DALTON PERSPECTIVE

Platinum binding to DNA: structural controls and consequences

Trevor W. Hambley

Centre for Heavy Metals Research, School of Chemistry, University of Sydney, Sydney, NSW 2006, Australia. E-mail: t.hambley@chem.usyd.edu.au

Received 20th June 2001, Accepted 14th August 2001
First published as an Advance Article on the web 13th September 2001

In recent years there have been numerous major advances in the understanding of the factors controlling all aspects of platinum binding to DNA and of the consequences of DNA binding. The interaction with DNA involves numerous stages, including: aquation of the complex, preassociation with the DNA, monofunctional adduct formation, closure to a bifunctional adduct, distortion of the DNA and recognition of this distortion. In this review we outline how the recent advances have increased our understanding of the factors controlling each of these steps and the implications for rational drug development.

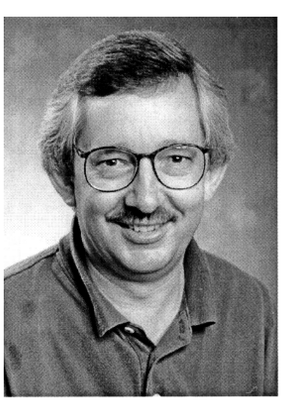
Introduction

The platinum-based anticancer drugs ("the platinums") are already arguably the world's most extensively used anticancer drugs and their use is continuing to grow at a remarkable rate. In 1998 sales of cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) and carboplatin ($[Pt(CBDCA)(NH_3)_2]$, CBDCA = cyclobutane-1,1-dicarboxylato) were US$ 525 and US$ 132 million respectively.[1] Only Taxol outsold this pair. Oxaliplatin, still only registered for use in Europe and Japan, generated an extraordinary US$ 67 million in the first half of 1999. Figures for the first three months of 1999 suggested annualised increases over these 1999 figures of 31% for cisplatin and 16% for carboplatin suggesting total annual sales for the platinums approaching US$ 1 billion. The emergence of novel drugs such as BBR3464 ($[(trans\text{-}PtCl(NH_3)_2)_2\{\mu\text{-}trans\text{-}Pt(NH_3)_2(NH_2(CH_2)_6NH_2)_2\}]^{4+}$) and ZD0473 ($[PtCl_2(2\text{-picoline})(NH_3)]$), which show promise against cancers resistant to the current platinums,[2–4] and the rapidly increasing use of cisplatin and carboplatin in combination therapies give rise to expectations of further increases in the use and success of the platinums.

Cisplatin has turned testicular cancer from a deadly disease into an eminently curable one, to the extent that more than 90% of sufferers of testicular cancer are now cured.[5] Perhaps the most celebrated case is that of American cyclist Lance Armstrong whose testicular cancer had metastasised to the brain yet was cured to the extent that he has gone on to win 3 Tours de France (so far)—even if he was reported as saying that he had been treated with plutonium![6] Unfortunately, success at this level is not uniform across all cancers and this coupled with the undesirable though manageable toxicities of the platinums is continuing to drive the search for new analogues.

The focus of efforts based on rational design is the interaction of the platinums with DNA since it is almost certainly this that leads to cell death and the anticancer effects. The interaction with DNA is a multistage process as shown in Fig. 1 and involves at least: aquation, preassociation, monofunctional adduct formation, closure to a bifunctional adduct perhaps preceded by aquation, distortion of the DNA and recognition of this distortion by a variety of proteins. A number of different adducts are formed that evidently contribute differently to the anticancer effect and to genotoxic side effects. Platinum binding to DNA ultimately leads to cell death, most probably *via* apoptosis (programmed cell death).[7] The sequence of events involved in the induction of apoptosis is not fully understood, but the current understanding has been well summarised by Eastman.[7] An important aspect of this process is believed to be high-mobility group (HMG) domain protein recognition of and binding to DNA bent by the platinum adduct.[8] HMG-domain protein binding is believed to interfere with the repair and removal of the platinated site by the normal repair mechanisms and this interference probably initiates the apoptosis process. In support of HMG-domain proteins playing a major role is the observation by Lippard and colleagues that upregulating HMG-domain protein production with hormones such as oestrogen increases the sensitivity to cisplatin.[9] This observation has led to a clinical study of the use of cisplatin and oestrogen or progesterone in combination therapy for the treatment of ovarian cancer. Thus, it emerges that an understanding of the nature and consequence of DNA platination can lead to potential new therapies.

In this review we describe the current understanding of the factors that control the various stages of platinum binding to DNA shown in Fig. 1 and of the consequences of this binding both in terms of its effects on DNA structure and the recog-



Trevor W. Hambley

Trevor Hambley was born in Albany, Western Australia and received his BSc(Hons) degree from the University of Western Australia in 1977. He then moved to Adelaide and undertook his PhD work on molecular modelling of metal complexes with Michael Snow. Postdoctoral studies followed at the Australian National University, in 1982 with Glen Robertson and Alan Sargeson, and in 1983 at CSIRO Energy Chemistry, Lucas Heights. His move east across Australia was completed in 1984 when he moved to the University of Sydney where he is currently an Associate Professor in the School of Chemistry. His scientific interests are in the area of medicinal inorganic chemistry with emphases on platinum anticancer agents, DNA binding agents, MMP inhibitors and metal-based anti-inflammatory drugs. He also has interests in theoretical modelling of metal complexes, both developmental and applications to bioinorganic chemistry, and heads the crystallography facility in the School of Chemistry. He has won awards for research and for postgraduate teaching and has published nearly 400 books, reviews and papers.

DOI: 10.1039/b105406f

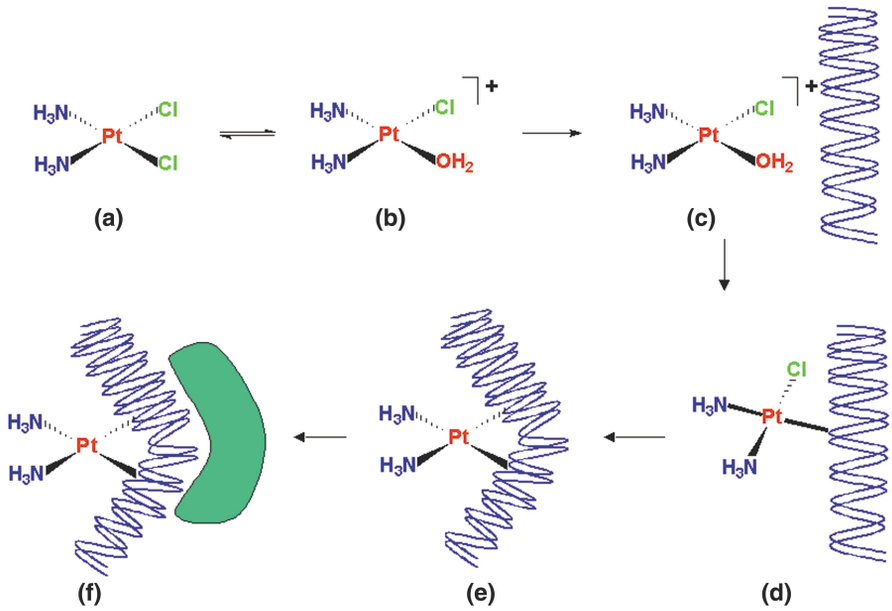


Fig. 1 The sequence of events in cisplatin binding to DNA.

