Why does Cisplatin Bind to ApG but not GpA Sequences of DNA? A Molecular Mechanics Analysis

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Molecular mechanics modelling of the bifunctional binding of cisplatin to d(GpApG) : d(CpTpC) and d(GpGpA) : d(TpCpC) sequences shows that attachment to ApG sequences is favoured by 28 kJ mol^{-1} over attachment to GpA sequences as a result of a favourable hydrogen bond between an ammine ligand and O^6 of the 3′ guanine in the former case, and a highly unfavourable interaction between the ammine ligand and the amine group of the 3′ adenine in the latter.

The primary mode of interaction between the anti-cancer drug cisplatin [*cis*-diamminedichloroplatinum(II)] and its putative intracellular target, DNA, is a bifunctional attachment to the N^7 atoms of adjacent purine residues of a single strand.[1,2] Little evidence is available on the details, at a molecular level, of these bifunctional interactions. Molecular mechanics calculations[3—5] and investigations of crystal structures[6] indicate formation of a hydrogen bond from one of the NH_3 ligands to the phosphate on the 5′ side of the bonded pair of nucleotides. Also, molecular mechanics calculations indicate a hydrogen bond between the other NH_3 ligand and O^6 of the guanine on the 3′ side of the pair.[3—5] Calculations on the interactions between the *R*,*R* and *S*,*S* enantiomers of cyclohexane-1,2-diamineplatinum(II) and DNA show that these two hydrogen bonds may play a significant role in determining binding ability of different diamineplatinum(II) species.[5]

Recently Reedijk *et al.* have shown that in binding to both DNA and the trinucleotide d(GpApG), cisplatin forms adducts to ApG but not GpA sequences.[2,7] It occurred to us that the reason binding to GpA sequences does not occur might be that the purine on the 3′ side of the pair, adenine, has an NH_2 group in the 6 position rather than the O atom present in guanine and, therefore, the second of the hydrogen bonds referred to above cannot form. Rather, a highly unfavourable interaction between the co-ordinated NH_3 group and the adenine NH_2 group would be expected. We therefore set out to determine the difference in the steric cost associated with binding of cisplatin to GpA and ApG sequences in the

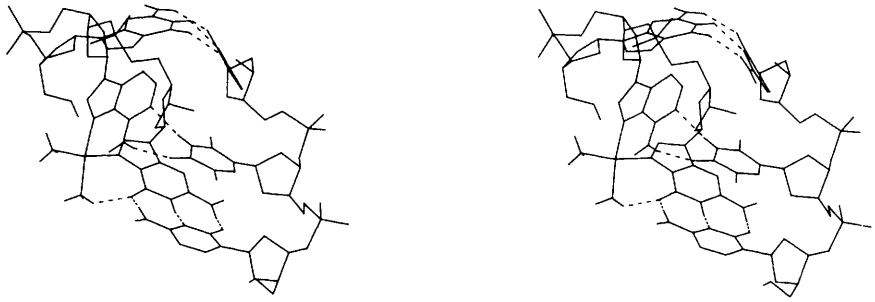

Figure 1. $Pt(NH_3)_2$ attached to the ApG sequence of d(GpApG) : d(CpTpC). --- indicates hydrogen bonds.

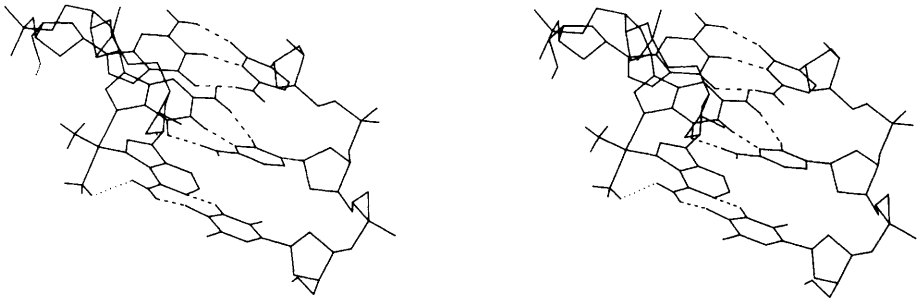

Figure 2. $Pt(NH_3)_2$ attached to the GpA sequence of d(GpGpA) : d(TpCpC). --- indicates hydrogen bonds, · · · indicates an unfavourable interaction between ligand NH_3 and adenine NH_2 groups.

deoxytrinucleotide duplexes d(GpGpA) : d(TpCpC) and d(GpApG) : d(CpTpC).

