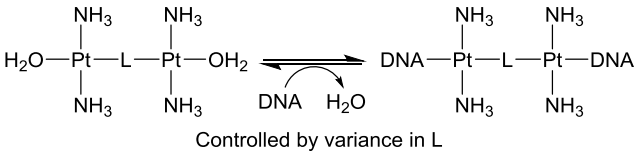
Ligand Substitution in Novel Platin Cancer Therapies

Eli Mlaver, Amherst College, 2014, Fall 2013 CHEM 371

Ligand Substitution, Cisplatin, Platin Drugs, Drug Design

ABSTRACT: Developing novel platin cancer therapies, Soldatovic *et al*[1] synthesize and characterize reactions of three dinuclear azole-bridged Pt(II) complexes with three biologically relevant ligands to learn more about chemo-protection and platinum metabolism. This research relies on the core concept of ligand substitution— a process by which organometallic compounds exchange their bound substituents—discussion of which will shed light on the mechanism of platin drugs and their design. The authors explore how factors such as solvent acidity, metal complex electrophilicity, and nucleophilic strength contribute to the rates of such reactions. Results indicate that the bridging ligand connecting the two platinum centers greatly influences the reactivity of the dinuclear complexes towards nucleophiles, speaking to potential drug activity.



Introduction

Cisplatin is one of the most potent antitumor agents available on the market. Its cytotoxic mechanism of action is mediated by its interaction with DNA nucleosides to form intrastrand crosslink adducts, which in turn calls for apoptosis.[2] As shown in Figure 1, intrastrand crosslinking occurs when an agent covalently bonds to two different positions on one strand of DNA, which most commonly occurs at two adjacent guanine bases.[3]

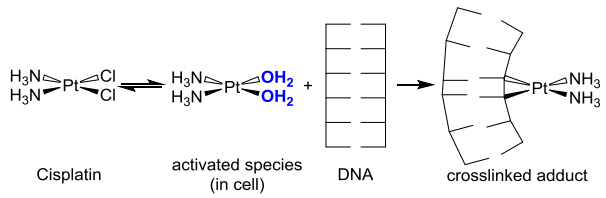


Fig. 1 General mechanism of action for cisplatin and other alkylating agents. After entering the cell the drug is activated through aquation, and the activated species then undergoes a second ligand substitution reaction with DNA, contorting the DNA. This covalent adduct cannot undergo replication.

Like in mustard gas derivatives, crosslinks block DNA replication, which causes cell death if the crosslink is not repaired. Unfortunately, negative side effects such as vomiting, resistance (attenuation of apoptosis signals[2]), and various potentially lethal toxicities have inspired researchers to design new classes of platinum complexes to ameliorate these side effects while improving anti-tumor properties. In fact, since cisplatin's FDA approval in 1978, a myriad of second-generation platin drugs (carboplatin, oxaliplatin, *et al.*) have been implemented for cancer treatment.

Researchers now aim to develop third generation platin drugs that incorporate new mechanisms in order to minimize side effects and maximize efficacy. Sterically hindered, sulfur containing , and poly-nuclear Pt(II) complexes are being developed for Phase 1 trials as are orally active Pt(IV) prodrug complexes which would naturally be reduced during metabolism.[4] Polynuclear Pt(II) complexes are of specific interest due to their ability to form DNA adducts that differ from those formed by cisplatin in that they can stretch further across the DNA strand. This could yield the capability to attach to the major groove, a new target that could be utilized for advanced specificity.[5] Others like Soldatovic suggest that dinuclear compounds also have the potential to be more customized for specificity and control. Activity of such complexes has been shown in vitro in cisplatin-resistant cell lines, and previous research asserts that azole bridged compounds (like those investigated by Soldatovic) can provide the 1,2-intrastrand cross-links needed to block replication even when cisplatin therapies have failed.[3]

As a main structural feature, the azole-bridged complexes possess a leaving hydroxo group for ligand substitution as well as the appropriate Pt—Pt distance and flexibility to provide the 1,2-intrastrand cross-links with a minimal distortion of the DNA.[1] This flexibility speaks to more controllable anti-tumor activity[5], and would open the door for more research in that control. Thus, the understanding of the chemical transformations of dinuclear Pt(II) complexes with biologically relevant nucleophiles under physiological conditions is of special concern for pharmaceutical and biomedical research.

This mini-review highlights the efforts by Soldatovic to shed light on the mechanism of the interactions of dinuclear platinum anti-tumor complexes with N- and S-donor ligands that are representative of the targets and competition of platin drugs, respectively. Through the work described herein the authors' investigations provide insight into the mechanism of chemo-protection and platinum metabolism.