Table 1 Rate constants for the first stage of cisplatin aquation in water and in the presence of 14-mer oligonucleotides with different binding sites[a]

$$cis\text{-}[PtCl_2(NH_3)_2] + H_2O \rightleftharpoons cis\text{-}[PtCl(NH_3)_2(OH_2)]^+ + Cl^-$$

Conditions	$10^{-5}k_{1H}/s^{-1}$	Ref.
H_2O, 9 mM $NaClO_4$	2.38(4)	28
5′-d(ATACATGGTACATA)-3′ : 5′-d(TATGTACCATGTAT)-3′	1.83(3)	31
5′-d(AATTGGTACCAATT)-3′	1.62(2)	28
5′-d(AATTAGTACTAATT)-3′	1.57(2)	32
5′-d(AATTGATATCAATT)-3′	1.23(2)	32
5′-d(AATTAGTACTAATT)-3′/82 mM NaCl	0.33(2)	33
5′-d(AATTGATATCAATT)-3′/65 mM NaCl	0.226(4)	33

[a] Standard deviations are given in parentheses and apply to the last significant figure.

nition of this bending. The many reactions with extracellular and intracellular species that the platinums may undergo prior to encountering DNA have been the subject of a recent review by Reedijk[10] and the downstream effects of DNA platination have been reviewed by Jamieson and Lippard.[11] We have focused on the formation of the bifunctional intrastrand 1,2-adducts since a broad range of information is available for these adducts. The interstrand and 1,3-adducts are potentially equally important,[12] but, as yet, less complete information is available on their formation.

Aquation

The binding to DNA of Pt complexes with halogeno leaving groups is almost certainly preceded by aquation, with loss of one or more of the leaving groups. Direct binding has not been ruled out, but Bancroft *et al.* have shown that it is the monoaquated form of cisplatin that is probably responsible for the bulk of DNA binding,[13] and we have shown by DFT studies of model systems that the direct pathway is substantially less favoured.[14] Most of the available evidence is consistent with aquation being the rate determining step[15] and consequently it has been the subject of intensive study since the first report of the aquation rate of cisplatin by Reishus and Martin.[16] For instance, House and colleagues have studied the aquation of cisplatin and related compounds under a variety of conditions.[17–25] However, even the apparently simple process of cisplatin aquation involves many species[26,27] and the many reactions have not been followed in detail until the recent application of [^{1}H,^{15}N] HSQC NMR methods.[28] It was found that the rate of aquation was slowed by 30–50% in the presence of oligonucleotides with an apparent dependence on the sequence of these oligonucleotides (Table 1).[28] This suggests that there is a preassociation between cisplatin and DNA, possibly a hydrogen bonding interaction with the phosphate groups or bases or an intercalative interaction with the bases. Cox *et al.* have also observed a significant slowing of the rate of aquation of 1,1/t,t (n = 6) (1,1/t,t = [{*trans*-$PtCl(NH_3)_2$}$_2$-(μ-$NH_2(CH_2)_6NH_2$)]$^{2+}$) in the presence of a 12-mer oligonucleotide.[29,30] They also observed shifts in the NMR resonances of the Pt complex on mixing with oligonucleotide and interpreted these results as indicating a preassociation.[30] Stronger preassociation is expected for 1,1/t,t than for cisplatin because of its positive charge, consistent with these observations.

Sadler and colleagues have shown that the rate of aquation of chloro ligands depends on the nature of the amine ligands *trans* to them.[34,35] For instance aquation occurred *trans* to a primary amine at approximately twice the rate as *trans* to an ammine.[34,35] It has also been shown that aquation rates can be affected by steric bulk with aquation of [$PtCl_2(NH_3)$-(3-picoline)] being 2–4 times faster than aquation of [$PtCl_2(NH_3)$(2-picoline)] (ZD0473), where the methyl group of the picoline inhibits attack at the platinum.[36] Such differences between aquation rates could significantly influence the relative rates of binding to DNA with consequences for anticancer activity.

The extent of aquation can also be influenced strongly by other changes in the coordination sphere. For example, in complexes such as [PtCl(dien)]$^+$ (dien = *N*-(2-aminoethyl)-ethane-1,2-diamine), [$PtCl(NH_3)_3$]$^+$ and 1,1/t,t, each with an N_3Cl coordination sphere, aquation is more rapid than in cisplatin, but anation of the aquated forms of these complexes is substantially more rapid than for *cis*-[$PtCl(NH_3)_2OH_2$]$^+$.[29,37] Presumably, the increased rate of anation is in part a consequence of the dipositive charge of the aquated complexes (*e.g.* [$Pt(NH_3)_3OH_2$]$^{2+}$) and it is noteworthy that there is a similarity in the rates of anation for these complexes and that for the diaquated form of cisplatin *cis*-[$Pt(NH_3)_2(OH_2)_2$]$^{2+}$.[28,29,37]

In order to understand the factors influencing Pt/DNA interactions it is essential to establish what species binds to the DNA. Since aquation almost certainly precedes the binding of cisplatin, the question that remains is whether it is the binding of the mono- or di-aquated species that predominates.

Chottard, Kozelka and colleagues have provided evidence that they have interpreted as showing that it is the diaquated form of cisplatin, *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$, or its deprotonated form *cis*-$[Pt(OH)(NH_3)_2(OH_2)]^+$, that is the primary route to DNA binding.[38–40] However, the [^{1}H,^{15}N] HSQC NMR data from a number of studies is inconsistent with this in that the monofunctional adducts that form have a chloro ligand bound.[28,31,32] Using the detailed aquation data referred to above, Davies *et al*. were able to show that the monoaquated species, *cis*-$[PtCl(NH_3)_2OH_2]^+$ accounted for at least 98% of the Pt binding to DNA.[28]

Deprotonation of the aquated complexes to give hydroxo complexes is important because these products are less reactive than the aqua complexes, have a lower charge and will associate differently with the DNA. Consequently, there have been a number of determinations of the pK_a's of aquated platinum complexes reported in recent years.[26,29,34,41] These show that for monoaquated cisplatin and close analogues, the pK_a of the bound water is between 6 and 7. Consequently, at biological pH, 5–50% of the complex will be in the reactive aqueous form rather than the substantially less reactive hydroxo form and, therefore, reactions of the latter are not likely to be significant. However, such reactions cannot be ruled out and the amount of the hydroxo form will influence the net rate of the reaction.

Preassociation

The binding of Pt to DNA is irreversible and is therefore kinetically controlled. As a consequence, it is to be expected that the distribution of adducts that results is determined at the level of monofunctional adduct formation.[42] Thus, the formation of the monofunctional adducts is of primary importance and, in this context, it has received surprisingly little attention. Given that the different adducts have different biological effects, positive and negative, it is important to develop an understanding of the factors that lead to a preference for binding at one sequence over another. Such sequence selectivity could be due to sequence recognition *via* outer-sphere binding or to lower barriers to binding at nucleotides in the favoured sequences.

