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Molecular mechanics modelling of Pt/nucleotide and Pt/DNA interactions

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Abstract

Applications of molecular mechanics modelling to the study of Pt/nucleotide and Pt/DNA interactions are reviewed. Difficulties associated with modelling the Pt moieties and their interactions with biomolecules are discussed. The use of molecular mechanics to study small molecule Pt/nucleobase and Pt/nucleotide complexes is analysed. Models of Pt/oligonucleotide interactions, used for a variety of purposes, but primarily to aid in the analysis of experimental results or to augment experimental data as in structural studies by NMR spectroscopy, are described.

Keywords: Molecular mechanics; Anti-cancer complexes; Cisplatin; Drug design

1. Introduction

Since the earliest development of the molecular mechanics (MM) method, it has been applied to the fields of biological and medical inorganic chemistry. The complexity of biological systems has often limited the application of MM to these areas but it has also encouraged the development of new techniques. In recent years there have been significant developments on a number of fronts, in terms of both the types of problems being addressed and the techniques being applied. For example, the rapidly expanding use of spectroscopies such as 2D NMR and EXAFS — techniques that provide structural data, but at a level that is insufficient to allow full 3D structural determinations — has resulted in the application of MM techniques to aid in definition of the structures. The similarly rapid expansion in macromolecular crystallography has, somewhat unexpectedly, led to an increase in the number of problems needing additional insight from techniques such as MM. This is particularly true in the case of enzyme structures where usually only a single state of the active site is characterised and more information is needed to elucidate the mechanism of action. Increases in the power of readily available computers has made possible the combination of quantum mechanics with MM and this is allowing for the modelling of transition states and other experimentally inaccessible species.

Platinum-based anticancer drugs have been a particular focus of molecular mechanics studies for the last 15 years. Drugs, such as cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) are believed to effect their action by binding to DNA and, until recently, structural characterisation of even modest sized Pt/oligonucleotide complexes had proven impossible. Consequently, molecular modelling has been employed to provide models for the visualisation of Pt/DNA interactions. Even now, only a small number of adduct types have been structurally characterised and molecular modelling can still provide much insight into structures and increasingly, into the factors that mediate formation of the adducts.

Previously, Hambley has reviewed the potential role of molecular modelling in the study of Pt/DNA interactions [1]. At that time we suggested that the combination of NMR spectroscopy and molecular mechanics would prove to be a powerful

and popular approach. This has proven to be true as is described below. Also, in this review we summarise the application of molecular mechanics to Pt-based anticancer drugs with an emphasis on the new problems being addressed and the new techniques being brought to bear. We begin by considering the particular problems associated with modelling Pt/DNA interactions.

2. Development of force-field parameters for systems containing Pt

Force fields for modelling the interactions of Pt moieties with nucleotides can be thought of as having two components, that for the Pt moiety and that for the nucleotide. Both components have presented unusual problems to those developing force fields, as have the new interaction types that result from the binding of Pt to the nucleotide. Consequently, the development of force-field parameters to accurately predict the interactions between platinum complexes and nucleic acids has been an ongoing process, with modifications made to the parameters following the publication of new experimental data such as the recently reported X-ray crystal structures of Pt/oligonucleotide adducts [2–4]. In this section we describe the developments of the force fields and comment on some of the problems facing those developing these models.

2.1. Development of the basic force fields

The AMBER force field, first published in 1984, was developed for simulating the structural and conformational energies of nucleic acids and proteins [5] and has formed the basis of most force fields developed for the modelling of Pt/DNA systems. Apart from minor modifications to the parameters for nucleobases directly bound to Pt, the AMBER parameters for nucleotides have generally been adopted unchanged.

The first study of Pt/DNA interactions that used molecular mechanics methods was reported by Kozelka et al. [6]. They based the geometrical parameters for the platinum complex on that of an idealised square planar complex [6]. The Pt–N7(purine) and Pt–N(am(m)ine) bond lengths were given values of 2.00 Å, and the N < Pt < N angles given values of 90° or 180° The force constants for bond stretching and bending were based on the IR spectroscopic study of $[Pt(NH_3)_4]^{2+}$ [7]. Most other parameters were taken from the AMBER database [8]. The charge on the *cis*-$\{Pt(NH_3)_2\}^{2+}$ moiety was distributed between the N7 and NH_3 ligands in accordance with calculations taken from the AMBER database [8] and the residual charge assigned to the Pt atom. In order to maintain the planarity of the coordinated nucleotides, constraints were applied to the torsional angles within the base. Similarly, torsional constraints were applied to prevent unreasonably large deviations of the Pt atom from the plane of the coordinated nucleotides [6].

In our laboratory, the first step in the development of a force field to model Pt–DNA interactions was the examination of the crystal structures of small bis(nucleobase)diammineplatinum(II) complexes, such as *cis*-$[Pt(NH_3)_2(G)_2]$ [9] and

cis-[Pt{d(pGpG)}$(NH_3)_2$] [10]. Geometrical parameters were taken from these structures and used as starting points for the derivation of ideal values in the force field [11]. Force constants for the platinum interactions were derived by considering IR data [12]. The Pt–N7(purine) bond was assigned the same force constant as the Pt–N(am(m)ine) bond and values for undeformed bond lengths and angles were modified to give the best fit for reported crystal structures [11]. The angle bending terms predicted by IR data relate to small distortions from molecules in a strain state, whereas in molecular mechanics, large distortions from a 'strain free' state are considered. Thus, the spectrally derived parameters were considered to be too large and the value of the angle bending force constant was reduced by 50% [12]. Valence angle bending terms involving the platinum and two ligands were also considered to be too large so the force constant was given a value of zero and the terms were replaced by non-bonded interactions between ligating atoms [11,12]. The C–H and N–H bond lengths were reduced to 0.97 and 0.91 Å, respectively, in order to locate the hydrogen atoms at the centre of electron density, as determined by X-ray studies [12]. The square-planar geometry of the Pt complex was maintained using out-of-plane deformation functions. These parameters have been shown to accurately reproduce structures of a range of platinum(II) complexes [11,13]. The charge on the complex was distributed between the Pt atom and the ammine hydrogen atoms and was found to give sensible hydrogen bonding geometries [11]. These force-field parameters were applied to larger systems and produced sensible models of various platinum(II) complexes bound to DNA [11,13,14].

A new force field, optimised for modelling Pt am(m)ine complexes bound to guanine via N7 has recently been developed [15]. This force field introduces six new atom types to the AMBER force field including NB1 and NB2 for the N7 atoms of the bound guanines and N31 and N32 for the N atoms in the am(m)ine ligands, PT and H3. The new force-field parameters were developed by comparing literature parameters with the structural features of numerous published crystal structures. The new parameters can be incorporated into the existing AMBER force field.

The AMBER force field was further expanded in order to apply molecular modelling calculations to a platinum complex containing quinoline as a ligand [16]. Fractional charges for quinoline were calculated using a combination of Gasteiger-Marsili and Hückel methods and assigned using literature methods [6]. The N1 atom of quinoline that the platinum is bound to was treated analogously to the N7 of bound 9-ethylguanine.

