapeutic drugs. Cisplatin (**1**), carboplatin, and oxaliplatin are collectively used to treat approximately half of all cancer patients receiving chemotherapy.[1] Recognition of the biological activity of platinum compounds is a prime example of the role that serendipity can play in scientific discovery.[2] In the present study we first repeated the landmark experiment performed by the Rosenberg group demonstrating that low concentrations of cisplatin inhibit division in bacteria, lead to filamentous growth, and cause induction in lysogenic bacteria.[3] We then investigated whether similar effects were observed using monofunctional complexes (**2** and **3**, see Chart 1), including the novel and potent compound phenanthriplatin (**4**).[4] Remarkably, we discovered that **4**, like **1** but unlike **2** or **3**, is able to slow the division of bacteria, produce a filamentous phenotype, and induce lysis in lysogens. These results indicate that the biological activity of phenanthriplatin is elicited through interaction with DNA. Both

1 and **4** share the same target despite the inability of the latter to form the cross-links that characterize the mechanism of action of **1** and that of the other clinically employed platinum drugs.[5] Until the development of phenanthriplatin,[4] and a few other notable examples,[6] it was unclear whether compounds that could only form monofunctional DNA adducts would have significant chemotherapeutic potential and medicinal inorganic chemistry applications.

From our long history of investigating the chemistry and biochemistry of **1**, we have more recently focused attention on Pt(IV) prodrugs, nanodelivery devices, and monofunctional Pt(II) complexes.[7] Monofunctional compounds are those that have only one labile ligand, the loss of which allows them to form a single bond with a DNA nucleotide. Bifunctional compounds like **1** have two labile ligands and can form cross-links in DNA. Of the monofunctional compounds we have prepared, **4** displays the most promising activity.[4] The impetus to explore nonclassical platinum compounds,[6] such as **4**, arose from interest in overcoming significant drawbacks to the use of platinum drugs.[8] Included are nausea, emesis, peripheral neuropathy, alopecia, hearing loss, nephrotoxicity, and myelosuppression. In many cases, one of these toxicities is dose-limiting. Moreover, tumors can display inherent or acquired resistance to treatment with conventional platinum agents.[9]

In this current comparison of the effects of **1** and **4** on bacteria, two other monofunctional compounds, **2** and **3**, the latter of which is also called pyriplatin,[11] were investigated as well. The effects of **1**−**4** on the rate of bacterial division were investigated by culturing cells in media containing one of the platinum compounds. Specifically, aliquots of a suspension of exponentially dividing K-12 *E. coli* (ATCC 11303) grown in liquid LB were used to inoculate LB containing 15 μM concentrations of one of **1**−**4**. This concentration was chosen because it was sufficient to permit the observation of differences between sample and control groups with a minimal amount of bacterial death (Figure S1 in Supporting Information [SI]). The rate of bacterial growth was assayed by measuring turbidity, OD_{600}, over time (Figure 1, details may be found in the SI). It should be noted that optical density scales as the logarithm of the number of cells in suspension.
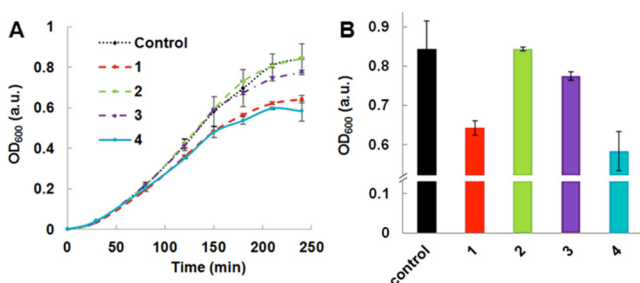
The growth of cells cultured in the presence of **1** is significantly less than that of the control without any platinum. An identical effect was observed using **4**. Compound **2**, on the other hand, had no effect, and **3** elicited an intermediate degree

**Chart 1. Chemical structures of the platinum complexes investigated**
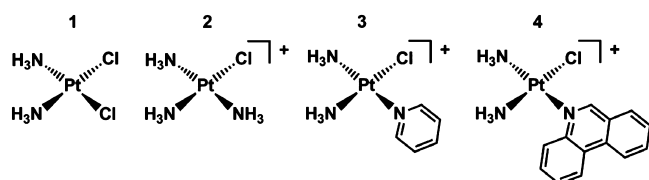
Figure 1. Effect of 15 μM concentrations of **1**−**4** on the growth of *E. coli*. (A) Growth over time. (B) Comparison of growth inhibition at the final time point from (A). Data points represent the average of two experiments. Error bars depict the square root of the variance of the two measurements.

