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Biological relevance of interaction of platinum drugs with *O*-donor ligands

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Abstract

Platinum complexes with S and N-donor small molecule ligands have received much attention with respect to understanding of Pt-protein and Pt-DNA(RNA) interactions in biology. Oxygen-donor ligands have received less attention, partly due to the fact that as a hard Lewis base, oxygen-donor interactions are expected to be less favourable for the soft Lewis acid properties of Pt(II), especially. Yet, it is now clear that for a full understanding of the cellular fate of platinum complexes, a plethora of oxygen-donor interactions are possible, considering extracellular and intracellular concentrations of simple anions in buffer. Further, the importance of the general class of glycans, the third major class of biomolecules after proteins and nucleic acids, contain many specific examples of important biomolecules such as sialic acids and sulphated glycosaminoglycans capable of metal complex interactions. In this contribution we summarise some important kinetic and thermodynamic aspects of platinum-oxygen-donor ligand interactions and their relevance to examples of biomolecular interactions contributing to the overall profile of platinum (and metal complexes in general) biology.

1. Introduction.

The molecular mechanism of the anticancer activity of platinum drugs has focused on their interactions with DNA as target. Metabolic interactions with proteins, especially Human Serum Albumin, and small peptides such as glutathione are considered deactivating. The vast majority of discussion on cellular formation of cisplatin-DNA adducts focusses on the necessity to produce monoaqua or diaqua species upon intracellular aquation of the Pt-Cl bonds or the *O*, *O*-dicarboxylates of carboplatin and oxaliplatin. Synthesis of generic [PtN₂O₂] donor sets has been used extensively to produce water-soluble species with reduced substitution lability compared to Pt-Cl bonds. However, weak interactions with endogenous oxygen donors are not considered to be essential to cisplatin activity. As a soft acid Pt is expected to form covalent bonds easily with *S* and/or *N*-donors and the detailed pathways for interaction with nucleic acids and proteins are by now well documented. Interactions with *O*-donor groups have not been explored to the same extent – in part because monodentate *O*-donor groups are considered as substitution-labile ligands in the bioinorganic chemistry of Pt(II) [1].

Depending on extracellular and intracellular concentrations these weakly coordinating anions – carboxylate, carbonate, phosphate and sulfate - should be considered as potential ligands in the cellular and biological milieu. While these interactions emphasize the *O*-donor groups as individual ligands, in recent work, we have suggested that the *O*-donor polyanionic biomolecules may be receptors and possible targets in their own right for platinum complexes and indeed for coordination compounds in general [2]. *O*-donor polyanionic biomolecules include phospholipids, and the large and diverse array of glycans including heparan and chondroitin sulfates, and the non-sulfated hyaluronic and sialic acids. This review covers the fundamentals of binding of mononuclear and polynuclear platinum complexes (Fig. 1) with individual *O*-donors and specifically how these fundamental chemical and biophysical studies can inform the fate of platinum-based anticancer agents in the biological milieu. We will not

consider in detail “preformed” complexes with the *cis*-[PtN₂O₂] chemotype and we will limit the many examples of N,O or even S,O chelates formed upon reaction of small platinum molecules with amino acids and polypeptides, or even the “pyrimidine blues” to illustrative examples.

<Insert Figure 1 here>

2. Reaction of mononuclear platinum anticancer complexes with *O*-donor ligands: buffer components and amino acids.

Early seminal multinuclear (¹⁹⁵Pt, ¹⁵N, ³¹P) NMR studies by Appleton characterized the products of reactions in aqueous solutions between the diaqua form of cisplatin (*cis*-[Pt(NH₃)₂(OH₂)₂](ClO₄)₂) and several weak anions including phosphate, acetate and sulfate [3]. The work highlighted that significant concentrations of phosphato and acetato complexes could be present in buffer solutions containing these anions, which could affect the interpretation of kinetic results [3].

