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Platinum(IV) antitumour compounds: their bioinorganic chemistry

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Abstract

This article reviews the investigations undertaken into platinum(IV) antitumour compounds since Barnett Rosenberg first noted the activity of platinum(IV) complexes. The chemical and pharmacological properties of the drugs are discussed and both the reactions with individual biomolecules, which have received attention and the characterisation of biotransformation products from animal and clinical trials are reviewed. The bioinorganic chemistry of platinum(IV) complexes has not previously been reviewed, and the purpose here is to provide insight into the requirements for the antitumour activity of platinum(IV) complexes. © 2002 Published by Elsevier Science B.V.

Keywords: Platinum(IV); Bioinorganic; Metabolite; Biotransformation

1. Introduction

The anticancer activity of platinum(IV) complexes such as *cis*-$[PtCl_4(NH_3)_2]$ has been known since the discovery of cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) by Rosenberg and colleagues [1,2]. Although substantially fewer platinum(IV) complexes have been studied as potential

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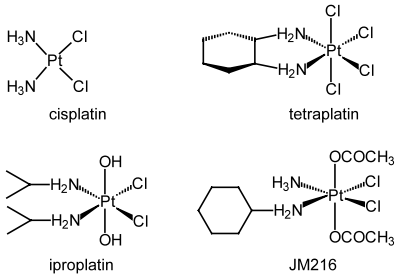
anticancer agents than have platinum(II) complexes [3–6], some have shown sufficient promise to enter clinical trials. Iproplatin (CHIP, JM9, *cis*,*trans*,*cis*-[$PtCl_2(OH)_2$(isopropylamine)$_2$]) was selected from a range of platinum(IV) complexes synthesised by Tobe and co-workers for its high solubility [3]. Iproplatin was sufficiently well tolerated to enter phase II and III clinical trials [7], but was ultimately found to be less active than cisplatin and so has not entered widespread clinical use [8]. Tetraplatin (ormaplatin, [$PtCl_4$(D,L-cyclohexane-1,2-diamine)]) showed great promise in preclinical studies but caused severe neurotoxicity in treated patients and the trials were subsequently abandoned at the phase I level [9]. JM216 (satraplatin, *cis*,*trans*-[$PtCl_2(OAc)_2(NH_3)$cyclohexylamine] is a rationally designed drug which was recently in phase III trials, but trials were abandoned due to variability in drug uptake [10].



That none of the platinum(IV) compounds trialled clinically have revealed significantly greater activity in humans than that of cisplatin is particularly disappointing in light of the report by Kelland et al. that analogues of JM216 are up to 840-fold more active than cisplatin in in vitro assays [11]. The high activity was ascribed to high cellular uptake, but in vivo reduction alters the pharmacological properties and thus the effectiveness of the drug. However, platinum(IV) complexes have enormous potential as anticancer agents in terms of both high activity and low toxicity, but this potential has not been realised by the drugs investigated to date, probably because they are reduced too readily in the bloodstream. The potential advantages of platinum(IV) complexes that remain in the higher oxidation state in the bloodstream are that their lower reactivity would diminish loss of active drug and lower the incidence of unwanted side reactions that lead to toxic side effects. In addition the higher lipophilicity of some platinum(IV) complexes would be retained, leading to potential improvements in cellular uptake.

The primary purpose of this review is to summarise the reported studies on the in vitro and in vivo chemistry of platinum(IV) complexes and their interactions with biomolecules.

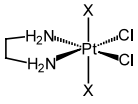
2. Structure activity rules

2.1. Relationships between structure and reduction potential

It is widely believed that reduction to platinum(II) is essential for the anticancer activity of platinum(IV) complexes to be effected [12–14]. If this is the case then the ease with which a platinum(IV) complex is reduced can be expected to influence its biological activity. There are a number of factors to consider when assessing the possible effects of administering the platinum(IV) analogue of a platinum(II) complex. The kinetic inertness of platinum(IV) complexes means that there is increased opportunity for the complex to arrive at the cellular target intact. Modifying the axial ligands[1] of platinum(IV) complexes alters the solubility of the complex (lipophilic vs. hydrophilic) and thus its ability to enter tumour cells before being reduced to yield the active platinum(II) drug. Preventing side reactions by administering platinum(IV) complexes has the potential to lead to a drug that has reduced toxicity and high activity.

The reduction potentials[2] of diam(m)ine platinum(IV) complexes are dependent on the nature of the axial and equatorial ligands, but the axial ligands generally exert

Table 1
Reduction potential with variation of axial ligands, for complex shown



Ligand (X)	E_p (mV)
Cl	−4
$OC(O)CH_3$	−326
$OC(O)CH_2CH_3$	−301
$OC(O)CH_2CH_2CH_3$	−273
OH	−664

The reduction potentials presented in this review have been adjusted such that all values are against Standard Hydrogen Electrode, to facilitate comparison.

[1] Herein, the ligands that lie above and below the plane of the am(m)ine ligands in platinum(IV) complexes will be referred to as the 'axial' ligands, and those that lie in the plane of the am(m)ine ligands will be referred to as the 'equatorial' ligands. This is in keeping with common usage in literature when discussing platinum(IV) complexes [15,16].

[2] Generally, cyclic voltammetry (CV) is used for measuring the reduction potential (E_p) of platinum(IV) complexes. The reduction process results in loss of the axial ligands and as such the reduction processes are irreversible and the E_p values obtained by CV are not thermodynamic, however a correlation between E_p and reduction rate has been established, *vide infra* [17].

the stronger influence [18]. Reduction occurs most readily when the axial ligands are chloro, least readily when they are hydroxo and is intermediate when they are carboxylato, as shown in Table 1 [15]. These results have recently been confirmed by Choi et al. who also showed that reduction rates correlate with reduction potentials [17]. Hambley et al. examined a large series of platinum(IV) complexes containing anionic polyfluoroaryl ligands [18]. As with the complexes in Table 1, small variations in carboxlato ligand chain length resulted in small changes in reduction potential, but changing the nature of the donor, e.g. to a hydroxo or chloro ligand, had a large effect.

An early study found that varying the equatorial amine ligands, in complexes of the general form *cis*-$[PtCl_4(NRH_2)_2]$ (R = H, Me, Et, Pr^n, Pr^i, Bu^n, Bu^i, Bu^s, Bu^t, CH_2Bu^t, CH_2Ph, *c*-C_6H_{11}) had a measurable, but not large effect on the rate of reduction of the complexes [19]. Both σ-donor ability and steric hindrance of R were given as factors influencing reduction rate. Choi et al. studied a range of complexes and suggested that the rate of reduction of platinum(IV) complexes depended on the bulkiness of the equatorial ligands [17]. For example, JM216 and iproplatin are reduced more rapidly than *cis*,*trans*-$[PtCl_2(en)(OCOCH_3)_2]$, and this was ascribed to the steric bulk of the amine ligands on JM216 and iproplatin destabilising the complex relative to the ethane-1,2-diamine ligand complex. However, more recent work has called into question this form of steric influence [20].

The structural variation of the am(m)ine ligands has a small effect on reduction potential, similar to the effect of altering the chain length of axial carboxylato ligands. Thus, the design of an optimum platinum(IV) complex need not be concerned with variation of the equatorial ligand—design of an effective platinum(II) compound should take precedence. The axial ligands should then be tailored to provide the stability required to ensure a good proportion of the drug arrives at the target site intact.

2.2. Relationships between E_p and biological activity

It is clear from the experimental data that a simple and general relationship between the reduction potentials of platinum(IV) complexes and either their in vitro or in vivo anticancer activity cannot be expected. This is because complexes such as tetraplatin are reduced rapidly yielding highly active platinum(II) complexes and complexes such as iproplatin, that are far less readily reduced, have also proven to be highly active [17]. However, the pharmacology and pharmacokinetics of the three platinum(IV) complexes that have been subjected to extensive clinical trials (iproplatin, tetraplatin and JM216) do provide insights into how reduction potential influences biological behaviour.

Tetraplatin, with axial chloro groups and a higher reduction potential, is reduced very rapidly in vivo and all biotransformation products are platinum(II) species [21]. JM216, with axial acetato groups and an intermediate reduction potential, is also reduced rapidly and does not remain intact. However, reduction is evidently slowed sufficiently to allow a small amount of hydrolysis of the equatorial chloro ligands of JM216 [22]. Iproplatin has hydroxo ligands in the axial positions and a more negative reduction potential [17]. Consequently, large amounts of iproplatin survive unchanged in vitro and in vivo and this correlates with low toxicity. Indeed it has been proposed that some iproplatin is reduced intracellularly rather than in the blood plasma [23]. Thus, there is a clear correlation between reduction potential and the pharmacology of the complex confirming that it is possible to tune these properties.

Choi et al. found that when the equatorial ligands were unchanged and only the axial ligands varied, cytotoxicity increased with increasing reduction potential [17]. However, variation of the amine ligand, and, therefore, the propensity for the platinum(II) analogue to bind to DNA, led to a low correlation between cytotoxicity and reduction rate. Kratochwil and Bednarski found that reduction potentials were a good predictor of the stability of complexes but did not find a relationship with cell growth inhibition activity for *cis*-dichloro- and *cis*-diiodo-(en)platinum(IV) complexes [24]. Hambley et al. also found no correlation between reduction potential and cytotoxicity in a large series of organometallic platinum(IV) complexes [18]. Rotondo et al. demonstrated that while platinum(IV) complexes with active platinum(II) analogues display activity in vivo, complexes such as *mer*-$[PtCl_3(dien)]$ with inactive platinum(II) reduction products are inactive [25].