of inhibition. These data are consistent with the general ability of these platinum agents to kill cultured cancer cells: **1** and **4** are potent, **3** is less so, and **2** is essentially inactive.[4,10,11]

The correspondence between bacterial growth inhibition and toxicity in cultured mammalian cancer cells is gratifying but does not reveal the more important similarities that exist between the behaviors of **1** and **4**. The effect of a compound on *E. coli* can also provide mechanistic insight, as was the case with the discovery of the anticancer activity of **1**. While investigating the effect of electric fields on bacterial cell division, the Rosenberg group discovered that addition of certain platinum complexes to the growth medium of bacteria resulted not only in retarded growth, as evidenced by decreased turbidity, but also induction of a filamentous morphology in the cells.[3] The most active compound investigated was **1**, a molecule that had first been described by Peyrone over 120 years earlier.[12] The filamentous growth results from the ability of **1** to inhibit bacterial cell division, but not all other cellular processes.[13]

We therefore investigated the ability of monofunctional compounds **2**−**4**, as well as **1**, to induce bacterial filamentation. Specifically, K-12 *E. coli* cells were cultured as described above in the presence or absence of one of these platinum agents. After 5 h of growth at 37 °C, 2 μL aliquots of each cell culture suspension were mounted on microscope slides and analyzed by light microscopy (details may be found in the SI). Representative results are depicted in Figure 2. The control group displays the morphology expected of *E. coli* cells, short
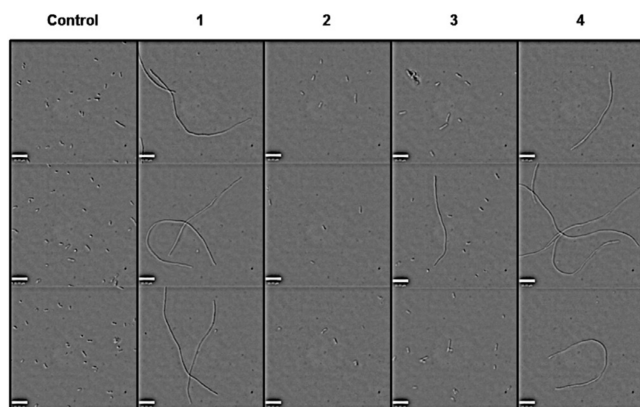

Figure 2. Effect of treatment with 15 μM concentrations of **1**−**4** on the morphology of *E. coli* cells. Scale bars are 10 μm. Images were processed as described in the SI. The raw images acquired from the microscope are shown in Figure S2 of the SI.

rods approximately 2 μm in length. Treatment with **1**, on the other hand, results in filamentous cell morphology and cells more than 100 times longer than those in the control group. No bacteria exhibited a normal length across all fields of view investigated. Compound **2** induced no filamentous growth, producing cells that are indistinguishable from those in the control group. Compound **3** produced a result very similar to that of **2**, but in some fields of view an isolated bacterium had grown into a filament. The lengths obtained by these filaments were comparable to those seen following treatment with **1**. The number of abnormal bacteria observed in the sample grown in the presence of **3**, however, was very low (<1%). Compound **4** elicited a response identical to that of **1**.

To identify the origin of the inhibition of bacterial cell division, the Rosenberg group investigated the effects of **1** on lysogenic bacteria.[2] These lysogens are bacteria infected with a temperate bacteriophage and stably reproduce via the lysogenic cycle. In lysogeny, viral genes are silently duplicated along with genetic material of the host organism during mitosis. The lytic phase, on the other hand, is characterized by production of viral proteins that form the capsid, package the viral genetic material into the capsid, and lyse the cell. Rupture of the cell membrane releases newly formed virions into the extracellular space where they can infect other bacteria.

During the lysogenic cycle of bacteriophage λ, a well-characterized virus that can infect *E. coli*,[14] expression of the proteins that carry out functions characteristic of the lytic cycle is suppressed by the constitutively expressed protein, cI. If the bacterium senses DNA damage, it will initiate the SOS response, in which the protein RecA is activated to form RecA*. RecA* relieves the constitutive inhibition of DNA repair genes carried out by LexA. Bacteriophage λ hijacks this cellular response by virtue of the similarity of the interactions of both cI and LexA with RecA*. When RecA* is produced to interact with LexA, it also interacts with cI, lifting the inhibition of the expression of viral genes needed to enter into the lytic phase.[14] Rosenberg found that **1** could induce lysis in *E. coli* infected with bacteriophage λ.[2] The ultimate cellular target of **1** was unknown at the time. The results obtained with lysogenic bacteria, *inter alia*, indicated that an interaction with DNA could be the origin of the inhibited cell division.