The introduction of 2D [¹H,¹⁵N] NMR techniques in the early 1990s made it possible to follow the reactions of cisplatin in aqueous solution at much lower (physiologically relevant) concentrations [4, 5]. The aquation of ¹⁵N-cisplatin was monitored in the presence of a 4.5 fold excess of phosphate (pH 5.9) [6]. Whilst the initial rate of aquation was not affected by the presence of phosphate, more than seven aquated and phosphate-bound species were present after 24 h of reaction. All major phosphate-bound products were attributed to binding to the diaqua species *cis*-[Pt(NH₃)₂(OH₂)₂]²⁺ and binding of phosphate to the monoaqua species was not significant [6]. Pyrophosphate compounds with a *cis*-[PtN₂O₂] coordination sphere as exemplified by phosphaplatin have found use as potential anticancer agents [7].

2D [¹H,¹⁵N] NMR spectroscopy also revealed interesting reactions of cisplatin with carbonate [8]. In the presence of 5 mM HCO₃⁻, the long lived complex *cis*-[PtCl(CO₃)(NH₃)₂]⁻ is formed, which reacts further to form the dicarbonato adduct [Pt(CO₃)₂(NH₃)₂]²⁻.

Biotransformation reactions in the presence of physiological concentrations of carbonate have been observed even for the substitution-inert carboplatin, including ring-opening of the dicarboxylate (CBDCA) ligand [9-11]. Since bicarbonate is present in blood at high concentrations (~25 mM), and these transformation reactions have been observed in cells [12], formation of carbonato-platinum intermediates may have a role in the cellular accumulation, cytotoxicity, and even resistance mechanisms of mononuclear platinum complexes [8, 10, 13]. Studies in RPMI-1640 cell culture medium showed that bicarbonate may also play a role in displacement of the bidentate malonate ligand in [Pt(malonato)(dach)] (dach = *trans*-1,2-diaminocyclohexane) [14].

The oxygen atoms of protein side chains and individual amino acids are also potential donor atoms for platinum, but a full description is beyond the remit of this article [15, 16]. ESI-Mass Spectrometry has helped elucidate donor atom preferences in amino acids, where generally there is a preference for α -NH₂ over the α -COOH groups [15]. Appleton used ¹⁹⁵Pt and ¹⁵N NMR to study the reaction of cisplatin with a series of amino acids ⁺NH₃(CH₂)_nCOO⁻, with variable chain length: n = 1, (glycine), n = 2 (β -alanine) and n = 3 (γ -aminobutyric acid) [17]. Initially glycine forms an *O*-bound complex with cisplatin, which ring closes to form an *N,O* chelated species, which is thermodynamically more stable. As the chain length increases ring closure becomes more difficult. Similar chemistry is seen for serine where the interchange between the *cis/trans* [Pt(*N,O*)₂] isomers can be monitored by ¹⁹⁵Pt NMR spectroscopy [18]. Even in the presence of strong S donors, Pt-O species may be transient reaction intermediates in reactions. *S, O- versus S, N*-chelation in the reaction of *cis*-[Pt(NH₃)₂(H₂O)₂]²⁺ with ¹⁵N-labelled S-methyl-L-cysteine and L-methionine is pH dependent and under acidic conditions the *S,O* chelate transforms to the *S,N* form [19]. Finally, monitoring of the intracellular biotransformation of the [Pt(malonate)(dach)] compound in L1210 cells showed the presence of minor amounts of amino acid-bound platinated species with *O*-bound aspartate and

glutamate, in addition to those from expected *S*-donor metabolites (methionine, cysteine and glutathione) [20].

