TRANSITION STATES OF CISPLATIN BINDING TO GUANINE AND ADENINE: *ab initio* REACTIVITY STUDY

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Received September 18, 2002
Accepted March 11, 2003

Fully optimised HF and DFT transition states of cisplatin binding to adenine and guanine are presented for the first time. They have similar structure as the recently published transition states for cisplatin hydrolysis with the angle of about 70° between entering and leaving ligands and corresponding bonds prolonged up to 0.5 Å. Calculated activation energies are in the range of 10.5–18 kcal/mol. The lowest activation energies were found for the binding of *cis*-Pt[$(NH_3)_2(H_2O)(OH)]^+$ to guanine. The role of hydrogen bonds in recognition of binding sites, stabilisation of reactants and final yields of individual cisplatin–DNA adducts is discussed.

Keywords: Nucleobases; Purines; Platinum; Antitumor drugs; Hydrogen bonds; Chelates; DNA; Recognition; Reaction mechanisms; *Ab initio* calculations; DFT.

In recent years much work has been done to improve the understanding of cisplatin–DNA reactivity and recognition. It is known that 60–65% of cisplatin is bound to d(GG) moiety[1,2]. Experimental results show that the rate of the platination reaction is higher for double-stranded oligonucleotides than for the single stranded ones[3], and it is increasing with the length of the oligonucleotide[4,5]. Furthermore binding to 5′-G is slightly preferred before 3′-G in GG sequences[3,4,6]. Therefore the DNA environment influences substantially the binding of cisplatin. The nature of the adduct is given mostly by structural properties of both Pt(II)-complex and DNA. Structurally very distinct adducts can be formed. However in all cases the first target of the complex attack is the same – the guanine base.

The dichloro form of cisplatin is not able to attack DNA and has to be modified by hydrolysis or by some S-bound groups of proteins before binding to DNA. Hydrolytic changes were already studied by Miller *et al.*[7] under physiological conditions. It was concluded that the most probable species inter-

acting with DNA are mono-charged complexes of *cis*-Pt[$(NH_3)_2(H_2O)Cl]^+$ (**1**) and *cis*-Pt[$(NH_3)_2(H_2O)(OH)]^+$ (**2**). Ratio of double-charged *cis*-Pt[$(NH_3)_2(H_2O)_2]^{2+}$ complex (**3**) is negligible (less than 1%)[7] under physiological pH of 7.4 (compare also with pK_a values of 5.37 and 7.21 [8]). However it was shown for Co-complexes by Black *et al.*[9] that with increasing positive charge the affinity of metal species increases by an order of magnitude or even more. That is why double-charged cisplatin can be important in the DNA binding and we have considered it in our study.

Despite the relative simplicity of the cisplatin molecule, the above-mentioned aspects of DNA–cisplatin reactivity result from complex interactions, where besides the role of the DNA double helix the influence of solvent should be considered, too. Water and counterions play an important role in cisplatin binding to DNA.

COMPUTATIONAL METHODS

Choice of Model System

The choice of the model system always reflects a compromise between computational possibilities and natural complexity of the molecular system. Our model system includes the whole nucleic acid base (adenine or guanine) and one of the three above-mentioned platinum structures (**1**, **2**, **3**).

Computational Details

Quantum calculations employ the GAMESS [10] and GAUSSIAN94 [11] program packages. LANL2DZ [12] and SBK [13] valence basis (VB) sets and corresponding relativistic effective core potentials (ECPs) were used for the platinum atom. Pt is treated as the 18-electron system in all calculations, with both the $n = 5$ and $n = 6$ shells considered as valence electron shells. Due to complexity of our system we performed all optimisations only on HF/SBK/SBK level, it means we replaced also all core electrons of main group elements and described them by SBK pseudopotentials. Valence electrons are described by split valence -31G SBK basis set. HF method was also recommended for cisplatin calculations on larger systems by Pavankumar *et al.*[14] It gives very reasonable agreement with MP2 and DFT calculations for bond and dihedral angles. Pt–X (X = Cl, N, O) were overestimated by about 0.1 Å but relative bond changes were well preserved when comparing minima and transition-states structures[15]. To prove this statement we have also performed B3P86/LANL2DZ/6-31G* optimisations for selected TS structures.

To give consistent and comparable picture for all transition states we based our results on HF structures. B3P86/LANL2DZ/6-31G* and HF/SBK/SBK optimised structures are compared in a special paragraph.