The MM2 force field [17] was also recently expanded to include three new Pt ligating atom types; chloride, carboxylate oxygen and coordinated nitrogen [18]. These new parameters allow models to be created for many of the Pt drugs being investigated for anti-cancer activity. The authors found that the new force-field parameters accurately predicted the geometry of both the Pt inner and outer coordination sphere of a number of square planar Pt(II) complexes [18].

2.2. Out-of-plane force-field parameters for deviation of the Pt–N7 bond from the plane of the coordinated purine

There has been considerable discussion regarding the degree of bending of the Pt–N7 bond out of the plane of the coordinated purine base (δ, Fig. 1) because the barrier is not directly accessible by experimental measurements. The parameters used for the modelling of DNA interactions were developed by considering the geometry of simple bis(nucleobase)diammineplatinum(II) complexes in which the Pt atom lies in or close to the plane of both coordinated purine bases. Therefore, force fields were developed that used torsional constraints or out-of-plane deformation terms to ensure the Pt atom remained within the plane of the coordinated purine. When applied to Pt/DNA systems these force fields produced models with substantial distortion of the DNA structure [6,19]. Obviously, geometric constraints will restrict the mobility of coordinated purine bases and consequently derivation of an accurate parameter describing out-of-plane deviation of the Pt is crucial.

The problem of accurate parameterisation of the Pt–N7 bond bending from the guanine plane was first addressed in studies on the distortion induced in DNA by an intrastrand d(GpG) adduct [20]. Parameterisations relating to the distortion of the Pt–N7 bond from the guanine plane were based on calculations determining the energy cost of bending the Pt–N bond out of the pyrimidine plane in $[Pt(NH_3)_3(pyrimidine)]^{2+}$ [21]. The four torsional angles about the Pt–N7(guanine) bond were modified to hold the platinum atom within the plane of the guanine with a combined energy cost of distortion from the guanine plane equivalent to that calculated for Pt–N(pyrimidine) [20]. Later studies used ab initio calculations with relativistic pseudopotentials to determine the force constant for the bending of the Pt–N7 bond out of the adenine plane using the model complex $[Pt(NH_3)_3(adenine)]^{2+}$ [22]. The force constant obtained by these calculations was shown to be similar to that obtained by the earlier calculations [21], suggesting the parameterisation used in the earlier models [20] was realistic [22].

Chval and Sip used ab initio quantum mechanical calculations using $[Pt(NH_3)_3(purine)]^{2+}$ (where purine = adenine or guanine) as a model system to show that the assumption of planarity of nucleic acid bases leads to an overestimation of the platinum out-of-plane bending force constant [23]. They questioned whether the purine ring is stiff enough to withstand large out-of-plane bending of the Pt–N7 bond without puckering of the five-membered ring containing N7. The calculated force constants for puckering of the base and out-of-plane deviation of the Pt atom indicate that the purine bases are likely to undergo significant

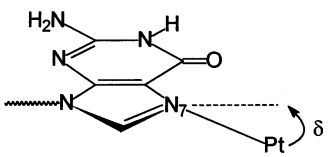

Fig. 1. Deformation of Pt out of the plane of the guanine base.

puckering. The authors recommended that instead of modelling the puckering explicitly, a convenient way of implementing the new parameters is to reduce the value of the out-of-plane force constants [23].

The publication of the crystal structure of a cisplatin/DNA intrastrand d(GpG) adduct by Takahara et al. [3] revealed that when the complex is bound to DNA, the platinum atom does not lie in the plane of the guanine bases. The Pt atom resides in a position 1.2 Å below the plane of the 5′ guanine and 0.8 Å above the plane of the 3′ guanine. The dihedral angle between the two guanine rings is ca. 26°, much less than the ca. 80° angle found in the crystal structure of *cis*-[Pt{d(pGpG)}$(NH_3)_2$] [3,10]. It is not possible to establish from the crystal structure of the oligonucleotide complex whether or not the guanine bases are puckered and these deviations should therefore be considered as deviations from the mean plane of the bases.

The models developed in these laboratories had also relied on crystal structures of $[Pt(dG)_2(NH_3)_2]^{2+}$, which showed the guanine bases to be tilted towards each other and the Pt atom lying in the planes of the bases [24]. Thus, in these models, the force constant for the deviation of Pt from the planes of the adenine or guanine bases were overestimated, producing unrealistic models when applied to Pt/DNA adducts.

Comparison between the molecular models and crystal structures of the platinum(II) complexes $[PtX_3$(quinoline-8-carbaldehyde)] and $[PtX_3$(benzoquinoline)] showed that the modelling parameters defining the energy cost associated with distortion of the Pt atom out of the plane of the aromatic ligand were deficient [19]. These complexes have short Pt···H contacts that cause the Pt to deviate from the aromatic plane in order to relieve the interactions. Empirical modification of the out-of-plane force constant (k_δ) using these crystal structures as templates resulted in the value of the force constant being reduced by 90% and gave energy minimised models with very similar geometry to the crystal structures [19]. The out-of-plane force constant developed in this way was found to accurately reproduce the deviations of the Pt from the planes of the guanine bases observed in the crystal structure of the cisplatin/oligonucleotide intrastrand d(GpG) adduct [3,19].

2.3. Planarity of the Pt moiety

Platinum(II) complexes rarely deviate significantly from a square-planar geometry and it is necessary to include functions that impose this planarity in the force field. Functions that impose planarity about sp^2 hybridised moieties such as those present in carboxylato or phenyl groups have been included in force fields for many years and have been adopted successfully for maintaining planarity about Pt(II) [11,13]. Others have used what are known as improper torsion angles to impose planarity and these too have been successful [6,20]. There is no clear evidence to suggest that either method is to be preferred but both suffer from the problem that they deal with only four atoms at a time rather than all five that make up the

square plane. Consequently, they cannot be expected to truly mimic the forces that impose planarity. Also, although the force constants have been empirically modified to reproduce observed deviations from planarity, there is little direct experimental evidence available as to the 'accuracy' of these force constants. In the majority of Pt/nucleotide interactions, deviations from planarity are small and, therefore, the limitations in this aspect of the modelling are probably not significant, but modelling of highly strained systems may not be reliable.

2.4. van der Waals radius of the Pt atom

The great majority of molecular mechanics studies of metal complexes have not included van der Waals terms for the metal atom and this has rarely led to obvious inaccuracies. However, Pt(II) complexes present a particular problem because close contact with the metal centre is possible for groups that lie perpendicular to the square plane. Recently, two studies relating to the parameters appropriate to modelling these non-bonded interactions have been reported. Marzilli and colleagues found that is was necessary to invoke a van der Waals radius of 2.44 Å for the Pt(II) atom in order to reproduce the geometry of Pt/guanine complexes [15]. More recently, one of us has reported a study of highly strained systems in which close Pt···H contacts are unavoidable and therein derived a van der Waals radius of 1.7 Å [19]. A complicating aspect, that may contribute to the difference between these values, is the possibility of attractive agostic or hydrogen bonding interactions involving the Pt atom. Indeed, there is substantial evidence for weak hydrogen bonds forming between Pt atoms and the hydrogen atoms of acidic groups [25,26]. The role of attractive or repulsive interactions in mediating Pt binding to nucleotides is not clear but close contacts to exocyclic O and NH_2 groups on guanine and adenine respectively do occur on binding to the N7 atoms and may play a role in the preference for binding to guanine over adenine.