The structure-activity relationship (SAR) rules defined for platinum(II) then are a significant factor in determining the activity of platinum(IV) complexes. These results establish that the pharmacology of platinum(IV) compounds does depend on how readily they are reduced and this in turn depends on the nature of the axial ligands. However, their activity depends more on the activity of the platinum(II) analogue yielded.

2.3. Lipophilicity

The high cellular uptake of analogues of JM216 in vitro correlates with the high lipophilicity of the platinum(IV) complexes [11]. Reduction of these platinum(IV) complexes with loss of the axial ligands occurs rapidly in vivo and the consequent loss of lipophilicity probably accounts for the disparity in activity between in vitro and in vivo systems. For example, Chaney et al. have shown that tetraplatin is reduced in rat blood plasma to its platinum(II) analogue with $t_{1/2} \sim 3$ s [21]. Other complexes are reduced more slowly but, in

general, the potential advantages of platinum(IV) complexes are lost quite quickly. A partial exception to this is iproplatin, much of which survives intact in the blood plasma [23,26].

Lipophilicity (or hydrophobicity), as measured by relative solubilities in water and chloroform [3,5,6] or partition between these solvents [3] has been investigated as a factor relevant to chemotherapeutic activity of platinum(II) complexes since the activity of cisplatin was discovered. A systematic study of the lipophilicity of *cis*-diam(m)ineplatinum(II) complexes taken as the *n*-octanol-water partition coefficient, log P_{oct}, was carried out by Souchard et al., who reported values for 16 compounds [27]. The authors demonstrated that when the leaving groups are unchanged, the hydrophobicity of a platinum complex is linearly related to that of the am(m)ine, but also that this relation does not hold for complexes with different leaving groups and cannot, therefore, be used as a predictive method in general. The solubilities of the first platinum(IV) complexes in chloroform and water were also reported relatively early [5,6], and the improved solubility of iproplatin over its platinum(II) analogue was an important factor in its selection for further trials. In fact, when the *trans*-dihydroxoplatinum(IV) analogues of a series of platinum(II) complexes were synthesised, the aqueous solubilities of the platinum(IV) complexes were dramatically greater than those of the corresponding platinum(II) complexes [6]. More recently, log P_{oct} of a number of platinum(IV) complexes has also been measured [4,28,29]. Kidani et al. [4] determined the log P_{oct} values of platinum(IV) analogues based on the [Pt(oxalato)(1R,2R-chxn)] moiety. As expected, log P_{oct} increased with increasing carboxylato ligand chain length. When one axial carboxylato ligand is replaced by a chloro ligand, log P_{oct} diminished by an order of magnitude [4]. This was observed more recently by Lee et al. in a series of tetracarboxylato(chxn)platinum(IV) complexes with log P_{oct} values ranging from -1.59 (tetraacetato) up to 3.03 (tetrapentyrato) [29]. Screnci et al. reported partition coefficients of seven Pt-containing drugs, including four platinum(IV) complexes. They went on to show that log P_{oct} has a close inverse correlation with the accumulation of platinum in peripheral nerve tissue of rats [28].

The mechanisms of resistance to cisplatin have been identified as decreased drug accumulation, increased cytoplasmic detoxification (e.g. overexpression of glutathione, metallothionein etc.) and increased DNA repair [30,31]. More lipophilic drugs are believed to assist in circumventing cisplatin resistance by an increase in passive diffusion through the cell membrane rather than relying on active uptake. Recently Song et al. examined the oral antitumour activity of a number of tetracarboxylato- and dicarboxylatodihydroxo-(1,2-dach)platinum(IV) complexes with varying log P values and could not establish a clear correlation between log P and activity [32]. The complexes which are too hydrophilic or lipophilic in character displayed poor activity, probably due to difficulty passing biological membranes or poor solubility, respectively. This suggests an important factor in platinum(IV) drug design may be an optimal 'window' of log P_{oct} values in which activity may be expected from a series of complexes.

Clearly, the lipophilicity of platinum drugs is of substantial current interest, and a general method for its prediction would be a highly useful tool in the rational design of new drugs. This has been achieved recently using calculations of polar surface area [33]. The experimental log P_{oct} reported for 24 platinum(II) and platinum(IV) complexes with a variety of am(m)ine and leaving groups were closely predicted. It was also shown that the calculated log P_{oct} correlates well with observed cellular uptake for five platinum complexes [33].

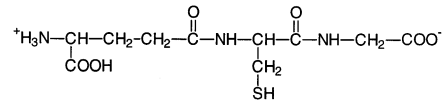
Among the important properties for platinum(IV) antitumour complexes are stability (E_p) and lipophilicity, and these should be a basis for rational design, provided the platinum(II) moiety is active.

3. Interactions of platinum(IV) complexes with biomolecules

The biomolecules present in blood and cells are varied in their structure and chemical reactivity. Any number of small molecules, proteins and enzymes have the potential to react with platinum(IV) complexes, primarily via reduction. As platinum(IV) complexes are relatively inert [34], substitution reactions are not often reported, though significantly, platinum(IV) binding with DNA has been reported. A consideration when investigating reactions of platinum(IV) complexes with biomolecules is the reaction rates for reduction versus substitution. Reactions with biomolecules that require weeks for substantial reaction are irrelevant when considering the fate of platinum(IV) drugs in the body, as the clearance rate of such drugs from the body is on a scale of days not weeks [35,36].

The number of small molecules involved in redox balance in the body is extensive, and many have the potential to reduce platinum(IV) complexes. Despite this, many such molecules have received little or no attention in this respect; for example, glucose and other sugars and starch, NADH, NADPH and FADH. The biomolecules considered below are those whose reactivity with platinum(IV) complexes have received substantial attention.

3.1. Glutathione



glutathione

The tripeptide glutathione (GSH), γ-glutamylcysteinylglycine is the most prevalent intracellular non-protein thiol, with concentrations of up to 8 mM [37]. GSH is one of the primary defences against toxins and oxidants present in the cell, and can deactivate electrophilic drugs including chemotherapeutic agents. For example, the platinum(II) drug cisplatin is coordinated and 'deactivated' by glutathione, diminishing the amount of active species before arrival at the target site (DNA), and one of the major mechanisms of resistance towards platinum chemotherapeutic agents is increased cellular expression of glutathione [31,38–41]. While the coordination chemistry of many mono-, di-, tri- and larger peptides with platinum(II) has been investigated extensively [42,43] (and references therein), reaction with platinum(IV) complexes has received little attention [44–46].

In the instance of platinum(IV) complexes, which are believed to be activated on reduction, their interaction with glutathione is important. The sulfhydryl group of glutathione is readily oxidised, and the E° value has been reported at -240 mV at pH 7.0 (close to biological pH): [37]

$$2\mathrm{GSH} \Leftrightarrow \mathrm{GSSG} + 2\mathrm{H}^+ + 2\mathrm{e}^- \quad (1)$$

This is in the range for oxidation by platinum(IV) complexes, which have E° values up to -1000 mV [15].

The reduction of *trans*-$[PtCl_2(CN)_4]^{2-}$ by glutathione was shown by stopped-flow spectrophotometry to proceed directly without substitution at platinum(IV). With a stoichiometry of $[GSH]_{tot}$:$[Pt(IV)] = 2{:}1$, the rate Eq. (2) for reduction is: [47]

$$\mathrm{d}[Pt(CN)_4^{2-}]/\mathrm{d}t = k_{obsd}[PtCl_2(CN)_4]^{2-} = k'[GSH]_{tot}[PtCl_2(CN)_4]^{2-} \quad (2)$$

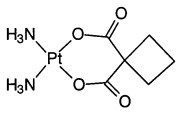
The use of *trans*-$[PtCl_2(CN)_4]^{2-}$ ensured that aquation or reaction of GSH with the reduced platinum(II) analog ($[Pt(CN)_4]^{2-}$) did not interfere with the reaction kinetics. The reduction was found to be pseudo-first-order, and variation of the chloride concentration had no effect on the rate of reduction. The rate of reduction increased with increasing pH, suggesting that the deprotonated thiol group is the more reactive species towards platinum(IV) complexes.

Eastman reported that the reaction of tetraplatin with DNA was slow in vitro, but the reaction became rapid upon addition of two stoichiometric equivalents of GSH, indicating reduction of the platinum(IV) complex to its platinum(II) analogue; [44]

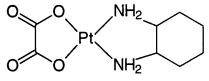
$$\mathrm{Pt(IV)} + 2\mathrm{GSH} \Leftrightarrow \mathrm{Pt(II)} + \mathrm{GSSG} + 2\mathrm{H}^+ \quad (3)$$

Decreased DNA binding was observed at GSH concentrations greater than those stoichiometrically required to reduce the platinum(IV) present due to subsequent binding and deactivation of the resulting platinum(II) analogue by excess GSH [48]. Kido showed that addition of GSH with tetraplatin to L1210 cell lines increased the cytotoxicity to that of its platinum(II) analogue dichloro(*R*,*R*-cyclohexane-1,2-diamine)(II), probably due to reduction of the platinum(IV) complex. The fact that GSH did not increase the cytotoxicity of the platinum(II) complex or itself exhibit activity adds further weight to the hypothesis that reduction is required for activity [49]. Binding of tetraplatin to salmon sperm DNA was only observed on addition of glutathione, and the platinum(IV) complex was shown to be completely reduced by the additional glutathione in vitro in 5 min.