As mentioned above, there is evidence from NMR data and decreased rates of aquation in the presence of oligonucleotides that association occurs between platinum complexes and DNA.[28,29] This is a minor effect and is unlikely to generate significant sequence selectivity whereas preassociation between the positively charged aquated species, *cis*-$[PtCl(NH_3)_2OH_2]^+$, is likely to be much stronger. Recently, Wang *et al.* have obtained direct evidence for preassociation between *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ and surface immobilised oligonucleotides by using quartz crystal microbalance electrodes.[43] It is most likely the aquachloro species that binds to the DNA[28] and, therefore, we have recently used molecular mechanics and molecular dynamics to investigate the preassociation of this cation with DNA.[44] Preliminary results show that $[PtCl(NH_3)_2OH_2]^+$ can form strong associations with bispurine sequences on DNA *via* as many as four hydrogen bonds. However, similar numbers and types of hydrogen bonds can form irrespective of whether the sequence is GpG, ApG or GpA as shown in Fig. 2. Thus, these interactions do not *per se* provide an obvious rationale for the well established preference for the GpG and ApG sequences and the lack of observable levels of binding at the GpA sequence.[45–48] However, what is notable in all of these models is that irrespective of the sequence, the Pt lies in or close to the plane of the purine on the 3′ side of the pair. This is a consequence of the relative orientations of the two purines and results in the Pt being favourably positioned with respect to the lone pair of the N7 atom of the 3′ purine, one of the two binding sites. This is consistent with the observation that binding to the 3′ base is preferred over the 5′ base at

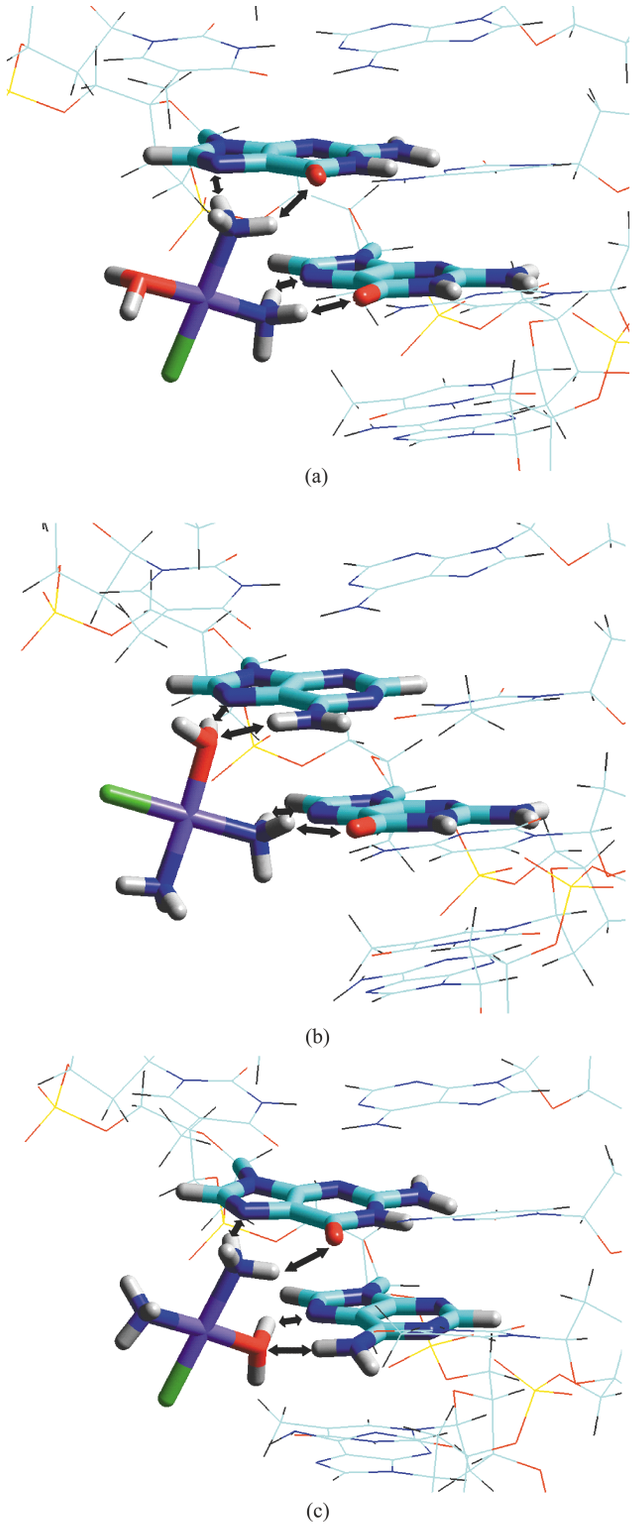


Fig. 2 Models of the preassociation between *cis*-$[PtCl(NH_3)_2OH_2]^+$ and the sequences (a) GpG, (b) ApG and (c) GpA. Hydrogen bonds are indicated with black arrows.

GpG and ApG sequences flanked by thymine bases.[28,31,32] It is also consistent with the lack of binding to the GpA sequences, since the adenine on the 3′ side is inherently a less favoured binding site compared to guanine. However, it is not a complete explanation because binding to the adenine of the GpA site is slower than binding to the adenine of the ApG site[32] despite the more favourable positioning with respect to the former. Thus, there is something inherently less favourable about the GpA site and more modelling studies of association and transition state formation will be required to unravel this.

Table 2 Rate constants for monofunctional adduct formation with 14-mer oligonucleotides with different binding sites[a]

Sequence	$k_{3'}$/s^{-1} M^{-1}	$k_{5'}$/s^{-1} M^{-1}	Ref.
5′-d(ATACATGGTACATA)-3′ : 5′-d(TATGTACCATGTAT)-3′	0.47(8)	0.15(3)	31
5′-d(AATTGGTACCAATT)-3′	0.48(15)	0.16(6)	28
5′-d(AATTAGTACTAATT)-3′	0.37(2)	0.061(7)	32
5′-d(AATTGATATCAATT)-3′	0.018(2)	0.046(3)	32
5′-d(AATTAGTACTAATT)-3′/82 mM NaCl	0.047(8)	0.011(2)	33
5′-d(AATTGATATCAATT)-3′/65 mM NaCl	0.0017(4)	0.0106(6)	33

[a] Standard deviations are given in parentheses and apply to the last significant figure.

Monofunctional adduct formation

As mentioned, platinum binding to DNA is kinetically controlled and therefore, the adduct profile—and the biological consequences—are determined at the time of monofunctional adduct formation. This has only recently been confirmed experimentally by examining the rate of disappearance of cisplatin in the presence of oligonucleotides of different sequences.[28] In this section, we outline recent progress in determining the rates of monofunctional adduct formation.

Bancroft *et al.* used ^{195}Pt NMR spectroscopy to study the binding of cisplatin to chicken erythrocyte DNA at pH 6.5 and 37 °C and obtained a net rate of monofunctional adduct formation of 10.2(7) × 10^{-5} s^{-1} that encompassed aquation and monofunctional binding.[13] Bodenner *et al.* and Ushay *et al.* obtained similar results[49,50] but in none of these studies were the aquation rates and monofunctional adduct formation rates obtained in a single experiment. As mentioned above, it is only the application of HSQC techniques that has allowed the rates of each step to be obtained separately.