Relative energies were evaluated by single point MP2/LANL2DZ*/6-31G* calculations. LANL2DZ valence basis set on the platinum atom was modified by an extra set of *f* functions of exponent 0.78 [16].

The procedure of determining the transition states has been described elsewhere[15]. All structures were carefully optimised (with the maximum gradient of 0.00001 a.u.) and the nature of the stationary points obtained was always checked by frequency analysis.

The activation energies are computed with respect to the appropriate H-bonded minima structures that we call "pre-substitution" complexes.

Labelling Scheme

Structures are referred to as XxxYz, where Xxx is either Ade (adenine) or Gua (guanine), Y is one of the three above-defined platinum structures **1**, **2**, **3** and the italic letter *z* distinguishes various conformers of the same structure. Transition states are referred to as TS_XxxYz, adopting the same scheme as the corresponding structures. The TS structure corresponding to guanine O6 atom bonding in the first step is designated as TS_Gua**1***a*-O6.

RESULTS

*Interaction of **1** with Guanine*

The "pre-substitution" complex Gua**1***a* can be formed before reaction. In Gua**1***a* structure the first H-bond connects the N7 atom of guanine with a H_2O ligand (r_{N7-H} = 1.602 Å) and the second one connects O6 atom of guanine with the NH_3 ligand (r_{O6-H} = 1.786 Å) (Fig. 1). The first H-bond helps to keep the platinum central atom of the complex in a relative vicinity of the future reaction center of guanine – the N7 atom (r_{Pt-N7} = 4.099 Å). Thus, a good relative position with respect to the transition-state geometry is also ensured and the probability of the reaction is increased effectively. The chloro ligand is a "bad" leaving ligand since its interaction with N7 atom of guanine is repulsive. Although in the X-ray and NMR studies of the 1,2-intrastrand adduct cisplatin–DNA the second H-bond O6···NH_3 has not been observed[17,18] its formation is well known in the monofunctional complexes of cisplatin with guanine[3]. This H-bond is preserved in the course of the substitution and it further stabilises the complex. In the reaction with

DNA there is also the third H-bond to the oxygen of the phosphate which was observed[17,18]. Its stabilising role is important[19] but it is in our opinion well preserved in the course of the reaction due to flexibility of the phosphate chain and it should not affect the mechanism of the substitution. It may only change the relative conformation of the platinum complex with the purine base.

The shape of the transition-state structure TS_Gua**1***a* is a distorted trigonal bipyramid with longer bonds to the entering guanine (r_{Pt-N7} = 2.588 Å comparing with 2.091 Å in the *cis*-Pt[$(NH_3)_2$GuaCl]$^+$ complex) and to the leaving water (r_{Pt-O} changes from 2.022 to 2.451 Å). The angle entering ligand, platinum atom, leaving ligand is only 71.7°. The angle between the plane of guanine and that of Pt(II)-complex is about 51°. However this value can be very different in a real DNA environment (compare also φ values in Table I). The structure is stabilised by a strong O6···HNH_2 H-bond.

An internal rotation around the Pt–N7 bond gives another (by about 0.5 kcal/mol less stable) conformation of the transition state TS_Gua**1***b* with two weaker and non-linear H-bonds resulting from the interaction of O6 atom with the NH_3 ligand of cisplatin and the hydrogen of the leaving water molecule (see Table I).

We have also checked for possibility of formation of the Pt–O6 adduct in the first step. Formation of the Pt–O6 bond causes significant changes in the interaction between guanine and cytosine compared to the Watson–Crick H-bonding pattern[20]. It may lead to a point mutation of DNA[21]. The corresponding transition state TS_Gua**1***a*-O6 is by about 2.9 kcal/mol less