Glutathione levels are inversely proportional to cellular sensitivity towards platinum(II) and platinum(IV) drugs, and Mistry and co-workers showed for iproplatin and tetraplatin that GSH plays a significant role in resistance to platinum(IV) drugs [50]. The relationship between cellular glutathione levels and drug resistance for a range of platinum(II) drugs and the platinum(IV) drugs tetraplatin and iproplatin was demonstrated by Meijer [51]. Similarly, Pendyala et al. demonstrated that the positive correlation between intracellular glutathione concentration and cytotoxicity was significant for iproplatin ($P = 0.002$) and tetraplatin ($P = 0.06$) but not for the platinum(II) drugs cisplatin, carboplatin (*cis*-diammine(CBDCA)platinum(II), CBDCA = cyclobutanedicarboxylato) and oxaliplatin ((*R*,*R*-cyclohexane-1,2-diamine)(oxalato)platinum(II)) [52].



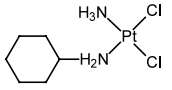
carboplatin



oxaliplatin

This observation supports the theory that platinum(IV) complexes must be reduced in vivo, yielding the active platinum(II) analogue [12]. The more significant correlation for iproplatin than tetraplatin can be accounted for when their reduction potentials are considered. As iproplatin is less easily reduced, it would be more reliant on the presence of strong reductants such as glutathione, while tetraplatin could be reduced by weaker reductants such as nucleobases, proteins and ascorbate [53]. This also suggests that complexes with lower reduction potentials remain stable in vivo as the platinum(IV) complex for greater periods of time.

Reduction of JM216 has also been shown by Raynaud et al. to correlate with intracellular glutathione levels in a range of cell lines, but the glutathione concentration was reduced by addition of buthionine sulphoximide and, therefore, the concentrations could not be accurately determined [54]. The presence of the platinum(II) biotransformation product of JM216, JM118 (*cis*-amminedichlorocyclohexylamineplatinum(II)) was increased in concentration in cell lines expressing glutathione at higher concentrations. The authors note that when glutathione levels in the cell are low, more platinum(IV) biotransformation products are formed, correlating with greater cytotoxicity. It is likely that higher glutathione levels both reduce the platinum(IV) complex and deactivate the resulting platinum(II) biotransformation products and, therefore, reduce the cytotoxicity.



JM118

The reduction of platinum(IV) complexes by glutathione involves no observable intermediates and the reductive elimination has been postulated by a number of researchers to proceed via a halide bridged electron transfer [34,55–58]. The reduction mechanism can be considered as an electrophilic attack by the highly polarised chloro ligand of the platinum(IV) complex on the thiol. The transition state is shown in Fig. 1.

The reductive elimination results in the production of GSCl, which is consumed rapidly according to Eqs. (4)–(6) [47], and expulsion of the *trans* ligand resulting in the square–planar platinum(II) square planar product.

$$GSCl + H_2O \rightarrow GSOH + Cl^- + H^+ \quad (4)$$

$$GSOH + GSH \rightarrow GSSG + H_2O \quad (5)$$

$$GSOH + GS^- \rightarrow GSSG + OH^- \quad (6)$$

The reduction of *trans*,*trans*,*trans*-$[PtCl_2(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$ (JM335) by 2 molar equivalents of glutathione again produced GSSG and the *trans*-dihydroxo (or more likely the aquahydroxo) platinum(II) analogue. Interestingly, no *trans*-dichloroplatinum(II) product was detected, implying that the chloro ligands are lost preferentially in the reduction process. The labile platinum(II) analogue formed would then rapidly coordinate glutathione. The isomer of JM335, *cis*,*trans*, *cis*-$[PtCl_2(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$ (JM149), with a much lower reduction potential, is not reduced under the same conditions by glutathione [59].

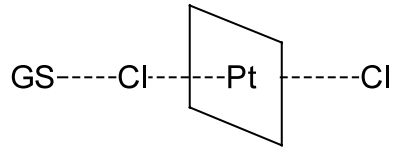
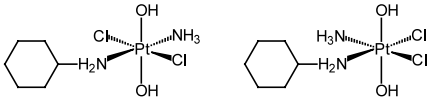
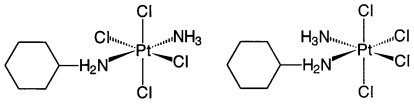


Fig. 1. Transition state of glutathione reduction of a *trans*-dichloro platinum(IV) complex.



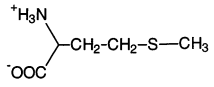
JM335 JM149

While the chloro ligands are *trans* to one another in JM335, the chloro ligands in JM149 are coordinated *trans* to strongly bound a(m)mine ligands, which would not be easily eliminated reductively. The production of only the *trans*-dihydroxo platinum(II) analogue of JM335 and the non-reaction of JM149 suggests that the *trans*-dihalo moiety may be required for glutathione to reduce platinum(IV) complexes efficiently. This is reflected in the relatively slow reduction rates observed for *trans*-dihydroxo complexes such as iproplatin. Support for this is also provided by Lemma et al. who demonstrated that the rate of reduction of *trans*-$[PtCl_4(c\text{-}C_6H_{11}NH_2)(NH_3)]$ (**1**) by GSH is twice as fast as that for *cis*-$[PtCl_4(c\text{-}C_6H_{11}NH_2)(NH_3)]$ (**2**), which possesses half the number of mutually *trans* chloro ligands [60].



(1) (2)

3.2. L-Methionine



L-methionine

The amino acid L-methionine (L-met) contains a thioether side-chain, is a component of many biomolecules (peptides, proteins etc.) and is present as the free amino acid in the blood. The possibility of thioethers as potential reductants of platinum(IV) chemotherapeutic complexes has resulted in a number of investigations into the interactions of platinum(IV) complexes with L-methionine. Thioethers are less powerful reductants than thiols, and the lack of a labile proton in the functional group of methionine (RSMe) results in little change in the reduction rate of platinum(IV) complexes as a function of pH. This is in contrast to thiols whose reaction rates are strongly pH dependent.

The reduction of platinum(IV) complexes by L-methionine is reported to be a 1:1 reaction, yielding the respective platinum(II) analogue and methionine *S*-oxide, as shown in Eq. (7), where X = Cl or Br [61].

$$trans[PtX_2(CN)_4]^{2-} + MeSR + H_2O \rightarrow [Pt(CN)_4]^{2-} + MeS(O)R + 2X^- + 2H^+ \quad (7)$$

The platinum(II) analogue $[Pt(CN)_4]^{2-}$ is substitution inert due to the cyano ligands and the methionine *S*-oxide does not further interact with platinum(IV). A halide-bridged reductive elimination by L-methionine is suggested by Shi et al. [61], the short-lived intermediate formed being MeS(X)R which is rapidly hydrolysed to MeS(O)R. It is interesting to note that the *trans*-dibromo platinum(IV) complex was reduced at a rate one order of magnitude greater than that for the *trans*-dichloro complex.

The reduction of the JM216 analog *cis*,*trans*,*cis*-$[PtCl_2(OCOCH_3)_2(NH_3)_2]$ by L-methionine has also been investigated [62]. The kinetically labile chloro ligands on the initial platinum(II) product complicate the reaction. Reduction of the platinum(IV) complex was indicated by the appearance of free acetate in the ^{1}H-NMR spectrum of the reaction solution, and concurrent appearance of the peaks for L-methionine *S*-oxide. An excess of L-methionine resulted in the platinum(II) products coordinating with L-methionine.

The rate constant for the reaction of L-methionine with *trans*-$[PtCl_2(CN)_4]^{2-}$ is an order of magnitude greater than that for reaction with *cis*,*trans*,*cis*-$[PtCl_2(OCOCH_3)_2(NH_3)_2]$ at pH 7. Chen et al. account for this in terms of the soft (with respect to HSAB rules) cyano ligands stabilising the platinum(II) analogue, the reduction potential of *trans*-$[PtCl_2(CN)_4]^{2-}$ is -399 mV, compared with -689 mV for *cis*,*trans*,*cis*-$[PtCl_2(OCOCH_3)_2(NH_3)_2]$ [62]. The reductive elimination of the acetate ligands from *cis*,*trans*,*cis*-$[PtCl_2(OCOCH_3)_2(NH_3)_2]$ is expected to proceed via reduction through an acetate ligand, which is reasonable as the conjugated acetate ligand can be easily polarised, as shown in Fig. 2.

A factor not considered by Chen et al. is the steric bulk of the ligands. The mechanism shown in Fig. 2 reveals the difficulty for a thioether such as methionine in that not only must the steric hindrance in the orientation of the thioether be satisfied, but the steric bulk of the acetate ligand must be overcome.

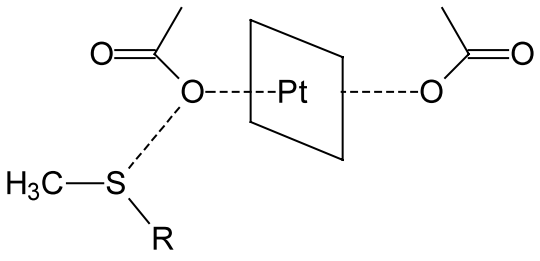
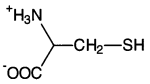


Fig. 2. Possible mechanism for reduction of a *trans*-diacetato platinum(IV) complex by a thioether [62].

Methionine metabolites of platinum(II) complexes such as cisplatin have been well characterised [63–65], and clinical trials, e.g. for tetraplatin, report the excretion of such biotransformation products as [Pt(dach)(L-met)] [21,66]. These biotransformation products must not be confused with the reduction step products by biomolecules such as L-methionine as they are formed by coordination of the platinum(II) product such as $[PtCl_2(dach)]$ with methionine following reduction. This has been shown by Shi et al. by reaction of excess methionine with platinum(IV) [47]. The outer sphere reduction of platinum(IV) complexes to yield a labile platinum(II) analogue means identification of the reductants and their relative importance is difficult to establish.