Berners-Price, Sadler and colleagues were the first to extract rates for each of the steps associated with cisplatin binding to DNA.[31,51] They found that binding at the 3′-base of a GpG pair was approximately three-fold faster than for binding to the 5′-base (Table 2).[31,51] A possible rationale for this selectivity, based on preassociation, has been outlined above, but it could also be due to differences in the energies of the transition states leading to the adducts. In either case, it is clear that the DNA structure exerts a strong influence on binding rates because the two guanine bases are chemically identical. Davies *et al.* obtained very similar results for binding at the GpG site of a different sequence—though one that also had a TpGpGpT binding site.[28] A similar preference for the 3′-base is observed in the binding of cisplatin to a single stranded oligonucleotide[31] and in the binding at an ApG sequence, though in the latter case the rate constant for binding to the 3′-base (the guanine) was six-fold greater than that for binding at the 5′-base (the adenine).[32] This amounts to a two-fold decrease in the rate of binding at the adenine compared to the equivalent guanine of a GpG sequence, presumably reflecting in part the lower preference for binding to adenine. However, in free bases the preference is 7–20 fold in favour of guanine[52] so the DNA structure remains the controlling factor. In binding to the GpA sequence, the 5′-guanine is preferred over the 3′-adenine by a factor of three[32] so the preference for binding to the 3′-base is reversed. It is not clear whether this is due to the inherently lower preference for adenine or the properties of the GpA sequence as a whole. The presence of 60–80 mM chloride slowed the formation of the monofunctional adducts by about a factor of ten.[33]

The nature of the complex can also have a profound effect on the rate and selectivity of the DNA binding. For instance, the diaquated form of cisplatin, $[Pt(NH_3)_2(OH_2)_2]^{2+}$, displays no preference for the 3′- or 5′-guanine of a TpGpGpT sequence of a hairpin duplex oligonucleotide[39] or a single-stranded oligonucleotide.[53] However, when the sequence is TpGpGpC, the 5′-guanine is preferred by a factor of two in the case of a single-stranded oligonucleotide and by a factor of 12 in the case of duplex oligonucleotide.[38] $[Pt(NH_3)_2(OH_2)_2]^{2+}$ also binds to ApG and GpA sequences at almost the same rate and in both cases binds only to the guanine bases[32] in contrast to cisplatin which, as outlined above, strongly prefers the ApG sequence and binds to both the adenine and the guanine. $[PtCl(dien)]^+$ binds to the 5′- and 3′-guanines of a –TpGpGpT– sequence in a 14-mer at the same rates,[54] again in contrast to cisplatin which binds to the 3′-guanine at nearly three times the rate at which it binds to the 5′-guanine.[31] These differences may be due to interactions between the complex and the duplex DNA structure, but there are also differences in rates of binding of simple nucleotides to different complexes and different sites within a complex. For instance 5′-GMP binds *trans* to the cyclohexylamine of $[PtCl_2(NH_3)(cyclohexylamine)]$ at five times the rate at which it binds *trans* to the ammine.[35]

There is little information available on the structural consequences of monofunctional adduct formation. Given the longevity of some monofunctional adducts,[31] they may have biological significance. Brabec and co-workers have shown that monofunctional adducts, particularly at pyrimidine–guanine–pyrimidine sequences can cause a local denaturation.[55] Jones and others have shown by molecular modelling that monofunctional adducts cause substantial destacking of the base on the 5′ side of the adducts (Fig. 3).[53,56]

Bifunctional adduct formation

Closure of the monofunctional adduct to a bifunctional intrastrand or interstrand adduct is believed to be essential for the anticancer activity to be effected. Bancroft *et al.* obtained a net rate of closure for cisplatin of 9.2(14) × 10^{-5} s^{-1} at 37 °C using ^{195}Pt NMR spectroscopy.[13] However, it is only recently that precise rates of formation of the intrastrand bifunctional adducts from different monofunctional adducts have been determined: by the application of HSQC techniques by Berners-Price, Sadler and colleagues[31] and by HPLC techniques by Chottard, Kozelka and colleagues.[38] Interestingly, these and other studies show that closure from a 3′ monofunctional adduct is approximately an order of magnitude faster than closure from a 5′ adduct, irrespective of whether the sequence is GpG, ApG or GpA.[28,31,32,38] Thus, the DNA structure is a primary influence on the rate of this process. The inherent preference for guanine over adenine is only a minor factor—approximately a factor of two, which interestingly, is similar to the preference noted for monofunctional adduct formation (see above).

It is not clear whether closure to the bifunctional adduct occurs directly from the halogeno form of the monofunctional adduct or whether it is preceded by aquation. The rates of closure are consistent with aquation being the rate determining step,[40] but this suggestion is based on the assumption that the rate of aquation of a complex bound to DNA is similar to that of an unbound analogue in solution. In none of the HSQC studies of cisplatin binding to GpG sequences were aquated monofunctional intermediates observed,[28,31] so if they do form then closure must occur rapidly thereafter. However, small amounts of aquated monofunctional adducts were observed in studies of cisplatin binding to the ApG and GpA sequences even though the net closure rates were not greatly different to

Table 3 Rate constants for closure from monofunctional adducts to bifunctional adducts on oligonucleotides with different binding sites[a]

Sequence	$10^{-5}k_{3'c}/s^{-1}$	$10^{-5}k_{5'c}/s^{-1}$	Ref.
cisplatin			
5′-d(ATACATGGTACATA)-3′ : 5′-d(TATGTACCATGTAT)-3′	3.2(1)	0.24(18)	31
5′-d(AATTGGTACCAATT)-3′	2.55(7)	0.171(11)	28
5′-d(AATTAGTACTAATT)-3′	1.46(5)	0.29(9)	28, 32
5′-d(AATTGATATCAATT)-3′	2.5(5)	0.07(2)	28, 32
cis-$[Pt(NH_3)_2(OH_2)_2]^{2+}$			
5′-d(ATACATGGTACATA)-3′ : 5′-d(TATGTACCATGTAT)-3′	25(3)	4.9(4)	53
5′-d(AATTAGTACTAATT)-3′	3.71(5)	—	32
5′-d(AATTGATATCAATT)-3′	—	0.162(5)	32
5′-d(TTGGCCAA)-3′	0.8(2)	0.06(4)	38
5′-d(TATGGTATATACCATA)-3′	0.9(2)	0.18(1)	57
5′-d(TATAGTATATACCATA)-3′	0.08(1)	0.3(3)	57

[a] Standard deviations are given in parentheses and apply to the last significant figure.