3. Kinetics of Pt-Carboxylate Substitution. The *trans* effect of carboxylate.

To study the variation of electronic properties by changes in ^{195}Pt and ^{15}N chemical shifts Appleton *et al.* prepared $\text{cis-}[\text{PtX}_2(\text{NH}_3)_2]$ compounds with $\text{X}_2 = (\text{OAc}^-)_2$, PO_4H^{2-} or SO_4^{2-} [3]. Interestingly, the sulfate species was the only one that did not show blue or green colouration on exposure of the solid to air or light. The aqueous behaviour of the acetate system is quite complex with $\text{cis-}[\text{Pt}(\text{OAc})_2(\text{NH}_3)_2]$ slowly coming to equilibrium with $\text{cis-}[\text{Pt}(\text{OAc})(\text{NH}_3)_2(\text{H}_2\text{O})]$ and free acetate. The kinetic and thermodynamic properties of substitution of the $\text{Pt-O}_2\text{CR}$ bond by nucleophiles are consistent with these observations [21, 22]. In $[\text{Pt}(\text{RCO}_2)(\text{dien})]^+$ ($\text{R} = \text{CH}_2\text{Cl}$, CHCl_2) substitution reactions are catalysed by acid by way of a pre-equilibrium protonation of the carboxylate ligand [21]. The displacement followed a normal linear relationship between $\log k$ and the nucleophilicity of the entering group. In the absence of added acid the cationic monocarboxylato complex has a normal nucleophilic discrimination similar to the analogous chloro complex [22]. For $\text{cis-}[\text{Pt}(\text{ClCH}_2\text{COO})_2(\text{Pr}^i\text{NH}_2)_2]$ the nucleophile-independent pathway dominates the substitution by the weak nucleophile, Cl^- , and a relatively strong nucleophile, such as SCN^- , is required to make the direct substitution pathway important [22]. An explanation for this observation is the loss of one carboxylate ligand in the bis(substituted) compound and formation of an intermediate aqua or even chelated monocarboxylate intermediate [22]. The crystal and molecular structure of $[\text{Pt}(\text{OAc})_2(\text{dach})]$ showed average Pt-N and Pt-O distances of 2.00(3) and 2.02(3) Å, with the similarities in bond lengths also reflecting the weak *trans* influence of N donors [23]. The small N-Pt-N angle of $85(1)^\circ$ is explained by the small bite of the chelate whereas the equally small O-Pt-O angle of $85(1)^\circ$ is attributed to stacking effects. In summary, the results suggest that,

overall and in the absence of a strong nucleophile, Pt-O₂CR species may be more long-lived in biological medium than initially thought.

An interesting example of taking advantage of a fundamental property of *O*-donor carboxylates, its weak *trans* effect, can be seen in transplatinum chemistry. A fundamental mainstay of the original structure-activity relationships on the anticancer activity of platinum complexes is that the *cis* geometry is active while the *trans* geometry is tumour inactive. When one or both of the NH₃ groups in *trans*-[PtCl₂(NH₃)₂] are changed to a sterically hindered planar amine ligand, the compounds exhibit micromolar cytotoxicity equivalent to that of cisplatin and retain cytotoxicity toward resistant cells [24, 25]. Possible explanation for this retained cytotoxicity is associated with the differences in DNA binding profile exhibited by the *trans*-platinum compounds [26, 27]. To further optimize the pharmacological properties of the *trans*-platinum planar amine (TPA) compounds, the chloride leaving group can be replaced with carboxylate leaving groups, which enhances aqueous solubility and resistance to aquation while retaining the cytotoxicity of the parent chloride compounds (Fig. 2) [28, 29]. Replacing one or two NH₃ ligands with an aromatic or even aliphatic amines diminishes the aqueous solubility and use of *O*-donors negates this effect but more importantly makes the compounds significantly less substitution-labile in general. The fundamental reason behind this effect lies in fundamental aspects of platinum kinetics. The mutual *trans* effect of two *trans*-chloride ligands means that the *trans* geometry in general is considered more reactive than the *cis* isomer - indeed enhanced metabolic deactivation of the *trans* isomer is a contributing factor to its lack of meaningful antitumour activity. In the case of two mutually *trans*-O-carboxylates however, the inherently weak *trans* effect means there is no strong driving force for substitution and the complexes become kinetically similar to the *cis* isomer, even with respect to simple aquation (Table 1). While detailed substitution reactions with standard nucleophiles have not been carried out in detail on these systems, their resistance to degradation in plasma and lower

reactivity with methionine in comparison to the parent dichlorides further confirm the creation of substitution-inert species using the *trans*-carboxylate axis [30]. The reactivity can be modulated by changing the nature of the carboxylate group.