3.3. L-Cysteine



L-cysteine

The amino acid L-cysteine possesses a thiol functional group with a pK_a of 8.40. The amino acid is present in many proteins, enzymes and smaller biomolecules such as glutathione described above. The functional group is easily oxidised to the disulfide bridged cystine, RSSR, and in vivo the thiol and disulfide couple serve as both a redox balance in the cell and provide a structural role in protein intramolecular bonding.

Shi et al. investigated the reaction of *trans*-$[Pt(CN)_4X_2]$ (X = Cl, Br) with L-cysteine [47]. Reduction occurs without substitution into the platinum(IV) coordination sphere, in a similar fashion to that for glutathione described in Eq. (1), the reaction stoichiometry for L-cysteine:platinum(IV) being 2:1. The *trans*-dibromo complex ($E_p = -750$ mV) was reduced 47 times more rapidly than the dichloro complex ($E_p = -962$ mV), and this increase in reduction rate cannot be accounted for in terms of the difference in reduction potentials of the respective complexes. The rate of reduction of both the dibromo and dichloro complexes was most rapid at physiological pH, where the thiol is deprotonated. The general reaction mechanism for thiol attack and reduction of the dihalide platinum(IV) complexes is via a halide bridged reductive elimination mechanism as described for glutathione.

Reduction of the JM216 analogue *cis*,*trans*,*cis*-$[PtCl_2(OCOCH_3)_2(NH_3)_2]$ by excess L-cysteine yields two acetato ligands and the formation of cystine, confirming the oxidation of L-cysteine during the reaction. Use of an excess of cysteine leads to coordination of cysteine to the platinum(II) product *cis*-$[PtCl_2(NH_3)_2]$ (cisplatin), as has been previously re-

ported [67,68]. Again the reaction occurs more rapidly at pH 7.0 than at acidic pH, the deprotonated species being the most effective reductant, however, the rate of reaction is much slower than with *trans*-$[PtX_2(CN)_4]$ (X = Cl, Br) due to the higher reduction potentials of the tetracyano platinum(IV) complexes.

Lemma et al. investigated the reduction of *trans*, *trans*,*trans*-$[PtCl_2(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$ (JM335) by a number of thiols including L-cysteine. The reduction of the complex in a 2:1 ratio with cysteine is first order, k being dependent on pH because of the pK_a of the thiol, the half-life of JM335 being 23 s under physiological conditions. The preference for the reductant attack on a halide group *trans* to a good leaving group such as another halide ligand is demonstrated by the only platinum(II) biotransformation product being; *trans*-$[Pt(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$ (or the aquahydroxo equivalent) with no reduction through the hydroxide ligands. This is confirmed by *cis*,*trans*,*cis*-$[PtCl_2(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$ (JM149), which does not undergo reduction by L-cysteine under the same conditions, the am(m)ine ligands being too strongly bound for reductive elimination with their respective *trans*-chloro ligands. The cytotoxicity of JM149 is lower than that of JM335, and this could be due in part to the requirement of reduction for activity [69]. Interestingly, the platinum(II) analogue yielded by JM335, *trans*-$[Pt(OH)_2(c\text{-}C_6H_{11}NH_2)(NH_3)]$ (JM334) is completely inactive [70,71], as the 'diaqua' platinum(II) complex is highly reactive and would be rapidly chelated by biomolecules.

The importance of thiols in their reduction of platinum(IV) complexes and the potential for concurrent structural disruption of proteins by platinum(IV) complexes through oxidation of cysteine residues has been taken advantage of by Rabentstein and co-workers, who have employed *trans*-$[PtCl_2(en)_2]$ for selective formation of intramolecular disulfide bonds in peptide chains [72].

3.4. Metallothionein

Metallothionein (MT) is a small metal-binding protein of 61 amino acids containing 20 cysteine residues and a single acetylated methionine at the N-terminus [73]. Metallothionein is known for its role in scavenging heavy metals such as Hg^{2+} and Cd^{2+}, and the metal content of native MT is variable. Most *d*-block metal ions have been shown to be able to bind strongly to the thiolate clusters, including platinum(II) [74–76]. It is suggested that metallothioneins also serve in metal ion transport and storage. Mammalian metallothionein exhibits saturation binding with most *d*-block metals at seven molar equivalents, forming M_7-metallothionein. The seven metal ions are generally contained in two domains (α and β) which each contain a metal–cysteine thiolate cluster. The COOH-terminal α domain accepts up to four metal ions in a M_4–Cys_{11} complex and the NH_3-terminal β domain holds three metal ions in a M_3–Cys_9 complex. There are exceptions to the M_7–MT stoichiometry, for example, Ag(I) and Cu(I) bind with a M_{12}–MT stoichiometry [74]. Metal ions usually bind in the coordination clusters in a tetrahedral arrangement to four cysteine residues.

A number of papers dealing with the binding of platinum(II) complexes (in particular cisplatin and its *trans* congener) to metallothionein have been published [75–81], and platinum(II) complexes have been shown to bind to apo-MT or displace Zn(II) in Zn_7–MT to form $Pt(II)_7$–MT. Hoeschele and co-workers showed that there is little or no induction of metallothionein in vivo by cisplatin [76]. However, increase in MT levels has been demonstrated to represent a mechanism of resistance towards cisplatin [82]. Reaction with native MT results in binding of only three to four Pt(II) moieties, replacing zinc, cadmium and copper [77]. EXAFS was used to show that Pt(II) bound in MT was bound to four S (cysteine) donor atoms, and the bidentate ligand in dichloro(ethane-1,2-diamine)platinum(II) was shown to be displaced during the reaction with metallothionein, by using ^{14}C-radiolabelled ethane-1,2-diamine [75].

Comparatively little work has been undertaken on the interactions of platinum(IV) complexes with metallothionein. A complicating factor that could be expected is the reduction of platinum(IV) complexes by the cysteine thiols leading to cystine (RSSR) formation—either intra- or inter-molecularly. Zhang et al. reported that sodium hexachloroplatinate is reduced on reaction with Cd,Zn–MT. Oxidation of the cysteine thiols during reduction of the platinum(IV) forms Cys–S–S–Cys linkages including the generation of dimers and precipitated oligomeric species [83]. The monomeric MT remaining was found to contain 1–2 platinum(II) atoms per molecule, along with ca. five cadmium atoms. The zinc was almost completely displaced by platinum(II), which raises interesting possibilities with respect to zinc fingers and other zinc enzymes. The slightly lower metal content in the metallothioneins suggests that intramolecular thiol bridges are formed interfering with complex formation. Increasing the Pt(IV):MT ratio increases the amount of precipitated oligomeric species. CD spectra showed that the clusters were destroyed during reduction of platinum(IV) due to the formation of the intramolecular thiol bridges, but the presence of platinum(II) coordinating in a square-planar geometry would also be expected to distort the tetrahedrally predisposed cysteine clusters.

Sodium hexachloroplatinate and iproplatin induce production of metallothionein in rabbits, though at a lesser rate than zinc(II) does, and the in vivo binding of

platinum(II) to native metallothionein was confirmed [84].

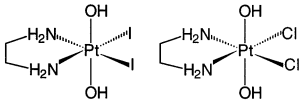
3.5. Serum albumin

Serum albumin (66.5 kDa) is a small protein (585 amino acids) and one of the major thiols in the blood, with a concentration of 5 g per 100 ml [85]. It is responsible for maintaining blood pH and also serves as a detoxicant and transporter of fatty acids [86]. The amino acid sequence of albumin contains 35% cysteine residues, yet only one, Cys34, is a sulfhydryl, the rest being present as disulfides serving a structural role (17 disulfide bridges). Interactions between Cys34 and neighbouring residues maintain its pK_a at five (lower than that of cysteine). In the blood, 30% of Cys34 is involved in a disulfide bridge with cysteine or glutathione, and further albumin is dimerised, or has a metal–ion bound. Human serum albumin also has six methionine residues (four in bovine serum albumin) that can reduce or coordinate metal ions, subject to steric considerations [87].

Albumin can bind a large number of ligands including fatty acids, cysteine, glutathione, and negatively charged aromatic compounds. It can also bind metal ions including Cu(II), Ni(II), Hg(II), Ag(II), Au(I) and Pt(II) [86,88]. There are six main binding sites on serum albumin, two of which are relevant to inorganic compounds; sites I and II bind small organic compounds, sites III and IV bind long chain fatty acids. Site V is the Cys-34 residue described previously and site VI is the N-terminus; both of these can bind metal ions [89].

The Cys-34 residue is located in a cleft and the non-oxidised thiol is generally deprotonated at physiological pH, rendering it more reactive towards platinum(IV) complexes as has been demonstrated for cysteine [47]. Several groups suggested that the main binding site of cisplatin to albumin was at Cys-34 [90–92]. However, Sadler and co-workers determined that the major fate of cisplatin on albumin was a methionine *S*,*N* macrochelate, with minor monofunctional binding of cisplatin to further methionines and to Cys-34 [88]. Iproplatin was shown not to have bound to human serum albumin in vitro after 24 h, while the platinum(II) drugs cisplatin, spiroplatin, JM-40 and carboplatin bound to varying degrees. JM216 and its platinum(IV) metabolites JM518 and JM383 were found not to have bound to albumin after 24 h, while cisplatin and the JM216 platinum(II) biotransformation product JM118 were almost completely bound [93].