2.5. Charges on the Pt moiety

Related to the van der Waals radius of the Pt atom is its residual charge and those of other atoms making up the complex that binds to DNA. The charges on DNA, overall and residual, make inclusion of electrostatic terms essential but in order to do so charges must be available. Calculation of reliable charges necessitates the use of high level quantum-mechanical methods and these are not yet available for heavy metal complexes. To date, most studies have employed charges calculated based on lower level quantum-mechanics calculations or on estimates [14]. Most calculations, particularly the higher level calculations have given low values for the residual charges on the nominally dipositive Pt(II) ion, in accord with Pauling's electroneutrality principle and therefore, inclusion of a charge for the Pt may not be critical. However, coordinated amine or ammine groups are frequently involved in hydrogen bonds and in these cases the electrostatic interactions will be more important.

3. Molecular modelling of small molecules containing Pt as model systems

Molecular modelling has been extensively applied to the study of simple Pt(II) complexes designed to model Pt/nucleobase interactions within DNA. The structural features of these model complexes can aid in the interpretation of experimental data from interactions with DNA.

An analysis, using molecular mechanics, of the steric factors influencing isomeric preferences and barriers to isomer interconversion for a range of *cis*-di(-am(m)ine)bis(purine)platinum(II) complexes was undertaken [11]. The nature of the interaction between the am(m)ine ligands of the complex and the exocyclic group of the purine ligands was found to be the major factor contributing to differences in rotational barriers.

In order to slow the rapid conformational interconversion of typical Pt/d(GpG) adducts in solution, Marzilli et al. designed chirality controlling chelate (CCC) ligands [27]. One such CCC ligand is 2,2′-bipiperidine (bip) and the complex $[Pt\{(R,S,S,R)\text{-bip}\}(H_2O)_2]^{2+}$ was found to slow the dynamic processes after product formation [27]. NMR, mass spectral and HPLC analysis were used to show that a novel head-to-head (HTH) conformer, denoted HH2, was formed when this complex was reacted with d(GpG) [28]. Molecular modelling calculations were used with NMR restraints to show that the normal HTH conformer, HH1, and HH2 have comparable energies and no unusual features that would preclude the existence of the HH2 conformer [28].

A model complex for the d(GpA) adduct, *cis*-$[Pt(NH_3)_2$(9-methyladenine)(9-ethylguanine)$](NO_3)_2$, was synthesised and the conformational behaviour examined by molecular mechanics [29]. A molecular model of the complex *cis*-$[Pt(NH_3)_2(A)(G)]^{2+}$ (A = adenine, G = guanine) was built with the two bases being coplanar with the platinum coordination plane. The two bases were then rotated about the Pt–N7 bond in multiples of 10° and the relative non-bonded energies calculated. An energy map was produced and showed that four minimum energy zones were produced, which were interpreted as HTH or head-to-tail (HTT) minima [29]. This study yielded results in close agreement with those derived for the complex *cis*-$[Pt(NH_3)_2(G)_2]^{2+}$ [30]. The modelling results, in conjunction with the crystal structure of *cis*-$[Pt(NH_3)_2$(9-methyladenine)(9-ethylguanine)$](NO_3)_2$, allowed the structure of two conformers observed in the NMR analysis of *cis*-$[Pt\{d(ApG)\}(NH_3)_2]^{+}$ [31] to be characterised as two forms of HTH conformers.

The complex *cis*-$[Pt\{d(TpG)\text{–}N3(1),N7(2)\}(NH_3)_2]$ was found to exist in equilibrium between two conformers due to hindered rotation of the bases and the structure of these isomers was studied by ^1H NMR and molecular modelling [32]. The NMR results showed that the rotamers were both HTH conformers with *syn*/*anti* and *anti*/*anti* sugar–base orientations. Molecular modelling and dynamics were used to investigate a number of different conformations of the bases and the generated models compared with the NMR data. Only one model was found to be viable for each rotamer and was able to plausibly explain the main chemical shift differences between the two rotamers.

Sadler et al. have made extensive use of molecular modelling to aid in the interpretation of results obtained by [^{1}H, ^{15}N] heteronuclear single quantum coherence (HSQC) NMR spectroscopy. This technique was used to examine ring-opened adducts of *carboplatin* analogues in reactions with methionine derivatives in an attempt to determine possible mechanisms of action in vivo [33]. Molecular modelling of the ring-opened intermediates suggested that hydrogen bonding between the ligands may contribute to their stability and longevity, as seen in the NMR experiments.

Molecular mechanics calculations, in conjunction with NMR experiments, have also been applied to show that platinum(II) is able to form macrochelate complexes of 5′-ATP and other 5′ nucleotide triphosphates [34]. In the absence of a crystal structure of a complex involving coordination of both purine and phosphate groups to the same metal, molecular modelling was used to evaluate chelates in which the platinum was bound to the N7 of a 6-oxopurine and an α, β, or γ phosphate group. The molecular model of *cis*-[Pt(5′-GTP-N7, γPO)$(NH_3)_2$] was found to be in agreement with the observations from the NMR data [34].

The aquation of cisplatin, which is the rate determining step in the reaction with DNA [35], has been the subject of a molecular mechanical and quantum chemical study [36,37]. Cisplatin, and a number of substituted ethylenediamine derivatives were studied with respect to the first step of their hydrolysis reaction [36]. The energy minimised structures of the reactants and products were used to calculate the charge distribution and relative electronic energies by the extended Hückel method. These studies showed that the thermodynamic stability of the complex correlated with the rate of hydrolysis due to the stabilisation of the square planar geometry [36]. The hypothetical transition states of these complexes were also examined using similar techniques [37]. Transition state complexes with trigonal-bipyramidal and square-pyramidal geometry were studied by molecular mechanics and extended Hückel methods. These studies showed that the cisplatin transition state preferred to adopt the trigonal bipyramidal geometry, whilst the ethylenediamine derivatives preferred the square pyramid geometries [37].

4. Molecular modelling of cisplatin/DNA interactions

4.1. The intrastrand d(GpG) adduct

The first application of molecular mechanics-like calculations to platinum anticancer research were carried out in an attempt to explain the experimentally observed shrinkage of DNA after platination [38,39]. These models were highly simplified and each base, sugar and phosphate in the DNA was represented as a point residing at the centre of gravity of each group. No attempt was made to take into account the conformations of the deoxyribose ring and therefore only limited conclusions could be drawn.