<Inert Figure 2 and Table 1 here>

4. Polynuclear platinum complexes (PPCs). Aquation studies in the presence of phosphate, acetate and sulfate.

Polynuclear platinum complexes (PPCs) represent a broad discrete structural class of clinically relevant Pt drugs where one or more {Pt(amine)} coordination units are linked through flexible diamine chains (Fig. 1) [31]. The leaving groups can be monodentate for a {Pt(triam(m)ine)} coordination sphere or bidentate for a {Pt(am(m)ine)₂} group [31]. The wide array of structures have good antitumour activity and Triplatin (BBR3464) is the only “non-classical” platinum-based anticancer drug to have entered human Phase II clinical trials [31]. The chemistry has been extensively studied [4, 31]. In this review we will emphasize the structures with [PtCl(am(m)ine)₃] coordination spheres, which can be considered analogous to mononuclear [PtCl(dien)]⁺ and the steric and electronic effects of the linker can be examined in comparison to mononuclear species.

2D [¹H·¹⁵N] HSQC NMR methods have been used to compare the aquation profiles of Triplatin (1,0,1/*t,t,t*) [32] with the dinuclear analog (1,1/*t,t*) both in the absence [33] and presence of phosphate buffer [34]. For both PPCs equilibrium conditions are established in the presence of phosphate far more rapidly than for cisplatin [6], due to a higher aquation rate constant, keeping the equilibrium more to the chloride form. In the case of 1,0,1/*t,t,t*, the equilibrium constant for the aquation (*pK*₁) is lower than for the dinuclear compound, attributed to the presence of the charged central {PtN₄}²⁺ linker which engages in non-productive ion pairing with chloride ions, resulting in a reduction in magnitude of the aquation rate constant [32]. No slowing of the initial aquation was observed in the presence of 15 mM phosphate,

but reversible reaction between aquated species and phosphate was observed. Whilst for both complexes equilibrium in the presence of phosphate was attained after a similar time (12 h), a greater percentage of phosphate species were present for the trinuclear (*ca.* 50%) compared to the dinuclear (22%) compound [34]. Comparison of the rate constants for these aquation reactions in phosphate (Table 2) shows that the rate constant for phosphate displacement of the aqua ligand (k_L) is 2-fold higher for the trinuclear complex, but the rate constants for the reverse reaction (k_{-L}) are similar.

< Insert Table 2 and Scheme 1 here >

For the dinuclear 1,1/*t,t*, aquation reactions been studied by [¹H, ¹⁵N] NMR methods in the presence of acetate, phosphate [34] and sulfate [35] under similar conditions, revealing interesting differences in the reactions with these three physiologically relevant weak anions. The rate and equilibrium constants are compared in Table 2. Firstly, in comparing acetate and phosphate, while the rate constants for displacement of the aqua ligand (k_L) are quite similar, the rate constant for the reverse reaction (k_{-L}) is close to 10-fold higher in the case of phosphate. The high lability of bound phosphate, seen also in the case of the trinuclear 1,0,1/*t,t,t* [32], led to the suggestion [32, 34] that reversible reactions with negatively charged phosphates of amphiphilic anionic phospholipids in membranes could provide a “shuttling” mechanism allowing uptake of these amphiphilic cations. Studies of the interactions of PPCs with phospholipids are discussed below (section 5.1).

Secondly, the comparison of phosphate and sulfate [35] shows that the rate constant for sulfate displacement of the aqua ligand (k_L) is approximately 3-fold higher than that of phosphate, whilst aquation of the bound sulfato ligand (k_{-L}) is more than an order of magnitude faster in comparison to displacement of phosphate. The reaction of the trinuclear 1,0,1/*t,t,t* with sulfate similarly reaches equilibrium after 12 h and has a similar distribution at equilibrium of chloro, aqua and sulfato species [36]. However, inspection of the rate

constants (Table 2) shows interesting differences attributed to a balance of ion-pairing (Cl^-) and/or formation of “sulfate clamps” with the charged central $\{\text{PtN}_4\}^{2+}$ unit. The two $\text{p}K_1$ values are comparable as a consequence, for the trinuclear complex, of a slightly lowered forward rate constant (k_{H}) and 2-fold lower anation rate constant ($k_{-\text{H}}$) for the reformation of the chloro species. The two $\text{p}K_2$ values are also comparable due to a 2-fold lowering (for 1,0,1/*t,t,t*) of the rate constants for both sulfate displacement of the aqua ligand (k_{L}) and the aquation of the sulfato ligand ($k_{-\text{L}}$). Given the physiological concentrations of sulfate (the fourth most abundant anion in human plasma with serum concentration typically in the range 0.3-0.4 mM) [35, 37-39] and the presence of many sulfonated sites on biological membranes, binding to sulfate is likely to have physiological significance, with sulfato species forming quickly and being highly substitution labile. These simple studies with sulfate anion have aided in the analysis of more complex reactions with defined fragments of heparan sulfate (see section 5.2).