LeRoy et al. found that the rates of binding of cisplatin and tetraplatin to BSA were similar, however tetraplatin was noted to aquate at a rate 15-fold slower than that of cisplatin [94]. The authors concluded that cisplatin requires aquation before reacting with BSA, whereas for tetraplatin to react at a similar rate it must react directly with BSA without prior aquation. The investigators did not consider the possibility of reduction of the platinum(IV) complex, however, which has been established as the likely mechanism for reaction, as described within. It is also interesting to note that the measured rate of aquation for tetraplatin varied for each batch measured, and the authors could not account for this by chemical analysis or other means. This occurrence had been described previously by researchers [95–97], and was established by Basolo and Pearson [56,57,98] to be due to the presence of trace amounts of platinum(II) which have a catalytic effect on the rate. This trace quantity varies with each batch, leading to the observed discrepancy.



(3) (4)

The low reactivity of albumin for some platinum(IV) complexes could be expected to be partially due to steric hindrance in approaching Cys-34 or methionine residues. The reaction of platinum(IV) complexes with cysteine versus methionine residues in human serum albumin would be expected to be kinetically favoured by the deprotonated cysteine, as discussed for the free amino acids earlier. Kratchowil et al. investigated the reaction of the photoactivatable complex *cis*,*trans*-[$PtI_2(OH)_2$(en)] (**3**) with Cys-34 of human serum albumin and showed a 1:1 stoichiometry resulted in a dramatic decrease in the LMCT band for Pt(IV)-I level after 24 h [99]. The reaction was confirmed to be at Cys-34 as no reaction with Cys-34 blocked albumin was detected. No disulfide bridged albumin dimer is formed during the reaction, so simple reduction of the complex to its platinum(II) analogue [PtI_2(en)] is not likely. The mechanism was found to involve Cys-34 attack on an iodo ligand, forming sulfenyl iodide which is hydrolysed to sulfenic acid. The platinum(II) metabolite would undergo subsequent ethane-1,2-diamine ring-opening as described earlier for glutathione attack and rapid ring closure to form [PtClI(en)], which is observed by

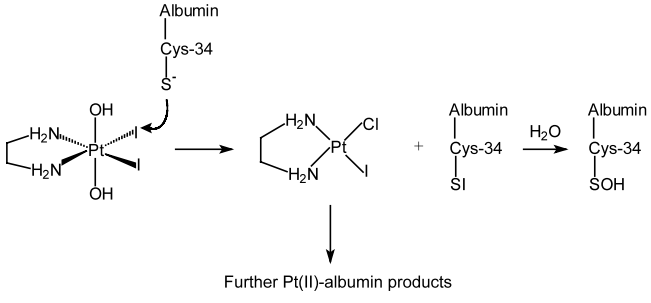
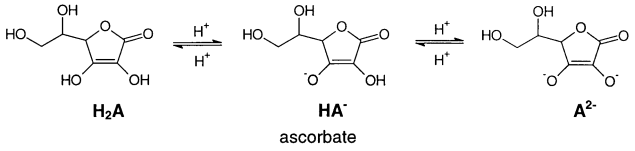


Fig. 3. Mechanism of reaction of *cis*,*trans*-[$PtI_2(OH)_2$(en)], from Kratochwil et al. [99].

NMR. This platinum(II) complex may then react further with albumin.

The dichloro analogue *cis*,*trans*-$[PtCl_2(OH)_2(en)]$ (**4**) did not affect the thiol concentration over the same period. The coordination capability of the diiodoplatinum species was reinforced by showing that the platinum(II) analog $[PtI_2(en)]$ was also reactive with Cys-34 (Fig. 3).

3.6. Ascorbate



Ascorbate is a vitamin (C) in humans which serves several roles. It is considered the primary small-molecule antioxidant in the body, but is also an excellent reductant, and is able to reduce catalytic metals such as Fe^{3+} and Cu^{2+} [100,101]. Ascorbate is also involved in enzyme recycling, collagen formation and Vitamin E recycling, and a deficiency in ascorbic acid leads to scurvy.

Ascorbic acid (H_2A) has two pK_a, pK_1 (3.95) and pK_2 (11.24). At physiological pH, 99.9% of ascorbate is present as the ascorbate monoanion HA^- [102]. The large difference between the pK_1 and pK_2 of ascorbate is due to the resonance stabilization of the monoanion, and the stabilising intramolecular hydrogen bonding [101].

Blatter et al. demonstrated that reduction of iproplatin and *cis*,*trans*,*cis*-$[PtCl_2(OH)_2(NH_3)_2]$ (oxoplatin) by ascorbate resulted in their respective platinum(II) analogues, *cis*-dichlorobis(isopropylamine)platinum(II) and cisplatin [103]. As a reductant, the ascorbate monoanion can undergo two one electron steps to yield dehydroascorbic acid as shown in Fig. 4, and, therefore, overall 1:1 ascorbate:Pt(IV) reaction stoichiometry is expected.

Iproplatin is reduced by ascorbic acid under pseudo-first-order conditions, and Evans et al. suggested that the reaction could be reversible [104]. The pH of the reaction was not reported, but the reaction pH must be below the pK_1 of ascorbate for ascorbic acid H_2A to be the dominant species in solution. Reaction of iproplatin with ascorbate at pH 7.0 results in the formation of dehydroascorbic acid, with the ascorbate radical intermediate observed by EPR at pH 7.0 but not below pH 4.0 [105]. This observation suggests that two one-electron transfer processes are involved. The reaction also displays an induction period followed by an increased rate of reaction which is believed to be due to generation of the platinum(II) analogue via Eq. (7′).

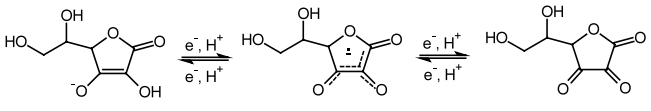


Fig. 4. Two-step two-electron reduction of the ascorbate monoanion which dominates at physiological pH.

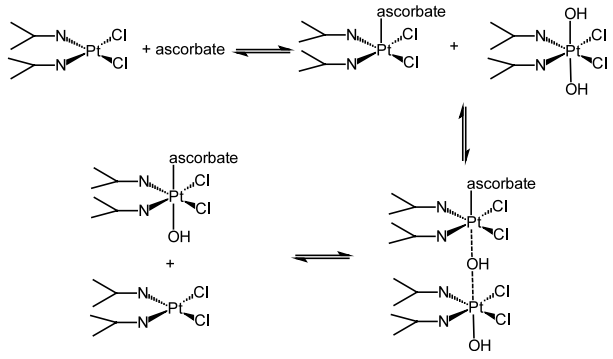


Fig. 5. Platinum(II) catalysed substitution of ascorbate onto platinum(IV) [105].

$$Pt(IV) + asc \rightarrow Pt(II) + \text{dehydroascorbic acid} \quad (7')$$

The subsequent increased reaction rate is best explained by platinum(II) catalysed substitution of ascorbate onto platinum(IV), a mechanism first described by Basolo [56,57,97,98] (Fig. 5).

The reduction of the platinum(IV) ascorbate-species would take place in two one-electron steps by two ascorbate molecules—the first inner sphere by the ligand and the second outer sphere by another ascorbate. The ascorbate radicals produced would then react to form an ascorbate and a dehydroascorbic acid molecule, as shown in Eqs. (8) and (9).

$$Pt(IV)asc + asc \rightarrow Pt(II) + 2\ asc^{\bullet} \quad (8)$$

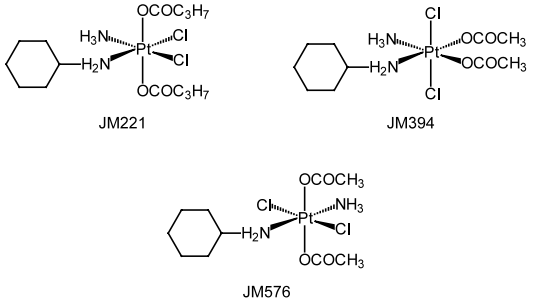
$$2asc^{\bullet} \rightarrow asc + \text{dehydroascorbate} \quad (9)$$

The reaction scheme suggested by Bose is complicated and it is important to consider that a number of mechanisms could be involved in the reduction of platinum(IV) complexes in vivo. The direct reduction of complexes with very low reduction potentials such as iproplatin may initially be slow, but once platinum(II) is produced it could increase the rate of reaction markedly. The platinum(II) catalysed substitution could indeed allow platinum(IV) binding to proteins including DNA as discussed below.

Choi and co-workers investigated the reduction of eight coordination complexes with five different axial groups ($OCOCF_3$, Cl, $OCOCH_3$, $OCOC_3H_7$, OH) by ascorbate at pH 7.0 [17]. The rate of reduction of the complexes increased in the order $OH < OCOCH_3 \approx OCOC_3H_7 < Cl < OCOCF_3$, which corresponds to the reduction potentials of the complexes. *cis*,*trans*,*cis*-$[PtCl_2(OH)_2(NH_3)_2]$ was not reduced at all by ascorbate, and iproplatin only very slowly; the bulkiness of the

isopropylamine ligands of iproplatin slightly destabilising the complex possibly accounting for the difference in reduction potential between the two complexes. While an induction period followed by deviation from pseudo first-order kinetics due to autocatalysis was confirmed by Choi for iproplatin, kinetic evidence for autocatalysis was not checked.

The reduction of four dicarboxylatoplatinum(IV) complexes, JM216, *cis*,*trans*,*cis*-[$PtCl_2(OCOC_3H_7)_2$-(cha)(NH_3)] (JM221), *trans*,*cis*,*cis*-[$PtCl_2(OCOCH_3)_2$-(cha)(NH_3)] (JM394), and *trans*,*trans*,*trans*-[$PtCl_2$(O-$COCH_3)_2$(cha)(NH_3)] (JM576) by ascorbate were studied at pH 7.0 (buffered) [106].