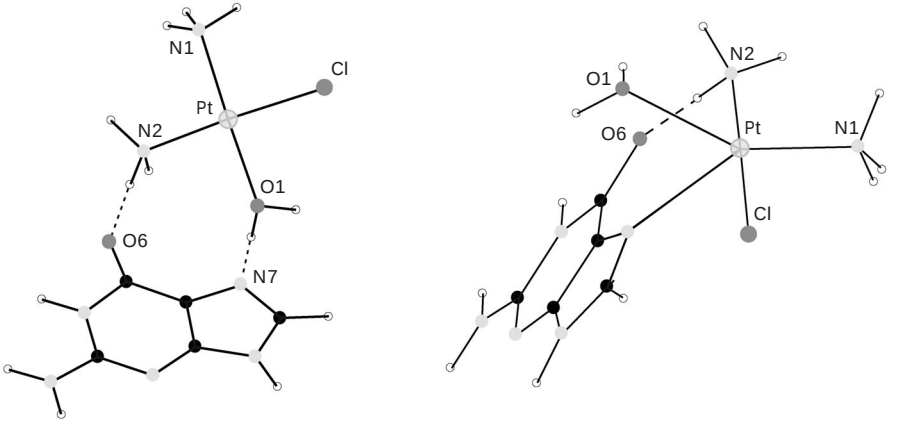

FIG. 1
Structure of the "pre-substitution" complex **1***a* (left) and the transition state TS**1***a* (right)[35]

TABLE I

Comparison of main structural characteristics of transition states for the cisplatin bonding with guanine and adenine computing by HF/SBK/SBK method (Structures denoted by DFT are optimised by B3P86/LANL2DZ/6-31G* method. See Fig. 1 for numbering of atoms of TS**1**–**3***a* structures; Fig. 2 for numbering of TS**1***a*-O6 and TS**II**-O6 structures; Fig. 3 for numbering of TSAde**1** structures. Pt-displacement, displacement of platinum atom from the guanine (adenine) base plane; φ, angle between planes of guanine (or adenine) and Pt(II)-complex; E_a, activation energy for corresponding substitution.)

Transition states	Pt–N7	Pt–O1	H-bonds Å	N7–Pt–O1 °	Pt-displacement Å	Φ °	E_a kcal/mol
TS**1***a*	2.59	2.45	1.75[a]	71.7	0.4	51	14.0
TS**1***b*	2.57	2.40	1.91[a]; 2.17[b]	74.5	0.0	50	14.5
TS**1***b*-DFT	2.48	2.38	1.90[a]; 1.90[b]	73.1	0.5	56	–
TS**2***a*	2.55	2.43	1.84[a]	71.1	0.6	55	10.4
TS**2***b*	2.58	2.41	1.91[b]; 2.14[a]	74.6	0.2	53	11.5
TS**3***a*	2.59	2.45	1.42[b]	71.2	0.6	70	17.9
TS**3***b*	2.50	2.48	2.02[a]; 2.47[a,g]	78.2	0.4	79	21.1
TSAde**1***a*	2.59	2.44	1.87[c]; 2.58[d]	75.1	0.0	66	14.5
TSAde**1***a*-DFT	2.50	2.38	1.75[c]; 2.39[d]	74.8	0.1	67	–
TSAde**1***b*	2.59	2.44	2.56[d]	69.6	0.7	88	16.1
TSAde**1***c*	2.62	2.42	2.09[e]	73.6	0.5	37	16.7

	Pt–O6 Å	Pt–O1 Å	H-bonds Å	O6–Pt–O1 °	Pt-displacement Å	Φ °	E_a kcal/mol
TS**1***a*-O6	2.43	2.49	1.77[f]; 2.55[b]	74.9	1.4	76	16.9
TS**1***b*-O6	2.46	2.50	2.06	63.1	0.6	79	17.1
TS**II**-O6	2.51	2.55	–	66.2	0.0	31	–

[a] O6···HNH_2 distance; [b] O6···HOH distance; [c] N6···HOH distance; [d] Cl···HN6 distance; [e] NH_3···N6 distance; [f] N7···HOH distance; [g] O6 atom forms H-bonds with both NH_3 groups of cisplatin.

stable than TS_Gua**1***a* structure. Substitution reactions of cisplatin are kinetically driven giving only a minor importance to the thermodynamic stability of the product structures. In spite of that we would like to mention that the reaction on the O6 atom is slightly endothermic (by about 2.2 kcal/mol) while the reaction on the N7 atom it is exothermic for all species. Our data clearly support the N7 atom as the preferential binding site for platinum but they are not able to explain fully the zero occurrence of Pt–O6 adducts. Other factors such as steric hindrance that is not included in our model can influence the results.

Very similar conclusions are probably also valid for the O6-binding in the second step after a previous binding of cisplatin to N7 atom to form N7–Pt–O6 chelate (with corresponding TS**II**-O6 transition-state structure) although in this case an increase of energy connected with the deformation of DNA has to be taken into account for the competitive GpG chelate. Structures of both transition states for the O6-adduct formation are shown in Fig. 2 and in Table I.