Finally, [^1H , ^{15}N] NMR studies have been used to explore the effects of geometric isomerism in dinuclear PPCs on aquation reactions in the presence of acetate and phosphate, by comparing 1,1/*t,t* with the geometric *cis* isomer 1,1/*c,c* [34]. The rate and equilibrium constants are compared in Table 2. In contrast to the reactions with 1,1/*t,t* (see above), for 1,1/*c,c* the rate constants for the forward and reverse reactions with acetate and phosphate are quite similar so that equilibrium conditions are reached very slowly and a greater proportion of phosphate-bound species are present. A major difference in the chemical reactivity of the geometric isomers is the ability of 1,1/*c,c* to form hydroxo- and phosphate-bridged macrochelate species, with the latter accounting for the reduced lability of the bound phosphate (Fig. 3).

< Insert Figure 3 here >

5. Interaction of platinum complexes with *O*-donor ligands in biomolecules.

5.1. Interaction of platinum complexes with phospholipids.

Negatively charged phospholipids (e.g. phosphatidylserine (PS), phosphatidic acid (PA) and phosphatidylglycerol (PG)) are one of the major components of cell membranes, hence, the interaction of platinum drugs with phospholipid fragments has been investigated as a mechanism for their cellular uptake. Reedjik and co-authors have investigated the interaction of cisplatin with a model fragment of PS at pH 6.0 and 37 °C [40, 41]. These studies showed that cisplatin covalently binds to the PS fragment through the amine *N*-donor and carboxyl *O*-donor of the serine moiety; the highest binding was observed in the absence of chloride ions indicating that positively charged aquated cisplatin is the reactive species.

In earlier work we investigated the binding of highly positively charged PPCs with the negatively charged phospholipids, DPPA, DPPS and DPPG (Fig. 4).

<Insert Figure 4 here>

The study showed that PPCs interact with both the phosphate head group and the fatty acid tail region of the liposomes and these interactions involve both covalent and non-covalent binding [42]. For both 1,0,1/*t,t,t* and 1,1/*t,t*, binding to the DPPA liposomes was higher compared to the DPPS liposomes. These studies also indicated that liposome binding to 1,0,1/*t,t,t* and 1,1/*t,t* was significantly stronger compared to that of cisplatin. To explore the molecular details of these interactions we have recently reported detailed 2D [¹H,¹⁵N] HSQC NMR and DFT modelling studies following the binding of ¹⁵N-PPCs with shorter water soluble phospholipid fragments 1,2-dihexanoyl-*sn*-glycero-3-phosphate (DHPA) and 1,2-dihexanoyl-*sn*-glycero-3-[phosphatidyl-L-serine] (DHPS) (Fig. 4) [43, 44]. These studies were carried out under similar conditions to our previously reported studies on the binding of these PPCs to simple phosphate and acetate (Section 4) allowing the kinetics of the reactions to be compared. Reaction of 1,0,1/*t,t,t* with the sodium salt of DHPS showed a very minor product formation *via*

coordination to the *N*-donor atom of the phosphoserine moiety. Analysis of the [¹H, ¹⁵N] HSQC NMR spectra (Fig. 5) in conjunction with DFT models showed that both 1,1/*t,t* and 1,0,1/*t,t,t* react with the sodium salt of DHPA to form an initial mono-adduct in which the DHPA is coordinated *via* the phosphate O atom, in a very similar conformational orientation. For the dinuclear 1,1/*t,t* coordination of a second DHPA, in two different orientations, gives rise to two different conformers of the bifunctional adduct (Fig. 6a and 6b). For 1,0,1/*t,t,t*, the two bound DHPA molecules are not close enough to have any influence on the H-bonding interactions of the other coordination sphere, however binding of the second DHPA molecule allows the central {PtN₄} coordination unit to bind electrostatically to two additional DHPA molecules *via* phosphate clamp interactions, in an extended network, in which 1,0,1/*t,t,t* interacts both covalently and non-covalently with the phosphate and carboxyl *O*-donors of four DHPA fragments (Fig. 6c) [43]. For both 1,1/*t,t* and 1,0,1/*t,t,t* equilibrium conditions are obtained more slowly (>35 h) than in the presence of phosphate (12 h); in each case the rate constant for the first step of DHPA binding is about 8 times higher than that for phosphate, whereas the rate constants for the reverse reactions are quite similar [43].