Ligand Substitution

The control of ligand substitution is inherent in all platin drug mechanisms of action. For example, all platin drugs

enter the body as chlorinated species, and the Cl—H_2O exchange is a ligand substitution reaction controlled by their relative concentrations. Complexes maintain their Cl ligands in blood where ion concentration is high (~100 mM). Having entered the cell, the complexes undergo aquation due to a much lower intracellular Cl^- concentration (3-20 mM).[7]

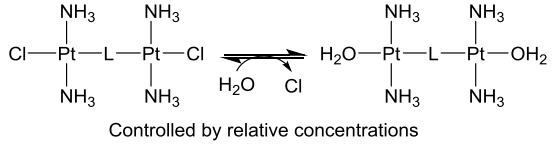


Fig. 2 Proposed mechanism of a dinuclear platin drug in vivo. As with all platin drugs, the replacement of Cl with H_2O occurs naturally as the prodrug passes from the bloodstream into the target cell where concentration of chloride ion drops dramatically.

This is a prime example of concentration-controlled ligand substitution, but many other factors contribute to both the thermodynamics and kinetics of this mechanism. The work in Soldatovic provides draws heavily on this key concept of Inorganic Chemistry, and highlights important practical applications.

The thermodynamics of substitution depend on the relative strength of the two metal-ligand bonds, and the stability of the departing and incoming ligands. The kinetics of such a reaction are affected by a large variety of factors including relative reactivites and concentrations of ligand, solvent, temperature, and pH.

The mechanism of substituting one ligand for another on a metal center sits somewhere on a spectrum of associative to dissociative addition. At the associative extreme, analogous to SN_2, the incoming ligand first forms a bond to the metal, then the departing ligand takes its lone pair and leaves. At the dissociative extreme, the order of events is opposite—the departing ligand leaves, then the incoming ligand comes in, analogous to SN_1.

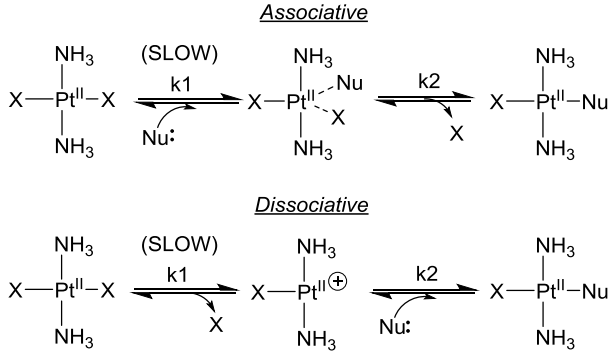


Fig. 3 Comparison between two theoretical pathways for ligand substitution. Note that as the first step is rate-determining in both cases, the rate of the dissociative mechanism is determinant only on the concentration of the platin drug, whereas the rate of the associative process is determinant on the concentration of both the platin drug and the attacking nucleophile.

The rate determining step determines the associative or dissociative nature of the overall substitution. Following the reaction mechanisms in Figure 3, the reaction rate for dissociative ligand substitution is dependent only on complex concentration:

$$\text{rate} = k1[PtX]$$

while the reaction rate for an associative ligand substitution is dependent on both complex and nucleophile concentration:

$$\text{rate} = k1[PtX][Nu]$$

Although dissociative is most common, the likelihood of an associative path increases for reactions involving (1) addition of highly nucleophilic ligands and (2) reactants containing metals of larger atomic radii that can more readily expand their coordination sphere.[8] Citing past research which analyzes the electronic configuration of Pt(II), Soldatovic assumes that the cisplatin reaction is associative, asserting that "the significantly negative activation entropies suggest that the activation process [is] strongly dominated by bond making." In order to avoid the complication of manipulating both concentrations, reactions are run under "*pseudo*-first order conditions" in which the nucleophile concentration is more than ten times that of the platin complex and thus will not vary through reaction.

Key Experiments and Data[1]

Soldatovic begins by synthesizing three novel dinuclear Pt(II) complexes (**Pt1**, **Pt2**, **Pt3**), differing only in the bridging ligand as shown in Figure 4. Spectral data was then collected and analyzed to determine the ligand substitution activities of the three complexes with "bio-relevant" nucleophiles.