A more typical application of molecular mechanics modelling of the interactions between cisplatin and DNA was reported by Kozelka et al. in 1985 [40]. They used

molecular models to visualise possible structures adopted by DNA having an intrastrand d(GpG) adduct. A number of structural changes in the DNA occurred when the drug bound, as well as significant interactions between the complex and DNA. In particular, hydrogen bonds between the ammine ligand and the phosphate backbone were seen, the pucker of sugar of the 5′ side of the adduct changed and the 5′ guanine was forced to tilt out of the base stack. These predictions were used to explain experimental results, albeit with acknowledgement of the limitations of theoretically generated models.

In further studies, models where the DNA was kinked at the site of an intrastrand adduct were constructed and shown to have similar minimised energies to the unkinked structures [41]. Again, the modelling results were used to postulate reasons for experimental findings such as the shortening and unwinding of superhelical DNA upon platination. The crystal structure of the complex *cis*-[Pt{d(pGpG)}$(NH_3)_2$] [10] provided detailed parameters for the platinum d(GpG) chelate and enabled more accurate models to be constructed [6,20,42].

An extensive ^{1}H and ^{31}P NMR and molecular mechanics study was carried out on a double stranded decanucleotide that contained a cisplatin intrastrand d(GpG) adduct [20,42]. The NMR data was correlated with molecular models and showed that the DNA has a kinked structure and that several conformations are in equilibrium. The degree of kinking predicted in these studies was greater than that calculated by electrophoretic mobility experiments of platinated DNA [43,44] but the discrepancy was explained as inaccuracies of both techniques [20].

The intrastrand d(GpG) adduct was also modelled by McCarthy et al. [45,46]. They considered models in which the ammine ligands of the *cis*-$\{Pt(NH_3)_2\}^{2+}$ moiety are hydrogen bonded to a phosphate oxygen atom and to the exocyclic oxygen atom of guanine. Other models considered involved hydrogen bonds between the ammine ligands and water molecules which are, in turn, hydrogen bonded to the phosphate oxygen atoms. However, it has been suggested [47] that the torsional angles in the DNA backbone predicted by these models are inconsistent with experimental evidence from related NMR studies [20].

In parallel with HSQC 2D NMR studies on the kinetics of binding of cisplatin and $[Pt(OH_2)_2(NH_3)_2]^{2+}$ to a d(GpG) site of single- and double-stranded DNA, molecular modelling was used to provide insight into the hydrogen bonding and destacking interactions of the monofunctionally bound complex [48]. The models produced were also used to postulate interactions responsible for the experimental observation that the reactivity of the 5′ and 3′ guanines were not equivalent.

Marzilli et al. used distance geometry structures, calculated from NMR data, to help determine the structural basis for an unusual type of d(GpG) adduct observed in a duplex dodecanucleotide [49]. The adduct was described as having a head-to-side type configuration, where the 3′ guanine has been removed from the base stack producing a hairpin-like structure at the lesion. The DNA conformation at the d(GpG) adduct was described as *anti*/*syn* head-to-side, whereas previous studies had observed the DNA conformation to be *anti*/*anti* HTH [20,50,51]. The induced distortion was localised to the central four base pairs around the adduct, the remainder of the oligonucleotide had more typical B-form structure [49].

4.2. The intrastrand d(ApG) and d(GpA) adducts

The structure of the intrastrand d(ApG) adduct, the second most prevalent lesion in cisplatin treated DNA and accounting for 20% of the total adducts [52], has been examined using molecular modelling in conjunction with NMR experiments [53]. The procedure followed was similar to that of the NMR/molecular modelling study of the d(GpG) adduct described previously [20]. It was found that the platinum d(ApG) adduct bends the DNA in a similar manner to the d(GpG) adduct, but significant differences between the adducts were seen in the bases close to the lesion. The major finding was that the base pairing of the 5′ platinated base is more severely disrupted in the d(ApG) models and that the complementary thymine remains stacked with the adjacent base on the 5′ side. In the d(GpG) models, the cytosine complementary to the 5′guanine was shown to oscillate between two positions. These results were used to postulate reasons for the different mutagenicities and cytotoxicities of the adducts [53].

The non-formation of the d(GpA) adduct in cisplatin treated DNA has also been examined using molecular mechanics [54]. Models of duplex tetranucleotides and, more recently [55], octanucleotides containing the d(GpA) and d(ApG) adducts were built and energy minimised. Examination of each model showed that in the case of the d(ApG) adduct a hydrogen bond was formed between an ammine ligand and the exocyclic oxygen of the guanine (Fig. 2). However, in the d(GpA) model, this hydrogen bond was replaced with a highly unfavourable interaction between the ammine ligand and the exocyclic amine group of the adenine. These models indicate that the unfavourable interaction may play a role in the non-formation of the d(GpA) adduct compared with the prevalence of the d(ApG) adduct.

4.3. The 1,3-intrastrand adduct

The 1,3-intrastrand adduct formed by cisplatin at d(GpNpG) sequences has also been examined using molecular modelling. Mazeau et al. examined the structural changes induced in a dodecanucleotide containing an intrastrand *cis*-[Pt{d(pGpCpG)}$(NH_3)_2$] adduct and compared the structural features observed with NMR studies [56]. Initially, the conformation of a platinated single stranded trinucleotide was examined in order to determine the conformation of the chelating guanines and potential for the phosphate oxygen atoms to hydrogen bond to the ammine ligand. A number of starting structures were built to model a wide range of conformations and the model with the lowest energy was found to have many hydrogen bonds between phosphate groups and ammine hydrogen atoms. The central cytosine was found to have a large degree of conformational flexibility, being able to move out of the plane of the two guanine bases or sit in the hollow between the guanines. A number of starting models for the dodecanucleotide duplex were created with kinked and unkinked structures, using geometrical constraints determined from the trinucleotide models. In all models, the hydrogen bonding of the central C:G base pair at the platination site is disrupted and the cytosine bulges out of the base stack. There is very little disruption to the stacking

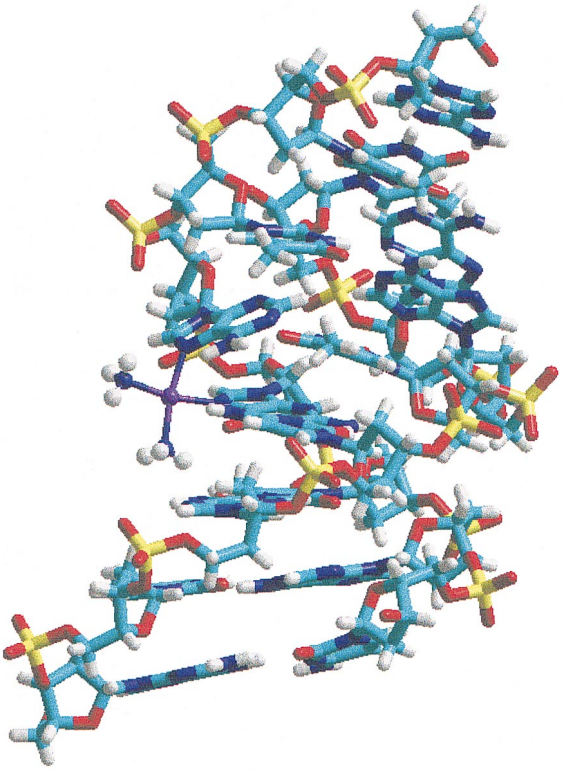

Fig. 2. Cisplatin bound at the ApG sequence of an oligonucleotide showing the bending induced in the DNA.

of the bases on the 3′ side of the platination site but the 5′ bases are more destacked in the unkinked models. The kink angle induced by the adduct was determined to be 60° by the modelling and the base stacking was less disrupted in these models. Hydrogen bonds between the ammine hydrogen atoms and the 5′ phosphate were observed in all models.