We investigated the ability of **1**−**4** to induce a lytic transformation in *E. coli* W3104, which are infected with bacteriophage λ. In a manner analogous to the earlier experiments using uninfected cells, aliquots of an exponentially growing cell culture of *E. coli* W3104 were used to inoculate LB containing 15 μM concentrations of one of **1**−**4** (details may be found in the SI). After 5 h of incubation at 37 °C, an aliquot of each suspension was diluted 1000-fold. A 10 μL drop of this diluted suspension was then spotted onto a lawn of nonlysogenic *E. coli* (ATCC 11303) grown in top agar (Figure 3). The formation of a plaque indicates the presence of viral particles in the cell suspension that arise from the induction of lytic phage reproduction. Plaques were only observed for cells treated with **1** and **4**. As controls, a spot of a 15 nM solution of each platinum complex was also applied to the plate, as was a spot of *E. coli* W3104 cells not treated with platinum (details may be found in the SI). In none of these controls were any plaques observed, indicating that the plaques described previously do not arise from any residual platinum in the solution nor any background production of viral particles by the cells in the absence of platinum treatment.
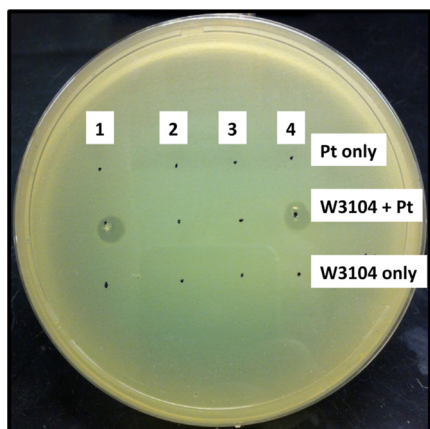
Figure 3. The development of plaques in a lawn of *E. coli* (ATCC 11303) following application of 10 μL drops of 1000-fold diluted suspensions of *E. coli* W3104 cells that had been treated with 15 μM concentrations of **1**−**4** (center). Control rows (top and bottom) were spotted with solutions containing only the corresponding platinum complex, 15 nM, or a 1000-fold diluted suspension of cells not treated with platinum.

The impact that **1** has had on the field of medicine resulted from Rosenberg's deduction that inhibition of bacterial cell division may imply an ability to inhibit the division of cancer cells. In 1969, a few short years after the publication of the effects of cisplatin in bacteria, it was reported that **1** could reverse the growth of tumors in mice,[15] and 9 years later the compound was approved for the treatment of genitourinary tumors.

Early studies with bacteria, cultured mammalian cells, and murine tumor models indicated that monofunctional compounds, such as **2**, did not show any desirable biological activity.[16] The inability of monofunctional compounds to form cross-links was thought to prevent them from having anticancer activity of the type observed for **1**. In 1989, however, Hollis, Amundsen, and Stern published a study indicating that monofunctional compounds of the form *cis*-[Pt(NH$_3$)$_2$(Am)-Cl]$^+$, where Am is an N-heterocyclic amine, displayed varied degrees of anticancer activity.[17] As previously described,[7,18] our group rediscovered the activity of **3**, where Am is pyridine.[11] The activity was further improved by replacing the pyridine ring with phenanthridine to generate **4**.[4]

The studies described here reveal that the unprecedented potency of the monofunctional complex **4** is manifest not only in mammalian cells, as previously reported,[4] but also in bacterial cells. Importantly, **4** induces formation of filamentous cellular morphology in *E. coli*. This phenotype indicates that treatment with **4** can inhibit the process of cell division. The hypothesis that **4** exerts a biological effect through DNA damage, as in the case of **1**, was corroborated by induction in lysogens. The Rosenberg group found that induction of filamentation in normal *E. coli* and induction of lysis in lysogenic bacteria were two indicators that a molecule could act as an effective anticancer agent;[2] **4** satisfies both of these conditions. Experiments are underway to investigate the details of phenanthriplatin−DNA interactions.

ASSOCIATED CONTENT

ⓈSupporting Information

Experimental details, dependence of growth inhibition on concentration of **4**, raw microscope images, NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
lippard@mit.edu

Notes
The authors declare the following competing financial interest(s): S.J.L. declares a financial interest in Blend Therapeutics.

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