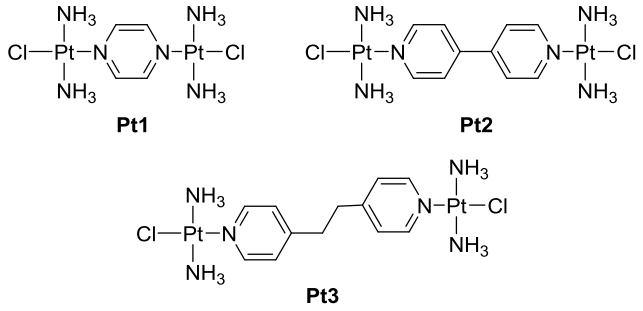


Fig. 4 Three dinuclear platinum complexes, differing only in bridging ligand. Note that the overall charge of each complex is 2^+, so Soldatovic reports each (Figure 1) as a salt with $(ClO_4)_2$

Three nucleophiles were chosen as representatives of the targets and competitors of platins in the cellular environment: The first, thiourea (Tu) is a strong sulfur-containing nucleophile with a high solubility which acts as a good model competitor[6]; notably Tu has been used clinically as a protecting agent to minimize nephrotoxicity following cisplatin treatment. Glutathione (GSH) is a reducing agent present in the cell, and has both nitrogen and sulfur centers to act as nucleophiles within its tripepetide structure. And guanosine 5'-monophosphate (5'-GMP) is used as a model for binding to nucleobases, which is the actual target of platin drugs.

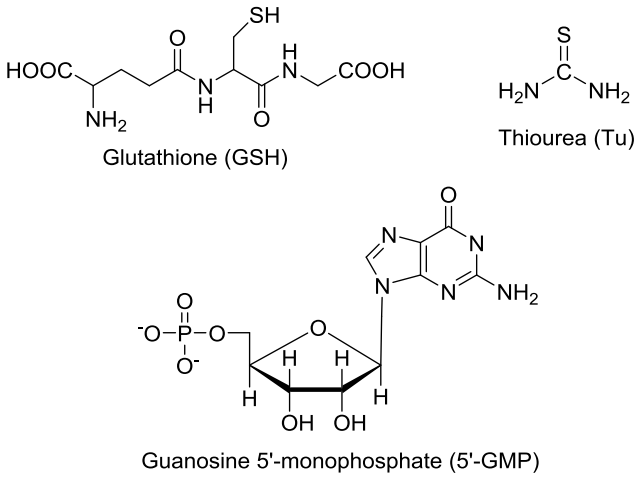


Fig. 5 Structures of the investigated nucleophiles. Thiourea is used as a good model nucleophile to bind to the platin drug. GSH is a tripeptide present in the cell. 5'-GMP is a model nucleobase.

Knowing the aquation mechanism (discussed above) would ensue *in vivo* and so is vital to a pre-pharmaceutical exploration, Soldatovic synthesized diaqua analogues of each complex by addition of $AgClO_4$ to solution of the chloro complexes. Since pharmaceutical researchers are interested in the eventual in vivo substitution of the water ligand, and not a deprotonated –OH ligand, the authors needed to find the pH at which their compounds would stay protonated. In order to determine the pKa, diaqua complex solutions were titrated with NaOH and $HClO_4$ within the pH range of 2-9. The spectral data was fit to an equation relating absorbance before, during, and after titration in order to identify pKa. The pKas found ranged from 3.94-5.69, with the general trends of **Pt1** having lower pKas than the other two and the pKa of the first ligand deprotonation being lower by about 1.0 than that of the second H_2O ligand. The latter trend is discussed at more length later, but is mainly attributed to the overall charge of the complex dropping from 4+ to 3+ making the second platinum center less electrophilic. From this data, the authors conclude to handle the aqua complexes at pH 2.5, while the chloro complexes are examined at physiological pH (7.2). The authors go on to perform spectral kinetic measurements on the complexes.

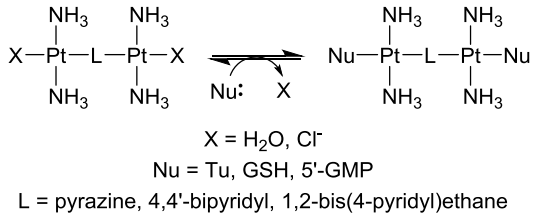


Fig. 6 Experimental design in Soldatovic. Note that variables that might contribute to ligand substitution rates include pH (experiments run at pH 2.5 for diaqua and pH 7.2 for chlorinated species), leaving nucleophile (X), incoming nucleophile (Nu), and-- the true focus of Soldatovic-- the size and nature of the bridging ligand between the Pt(II) centers.