The molecular mechanics force field used in all calculations was based on the all-atom force field reported by Weiner *et al.*[8] Functions which model the interaction of the nucleotide with platinum were derived by modelling a number of small molecule diaminebis-6-oxopurineplatinum(II) complexes as detailed elsewhere.[9] Strain energy minimization was achieved by full-matrix Newton–Raphson refinement using a program developed in these laboratories.[10] This method is considerably slower than methods generally employed for energy minimization of large molecules and therefore restricts the size of DNA fragment which can be reasonably modelled to one of three base pairs. However, it is a more precise method and allows a more reliable determination of strain energies,[11] an important factor when comparing binding energies for similar drug–substrate complexes. The binding energy is described as the difference between the strain energy of the cisplatin–DNA complex and the total strain energies of the two isolated components of this complex, cisplatin and DNA. This definition of the binding energy clearly only represents the change in strain energy which occurs on complex formation since the energy of the metal–ligand bond is not included. However, the implicit assumption, that the platinum–N^7 (guanine) and platinum–N^7(adenine) bonds are of similar energy, is probably reasonable. Binding energies were calculated for two cases: cisplatin bound to $N^7(2)$ and $N^7(3)$ of the d(GpApG) : d(CpTpC) sequence and to $N^7(2)$ and $N^7(3)$ of the d(GpGpA) : d(TpCpC) sequence, giving cisplatin bound to ApG and GpA sequences, respectively, each at the 3′ end of a strand and therefore comparable.

Binding of cisplatin to d(GpApG) : d(CpTpC) and d(GpGpA) : d(TpCpC) sequences results in an increase in strain energy in both cases, primarily as a result of the deformation of the DNA structure to enable the two purine ligands to bond to the platinum atom. The binding energy is much greater (45.8 kJ mol^{-1}) for complexing to GpA of the d(GpGpA) : d(TpCpC) sequence than to ApG of the d(GpApG) : d(CpTpC) sequence (17.7 kJ mol^{-1}). This difference (28.1 kJ mol^{-1}) is due almost entirely to the different interactions between one of the NH_3 ligands and the purine base on the 3′ side of the bifunctional attachment. In the case of the d(GpApG) : d(CpTpC) sequence the interaction is a favourable hydrogen bond [O^6 · · · N 2.960, O^6 · · · H(amine) 2.20 Å] as observed previously,[3–5] but in the d(GpGpA) : d(TpCpC) sequence the interaction is an unfavourable interaction between co-ordinated NH_3 and adenine NH_2 groups [N^6 · · · N(ammine) 3.382, H(amine) · · · H(ammine) 2.58 Å]. It is difficult to assess whether an increase of 28 kJ mol^{-1} is sufficient to account for cisplatin not binding to GpA sequences. However, the binding energy increase is likely to be underestimated in the present work because of the small size and consequently overestimated flexibility of the models considered for the DNA structure. In any case, an increase in binding energy of this size would certainly be a deterrent to binding. Views of the binding sites for the two sequences are shown in Figures 1 and 2.[12]

Van de Veer *et al.* have suggested that the reason for the failure of cisplatin to form attachments to GpA sequences is that it binds first to a guanine base, making the distance to N^7 of an adjacent purine in the 3′ direction greater than the distance to N^7 of an adjacent purine in the 5′ direction.[7] Our models confirm this suggestion; we calculated distances of 5.71 and 3.92 Å, respectively, so we do not rule out this difference as being a possible factor. However, DNA is not a rigid molecule and twisting about the dyad axis would reduce

the former distance considerably. Also, the fact that cisplatin binds to two guanine bases separated by a third base[7] (*i.e.* GpNpG sequences) suggests that intially large separations between two binding sites are not a serious deterrent to bifunctional attachment.

In conclusion, the molecular mechanics models indicate that a possible reason why attachment of cisplatin to GpA sequences does not occur is the effect of highly unfavourable interactions between an NH_3 ligand on the platinum and the adenine base. This result has implications for the design of new analogues of cisplatin. If an analogue of cisplatin could be designed with one ammine or amine group and one group able to hydrogen bond to the adenine NH_2 group, then a compound able to bind to GpA sequences might be produced. Such a compound could have considerably different anti-cancer properties to cisplatin.

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