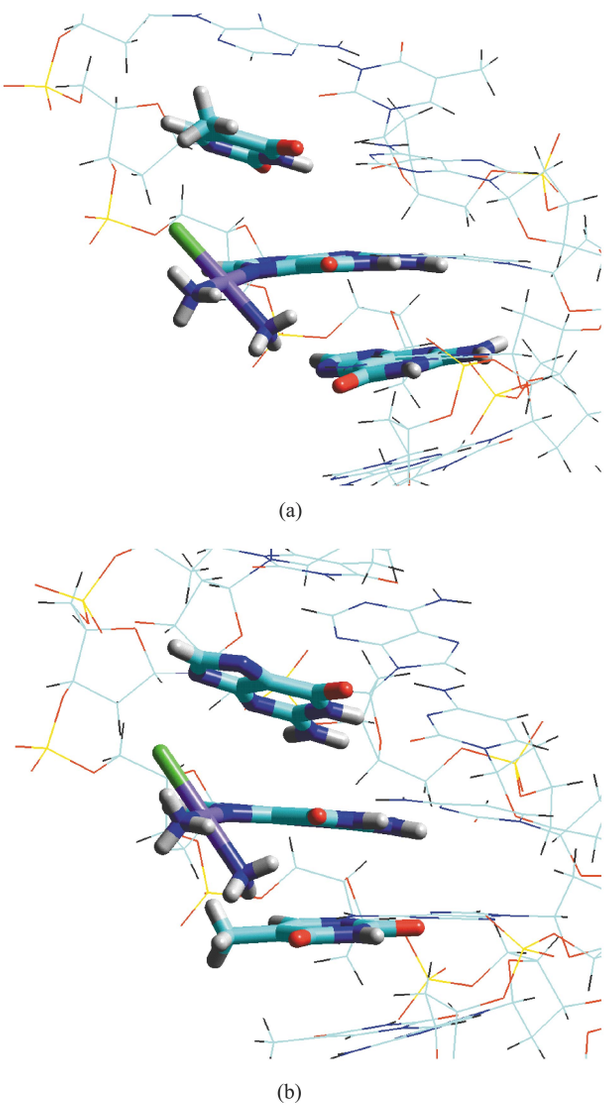


Fig. 3 *cis*-{$PtCl(NH_3)_2$} bound to (a) the 5′-guanine and (b) the 3′-guanine of a TpGpGpT sequence in a duplex octamer showing the destacking of the base on the 5′ side of the monofunctional adduct.

those for GpG.[32] This may be due to closure of the aquated form being slow at the ApG and GpA sequences, as is observed in the reactions of the diaquated complex with these sequences (Table 3).[32] If aquation is the rate determining step then the sequence dependence must arise from a structural control over this process, perhaps *via* inhibition of access to the platinum as suggested by molecular modelling (Fig. 3).[53]

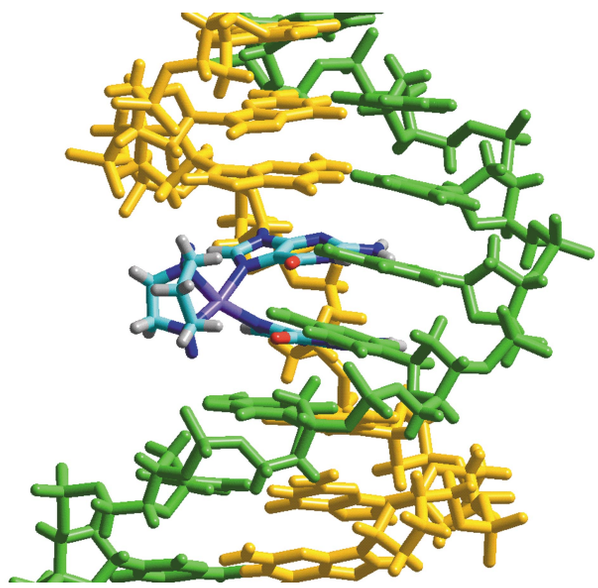


Fig. 4 The isomer formed by the binding of {Pt(hpip)} to a GpG sequence with the propylene chain adjacent to the floor of the major groove.

Studies using the diaquated form of cisplatin reveal a 10-fold higher rate of closure at GpG sequences (Table 3).[38,53,57] Closure from the 3′-side is again faster—by approximately a factor of five compared to closure from the 5′-side—when the diaqua form of the complex is used. Aquation cannot be the factor controlling this difference in closure rates and the fact that the faster closure is again from the 3′-side strongly suggests that the DNA structure is the major factor both here and probably in closure of the chloro adducts generated by cisplatin. Closure rates for single strand DNA are the same from the 5′- and 3′-sides for cisplatin, but, curiously, there is a five-fold difference for the aqua form, with closure from the 3′-side again being the more rapid.[38,53,57]

In work in our group we have shown that the extent of closure of monofunctional to bifunctional adducts can be substantially influenced by steric bulk. For instance the complex, $[PtCl_2(hpip)]$ (hpip = 1,4-diazacycloheptane) forms less than half the proportion of bifunctional GpG adducts that cisplatin forms and modelling shows that this is the result of steric interactions between the diamine ligand and the DNA.[58,59] Indeed, two isomeric forms of the GpG adduct are observed in a 1 : 3 ratio and modelling shows that the minor isomer, identified by 2D NMR as having the bulkier propylene chain adjacent to the floor of the major groove,[60] has more severe steric clashes between the ligand and the DNA (Fig. 4).[59] The data are consistent with steric clashes substantially inhibiting closure to the bifunctional adduct. Stereoselectivity and enantioselectivity

Table 4 Structural parameters in Pt/oligonucleotide complexes

Complex	Canting/°	Bending/°	Out-of-plane deviation/Å 5′, 3′	Ref.
Gel electrophoresis				
Pt/oligonucleotides		32–40		64, 65
X-Ray crystallography				
[Ptd(pGpG)$(NH_3)_2$]	80			74
[Ptd(CCTCTG*G*TCTCC)·d(GGACGACCAGAGG)$(NH_3)_2$]	26	39, 55	1.3, 0.8	70, 71
[Ptd(CCTCTCTG*G*ACCTTCC)·d(GGAGAGACCTGGAAGG)$(NH_3)_2$]	75	61		72
NMR spectroscopy				
[Ptd(CCTCTG*G*TCTCC)·d(GGACGACCAGAGG)$(NH_3)_2$]	49	78	0.8, 0.8	75
[Ptd(CTCTCG*G*TCTC)·d(GAGACCGAGAG)(4AT)(NH_3)]	47–50	≈80	0.5, 0.65	76
[Ptd(CCTG*G*TCC)·d(GGACCCAGG)$(NH_3)_2$]	23	58		77
[Ptd(ATACATG*G*TACATA)·d(TATGTACCATGTAT)$(NH_3)_2$]	44	52	0.8, 0.3	78

was also observed in the DNA binding of [$PtCl_2$(ahaz)] (ahaz = 3-aminohexahydroazepine).[61] Increased steric bulk as in the related complexes [$PtCl_2$(tmdz)] (tmdz = 5,5,7-trimethyl-1,4-diazacycloheptane) and [$PtCl_2$(meahaz)] (meahaz = *N*-methylahaz) can result in stereospecificity in the DNA binding with disfavoured isomers of the bifunctional adducts not observed at all.[62,63] Enantioselectivity in the DNA binding of [$PtCl_2$(tmdz)] indicated that steric bulk also had an influence on the formation of monofunctional adducts.[62] This dependence on steric bulk shows that the rate of aquation is almost certainly not the only factor controlling the rate of closure.

Structural consequences of platinum binding to DNA

Information on the consequences of platinum binding to DNA in terms of bending and unwinding has been available from electrophoresis studies for some years,[64–66] but it is only recently that structural details have begun to emerge from crystallographic and NMR structure determinations. Given that it has long been known that platinum binds to the N7 atoms of guanine, it might have been anticipated that little new information would emerge from these studies. However, differences have emerged in structural features that are now known to be critically important in terms of recognition of the adducts and the downstream consequences. Perhaps most important is the bend induced in the DNA since proteins such as those that contain an HMG-domain recognise bent DNA and so bind at the platinated site. Related to this bend is the angle between the two guanine bases, the extent to which the platinum lies out of the plane of these guanine bases and perhaps the planarity of the guanine bases.