*Interaction of **2** with Guanine*

This reaction needs the lowest barrier of about 10.4 kcal/mol to proceed. **2** is more stable than **1** [22] and it is also the most probable species to attack guanine according to our results. OH^- group is able to polarize more its bond with central platinum atom comparing to Cl^-. The Mulliken population analysis at the MP2/LANL2DZ*/6-31G* level shows that the charges on the Pt atom for **2***a* and TS**2***a* (or *b*) structures are approximately by +0.25 e

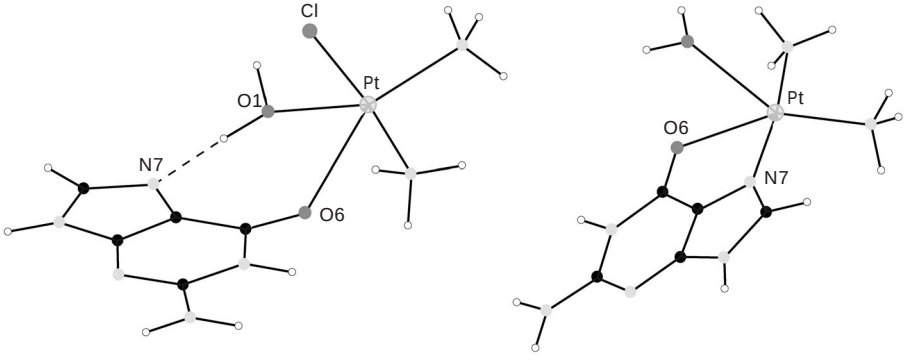

FIG. 2
Structure of transition states for the cisplatin binding on the O6 atom of guanine in the first step TS**1***a*-O6 (left) and in the second chelation step TS**II**-O6 (right)

higher than those for corresponding chlorine complexes **1***a* and TS**1***a* (or *b*) (+0.99 comparing to +0.72 e). However, this difference is overestimated for the 6-31G* basis set and it is expected to be lower with the extended basis set[22].

Otherwise, as expected, the substitution of the Cl^- ligand by an OH^- ligand does not change significantly the picture described in the previous section. Main structure characteristics of the transition states TS**2***a* and TS**2***b* for substitution of **2** with guanine are given and compared in Table I. The "pre-substitution" complex **2***a* can be found in Supporting Information.

*Interaction of **3** with Guanine*

The diaqua species **3** bearing two positive charges has the biggest affinity to guanine. However this structure differs not only in its charge but also in its capability to form H-bonds. The chloride and hydroxo ligands in **1** and **2**, respectively, are potential H-bond acceptor whereas the aqua ligand in **3** is a H-bond donor. Therefore there is one more ligand that is able to form an H-bond with O6 atom of guanine and several conformers of **3** with guanine can be formed. Only the most stable conformer with H-bonds to two aqua ligands was considered – **3***a* and **3***a*w structures (see further text). It leads to the TS**3***a* TS structure. The transition state TS**3***b* is less stable by about 3.2 kcal/mol due to a weaker O6···HNH_2 H-bond.

Due to the double positive charge of **3** the H_2O hydrogens are highly acidic here (pK_a value of 5.4 [8]) comparing with free water molecule hydrogens. It can lead to artificial differences between primarily equal hydrogens in our vacuum-phase calculations if the first of these acidic hydrogens is involved in hydrogen bonding to *e.g.* guanine or free water molecule and the second hydrogen is not. To make again both hydrogens equivalent, an additional water molecule, bound by H-bond to the second hydrogen of the water ligand, was included to our quantum chemical system. This approach was used with success for **3***a* complex to give complex designated as **3***a*w (see Supporting information).

No hydrogen transfer occurs in the TS structures since the H_2O···N7 hydrogen bond is already disrupted. We were able therefore to perform full TS optimisations without an additional water molecule (structures TS**3***a* and TS**3***b*). Optimisations with an additional water molecule were done only for TS**3***a* to give TS**3***a*w for a comparison with the **3***a*w minimum.

We have to note, however, that these structures are computed with the highest error due to the highest unscreened charge. Besides the previously described problems, it results in short H-bonds (HOH···O6 being only 1.42 Å

in the TS**3***a* structure). However, what is in our opinion more important, the covalent bonds are described properly as can be seen from the comparison in Table I.