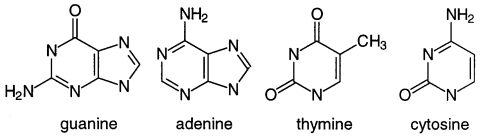


JM216 and JM221 are both reduced to the platinum(II) complex JM118 as has been previously reported from studies of metabolites formed in vivo [93,107], and JM394 was, as expected, reduced with loss of its chloro ligands to give *cis*-[$Pt(OCOCH_3)_2$(cha)(NH_3)]. JM576 could potentially be reduced through either the *trans*-carboxylato ligands or the *trans*-chloro ligands, however, as only *trans*-[$Pt(OCOCH_3)_2$(cha)(NH_3)] was observed in the reaction mixture, the reduction of JM576 occurs exclusively through the chloro ligands. The reduction potentials of complexes with *trans*-carboxylato ligands are greater than those with *trans*-chloro ligands [15], so the result is not counter-intuitive.

The first-order rate constants for reaction of the platinum(IV) complexes with ascorbate were linear at constant pH, but the second-order rate law revealed that the rate constant increased dramatically with increasing pH. As the platinum(IV) complexes examined do not possess labile protons, the pH dependence of the reaction was concluded to be due to the ascorbate ions. It was determined that A^{2-} was seven orders of magnitude more reactive than HA^-, and was the primary species responsible for reduction of the platinum(IV) complexes studied, despite representing less than 1% of the protolytic equilibrium at physiological pH. Hindmarsh et al. had observed the increasing rate of reduction of platinum(IV) complexes with increasing pH but proposed HA^- as the principal reductant [108]. The dicarboxylato complexes are proposed to undergo reduction by an outer-sphere mechanism, except for JM576, which can be reduced by a chloride-bridged reductive elimination mechanism through its *trans*-chloro ligands [106].

The reduced platinum(II) analogues do not undergo coordination with ascorbate or dehydroascorbate subsequent to reaction, however, despite the seemingly simple reaction process, the reduction by ascorbic acid has resulted in a number of papers disagreeing in key aspects. The long-lived radical observed during reduction of iproplatin by Bose [105] was ascribed to an autocatalytic mechanism, and this was supported by Choi [17]. However, the buffered system studied by Lemma revealed that the doubly deprotonated ascorbate anion was the major reductant; ascorbic acid H_2A (acidic solution) as a reductant as studied by Green et al. is not physiologically relevant. While the *trans*-dicarboxylato platinum(IV) complexes are more easily reduced than the *trans*-dihydroxo complexes, Lemma and co-workers did not investigate the presence of long-lived radicals in their reduction mixture. The presence of both halide-bridged and outer-sphere reduction mechanisms for ascorbate (depending on the nature of the platinum(IV) complex) suggest that several mechanisms of reduction might be at play in the body for a given complex.

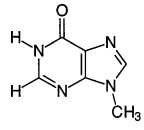
3.7. DNA nucleobases, nucleotides and their analogues



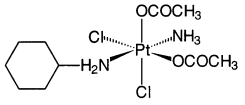
Studies with cisplatin show that it binds preferentially to the N7 position of the nucleobases guanine and adenine on DNA, with very little binding to thymine and cytosine [109]. Reduction of platinum(IV) complexes by DNA could occur from either the nucleobases or the phosphate-sugar backbone. For this reason comparison of reactions of (say) 5′-GMP (GMP = guanosine 5′-monophosphate) versus guanine are of interest. The platinum(IV) drug JM216 has been shown to be intact intracellularly [54], and so studying the reactions of platinum(IV) complexes with the DNA building blocks is relevant. It must be remembered that the phosphate-sugar backbone is sterically accessible to the platinum(IV) complex. Oxidation products of the nucleobases determined in isolation may not be relevant when only a portion of the nucleobase is either projected into the solvent in the major or minor groove to be available for reaction.

A number of platinum(IV) complexes with nucleobases, nucleotides and their analogues have been reported, however, these were synthesised by oxidising their platinum(II) analogues [110–113], and are, there-

fore, not necessarily representative of the substitution products of platinum(IV) complexes—particularly with reference to the donor atoms. Guanine coordinates to platinum(II) almost exclusively through N7 whereas platinum(IV) could have a preference for any number of sites, including N1 of guanine, and the sugar backbone. Oxidation of a platinum(IV)-cytosine complex results in ring closure to a four-member chelate through N3 and N4 not seen for the platinum(II) oxidation state [110].

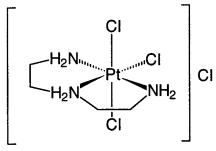


9-methylhypoxanthine

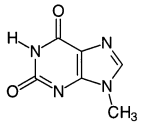


(5)

Initial investigations of a number of *cis*-diam(m)inetetrachloroplatinum(IV) complexes with the guanine analogue 9-methylhypoxanthine (mHyp) showed that at high temperature (80 °C) both platinum(II) and platinum(IV) nucleobase products are formed [114]. At physiological temperature (37 °C) no platinum(IV) products are observed from the reaction of mHyp and *trans*,*cis*,*cis*-[Pt(NH_3)(cha)Cl_2(OAc)$_2$] (**5**), the complex being reduced to its platinum(II) analogue and coordinated by mHyp through the N7 position [115]. Under the same conditions, *cis*,*cis*,*trans*-[Pt(NH_3)(cha)Cl_2-(OH)$_2$] and *trans*,*trans*,*trans*-[Pt(NH_3)(cha)Cl_2(OH)$_2$] are not reduced or coordinated by mHyp over 14 days, but mHyp does coordinate on addition of a reductant such as glutathione.



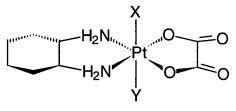
(6)



9-methylxanthine

mer-[$PtCl_3$(dien)]Cl (**6**) does not completely react with mHyp after 50 h at 37 °C, but results in a mixture of platinum(II) and platinum(IV)-mHyp products. The reaction mixture also contained 9-methylxanthine (9-mxan), which is the oxidation product of 9-methylhypoxanthine [116]. This result is important, as more than 20 modified purines and pyrimidines resulting from oxidative damage have been reported [117], and understanding the oxidation products caused by platinum(IV) complexes can allow an understanding of potential destabilisation of the DNA duplex structure.

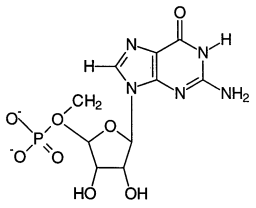
Another interesting implication for platinum(IV) chemotherapeutic agents is the catalytic effect for platinum(IV) reaction with nucleobases when a small amount of platinum(II) analogue is present. The substitution of 9-methylxanthine in *cis*-[$PtCl_4$(NH_3)$_2$], *cis*-[$PtCl_4$(NH_3)(cha)] and *cis*-[$PtCl_3$(dien)]Cl is catalysed by their respective platinum(II) analogues [118]. For example platinum(IV) 9-methylxanthine products appear after about 96 h at 28 °C for *cis*-[$PtCl_4$(NH_3)$_2$], but when the reaction mixture contains 10% cisplatin, platinum(IV) 9-methylxanthine products appear in only 3 h. A number of mechanisms are suggested by the authors, though only axially substituted platinum(IV) complexes are suggested. The coordination of 9-mxan is shown by ^{1}H-NMR to be via N7, but this is likely to be a result of 9-mxan coordinating to platinum(II), which is then oxidised by bridging to a platinum(IV) complex, and there does not appear to be any evidence presented which allows for discrimination between axially and equatorially coordinated 9-mxan. Products observed from uncatalysed reactions with platinum(IV) complexes are the same as those for catalysed reactions: two platinum(II) products (mono- and di-substituted platinum(II) analogue) but additionally the catalysed system has one platinum(IV)-9-mxan product. Further, platinum(II) products are formed in the uncatalysed platinum(IV) systems after long periods of time which are identical to those formed rapidly in the platinum(II) catalysed system. This indicates that trace amounts of platinum(II), either present from synthesis or formed by reduction of the platinum(IV) complex, are catalysing the reaction, but the minimal platinum(II) present means the rate is less than that of the 10% platinum(II) solution. The kinetically inert platinum(IV) complexes would not be expected to undergo direct substitution on this time scale.



(**7**) X = Y = Cl
(**8**) X = Cl, Y = $OCOC_5H_{11}$
(**9**) X = Y = $OCOC_4H_9$

The reaction of *trans*-[$PtCl_2$(ox)(*R*,*R*-dach)] (**7**) and *trans*-[$PtCl$($OCOC_5H_{11}$)(ox)(*R*,*R*-dach)] (**8**) with mHyp and 9-mxan ultimately results in the platinum(II) analogue coordinated to two bases, [Pt(dach)(base)$_2$] which is formed via a platinum(IV) intermediate [12]. However, the conceivable intermediates are not detectable in the reaction mixture, and the oxidised products of mHyp and 9-mxan with platinum(II) are not investigated or characterised by the authors. Despite a 1:2 reaction stoichiometry. *trans*-[Pt($OCOC_4H_9$)$_2$-(ox)(*R*,*R*-dach)] (**9**) is not reduced by either of the free bases.

A comparison of the reactivity of platinum(IV) complexes with nucleobases and nucleotides is instructive. The phosphate moiety of nucleotides is fully oxidised, so the sugar unit is the only unit other than the base which could potentially be oxidised.