<Insert Figure 5 and Figure 6 here>

In the case of the geometric *cis* isomer (1,1/*c,c*) [44] analysis of the NMR data, supported by DFT models, provide evidence that the monofunctional DHPA adduct exists in two different conformational orientations. Similar to the 1,1/*c,c* aquation product in phosphate (Fig. 3a), the aliphatic chain orientation in one conformer enables an interaction between the unbound {PtN₃Cl} moiety and the coordinated DHPA molecule, while the second conformer has a similar aliphatic chain elongation to that of 1,1/*t,t* – so that there is no interaction between the two Pt coordination spheres. Similarly, two bifunctional adduct conformers are identified, in which one has an interaction between the phosphate groups of the two bound DHPA molecules (Fig. 3b), whereas the other has an elongated orientation with no interaction.

Equilibrium conditions are reached much more slowly (120 h) than for the reaction with 1,1/*t,t* (similar to the reaction of the geometric isomers with phosphate [34]). The rate constant for the first step of DHPA binding is slightly lower (1.6 fold) for 1,1/*c,c* compared to the *trans*-isomer, whereas the rate constant for the reverse reaction is 4-fold lower, resulting in a much greater proportion of DHPA bound species at equilibrium [44]. As for the reaction in phosphate, interaction between the two {PtN₃Y} groups (a feature only accessible to the *cis* geometric isomer) provides an explanation for the slower release of the bound DHPA molecules.

5.2. Interaction of polynuclear platinum complexes with defined fragments of heparan sulfate.

Cleavage of extra cellular matrix (ECM) resident glycosaminoglycans (GAGs) such as heparan sulfate (HS) by the enzyme heparanase modulates tumour-related events including angiogenesis, cell invasion, metastasis and inflammation [45]. The glycosaminoglycans are highly anionic polysaccharides with a combination of sulfate and carboxylate residues contributing to the overall negative charge. Cell surface HS sequences consist of repeating 1-4 linked disaccharide units of D-glucosamine and glucuronic or iduronic acid residues that are variably substituted by sulfate at the 2-*N* or 6-*O* positions of D-glucosamine and by sulfate and acetyl at the 2-*O* position of iduronic acid and 2-*N* position of D-glucosamine units, respectively (Fig. 7a and 7b).

<Insert Figure 7 here>

As such, in biology, they are intimately associated with endogenous metal ions such as Ca²⁺ which may also affect their conformation. We have recently shown that 1,0,1/*t,t,t* (Triplatin) is capable of binding with the negatively charged *O*-donor polysaccharide fragments effectively inhibiting the physiologically critical HS functions such as growth factor recognition and the activity of human (heparanase) and bacterial (heparinase) enzymes on HS [46-48]. Using

Fondaparinux (FPX) as a model fragment for a highly-sulfated glycosaminoglycan, we have performed enzymatic cleavage inhibition studies in the presence and absence of 1,0,1/*t,t,t* by colourimetry and ^1H NMR spectroscopic assays [46, 47]. These studies showed significant inhibition of FPX cleavage in the presence of 1,0,1/*t,t,t* and the evidence for covalent binding interaction, possibly with the sulfate and carboxylate *O*-donor groups of FPX, was seen in ^1H NMR assays. The presence of a total of eight SO_3^- and two RCOO^- groups in FPX makes it difficult to understand individual binding tendencies so we have recently begun a systematic study on site-specifically altered mono and di-saccharides. To understand the kinetics of covalent binding with the monosaccharide fragments that represent those present within the repeating disaccharide units of HS, we have used 2D [^1H , ^{15}N] HSQC NMR spectroscopy to follow the interaction of ^{15}N -labelled 1,0,1/*t,t,t* with three D-glucosamine residues (GlcNS(6S), GlcNAc(6S) and GlcNS; see Fig. 7c) containing varied *O*-sulfate and *N*-sulfate or *N*-acetate substitutions at 6-*O* and/or 2-*N* positions [36]. The conditions were similar to those previously used for the reactions with anionic systems (see section 4). These studies showed that, along with binding to the sulfate *O*-donor, 1,0,1/*t,t,t* binds also to the *N*- and *O*-donors of sulfamate and *N*-acetyl groups, respectively. The results of these highly reversible covalent reactions of 1,0,1/*t,t,t* with the *O*-donor fragments that represent disaccharide sequences of HS, further emphasizes that cell surface glycans are efficient receptors for the PPC cellular internalization [36].