The substitution reactions of all the studied complexes proceeded in two subsequent steps that both depended on nucleophile concentration. The *pseudo*-first order rate constants, k_{obsd1} and k_{obsd2}, calculated from the kinetic traces fit the "typical" two-step reaction scheme in which each metal center acts independently of the other. The rate constants were plotted against concentration of nucleophile, and showed linear 1st-order dependence in all cases. In general, the linear fit passes through the origin, signifying that reverse reactions are insignificant.

As discussed, the diaqua complexes were studied at pH 2.5 to avoid deptrotonation. Reactions with Tu for all three platins turned out to be too fast to analyze, attributed to the strong S-donor character of the ligand. The other two nucleophiles were shown to be less reactive, showing two distinct reaction steps. In general, **Pt1** reacted 2 orders of magnitude faster than **Pt2** and **Pt3**. The higher lability of **Pt1** is ascribed to the pi-acceptor ability of the pyrazine ligand, which causes a decrease in electron density on the platinum center. Soldatovic notes that the second step for all three ligands is slower, and cites not only the decrease in metal charge as was witnessed in the pKa determination, but also introduces steric hindrance as a secondary contributor to rate reduction in the second substitution. After all, GSH is longer than the platin it is binding to, and might well hinder the binding of a second triepeptide.

Analysis of the dichloro complexes at physiological pH yielded similar patterns in much less extreme data than their diaqua counterparts (two orders of magnitude). This is in part due to the strong Pt—Cl bond and the lower electrophilicty of the overall complex due to the negative charge of Cl ligand. Again, the authors find that the first substitution step is 1-2 orders of magnitude faster than the second. Again, the reactivity of the complexes is **Pt1 > Pt2 > Pt3.**

Analysis of the data brings to light that ligand substitution rates vary based on distance between two platinum centers, and as such speak to stability of nucleophilic attack. Further, the authors find that each of the two nucleophile substitutions is its own independent step. The platinum centers in the study are thermodynamically and kinetically independent of each other, and for all the complexes two distinct rates were observed. In every case, the second substitution is slower than the first. The results show that the bridging ligand has an important influence on the reactivity of the platinum complexes, and specifically that the shorter the distance between the two Pt(II) centers the more electrophilic and acidic that center becomes.

Discussion

Many different factors could potentially affect the rate of ligand substitution. Soldatovic looks at the effect of changing electrophilicity at the platinum centers on ligand exchange activity. But Soldatovic also highlights some key questions that can be raised about this basic concept of inorganic chemistry.

Ground state thermodynamics provide a complementary explanation to the "distance effect" of the varying rates of

the three compounds. The *trans* effect is a phenomenon witnessed in octahedral and square planar complexes in which electron-rich ligands can direct substitution across the metal center due to their electron density pull. This effect can be quite strong for strong field ligands. Soldatovic, for examples, attributes the magnitudinal disparity in rates among the nucleophiles to the *trans* effect of the donating sulfur of thiourea.

Soldatovic asserts that the two centers are independent because they were able to identify distinct pKas. In the pKa determination, the slower rate of the second aqua ligand deprotonation is attributed to the fact that the first deprotonation lowers the overall charge of the complex from 4^+ to 3^+, decreasing the electrophilicity and acidity of the second platinum center. Parallel arguments are made in the rate-determination data. But it is notable that the change between each step's pKa ($pKa_1 - pKa_2$) is markedly lower for **Pt3** (-.92) than it is for **Pt1** (-1.06) or **Pt2** (-1.09). This means that the effect of manipulating one metal center on the other is smaller in non-aromatic species, and bridging ligand size difference cannot explain this discrepancy because **Pt2** actually has a slightly larger change than **Pt1**. The *trans* effect *would* seem to account for the data, especially the order of magnitude difference in the chloro substitutions. Rates of substitution on **Pt2** were faster than **Pt3** in both the aqua and chloro measurements, as **Pt3** is the only complex of the three that does not have a continuous pi-network between the platinum centers. The aromatic network connecting the two metal centers in **Pt1** and **Pt2** might allow the ligand substitution on one center to direct a second ligand to the other center. One way to explore this further would be to run similar testing on a compound with a longer aromatic bridge, such as the one proposed in Figure 7:

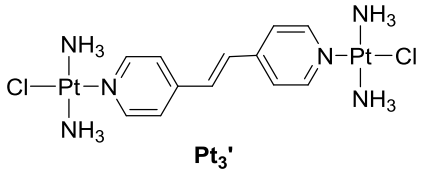


Fig. 7 Proposed dinuclear Pt(II) complex. This complex only differs from Pt3 in the double bond. This double bond extends the pi network, allowing for aromaticity, but should not change the overall structure of Pt3. If Soldatovic's conclusion that only ligand length determines activity, this should yield the same results s Pt3 with similar testing.