Early investigations in which nucleotides such as 5′-GMP were reacted with platinum(IV) complexes, e.g. $[PtCl_4(en)]$ revealed that the main product was a platinum(II) complex, $[Pt(5'\text{-}GMP)_2L_2]$ (L = am(m)ine ligand), and no platinum(IV) reaction products were observed by ^{1}H-NMR [119]. Under the same conditions, *cis*,*trans*-$[PtCl_2(OH)_2(en)]$ was not reduced by the nucleotide. The addition of a reductant such as ascorbate results in a higher yield of $[Pt(5'\text{-}GMP)_2L_2]$, as the ascorbate would reduce the platinum(IV) complexes at a greater rate than 5′-GMP, and the platinum(II) analogue would then coordinate 5′-GMP. The oxidised 5′-GMP resulting from the reaction is not described as taking further part in the reaction. However, if, as suggested, the electrons for reduction come from the sugar moiety, the subtle changes in ^{1}H-NMR of the non-exchangeable protons on the guanine of 5′-GMP may not be detectable. Reaction of *mer*-$[PtCl_3(dien)]Cl$ (**6**) with 5′-GMP by Roat et al. was shown to be incomplete after 7 days at 37 °C, however, both platinum(IV)- and platinum(II)-5′-GMP products were assigned by NMR [116]. The oxidised species again could not be identified.

trans,*cis*,*cis*-$[Pt(NH_3)(cha)Cl_2(OCOCH_3)_2]$ (**5**) is reduced slowly by 5′-GMP (detected by the appearance of free acetate in the ^{1}H-NMR spectrum), without platinum(IV)-5′-GMP formation, but a platinum(II)-5′-GMP complex is formed following reduction. *cis*,*cis*,*trans*-$[Pt(NH_3)(cha)Cl_2(OH)_2]$ (JM149) and *trans*,*trans*,*trans*-$[Pt(NH_3)(cha)Cl_2(OH)_2]$ (JM335) do not react with 5′-GMP in the same fashion as *trans*,*cis*,*cis*-$[Pt(NH_3)(cha)Cl_2(OCOCH_3)_2]$, but addition of glutathione results in reduction of the all *trans* isomer and results in platinum(II) products coordinated to both 5′-GMP and glutathione [115].

The reaction of *trans*-$[PtCl_2(ox)(R,R\text{-dach})]$ (ox = oxalato) with 5′-GMP results only in platinum(II) products (assigned by ^{1}H-NMR), mostly $[Pt(dach)(5'\text{-}GMP)_2]^{2+}$ ($t_{1/2} = 36$ h), however if a chloro ligand is replaced by an ($OCOC_5H_{11}$) ligand giving *trans*-$[PtCl(OCOC_5H_{11})(ox)(R,R\text{-dach})]$ (**8**) a more rapid reduction ($t_{1/2} = 12$ h) with the same platinum(II) product is observed [12]. *trans*-$[Pt(OCOC_4H_9)_2(ox)(R,R\text{-dach})]$ (**9**) did not react to any significant extent. No platinum(IV) products or intermediates are formed, which is in contrast to the reaction of nucleobases with platinum(IV) complexes, and again supports the notion that the ribose-sugar moiety is the reductant in DNA. The ^{1}H-NMR of 5′-GMP does not shift despite the oxidation of 5′-GMP in the reaction. Choi et al. did find a platinum(IV) intermediate product during the reduction of tetraplatin by 5′-GMP but it disappeared to be replaced by the platinum(II)-5′-GMP product [120]. The proposed mechanism is for slow substitution of an equatorial chloro position, reduction of the metal centre by the 5′-GMP ligand to oxidised 5′-GMP, and subsequent addition of a second 5′-GMP, resulting in [Pt(dach)(5′-GMP)(ox5′-GMP)], as shown in Fig. 6.

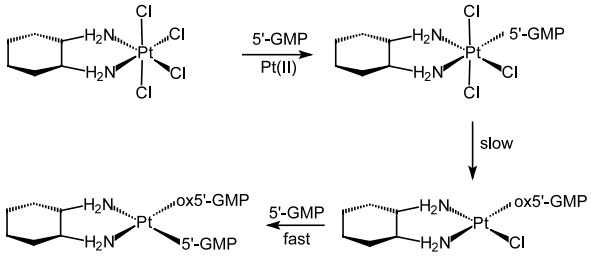
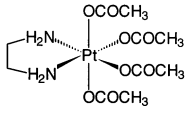


Fig. 6. Reaction of tetraplatin with 5′-GMP [120].

Despite the initial substitution step being slower than the inner sphere reduction step that follows in the proposed scheme, Choi proposes that the intermediate is long-lived, and observable by ^{1}H-NMR. Apart from this kinetic obstacle, the scheme also suggests direct substitution into the platinum(IV) complex. It is more likely that a platinum(IV) intermediate is formed from a 5′-GMP substituted platinum(II) complex oxidised by a platinum(IV) complex. This is supported by an induction time evidenced in kinetic plots of the reaction, and the obervation that the reaction is catalysed by addition of a small amount of platinum(II) [120]. The reaction of tetraplatin with DNA is not necessarily physiologically relevant as it is known to have a half-life in vivo of 3 s, and as such would not reach cellular DNA in the platinum(IV) form [21].



(**10**)

The reaction of 5′-GMP with a more inert complex, $[Pt(OAc)_4(en)]$ (**10**), resulted in a number of platinum(IV)-5′-GMP products (inferred by ^{1}H-NMR) which formed over a number of weeks [121]. In this instance the substitution product, $[Pt(OAc)_3(5'\text{-}GMP)(en)]$, is not expected to form by platinum(II) catalysed substitution as hydroxo ligands would be present in the axial positions following reoxidation, not acetato ligands. The formation of $[Pt(OAc)_3(5'\text{-}GMP)(en)]$ is also supported by mass spectroscopy, though this technique obviously does not provide isomeric information.

Studies where both nucleotides and nucleobases were examined separately with platinum(IV) complexes in-

dicate that reduction by the nucleotides is more rapid. Little change in the H8 shift in the NMR spectrum of 5′-GMP after reduction, and the absence of platinum(IV) intermediates suggest it is the sugar moiety which dominates the nucleotide reduction of platinum(IV) complexes. Certainly if platinum(IV) complexes arrived at DNA intact it has been shown by molecular modelling that there is little steric restriction to them approaching the major groove of DNA [15], though rates of the reactions described above suggest coordination is unlikely. Despite this, many studies of the reaction of platinum(IV) complexes with DNA have been carried out.

3.8. Deoxyribonucleic acid (DNA)

Deoxyribonucleic acid (DNA) is considered the ultimate target of cisplatin and its congeners [122]. The likelihood of platinum(IV) complexes arriving at the DNA intact is dependent on the stability of the platinum(IV) complex. All the biomolecules described can reduce or coordinate platinum(IV) complexes to varying degrees, such that the amount of platinum(IV) available for reaction with DNA might be a very small proportion of that which is administered. On arrival at the target site, the kinetic preference for reduction of the platinum(IV) complex by DNA versus the binding of DNA (or no reaction) is a final obstacle for platinum(IV) complexes. While it now seems likely that reduction of platinum(IV) complexes is required for activity [23,48], some evidence is consistent with platinum(IV) complexes binding directly to DNA [116,123].

Rosenberg et al. noted the activity of the *cis* form of the platinum(IV) complex $[PtCl_4(NH_3)]$ while determining the agent responsible for inhibiting *Escherichia coli* cell division [124]. In vitro however the *cis* form was found not to cross-link DNA [125], and Rosenberg accounted for this disparity (between lack of cross-linking and the high activity) in terms of reduction of the complex in vivo prior to binding to DNA [126]. Rosenberg also reasoned that platinum(IV) complexes were not able to bind on steric grounds. While no crystal or NMR structure of platinum(IV) complexes bound to an oligonucleotide have been published, platinum(IV) binding to DNA has been shown to be sterically feasible by molecular modelling [15], as shown in Fig. 7.

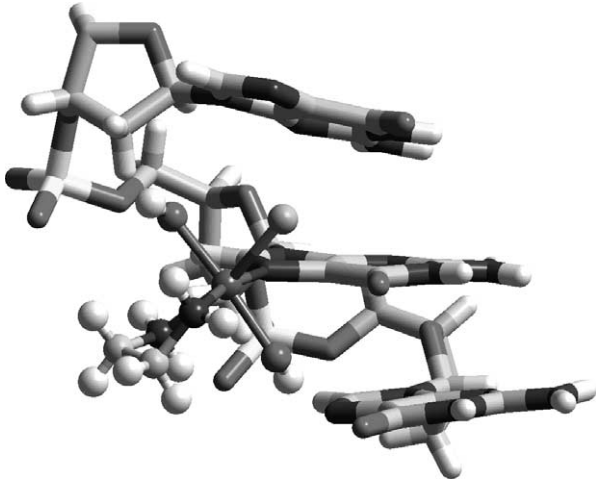
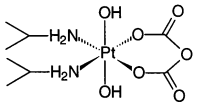


Fig. 7. Molecular mechanics model of the *trans*-$\{PtCl(OH)_2(en)\}^+$ moiety bound equatorially to N7 of gunanine in an 8-mer DNA strand, showing some distortion of the base stacking and potential hydrogen bonding of the hydroxo ligands [20].

The inactive *trans*-$[PtCl_2(NH_3)_2]$ and *trans*-$[PtCl_4(NH_3)_2]$ were shown by Harder to cross-link DNA in vitro [127]. This was reasoned by Rosenberg [126] to be due to the more labile *trans* complex reacting more rapidly with the DNA, and the lack of in vivo activity was because the more labile *trans* complex was being sequestered by other biomolecules before reaching the target.