The solution structure of the cisplatin/d(GpTpG) 1,3-intrastrand adduct in a double-stranded 13-base-pair oligonucleotide was examined using high resolution NMR studies and molecular modelling was used to construct potential models [57]. It was found that the structural distortion upon platination is localised to the platinated 5′ guanine and central thymine, the base pairing on both the 3′ and 5′ sides is intact. Interestingly, the central thymine base was positioned in the minor groove, stacked with the coordinated 5′ guanine which is greatly propeller twisted. The complementary strand was completely undistorted. The duplex is unwound and kinked at the platination site, but the kink angle is slightly lower than the experimentally determined value. The authors conclude that the presence of the thymine in the minor groove significantly alters the structure of the DNA and may be responsible for the non-recognition of the adduct by various proteins [57].

4.4. The interstrand GG cross-link

The interstrand GG adduct has also been examined using molecular mechanics. Gel electrophoresis, chemical probes and molecular modelling were used to determine a potential structure of a 22-base-pair oligonucleotide containing cisplatin bifunctionally bound to a central d(TpGpCpT) sequence [58]. Numerous starting models with varying axis curvatures were created and minimised and then compared with experimental data. Two models of lowest energy were found but only one was in agreement with the experimentally determined angle of curvature. This model suggests that the interstrand GG adduct bends the helix and that the distortion is localised at the lesion.

Recently published NMR [59,60] and crystal structures [4] of the interstrand GG cross-link show the conformation of the DNA to be greatly different to that predicted by the modelling studies. Although the NMR and crystal structures reveal different values for the DNA bending angles and directions as well as helix unwinding values, they all show that the deoxyguanosine-bridging *cis*-$\{Pt(NH_3)_2\}^{2+}$ moiety lies in the minor groove and the complementary deoxycytosines are extrahelical. In addition, the double helix is also reversed to a left-handed form around the lesion. Molecular modelling studies comparing the energy of such conformations with earlier predictions have not yet been performed.

5. Molecular modelling of *trans*-dichlorodiammineplatinum(II)/DNA adducts

A number of molecular modelling studies have been carried out on *trans*-diamminedichloroplatinum(II) (*trans*-$[PtCl_2(NH_3)_2]$, *trans*-DDP) bound to DNA. The inactivity of *trans*-DDP is believed to arise in part from its inability to form 1,2-intrastrand adducts, forming instead 1,3-intrastrand adducts and causing more severe disruption to the DNA which is recognised more readily by repair enzymes [61]. Lepre et al. used molecular dynamics calculations to generate a model for the 1,3-intrastrand d(GpApG) adduct in a duplex dodecanucleotide [62]. The model showed that *trans*-DDP can be accommodated within the double helix with minimal distortion of the phosphate backbone and only localised disruption of the base pairing and destacking of the platinated bases. The central adenine destacks from its neighbours and lies in the minor groove. The models showed that the adduct induces a kink of 18°, far less than the 40–70° reported previously for the cisplatin 1,2-d(GpG) adduct [6,20,42].

More recently, the distortions induced by *trans*-DDP forming a 1,3-intrastrand d(GpTpG) adduct in a duplex-octamer oligonucleotide were examined using an internal coordinate molecular modelling study [63]. The authors proposed structures in which unwinding is localised within a five-base-pair segment centred on the platinum binding site. Three families of structures with substantially different base-pair geometries at the platination sites, but all satisfying the experimental data on unwinding and curvatures, were developed. However, the authors concede that it is impossible to determine which (if any) of these models is correct [63].

The DNA distortion produced by the interstrand GC cross-link of *trans*-DDP has also been examined using molecular modelling techniques [64]. The experimental method used was similar to that used to determine the structure of the cisplatin interstrand GG cross-link described previously [58]. Two conformations of a duplex octanucleotide with *trans*-DDP coordinated to the central G:C base pair were constructed by varying the degree of rotation around the N7(G)–Pt–N3(C) axis. The energies of the conformers were compared and the most favourable one was examined, with respect to the curvature towards the major groove induced by the adduct by creating a series of conformations with varying initial kink angles. The model with the lowest energy corresponded to a curvature of 27° but the energy well was flat, suggesting some flexibility in the helix. The value of kink angle determined by the modelling studies was in close agreement with the value of 26° found by electrophoretic mobility experiments [58]. The combination of experimental and theoretical results allowed the authors to conclude that the duplex is distorted on both sides of the adduct, but the DNA bases are still paired. In addition, the distortion introduces greater flexibility into the helix and the helix is unwound and bent toward the major groove [64].

6. Molecular modelling of other anti-cancer active *trans*-platinum(II) complexes

Recently, a number of platinum(II) complexes with leaving groups in the *trans* position have been found to have anti-cancer activity [16,65], violating the classical structure–activity relationships [66,67]. In order to determine the mechanism of action of the complex *trans*-amminedichloroquinolineplatinum(II) (*trans*-$[PtCl_2(NH_3)(quin)]$, Fig. 3), NMR spectroscopy and molecular modelling were used to compare the interactions between both this complex and *trans*-DDP with nucleobases [16]. Models of both complexes monofunctionally and bifunctionally bound to 9-ethylguanine (9-EtG) were constructed and compared. Comparison of the bifunctionally bound models showed that the quinoline restricts the orientation of the guanine bases. The authors postulated that this steric demand may result in a preference for different sequences in DNA or longer lived monofunctional species, able to interact with competitive biological targets such as proteins.

The interactions between *trans*-$[PtCl_2(NH_3)(quin)]$ and DNA have also been examined recently [68]. Various chemical and biochemical probes of the DNA modified with *trans*-$[PtCl_2(NH_3)(quin)]$ have shown that the consequent distortion

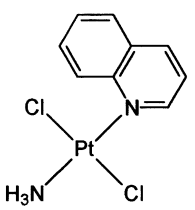

Fig. 3. *trans*-$[PtCl_2(NH_3)(quin)]$ — an anti-cancer active complex with *trans*-leaving groups.

of duplex DNA is similar to that induced by a cisplatin 1,2-intrastrand cross-link. Molecular modelling was used to assist in the search for potential DNA binding modes of *trans*-[$PtCl_2(NH_3)$(quin)] that might give rise to these distortions. It was found that when the complex was monofunctionally bound, two groups of structures were formed. The first group consisted of structures in which the quinoline ligand sat in the major groove, perpendicular to the stacked bases, with a number of unfavourable interactions. This binding mode had no effect on the curvature of DNA. However in the second group, which have lower minimised energies, the quinoline ligand intercalates between base pairs, resulting in a stacking interaction with the nucleobase on the 5′ side of the binding site. The most energetically favoured model in this group has the quinoline and guanine in a HTH configuration and a short hydrogen bond between the ammine ligand *trans* to the quinoline and a phosphate oxygen atom on the 5′ side of the binding site. The roll and kink angles of the DNA in these models were found to be similar to those in the crystal structure of the cisplatin d(GpG) adduct [3].