The energy barrier for this substitution is according to our calculations the highest: 17.9 kcal/mol. The reason may be a better stabilisation of the double-charged **3***a*w structure comparing to the stabilisation of the mono-charged **1***a* and **2***a* structures.

*Interaction of **1** with Adenine*

Amino group of adenine is intrinsically pyramidal due to a partial sp^3 hybridisation of its nitrogen atom, which allows forming non-classical interactions: out-of-plane H-bonds and amino-acceptor interactions[23]. It was already observed *e.g.* for the hydrated Mg^{2+}–Deoxyadenosine Monophosphate complex[19]. Another example is shown in Fig. 3. The transition state TS_Ade**1***a* for N7 binding to adenine has H1 atom of the NH_2 group substantially deviated by about 50° from the adenine plane. A double H-bond stabilisation of the TS structure and a little higher angle between entering and leaving ligands of about 75° are observed (see Fig. 3 and Table I). There is no out-of-plane force acting on the H2 hydrogen of the amino group. The AT base pair structure will not be therefore influenced. The activation energy is 14.5 kcal/mol.

Other possible TS-structure conformers are a little less stabilised by only one H-bond: TSAde**1***b* structure with the $NH_2\cdots Cl$ hydrogen bond and TSAde**1***c* structure with the $NH_3\cdots NH_2$ hydrogen bond are about 1.6 and 2.2 kcal/mol less stable, respectively. However, differences are not important and the most stable conformation will result from the stabilisations and/or destabilisations coming from the real DNA environment. The important feature of our model is the fact that all the structures have the same main structural characteristics as it is shown in Table I.

Comparing our results for guanine and adenine, it is clear that different reactivity is not caused by the difference in stability of their Pt–N7 bond. The difference of 100 kJ/mol of interaction energies of cisplatin adducts with guanine and adenine, respectively, was already calculated[24]. Our calculated value of activation energy for the platinum substitution of adenine is only by about 0.5 kcal/mol higher than the corresponding value of guanine. TSAde**1***a* structure is very well stabilised by $NH_2\cdots Cl$ and $H_2N\cdots H_2O$ H-bonds. In **2** OH^- group does not offer such an advantageous pattern, we expect a little higher difference of about 2.0 kcal/mol[25]. Anyway the "pre-substitution" complexes already include the large portion of electro-

static interaction, which was shown to be responsible for the stronger stabilisation of guanine adducts when compared to adenine[26].

DFT Optimised Structures

We have reoptimised TS_Gua**1***b* and TS_Ade**1***a* transition states using B3P86/LANL2DZ/6-31G* method. As shown in Table I, the DFT computations provide structures essentially identical with the Hartree–Fock results despite some systematic differences in bond lengths[19]. HF bond lengths Pt–X (X = Cl, N, O) are overestimated by about 0.1 Å what is in a good