6. Conclusions.

This review has covered covalent binding of platinum-amine complexes to a series of *O*-donor ligands. While the Pt-O bond may be considered labile, the presence of mutually *trans* *O*-donors in the case of transplatinum dicarboxylates or where the Pt-O bond is *trans* to a Pt-N bond with a weak *trans* effect, means that direct substitution reactions do not occur rapidly

except in the case of a strong nucleophile, which really is similar to the case for the Pt-Cl bond itself. The extension from simple individual *O*-donors to multiple neighbouring donors on a more complex polymeric, biomolecular template with many possible binding sites such as glycosaminoglycans (a Lewis base “sink”) emphasizes the necessity to consider Pt-O donor interactions in a full molecular description of the fate of platinum-amine complexes in biological medium. These species may still be relatively short-lived and in the presence of abundant *N*- and *S*-donors, the labile Pt-O bonds may be replaced over time.

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Declaration of Interest : none

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Table 1. Comparison of aquation rates of *trans*-platinum complexes and pK_a of the corresponding diaqua complexes.^{[a][a]}

Compound	$k_1(s^{-1})$	pK_{a1}	pK_{a2}
<i>trans</i> -Pt(OAc) ₂ (py) ₂ ^[b]	$3.2 \pm 0.3 \times 10^{-7}$	3.87	6.70
<i>trans</i> -[Pt(OAc) ₂ (NH ₃) ₂] ^[b]	$< 10^{-7}$	4.0 ^[c]	7.08 ^[c]
<i>trans</i> -[PtCl ₂ (NH ₃) ₂]	19×10^{-5}	--	--
<i>cis</i> -[PtCl ₂ (NH ₃) ₂]	5.18×10^{-5}	--	--
[PtCl(dien)] ⁺	$6.50 \pm 0.13 \times 10^{-5}$	--	--

Adapted from Ref. [28]. ^[b] Aqueous solubility 15.0 and 21.0 mg/mL for the NH₃ and pyr compounds respectively. ^[c] Literature values from NMR studies 4.35 and 7.40 for pK_{a1} and pK_{a2} , respectively [28].

Table 2. Rate and equilibrium constants for the aquation and ligation of 1,1/*t,t*, 1,1/*c,c* and 1,0,1/*t,t,t* in 15 mM acetate, phosphate and sulfate according to the kinetic model shown in Scheme 1.

compound	anion (L)	k_H (10^{-5}s^{-1})	$k_{-H}(\text{M}^{-1}\text{s}^{-1})$	k_L ($\text{M}^{-1}\text{s}^{-1}$)	k_{-L} (10^{-5}s^{-1})	pK_1	pK_2
1,1/ <i>t,t</i> ^[a]	OAc ⁻	1.83 ± 0.03	0.262 ± 0.009	0.0086 ± 0.0001	0.56 ± 0.02	4.16 ± 0.02	-3.19 ± 0.02
	PO ₄ ³⁻	2.49 ± 0.04	0.40 ± 0.01	0.0086 ± 0.0002	3.9 ± 0.1	4.21 ± 0.02	-2.34 ± 0.02
	SO ₄ ²⁻	3.85 ± 0.05	0.229 ± 0.04	0.025 ± 0.004	70 ± 10	3.77 ± 0.01	-1.6 ± 0.1
1,1/ <i>c,c</i> ^[b]	OAc ⁻	1.56 ± 0.02	0.134 ± 0.005	0.0066 ± 0.0001	0.38 ± 0.01	3.93 ± 0.02	-3.24 ± 0.02
	PO ₄ ³⁻	2.18 ± 0.03	0.145 ± 0.005	0.0071 ± 0.0001	0.70 ± 0.02	3.82 ± 0.02	-3.01 ± 0.02
1,0,1/ <i>t,t,t</i> ^[c]	PO ₄ ³⁻	3.4 ± 0.1	0.275 ± 0.009	0.0166 ± 0.0002	5.4 ± 0.1	3.73 ± 0.02	-2.51 ± 0.01
	SO ₄ ²⁻	3.43 ± 0.01	0.1324 ± 0.0007	0.0131 ± 0.0006	41 ± 2	3.587 ± 0.003	-1.50 ± 0.03