Electrophoretic studies have shown that cisplatin binding at a GpG sequence bends DNA by 32–40°,[64,65] and that the analogue [$PtCl_2$(1,2-chxn)] (1,2-chxn = cyclohexane-1,2-diamine) has a similar effect.[66] Binding at an ApG sequence is also reported to lead to a similar degree of bending.[67] Binding of the HMG-domain protein, HMG-1, to a platinated oligonucleotide has a dramatic effect, more than doubling the bending to 86° or higher.[68,69]

In the crystal structure of cisplatin bound to a dodecamer, the bending induced by the platinum is 39–55°, the guanine bases are canted by ≈26° with respect to one another and the platinum lies 0.8–1.3 Å out of the plane of these guanine bases.[70,71] In this structure, the DNA is B-form on the 3′-side of the adduct and is A-form on the 5′-side. It is possible that the appearance of the A-form is a consequence of the high ionic strength in the crystalline state or the presence of the platinum adduct, but it seems most likely that both factors play a role because, in solution, adduct formation causes A-form characteristics to be adopted, but only by the 5′-sugar of the adduct. In the crystal structure of the HMG-1 domain A adduct of a platinated DNA sequence, the DNA conformation is predominantly B-form, the bending is 61° and the guanine bases are canted by 75° with respect to one another.[72]

A number of structures determined using two-dimensional NMR data have been reported and bending angles, canting and out-of-plane deviations are collected in Table 4 along with those from other sources. In general, the NMR structures reveal bend angles that range from those found in the crystal structure of the cisplatin/dodecamer complex to those found in the crystal structure of the HMG/cisplatin/hexadecamer complex and greater. Marzilli and colleagues have argued[73] that most of the NMR determined structures are not a true reflection of the situation in solution and in reality, all are similar and have a bend angle similar to that in the HMG-domain protein bound complexes in solution. They base this suggestion on the similarity of the NMR shifts of numerous groups in the binding site and adjacent to it in all studies, including that with HMG-domain protein bound, arguing that such similarity could not arise from the range of structures that have emerged from these NMR studies.

It is curious that the bending determined from electrophoresis studies of platinated oligonucleotides is closer to the crystal structure value than the values determined by NMR methods (Table 4). The low degree of bending in the crystalline state is probably related to the adoption of A-form DNA on the 5′-side of the adduct and this in turn will be promoted by the high ionic strength at the point of crystallisation. It may be then, that the ionic strength and/or the environment in an electrophoretic gel similarly encourages the adoption of the A-form and a low bend. If this is the case, the bending determined electrophoretically is somewhat misleading.

The crystal structures of the platinated oligonucleotide[70,71] and of the HMG-1 domain A adduct of a platinated oligonucleotide[72] show unequivocally that a range of bending values is possible and this is supported by the electrophoresis and NMR studies (Table 4). As the bend angle increases the canting angle between the guanine planes decreases and the out-of-plane deviations decrease. This is consistent with increasing bending reducing stresses on the platinum binding site and the geometry at this site moving closer to that in the relatively unconstrained [Ptd(pGpG)$(NH_3)_2$] complex.[74] Since the energy cost of increased bending is balanced by reduced strain at the platinated site, it is highly likely that there is a continuum of conformations with different bends but similar energies. If this is the case, then in solution no one structure will predominate and consequently the NMR data will relate to a lifetime-weighted average structure. This would complicate the analysis of the NMR data because no one structure could be expected to reflect all aspects of the NMR spectra and this may explain in part the difficulties in obtaining consensus structures.

In order to answer some of the questions about how and why the DNA structure responds on formation of the 1,2-bifunctional adduct, we have modelled the sequence crystallised

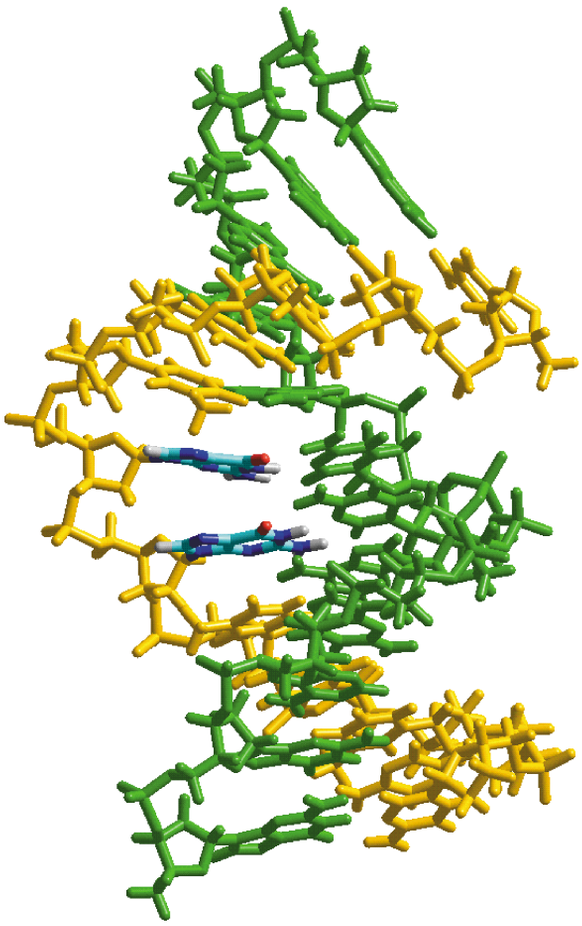


Fig. 5 Formation of an A-form/B-form junction at a GpG site, "predisposes" that site for platination.

by Takahara.[70,71] As a first step, we generated an unplatinated DNA structure with the gross conformational features observed in the crystal structure; A-form on the 5′-side of the GpG site and B-form on the 3′-side (Fig. 5). This produced a substantial bend in the DNA structure and also produced changes in the local geometry of the GpG site that would better accommodate cisplatin binding. Most notably, the two guanine bases are reoriented with respect to one another such that the lone-pairs of the N7 atoms are disposed in approximately the same direction. Thus, an A-form/B-form junction "predisposes" the DNA to bifunctional adduct formation. While this is not likely to occur spontaneously, it does demonstrate why bifunctional adduct formation is prone to promote the adoption of A-form DNA on the 5′-side of the adduct.

The other question we have addressed is the degree to which the platinum atom lies out of the planes of the guanine. The large deviations (up to 1.3 Å) observed crystallographically and in some NMR structures (Table 4) have been met with some scepticism. We first refined the force constants that limit such out-of-plane deformation using highly distorted small molecules where such deviations are observed.[79] Using these force constants and an 8-mer fragment of the A-form/B-form oligonucleotide mentioned above we generated a model for the bifunctional adduct. This is shown in Fig. 6 and the local geometry about the platinum adduct is remarkably close to that observed in the crystal structure.[70,71] In particular, the out-of-plane deviations are reproduced accurately (1.0 and 0.8 Å) demonstrating that such deviations are plausible. It should be noted that these deviations are from the least-squares planes through significantly non-planar guanine bases whereas in the crystallographic and NMR studies, planar guanine bases have been assumed. The out-of-plane deviations will not be significantly affected by any non-planarity but this does raise the question as to the planarity of the guanine bases in such adducts. Aromatic groups such as substituted quinolines are frequently distorted from planarity when bound to platinum and therefore, we believe that the models are realistic.