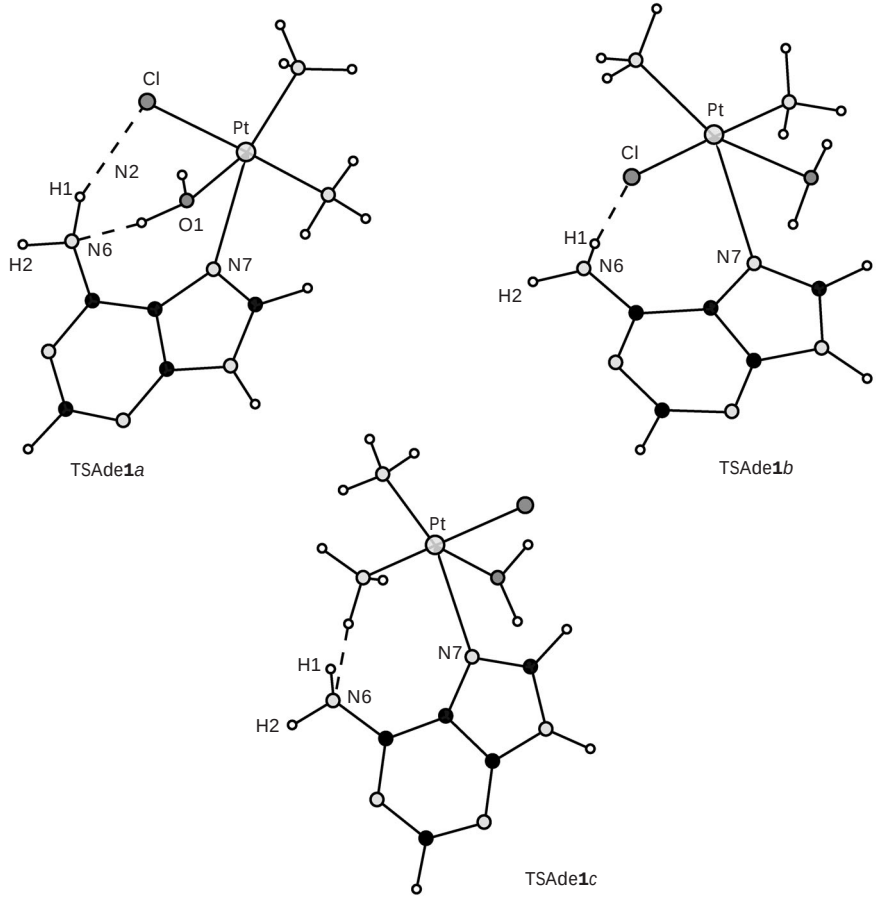

FIG. 3
Three different conformers of transition states for the cisplatin–adenine binding

agreement with results of our previous study[15]. H-Bonds are considerably shorter, too. The 6-31G* basis set enables much better orbital overlap between donor and acceptor atoms. TS_Gua**1***b* structure is stabilised by two strong H-bonds of 1.9 Å.

DISCUSSION

An agreement of *ab initio* calculation results of an isolated vacuum-phase molecular system with a real liquid-phase experiment is often achieved. It seems to be the case of Pt(II) transition-states structures as was already proposed by Deeth *et al.*[27], too. The question is whether and to what extent the results of the present study concerning a system formed by a whole nucleic acid base (adenine or guanine) and one of the three platinum structures (**1**, **2**, **3**) can be extrapolated to a real environment of DNA in solution.

First we would like to stress that all our results (including those from our previous study[15] concerning cisplatin hydrolysis) are in a good agreement with available experimental data. The second point is the conserved structure of these transition states, which does not seem to be dependent on the nature of leaving and entering ligands and of the ligands in the *cis*-positions. The TS structure does not even depend on the charge of the system – compare *e.g.* transition states for the first and the second step of cisplatin hydrolysis in our previous study[15] – or results shown in Table I of this study. In spite of large differences in the description of H-bonds, the double-charged system TS**3***a* has the same main structure characteristics which are connected with the Pt(II)-complex itself as the mono-charged complexes TS**1***a* and TS**2***a* or TSAde**1**.

Although nonbonding interactions do not have significant influence on the structure of transition states, they have a key role in molecular recognition affecting thus reactivity. We would like to touch this interesting and important theme from the point of view of our results.

In the "pre-substitution" complex structure, the H-bond, which connects the leaving ligand with N7 atom of guanine, can be significant in this particular geometry for two reasons: (i) helps to keep the platinum central atom of the complex relatively close to the future reaction center of atom N7 which may increase effectively the probability of the reaction and (ii) right mutual position of the reactants with respect to the structure of transition state is ensured in this case. To support the second point we would like to stress that the angle entering ligand-Pt-leaving ligand is low (about 70°) in the TS structures. We have not found any attempt of cisplatin to spread its coordination sphere. Even in the critical pentacoordinated struc-

ture of the TS, its electronic structure is indeed that of a slightly disturbed four-coordinated complex (see also Fig. 4 in our previous study[15]). Therefore the reaction could be more precisely described like "pushing away" of the leaving ligand by the entering one rather than a direct attack of the entering ligand from the direction perpendicular to the coordination plane.

The high electron density associated with N7 atoms in GG pairs appears to provide major driving force for these reactions with positively charged complexes[28]. And it was also stated that H-bonding plays a role in stabilisation of intermediates formed during the course of the reactions of cisplatin with nucleotides and it may provide a kinetic driving force toward GpG cross-links in oligonucleotides[29]. The question is the relative importance of different H-bonds, mainly of the H-bond with the O6 atom of guanine.