7. Molecular modelling of multi-nuclear platinum(II) complexes

The multinuclear platinum complexes designed by Farrell et al. have been extensively studied using molecular modelling [14,22,69]. The first complexes investigated were the bis(platinum) complexes [{*cis*-$PtCl_2(NH_3)$}$_2$(μ-$NH_2(CH_2)_4NH_2$)]$^{2+}$ and [{*trans*-$PtCl(NH_3)_2$}$_2$(μ-$NH_2(CH_2)_4NH_2$)]$^{2+}$ (abbreviated 2,2/c,c and 1,1/t,t, respectively) (Fig. 4) [14]. The modelling of the 2,2/c,c complex showed that interstrand binding, in which the Pt atoms are bound to opposite strands of DNA, is favoured over intrastrand binding. Each of the binding sites resembles cisplatin binding to DNA and hydrogen bonds form between the am(m)ine ligands and phosphate oxygen and exocyclic oxygen of guanine. The 1,1/t,t complexes displayed significantly different binding, due to the ability of each Pt moiety to form only monofunctional adducts. In the duplex sequence d(GpGpCpC), when the Pt was bound to the 5′ guanine on each strand, the Pt atoms were an ideal distance apart for a linking chain to join them. The effect that the length of the linking chain had on the cytotoxicity of the 1,1/t,t complexes was investigated and for $n = 3$–6, increasing the length of the chain correlated with increased activity [70].

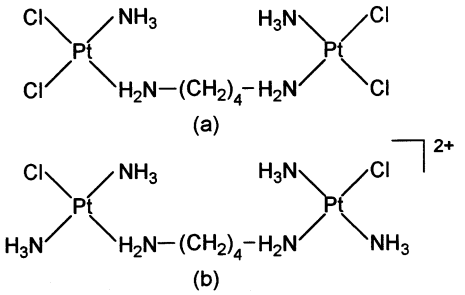

Fig. 4. Bis(platinum) complexes abbreviated (a) 2,2/c,c and (b) 1,1/t,t.

The kinetics of formation and structure of the intrastrand adduct of the 1,1/t,t complex ($n = 2–6$) with the dinucleotide d(GpG) were examined using NMR and molecular modelling [69]. Three starting models were created for the 1,1/t,t complexes ($n = 3$ and 6 only) with different base/sugar orientations in order to model a range of conformations. The experiments and modelling showed that the orientation of the glycosidic bond for the 5′ guanine changes towards a *syn* orientation, while the other guanine remains *anti*. This is different to the situation with cisplatin where the platinated d(GpG) moiety usually adopts the *anti*, *anti* conformation. These results help explain why the bent DNA resulting from binding bis(platinum) complexes is far less rigid than that produced by binding of cisplatin and may contribute to their increased cytotoxicity.

The trinuclear platinum complex, BBR 3464 (Fig. 5) is the first platinum complex with a completely novel structure to enter clinical trials [71,72]. The central platinum unit does not bind to DNA but incorporates a charge and hydrogen bonding capability in the backbone of the complex. DNA unwinding experiments showed that bifunctional binding of the complex occurred, but the amount of interstrand cross-linking was lower than expected. It was postulated that the remaining lesions are long range intrastrand adducts and molecular modelling was used to determine the relative stabilities of a 1,4 interstrand and 1,5 intrastrand cross-links by comparison of their minimum energy values, which were similar [73].

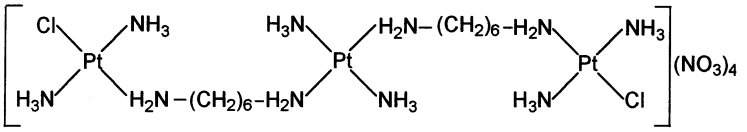

Fig. 5. BBR 3464, a trinuclear complex currently in Phase I clinical trials.

8. Steric probes of Pt/DNA interactions

The importance of hydrogen bonding between cisplatin and DNA has been examined using molecular modelling [74]. These studies revealed that two hydrogen bonds can form when cisplatin binds to a d(GpG) sequence, one between an ammine ligand and the phosphate backbone and the other between the second ammine ligand and the exocyclic oxygen of guanine. The formation of these hydrogen bonds correlates well with the observation that platinum complexes containing amine ligands with no hydrogen atoms generally show little anti-cancer activity [66,67]. Modelling calculations on the interactions between the *R*,*R*- and *S*,*S*-enantiomers of dichlorocyclohexane-1,2-diamineplatinum(II) and DNA showed that the bulky amine ligand did not interact with DNA but their amine hydrogen atoms had different orientations [74]. These orientations resulted in the *S*,*S*-enantiomer having hydrogen atoms better disposed to form hydrogen bonds and having slightly lower strain energy than the *R*,*R*-enantiomer. The anti-cancer activity of these enantiomers is marginally different [75,76] and this is consistent

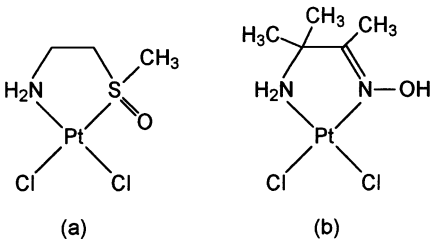

Fig. 6. Pt(II) complexes designed to bind to d(GpA) sites in DNA. (a) [$PtCl_2$(enso)] and (b) [$PtCl_2$(ambo)].

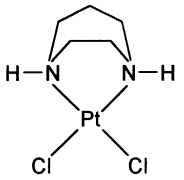

Fig. 7. [$PtCl_2$(hpip)], a complex designed to form interstrand adducts in preference to intrastrand adducts.

with the notion that hydrogen bonds may play a significant role in mediating the binding of platinum(II) complexes.

Molecular mechanics modelling has been used extensively in our laboratories to aid in the design of platinum complexes to target selected sequences or adducts. The first of these complexes was designed to test the postulate that unfavourable interactions between an ammine ligand and the exocyclic NH_2 group of adenine prevent the formation of the cisplatin/d(GpA) adduct. The complex *cis*-dichloro(methyl-2-ammoniumethylsulfoxide)platinum(II) ([$PtCl_2$(enso)], Fig. 6(a)) has a bidentate amine-sulfoxide ligand that is readily able to hydrogen bond with acidic hydrogen atoms [77]. Molecular models showed that when this complex is bifunctionally bound to a d(GpA) sequence in duplex DNA, a hydrogen bond is able to form between the oxygen on the complex and the exocyclic NH_2 group of the adenine. The DNA adducts formed by this complex were found to be unstable so an alternative complex, *cis*-dichloro(2-amino-2-methyl-3-butanoneoxime)-platinum(II) ([$PtCl_2$(ambo)], Fig. 6(b)) containing an oxime functional group as a hydrogen bond donor was synthesised [78]. The sequence specificity of [$PtCl_2$(ambo)] was tested and was shown to be markedly different to that of cisplatin including an increased reactivity towards adenine, but no evidence was found for significant binding at GpA sequences [78].