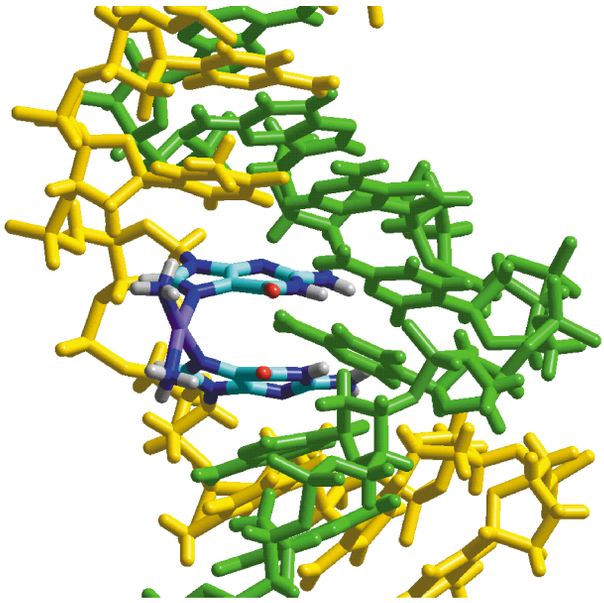


Fig. 6 A molecular model of the GpG adduct formed by cisplatin.

Conclusions and implications for drug design

In the foregoing, we have shown that over the last decade there have been many developments in our understanding of the factors that control platinum binding to DNA and of the consequences of this binding. How can we make use of this information to design compounds that bind to a different sequence and/or are more active? Clearly, the sequence selectivity is determined at the level of monofunctional adduct formation and is sensitive to the form of the complex that binds to DNA. Thus, there is scope for modifying the adduct profile by changing, in particular, the charge and hydrogen bonding properties of the complex and thereby modifying sequence recognition. The consequence of bifunctional intrastrand adduct formation is a distortion of the DNA structure and recognition of this distortion is probably critical in both the repair and the effecting of the activity of the adduct. It is not clear yet whether this process differs for different complexes, but it is likely that sterically demanding complexes or those with other DNA binding motifs could promote different degrees of bending and so modify activity.

Most of the foregoing relates to the mode of action of cisplatin and its close analogues and we are now at the point where rational design can be pursued. However, novel drugs such as BBR3464 and active *trans* compounds work by different mechanisms and will require the development of comparable levels of understanding before rational design can be extended to these promising new areas.

Acknowledgements

I wish to thank my many colleagues and research students for their contributions. In particular Professor Sue Berners-Price, Dr Murray Davies, Dr Barbara Messerle, Dr Ron Fenton, Dr Andrew Jones, Ms Vivienne Munk, Ms Connie Diakos, Dr Evonne Rezler and Mr Matthew Hall. Thanks also to Dr Peter Turner for producing the cover illustration. I wish to thank the Australian Research Council and the University of Sydney Cancer Research Fund for financial support.