^[a] Data from Ref. [34, 35]. ^[b] Data from Ref. [34]. ^[c] Data from Ref. [32, 36].

Scheme 1. Simplified kinetic model used to determine the rate constants for the aquation and ligation of PPCs with acetate, phosphate and sulfate as shown in Table 2.

Fig. 1. Structures of mononuclear and polynuclear platinum complexes.

Fig. 2. *trans*-Platinum planar amine (TPA) compounds containing planar ammine and carboxylate leaving groups.

Fig. 3. (a) Molecular model of a phosphate-bridged 1,1/*c,c* species (reproduced from ref. [34] and reprinted by permission from Springer) and (b) DFT optimized model for one conformer of the DHPA bound bifunctional adduct of 1,1/*c,c* in which there is an interaction between the two {PtN₃O} coordination spheres (reproduced from ref. [44] with permission from the Royal Society of Chemistry).

DPPA	1,2-dipalmitoyl- <i>sn</i> -glycero-3-phosphatidic acid; $n = 14$
DPPS	1,2-dipalmitoyl- <i>sn</i> -glycero-3-phosphatidyl-serine; $n = 14$
DPPG	1,2-dipalmitoyl- <i>sn</i> -glycero-3-phosphatidyl-glycerol; $n = 14$
DHPA	1,2-dihexanoyl- <i>sn</i> -glycero-3-phosphate; $n = 4$
DHPS	1,2-dihexanoyl- <i>sn</i> -glycero-3-phosphatidyl-serine; $n = 4$

Fig. 4. Structure and abbreviations of the phospholipid fragments.

Fig. 5. 2D [^1H , ^{15}N] HSQC NMR (600 MHz) spectra at 298 K from the reactions of ^{15}N -labelled PPCs (1 mM) with the phospholipid fragment DHPA (5 mM) in 100 mM NaClO_4 at equilibrium: (a) 1,1/*t,t* (b) 1,0,1/*t,t,t,t*. The NMR spectra recorded over the course of the reactions were interpreted with reference to DFT models (Fig. 6) and showed that both PPCs form similar monoadducts coordinated to DHPA via the phosphate O atom (peaks labelled ‘C’). The dinuclear 1,1/*t,t* forms a bifunctional DHPA adduct, which exists in two conformational forms (peaks D1 and D2), whereas the central $\{\text{PtN}_4\}$ coordination unit of 1,0,1/*t,t,t,t* binds electrostatically to two additional DHPA molecules *via* phosphate clamp interactions, which result in shifted resonances for the linker Pt-NH₃ and Pt-NH₂ groups. Adapted from ref. [43] with permission from John Wiley and Sons.

Fig 6. DFT optimized models for the DHPA bound bifunctional adducts of 1,1/*t,t* (a and b) and 1,0,1/*t,t,t* (c). The 1,1/*t,t* bifunctional adduct exists in two conformational forms (a and b). For the 1,0,1/*t,t,t* bifunctional adduct (c) two additional DHPA molecules (chains coloured in dark blue) are involved in phosphate clamp interactions with the central {PtN₄} coordination unit. Adapted from ref. [43] with permission from John Wiley and Sons.

Fig. 7. (a) Major repeating disaccharide unit of HS, (b) Variable repeating disaccharide unit of HS and (c) Structures of the monosaccharide fragments GlcNS(6S), GlcNS and GlcNAc(6S).