Molecular models of the intrastrand and interstrand adducts of cisplatin were used to design complexes readily able to form interstrand adducts but less suited to forming intrastrand adducts [79]. The complex *cis*-dichloro(1,4-diazacycloheptane)platinum(II) ([$PtCl_2$(hpip), Fig. 7) has amine protons disposed to potentially form hydrogen bonds when bound as an interstrand adduct. However, the geome-

try of the ligand causes unfavourable interactions between the complex and DNA when bound as an intrastrand adduct. This complex was found to form a similar quantity of interstrand adducts as cisplatin, but a substantially reduced number of intrastrand adducts [79]. The cytotoxicity of the complex was found to be very low *in vitro* and is evidence for the interstrand adduct not being primarily responsible for the anti-cancer activity of platinum(II) drugs.

The binding of $[PtCl_2(hpip)]$ to the dinucleotide d(GpG) was seen to produce two isomeric products [80]. These isomers were characterised using a combination of 2D NMR and molecular modelling which showed that the difference lay in the orientation of the ethane and propane chains of the ligand with respect to the guanine bases. The energies of each isomer calculated by molecular mechanics are similar, which is consistent with the experimental observation that the isomers form in approximately equal amounts [80]. Further experiments to determine the stereoselectivity of $[PtCl_2(hpip)]$ with DNA were carried out and showed that the isomers formed in a ratio of approximately 1:3 [78,80,81]. Molecular models of the two isomers revealed substantially stronger unfavourable interactions between the complex and the DNA in the minor isomer. This result suggests that steric interactions between the complex and DNA can exert a strong influence on the formation of adducts with DNA [80,81].

The interactions between DNA and chiral complexes have been investigated using molecular mechanics in order to probe the origins of the differences in cytotoxicity and DNA binding of enantiomeric complexes. The *R*- and *S*-enantiomers of *cis*-$[PtCl_2(3\text{-aminohexahydroazepine})]$ ($[PtCl_2(ahaz)]$, Fig. 8) were synthesised and tested for their DNA binding ability and proportion of monofunctional adducts [82]. It was found that bifunctional binding of the *S*-enantiomer to DNA is nearly twofold greater than for the *R*-enantiomer and the *S*-enantiomer forms less monofunctional adducts [82]. Molecular models of $[PtCl_2(ahaz)]$ bifunctionally bound to a d(GpG) sequence of duplex DNA were constructed to determine the role of steric interactions between the complex and DNA. Each enantiomer of $[PtCl_2(ahaz)]$ can bifunctionally bind to DNA in two ways, with the primary amine *cis* to the 5′ coordinated guanine, or with the primary amine *trans* to the 5′ coordinated guanine. The molecular models of both isomers of the *R*-enantiomer showed that the bulky ahaz ring of the complex has unfavourable interactions with the DNA. However, one of the isomers of the *S*-enantiomer was found to be able to reside in the major groove of DNA with minimal steric interactions with DNA

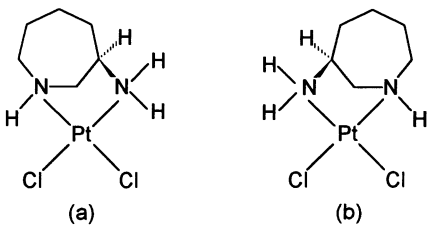

Fig. 8. The (a) *R*- and (b) *S*-enantiomers of $[PtCl_2(ahaz)]$.

[82]. It was postulated that the differences in the steric interactions with DNA are responsible for the difference in the extent of bifunctional adduct formation.

Other groups have used molecular mechanics studies to correlate the stability of Pt(II)/DNA adducts with biological activity. McCarthy et al. calculated the relative conformational energies of a series of complexes with monosubstituted am(m)ine ligands bound to the d(GpG) site in a pentamer duplex [45]. The favourable steric fit and slight decrease in overall relative energy change associated with decreasing steric bulk of the complex was reported to be consistent with the increase in the anticancer activity of the complexes.

Strain energy minimisation calculations of the three isomers of [$PtCl_2$(1,2-diaminocyclohexane)] (*R*,*R*-, *S*,*S*-(*trans*), *R*,*S*-(*cis*) [$PtCl_2$(dach)]) monofunctionally bound to the N7 of guanine in the duplex sequence d(pCpGpAp) were carried out to determine the relative minimum energies [83]. The authors proposed that the more stable systems are more anti-cancer active than the corresponding less stable ones and the models of the more active *trans*-[$PtCl_2$(dach)] were indeed found to be 37 kJ mol^{-1} more stable than the model *of cis*-[$PtCl_2$(dach)].

Molecular models of the *R*,*R*- and *S*,*S*-isomers of [$PtCl_2$(dach)] bound to the N7 of guanosine-5′-monophosphate (GMP) and cytosine-5′-monophosphate (CMP) were constructed and energy minimised [84]. The observation that the total energy of the *R*,*R*-enantiomer was less than that of the *S*,*S*-enantiomer was used to explain experimental findings, such as DNA binding constants and changes in DNA melting points.

[^{1}H, ^{15}N] HSQC 2D NMR techniques were applied to a study of the kinetics of formation and stability of the adducts formed by $[PtCl(dien)\}]^+$ (dien = diethylenetriamine) with d(GpG) sequences in DNA [85]. Molecular modelling showed that rotation about the Pt–N7 bond is more restricted when the complex is bound to the 5′ guanine than the 3′ guanine. The models also suggest that the orientation of the Pt-dien plane may be different for each adduct, adopting a parallel or perpendicular orientation to the guanine.

9. Molecular modelling of platinum(IV) complexes

The potential interactions between Pt(IV) complexes and DNA have been examined using molecular modelling [86]. In a series of studies on the effect axial ligands have on the reduction to Pt(II) and reaction with DNA, Ellis et al. used molecular modelling to show that the monofunctional binding of Pt(IV) complexes to DNA is sterically feasible. In these studies, the moiety $\{Pt(en)Cl_3\}^+$ was bound to the N7 position of guanine in a four-base-pair strand of duplex DNA, through both the axial and equatorial positions. In both cases, models were developed that had few short non-bonded interactions and produced only moderate DNA distortion.

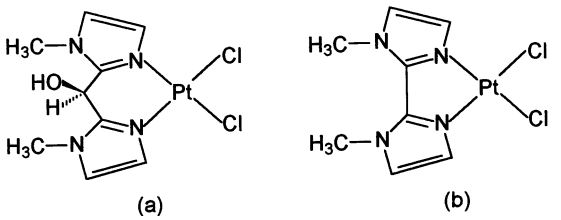

Fig. 9. Structures of the (a) anti-cancer active [$PtCl_2$(bmic)] and (b) inactive [$PtCl_2$(bmi)].