References

1 K. Siafaca, in *Oncology Trends Product Markets – Part I, Future Oncology*, New Medicine, Inc., Lake Forest, CA, 1999, pp. 1045–1071.
2 P. Perego, C. Caserini, L. Gatti, N. Carenni, S. Romanelli, R. Supino, D. Colangelo, I. Viano, R. Leone, S. Spinelli, G. Pezzoni, C. Manzotti, N. Farrel and F. Zunino, *Mol. Pharmacol.*, 1999, **55**, 528.
3 G. Pratesi, P. Perego, D. Pollizzi, S. C. Righetti, R. Supino, C. Caserini, C. Manzotti, F. C. Giuliani, G. Pezzoni, S. Tognella, S. Spinelli, N. Farrell and F. Zunino, *Br. J. Cancer*, 1999, **80**, 1912.
4 J. Holford, P. J. Beale, F. E. Boxall, S. Y. Sharp and L. R. Kelland, *Eur. J. Cancer*, 2000, **36**, 1984.
5 P. J. O'Dwyer, J. P. Stevenson and S. W. Johnson, in *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, ed. B. Lippert, Zurich, Helvetica Chimica Acta, 1999, pp. 31–69.
6 T. Sancton, in *From Despair to Glory, Time*, 1999, p. 49.
7 A. Eastman, in *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, ed. B. Lippert, Helvetica Chimica Acta, Zurich, 1999, pp. 111–134.
8 D. B. Zamble and S. J. Lippard, in *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, ed. B. Lippert, Helvetica Chimica Acta, Zurich, 1999, pp. 73–110.
9 Q. He, C. H. Liang and S. J. Lippard, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 5768.
10 J. Reedijk, *Chem. Rev.*, 1999, **99**, 2499.
11 E. R. Jamieson and S. J. Lippard, *Chem. Rev.*, 1999, **99**, 2467.
12 J.-M. Malinge, M.-J. Giraud-Panis and M. Leng, *J. Inorg. Biochem.*, 1999, **77**, 23.
13 D. P. Bancroft, C. A. Lepre and S. J. Lippard, *J. Am. Chem. Soc.*, 1990, **112**, 6860.
14 J. S. Toh, G. B. Bacskay, M. S. Davies, S. J. Berners-Price and T. W. Hambley, to be submitted.
15 N. P. Johnson, J. D. Hoeschele and R. O. Rahn, *Chem.-Biol. Interact.*, 1980, **30**, 151.
16 J. W. Reishus and D. S. Martin, *J. Am. Chem. Soc.*, 1961, **83**, 2457.
17 K. Hindmarsh, D. A. House and M. M. Turnbull, *Inorg. Chim. Acta*, 1997, **257**, 11.
18 E. Koubek and D. A. House, *Inorg. Chim. Acta*, 1992, **191**, 103.
19 E. Koubek and D. A. House, *Inorg. Chim. Acta*, 1992, **198**, 103.
20 S. E. Miller and D. A. House, *Inorg. Chim. Acta*, 1989, **161**, 131.
21 S. E. Miller and D. A. House, *Inorg. Chim. Acta*, 1989, **166**, 189.
22 S. E. Miller and D. A. House, *Inorg. Chim. Acta*, 1990, **173**, 53.
23 S. E. Miller, K. J. Gerard and D. A. House, *Inorg. Chim. Acta*, 1991, **190**, 135.
24 S. E. Miller, H. Wen, D. A. House and W. T. Robinson, *Inorg. Chim. Acta*, 1991, **184**, 111.
25 S. E. Miller and D. A. House, *Inorg. Chim. Acta*, 1991, **187**, 125.
26 S. J. Berners-Price, T. A. Frenkiel, U. Frey, J. D. Ranford and P. J. Sadler, *J. Chem. Soc., Chem. Commun.*, 1992, 789.
27 S. J. Berners-Price and T. G. Appleton, in *Platinum Based Drugs in Cancer Therapy*, ed. L. R. Kelland and N. Farrell, Totowa, NJ, 2000.
28 M. S. Davies, S. J. Berners-Price and T. W. Hambley, *Inorg. Chem.*, 2000, **39**, 5603.
29 M. S. Davies, J. W. Cox, S. J. Berners-Price, W. Barklage, Y. Qu and N. Farrell, *Inorg. Chem.*, 2000, **39**, 1710.
30 J. W. Cox, S. J. Berners-Price, M. S. Davies, N. Farrell and Y. Qu, *J. Am. Chem. Soc.*, 2001, **123**, 1316.
31 S. J. Berners-Price, K. J. Barnham, U. Frey and P. J. Sadler, *Chem. Eur. J.*, 1996, **2**, 1283.
32 M. S. Davies, S. J. Berners-Price and T. W. Hambley, *J. Am. Chem. Soc.*, 1998, **120**, 11380.
33 M. S. Davies, S. J. Berners-Price and T. W. Hambley, *J. Inorg. Biochem.*, 2000, **79**, 167.
34 S. J. Barton, K. J. Barnham, A. Habtemariam, R. E. Sue and P. J. Sadler, *Inorg. Chim. Acta*, 1998, **273**, 8.
35 S. J. Barton, K. J. Barnham, U. Frey, A. Habtemariam, R. E. Sue and P. J. Sadler, *Aust. J. Chem.*, 1999, **52**, 173.
36 Y. Chen, J. A. Parkinson and P. J. Sadler, *J. Chem. Soc., Dalton Trans.*, 1998, 3577.
37 N. Marti, G. H. B. Hoa and J. Kozelka, *Inorg. Chem. Commun.*, 1998, **1**, 439.
38 F. Reeder, F. Gonnet, J. Kozelka and J.-C. Chottard, *Chem. Eur. J.*, 1996, **2**, 1068.
39 F. Legendre, J. Kozelka and J.-C. Chottard, *Inorg. Chem.*, 1998, **37**, 3964.
40 J. Kozelka, F. Legendre, F. Reeder and J.-C. Chottard, *Coord. Chem. Rev.*, 1999, **190–192**, 61.
41 Z. Guo, Y. Chen, E. Zang and P. J. Sadler, *J. Chem. Soc., Dalton Trans.*, 1997, 4107.
42 A. Laoui, J. Kozelka and J.-C. Chottard, *Inorg. Chem.*, 1988, **27**, 2751.
43 Y. Wang, N. Farrell and J. D. Burgess, *J. Am. Chem. Soc.*, 2001, **123**, 5576.
44 T. W. Hambley, unpublished data.
45 A. Eastman, *Biochemistry*, 1983, **22**, 3927.
46 A. Eastman, *Biochemistry*, 1986, **25**, 3912.
47 A. M. J. Fichtinger-Schepman, P. H. M. Lohman and J. Reedijk, *Nucleic Acids Res.*, 1982, **10**, 5345.
48 A. M. J. Fichtinger-Schepman, J. L. v. d. Veer, J. H. J. d. Hartog, P. H. M. Lohman and J. Reedijk, *Biochemistry*, 1985, **24**, 707.
49 D. L. Bodenner, P. C. Dedon, P. C. Keng and R. F. Borch, *Cancer Res.*, 1986, **46**, 2745.
50 H. M. Ushay, T. D. Tullius and S. J. Lippard, *Biochemistry*, 1981, **20**, 3744.
51 K. J. Barnham, S. J. Berners-Price, T. A. Frenkiel, U. Frey and P. J. Sadler, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1874.
52 R. B. Martin, *Acc. Chem. Res.*, 1985, **18**, 32.
53 F. Reeder, Z. Guo, P. S. Murdoch, A. Corazza, T. W. Hambley, S. J. Berners-Price, J.-C. Chottard and P. J. Sadler, *Eur. J. Biochem.*, 1997, **249**, 370.
54 P. S. Murdoch, Z. Guo, J. A. Parkinson and P. J. Sadler, *J. Biol. Inorg. Chem.*, 1999, **4**, 32.
55 V. Brabec, V. Boudny and Z. Balcarova, *Biochemistry*, 1994, **33**, 1316.
56 A. R. Jones, PhD Thesis, University of Sydney, Sydney, 1999.
57 F. Legendre, V. Bas, J. Kozelka and J.-C. Chottard, *Chem. Eur. J.*, 2000, **6**, 2002.
58 E. C. H. Ling, G. W. Allen and T. W. Hambley, *J. Am. Chem. Soc.*, 1994, **116**, 2673.
59 T. W. Hambley, E. C. H. Ling, V. P. Munk and M. S. Davies, *J. Biol. Inorg. Chem.*, 2001, **6**, 534.
60 T. W. Hambley, E. C. H. Ling and B. A. Messerle, *Inorg. Chem.*, 1996, **35**, 4663.
61 H. M. Er, PhD Thesis, University of Sydney, 1996.
62 V. P. Munk, R. Fenton, B. A. Messerle and T. W. Hambley, unpublished data.
63 E. M. Rezler, PhD Thesis, University of Sydney, Sydney, 2001.
64 J. A. Rice, D. M. Crothers, A. L. Pinto and S. J. Lippard, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 4158.
65 S. F. Bellon and S. J. Lippard, *Biophys. Chem.*, 1990, **35**, 179.
66 V. Boudny, O. Vrana, F. Gaucheron, V. Kleinwachter, M. Leng and V. Brabec, *Nucleic Acids Res.*, 1991, **20**, 267.
67 A. Schwartz, L. Marrot and M. Leng, *Biochemistry*, 1989, **28**, 7975.
68 C. S. Chow, C. M. Barnes and S. J. Lippard, *Biochemistry*, 1994, **33**, 15124.
69 E. R. Jamieson, M. P. Jacobsen, C. M. Barnes, C. S. Chow and S. J. Lippard, *J. Biol. Chem.*, 1999, **274**, 12346.
70 P. M. Takahara, A. C. Rosenzweig, C. A. Frederick and S. J. Lippard, *Nature*, 1995, **377**, 649.
71 P. M. Takahara, C. A. Frederick and S. J. Lippard, *J. Am. Chem. Soc.*, 1996, **118**, 12309.
72 U.-M. Ohndorf, M. A. Rould, Q. He, C. O. Pabo and S. J. Lippard, *Nature*, 1999, **399**, 708.
73 (*a*) S. O. Ano, Z. Kuklenyik and L. G. Marzilli, in *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, ed. B. Lippert, Helvetica Chimica Acta, Zurich, 1999; (*b*) L. G. Marzilli, J. S. Saed, Z. Kuklenyik, K. A. Keating and Y. Xu, *J. Am. Chem. Soc.*, 2001, **123**, 2764.
74 S. E. Sherman, D. Gibson, A. H. J. Wang and S. J. Lippard, *Science*, 1985, **230**, 412.
75 A. Gelasco and S. J. Lippard, *Biochemistry*, 1998, **37**, 9230.
76 S. U. Dunham, S. U. Dunham, C. J. Turner and S. J. Lippard, *J. Am. Chem. Soc.*, 1998, **120**, 5395.
77 D. Yang, S. S. G. E. van Boom, J. Reedijk, J. H. van Boom and A. H.-J. Wang, *Biochemistry*, 1995, **34**, 12912.
78 J. A. Parkinson, Y. Chen, Z. Guo, S. J. Berners-Price, P. del S. Murdoch, T. Brown and P. J. Sadler, *Chem. Eur. J.*, 2001, **6**, 3636.
79 T. W. Hambley, *Inorg. Chem.*, 1998, **37**, 3767.