The H-bond to the O6 atom is well known from the monofunctional cisplatin–DNA adducts and its possible stabilisation role was already proposed[3]. Interaction with NH_2 group of adenine has a much lower stabilising effect[24]. The H-bond towards the O6 atom was not observed in the bifunctional adducts of cisplatin with DNA [17,18] but already bonded guanine may play its stabilisation role for the chelation step. In this case the Pt(II)-complex is already well anchored to the chain and the O6 atom is not necessary for the stabilisation. Therefore cisplatin can chelate very easily also to adenine if it is close enough. This is the case, indeed, for 1,2-intrastrand ApG adducts with the 25% occurrence ratio.

Previous consideration is in a good agreement with the common hypothesis that the presence of at least one N–H group to bind O6 atom of guanine is a prerequisite for the drug anticancer activity[30]. However this hypothesis was scrutinised recently. It was shown[26] that a net stabilisation due to this H-bond formation is only 4.2 kcal/mol for $[Pt(NH_3)_3$(9-methylguanine)$]^{2+}$. After experiments on DNA adducts with dichloro-*N*,*N*′-dimethylpiperazineplatinum(II), which lacks N–H functionalities, it was concluded that the very small size of the NH group, not its H-bonding ability, is responsible for the good activity exhibited by Pt compounds with ammine carrier ligands with multiple NH groups[31].

Moreover the theoretical study of interactions of hydrated divalent cations with purine nucleotides reveals that the interaction with the O6 atom of guanine is apparently weaker[19] comparing to strong bridges between the cation and anionic oxygen atoms of the phosphate group.

The H-bonding to the phosphate oxygen was observed for adducts of mononucleotides[32] up to adducts of real DNA [17,18]. It was shown *e.g.* that 5′-GMP reacts with *cis*-$[Pt(H_2O)_2(NH_3)_2]^{2+}$ more rapidly than 3′-GMP, G, or dG [33]. The interaction with the phosphate backbone changes certainly con-

formations of our optimised structures. We expect mainly some internal rotation of the platinum complex around Pt–N7 bond and the angle between the purine base plane and the plane of the platinum complex will be changed. However, the high flexibility of the phosphate chain should allow neglecting changes of interaction energies connected with these H-bonds in the course of the reactions.

The higher strength of the H-bond of N7 atom of guanine is directly connected with its higher electron density comparing to the electron density of N7 atom of adenine.

To conclude this section, we should like to stress the importance of the H-bonding with N7 atom in the "pre-substitution" complex followed by H-bonds with phosphate oxygens and the O6 (NH_2) atom. The latter two are well preserved during the reaction. The main role of the O6 atom in guanine is in our opinion in the enhancement of a negative electric field in the vicinity of the N7 atom as can be seen from a molecular electrostatic potential comparison[34]. N6 atom of adenine is a nucleophile, too, when the amino group becomes pyramidal-rotated but in the adenine plane the N6 atom is shielded by its hydrogens.

CONCLUSIONS

Our reaction model of the cisplatin binding to guanine supposes:

1) Formation of the "pre-substitution" complex which is stabilised by three H-bonds connecting N7, O6 and phosphate with proper ligands of Pt(II)-complex. However the H-bond to phosphate is neglected in our study supposing to be well preserved in the course of the reaction and not having significant influence on the mechanism of the reaction. The H-bond connecting the leaving ligand with the reacting center of the guanine (N7 atom) ensures a good mutual position of reactants with respect to the TS structure.

2) Substitution proceeds *via* a pentacoordinated transition state with a low angle of about 70° between entering and leaving ligands and corresponding bonds prolonged up to 0.5 Å. It has the same structural characteristics as our previously reported transition states for cisplatin hydrolysis[15].

3) The activation energy for Pt–adenine binding is slightly higher by about 0.5–2.0 kcal/mol than the activation energy for Pt–guanine binding. This number reflects mainly differences in orbital energy stabilisations and only a small portion of electrostatic contribution. The preferential binding site for Pt(II)-complexes resulting from our calculations is as expected the N7 atom of guanine.

Supporting Information Available

Tables containing HF total energies, thermal corrections, and Cartesian coordinates of all stationary points discussed in this study, in ASCII format, are available on request.

The research was partly supported by the Aktion 27p15 and Kontakt 2001-24 projects of scientific cooperation between Czech Republic and Austria and was included into the COST Action D20. We are very grateful to Supercomputing centers of the Technical University in Prague; the Masaryk University in Brno and the Charles University in Prague for providing their computational facilities. We are indebted to Dr J. V. Burda for helpful discussions and to Dr M. Kabeláč for the help with time-consuming DFT optimisations.

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