10. Molecular modelling of other platinum(II) complexes

Other recently discovered anti-cancer active complexes have been examined using molecular mechanics to aid the understanding of fundamental interactions between these complexes and DNA.

The structural aspects of (bis(*N*-methylimidazol-2-yl) carbinol)dichloroplatinum-(II) ([$PtCl_2$(bmic)]), which is anti-cancer active, and (*N*′,*N*′-dimethyl-2,2′-biimidazole)dichloroplatinum(II) ([$PtCl_2$(bmi)]) (Fig. 9), which is inactive, bound to nucleotides were investigated [87]. The complex [$PtCl_2$(bmic)] is of particular interest because it violates the classical structure–activity relationships [66,67] by not having a N–H moiety. Energy minimised models of [Pt(bmic)(GMP-*N*7)$_2$] (GMP = guanosinemonophosphate) were found to adopt the HTH orientation, which is unusual as most bis(oxopurine)platinum complexes (including [Pt(bmi)(GMP-*N*7)$_2$]) adopt the HTT conformation. Close inspection of the models revealed a number of hydrogen bonds between the OH group of the complex and the nucleobase resulting in a stabilisation of this form. A large number of conformers are also observed when the complex [$PtCl_2$(bmic)] binds to d(GpG), also due to these hydrogen bonds. This type of interaction is not observed for cisplatin and it is postulated that these interactions may induce significantly different distortions to the DNA structure in vivo. In addition, the activity of [$PtCl_2$(bmic)] despite the absence of a N–H moiety was postulated to be due to the absence of steric hindrance around the coordinating nitrogen.

A new class of Pt(II) complexes containing a diamine ligand having an affinity for the oestrogen receptor have been shown to be more selective and less toxic than cisplatin [88]. A study was undertaken to try and find correlations between geometry and thermodynamic stability and anti-cancer activity, based on the assumption that a more stable complex will reach the tumour cell in higher concentration and therefore have greater activity [89]. The influence of the type and the positions of the ring substituents on the conformational and thermodynamic stabilities of [PtL_2\{1,2-bis(hydroxyphenyl)ethylenediammine\}] (L_2 = 2Cl, 2I, SO_4) were examined. The calculated energies were found to be in agreement with experimental data on the reactivity and anti-cancer activity of the complexes [89].

Molecular models have been used to examine how cisplatin-like complexes connected by one methyl chain to intercalators, such as acridine and phenylquino-

lines, are able to interact with DNA [90]. The short linkage is expected to retard the covalent binding of the drug thus allowing the complex to be delivered to the core of a solid tumour mass. Models were generated representing two binding modes, intercalative and non-intercalative and found to have almost identical energies, suggesting that the DNA is flexible enough to accommodate the covalent d(GpG) cross-link and as well as the intercalation.

11. Other methods

An alternative approach to modelling the binding of Pt complexes to DNA has been developed by Yuriev and Orbell [91–93]. This technique quantifies the steric effect of interactions between platinum(II)–ammine complexes and nucleobases. A complex repulsive energy (CRE) strategy was developed where the steric requirements of a metal complex on approach to a site on a target molecule may be evaluated. In particular, the steric requirements for the monodentate approach of a variety of Pt(II) complexes to the N7 site of guanine were examined. A ligand repulsive energy (LRE) methodology was also developed which allows the relative repulsive energies of a series of target biomolecules to be assessed. The spherically symmetrical moiety $\{Cr(CO)_5\}$ is used as a steric probe to which the target biomolecule is hypothetically bound. The LRE is expressed by the gradient of the van der Waals repulsive energy between the ligand and the steric probe.

The general strategy for the calculation of both the CRE and LRE is similar and involves obtaining the energy minimised structure for the complexes [PtCl(9-ethylguanine)L_2] or $[Cr(CO)_5L]$, respectively (where L = amine ligand). The metal–ligand bond distance of both complexes is then varied, with all other internal coordinates frozen, creating a set of structures for each complex. Using non-bonded parameters, the repulsive portion of the van der Waals interactions is able to be calculated and converted into the repulsive energy [92]. A series of Pt(II) complexes with known biological activities were examined using CRE and LRE calculations and the trends examined [92]. The relationship between LRE and toxicity or anti-cancer activity of these complexes suggested an optimal steric requirement for similar series of complexes. The authors note that anti-cancer activity of Pt(II) complexes is dependent on many factors but once the molecule has reached the target site, these descriptors may explain their relative activities [92].

The LRE was also used to determine the relative steric parameters of all the potential binding sites on the common nucleobases [91]. The LRE for each of the sites was calculated and then normalised with respect to the value obtained for the N7 site of guanine, which was predicted to present the lowest relative steric hindrance to binding. Thus, a steric index for each of the sites is produced and is in accord with experimental results where steric effects are considered to be operative. The authors postulate that these steric parameters would be more useful than other parameters (such as molecular volume) in quantitative structure–activity relationship (QSAR) investigations.

The energy repulsive methodology was expanded to examine monofunctional binding to all endocyclic nitrogens of the nucleobases by complexes of variable 'flatness' [93]. Three Pt(II) complexes were used to probe the sites, with a gradual build-up of bulk on either side of the coordination plane, as well as the spherically symmetrical $\{Cr(CO)_5\}$ standard probe. The results obtained suggest that when less bulky (flatter) complexes bind to a nucleobase, the distribution of the neighbouring exocyclic groups is more important than their nature. However, when the $\{Cr(CO)_5\}$ probe coordinates to a nucleobase, the individual nature of the neighbouring exocyclic groups determine the steric effects. In addition, it is suggested that the binding of less bulky Pt(II) complexes to the N7 position of adenine or guanine is essentially similar. Upon coordination, rotation about the Pt–N7 bond of adenine is better able to absorb the resulting steric repulsion over a wider rotational range [93].

A combination of molecular mechanics, Monte Carlo and molecular dynamics methods was used to predict the geometries of square planar Pt(II) complexes which were then used for a prototypical QSAR study [94]. The emphasis of this work was on the modelling of *cis*-$[Pt(am(m)ine)_2X_2]$ pro-drugs in an attempt to discern the drug geometry corresponding with optimal anti-cancer activity. The results of the QSAR study suggested that toxicity is less for complexes with larger volumes and surface areas but no correlation was found linking anti-cancer activity and steric effects.

12. Conclusions

Molecular mechanics modelling has contributed substantially to our understanding of Pt/DNA interactions. In conjunction with experimental techniques such as NMR it has proven particularly powerful. Most adduct types have now been investigated but it is clear that reliable predictions of the bending induced in DNA by these adducts and of the different conformational geometries that the DNA can adopt to accommodate the adducts remains to be achieved.

Understanding of the processes that lead to adduct formation and the selectivity observed therein will require input from higher level methods. Kozelka, in a recent review [95], has summarised some of the recent applications of quantum mechanics to Pt containing systems. DFT calculations in particular are likely to be of benefit as shown by Deeth and Elding in their studies of ligand exchange processes at Pd and Pt [96].

Acknowledgements

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