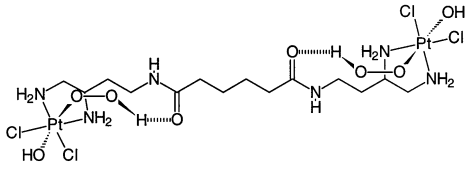
JM28

Mong et al. reported that the platinum(IV) complexes iproplatin (JM9) and *trans*,*cis*-$[Pt(OH)_2(mal)(ipa)_2]$ (JM28) induced breakage of closed-circular PM-2 DNA. The DNA breakage by iproplatin was not inhibited by chloride, suggesting that the axial hydroxo ligands were involved in the mechanism. The DNA breakage is in contrast to platinum(II) complexes which alter superhelical conformation. The possibility of contaminating peroxide from synthesis of the complexes being responsible for the DNA cleavage was considered by the authors, but dismissed due to a number of supporting factors, including the purported purity of the iproplatin used [128]. However, when the crystal structure of iproplatin was reported by Dabrowiak and co-workers there was in fact one hydrogen peroxide present per platinum(IV) complex [129]. Purified iproplatin was then found not to cause either DNA breakage or the deformation typical of platinum(II) binding. Dabrowiak and co-workers also used spin trapping to show that reduction of a number of platinum(IV) complexes including iproplatin was incapable of producing radical species which could be responsible for DNA damage [130]. Defais et al. later reported DNA strand breakage in vivo in bacteria treated with iproplatin and *cis*-$[PtCl_4(NH_3)_2]$, but the mechanism was not elucidated [131].

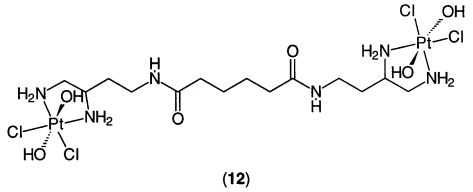
Despite the early work of Dabrowiak demonstrating that reduction of a platinum(IV) complex was required for DNA binding, several other papers have described platinum(IV) binding covalently to DNA. Brabec et al. observed DNA binding by iproplatin and oxoplatin in

vitro and concluded that the platinum(IV) complexes were coordinating monofunctionally without reduction [132], though the authors conceded that the platinum(IV) binding requires an excess of platinum(IV) and the time course for the direct binding is long (10% bound after 12 days) [133]. In a later paper concentrating on DNA binding by oxoplatin, digestion and HPLC was used to show that oxoplatin binds preferentially to guanine residues on DNA [123]. Further, not only monofunctional, but also inter- and intra-strand bifunctional adducts with DNA were reported. Recently, Choi and co-workers investigated the reaction of DNA with the relatively reactive tetraplatin which indicate direct DNA binding, followed by cleavage of the DNA on reduction [134].

Despite the in vitro evidence shown for DNA binding by platinum(IV) complexes, there is much evidence that reduction is required for effective antitumour activity. Early studies by Blatter et al. with iproplatin and oxoplatin showed that neither bound to PM2 DNA unless a reducing agent (Fe(II) or ascorbic acid) was added [103,135], leading to the conclusion that reduction is required for activity. The reduced iproplatin metabolite, *cis*-$[PtCl_2(ipa)_2]$ (CIP) was found to bind to DNA at a much greater rate than the parent drug in vitro [136], and the platinum(II) analogue of JM216 has similarly been shown to bind DNA [137].



(11)



(12)

Recently, Beck and co-workers have investigated the binding of a number of bis[platinum(IV)] complexes with DNA, and found that while the *trans*-hydroxo complexes (e.g.(**12**)) did not bind to DNA, products possessing a hydroperoxy ligand (**11**) did form Pt-DNA adducts reportedly without reduction though this is not confirmed [138]. While the possibility that binding is due to trace platinum(II) catalysis [34], the effect of the peroxo species contributing to DNA damage was not considered. Reaction with DNA at the coordinated hydroperoxy ligand or with peroxide yielded by the complex could take place, causing DNA oxidation and damage, though stable platinum(IV)-peroxo species have been reported [139].

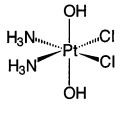
The more rapid DNA binding of platinum(II) metabolites of platinum(IV) drugs and the reported cytotoxic activity of platinum(II) analogues lend support to the notional requirement for platinum(IV) to be reduced [5]. Early reports of iproplatin binding to DNA were subsequently shown to be due to other effects, and the short half-life of complexes such as tetraplatin (3.5 s) indicates the drug would be reduced and consumed well before arriving at the target site. Development of complexes with very negative reduction potentials (e.g. iproplatin) could provide drugs that arrive at the target site intact.

4. Biotransformation products of platinum(IV) complexes

4.1. Iproplatin

When investigating a range of platinum(II) and (IV) *cis*-diamine complexes, it was found that iproplatin (CHIP) was two orders of magnitude more soluble than its platinum(II) congener (44.1 vs. 0.22 mM) [6]. It was selected for further examination, and preclinical pharmacokinetic and metabolic studies in the dog showed that the unchanged drug was excreted rapidly in the first hour after administration, followed by a number of unidentified metabolites [26]. The half-life of iproplatin in vivo was found to be 0.3–0.5 h, and iproplatin was shown not to bind to proteins in vitro. The platinum(II) analogue was subsequently identified as the major metabolite of iproplatin, and it was demonstrated that a number of biomolecules reduced iproplatin in vitro [23]. Metabolism of iproplatin in patients in clinical trials indicated that the iproplatin was reduced intracellularly, and a high proportion of total platinum in plasma is protein-bound [140]. The conclusion drawn from metabolic studies is that reduction is required for activity, as NMR of metabolites showed the only platinum(IV) species to be the intact iproplatin. The high solubility and inertness of iproplatin probably allows for distribution in the body before reduction to platinum(II).

4.2. Oxoplatin



oxoplatin

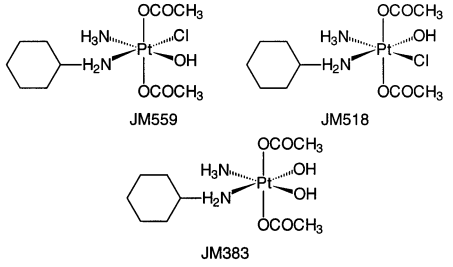
A small number of studies were conducted with a platinum(IV) analogue of cisplatin, *cis*,*trans*,*cis*-$[PtCl_2(OH)_2(NH_3)_2]$ (oxoplatin). Studies of the metabolism of ^{191}Pt-labeled oxoplatin in mice revealed that it is

converted into at least four metabolites, one of which is cisplatin [141]. The inertness and low reduction potential of oxoplatin is reflected in its slow plasma protein binding, and it was reported to be metabolised slowly [142]. Again reduction appears to be required for the activity of oxoplatin to be effected [143], however, the intact drug was also found, indicating the drug can arrive at the cell intact.

4.3. Tetraplatin

Tetraplatin (also known as ormaplatin) showed activity against cell lines resistant to cisplatin [144], and entered clinical trials. Unfortunately the clinical trials were abandoned at the phase I level due to high levels of neurotoxicity [9]. As discussed previously, platinum(IV) complexes with chloro ligands in the axial sites have relatively high reduction potentials, and the rapid reduction of tetraplatin to its platinum(II) analogue [$PtCl_2$(dach)] was noted early in in vitro and in vivo studies [21,145]. The half-life in blood plasma is 3 s at 37 °C [21,66]. Reduction is followed by slower substitution reactions of the chloro ligands by biomolecules such as methionine (forming [Pt(met)(dach)]) and cysteine (forming [Pt(cys)(dach)]) [21,66,146]. Phase I trials showed that reduced tetraplatin is the major metabolite excreted [147]. The rapid rate at which tetraplatin is reduced, and the ability of the primary metabolite [$PtCl_2$(dach)] to cross cell membranes [148] suggest that the tetraplatin metabolite [$PtCl_2$(dach)] is the active species.

4.4. JM216



Metabolic studies of JM216 show a variety of platinum(II) and platinum(IV) metabolites in plasma ultra-filtrate, indicating that ligand substitution on the platinum(IV) takes place under biological conditions [22]. No parent drug is detectable in patients after as little as 15 mins. The platinum(II) analogue of JM216, JM118, is the major biotransformation product in human plasma indicating straightforward reduction in vivo as the major fate of the administered drug [149]. Three platinum(IV) species, JM559, JM518 and JM383 provide evidence of aquation with loss of equatorial chloro ligands [93,150]. It is unclear whether the JM118 is derived from the parent drug or the other platinum(IV) metabolites, though it is likely that if the active species is from JM559, JM518 or JM383 that their respective aquaplatinum(II) analogues would be returned to the dichloro species due to the high chloride concentration in the blood. The aquation of JM118 would proceed via similar pathways to those for cisplatin. Increased glutathione levels result in a platinum–glutathione metabolite and a corresponding decrease in the platinum(IV) metabolites, confirming the role of thiols as biological reductants of JM216 [54].

5. Conclusions

Reduction of platinum(IV) complexes by biomolecules appears to be required for activity, and several parameters can be modified to tune the activity of the drug. Variation of the axial ligands of platinum(IV) complexes alters the lipophilicity of the complex which can allow for modified activity of platinum drugs against cisplatin resistant cell lines due to increased passive diffusion into cells. This variation of the axial ligands also alters the reduction potential of the complex, and the slower the rate of reduction (within reason, i.e. before clearance), the greater the chance of the drug arriving at the target site intact. While structural alterations of the axial ligands can alter the properties of the complexes, it is important that the platinum(II) analogue yielded on reduction is active in vivo as ultimately it is the platinum(II) complex which confers the cytotoxic mechanism of action. Efforts have been directed towards design of platinum(II) complexes which avoid binding to biomolecules described above, but this could come at the expense of activity. With careful design it is possible that platinum(IV) complexes can protect the more reactive platinum(II) moiety on the way to the target site before reduction allowing activity within the cell.

Acknowledgements

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