Considering the assertion that distance is the only factor, this fourth compound should yield data quite similar to that of **Pt3**. But if the aromaticty of the new compound does have an effect, we can expect data more similar to **Pt2**. Should the *trans* effect be identified as a more important factor than originally projected, this might be more strongly considered in future searches for third generation platin drugs.

One final challenge is that the authors do not consider physiologically relevant concentrations or possible metabolism of these dinuclear centers. In fact, in order to get *pseudo*-first order reaction rates each nucleophile is provided at ten times the concentration of the platinum complex. In order for this work to be more pharmaceutically significant, further studies will need to to investigate reaction rates not just at physiological pH but at physiological concentrations as well. Looking on to future explorations that might have pharmaceutical significance, it is easy to imagine a non-symmetrical diplatinum complex which might be more electrophilic on one end than the other, a characteristic that could not exist in contemporary platin drugs with only one metal center. Although this doesn't seem to be a target of current research, this might prove to control toxicities resultant from platin drugs.

Conclusion

The authors are able to identify the most active of the three novel platins. The relative activities are a result of variety of bridging ligands, defining the distance most favorable to create the DNA cross-links necessary for drug activity. This was a successful application of ligand substitution theory, and marks the first step towards a pharmaceutical improvement of anti-tumor treatments.

ABBREVIATIONS

Pt1, [{*trans*-$Pt(NH_3)_2Cl\}_2$(m-pyrazine)]$(ClO_4)_2$; **Pt2**, [{*trans*-$Pt(NH_3)_2Cl\}_2$(m-4,4'-bipyridyl)]$(ClO_4)_2$·DMF; **Pt3**, [{*trans*-$Pt(NH_3)_2Cl\}_2$(m-1,2-bis(4-pyridyl)ethane)] $(ClO_4)_2$; Pt(II), platinum with oxidation state 2+; Nu, nucleophile (such as Tu, GSH, 5'-GMP); Tu, thiourea; GSH, glutathione; 5'-GMP, guanosine 5'-monophosphate

AUTHOR INFORMATION

Contact: emlaver14@amherst.edu

REFERENCES

(1) Soldatović, T.; Jovanović, S.; Bugarčić, Ž. D.; van Eldik, R. Substitution Behaviour of Novel Dinuclear Pt(II) Complexes with Bio-Relevant Nucleophiles. *Dalton T*. **2012**, *41*, 876–884.

(2) Siddik, Z. H. Cisplatin: Mode of Cytotoxic Action and Molecular Basis of Resistance. *Oncogene* **2003**, *22*, 7265–7279.

(3) Poklar, N.; Pilch, D. S.; Lippard, S. J.; Redding, E. A.; Dunham, S. U.; Breslauer, K. J. Influence of Cisplatin Intrastrand Crosslinking on the Conformation, Thermal Stability, and Energetics of a 20-Mer DNA Duplex. *P*. *Natl*. *Acad*. *Sci*. *U*. *S*. *A*. **1996**, *93*, 7606–7611.

(4) Varbanov, H. P.; Valiahdi, S. M.; Kowol, C. R.; Jakupec, M. A.; Galanski, M.; Keppler, B. K. Novel tetracarboxylatoplatinum(IV) Complexes as Carboplatin Prodrugs. *Dalton T*. **2012**, *41*, 14404–14415.

(5) Brabec, V.; Kaspárková, J.; Vrána, O.; Nováková, O.; Cox, J. W.; Qu, Y.; Farrell, N. DNA Modifications by a Novel Bifunctional Trinuclear Platinum Phase I Anticancer Agent. *Biochemistry* **1999**, *38*, 6781–6790.

(6) Pil, P.; Lippard, S. J. Specific Binding of Chromosomal Protein HMG1 to DNA Damaged by the Anticancer Drug Cisplatin. *Science* **1992**, *256*, 234–236.

(7) Soldatović, T.; Bugarčić, Ž. D.; van Eldik, R. Influence of the Chloride Concentration on Ligand Substitution Reactions of [Pt(SMC)Cl2] with Biologically Relevant Nucleophiles. *Dalton T*. **2009**, 4526.

(8) Miessler, G. L. *Inorganic Chemistry*; Fifth edition.; Pearson: Boston, 2014.