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Alteration of Cytosine-Guanine Interactions due to N7 Metal Cation Binding: A Structure-Activity Relationship for Cisplatin Analogues

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SUMMARY

Metal cation binding to N7 of guanine (G) and the resulting altered interaction with cytosine (C) were investigated by *ab initio* quantum chemical calculations for the ions Li^+, Na^+, B^+, Be^{2+}, and Mg^{2+}. It was found that the CG H-bonding interaction becomes more stable and the stacking interaction less stable as the charge density for the ion (ion charge/ionic radius) increases. From this observation a hypothesis is presented for a structure-activity relationship, which is supported by *in vivo* animal data, that may permit the design of less toxic analogues of the antitumor agent cisplatin.

Cisplatin, *cis*-diamminedichloroplatinum(II), is a highly effective antitumor drug that has been shown to bind to the N7 position of guanine in DNA. The structure of DNA adducts and mechanisms of pharmacologic action are being actively studied for cisplatin and structurally related analogues in an effort to develop more effective chemotherapeutic agents (1–4). The cisplatin DNA adduct can be viewed as a coordinate covalent bond between the metal cation and the nitrogen lone-pair electrons, plus the altered distribution of valence shell electronic charge of the guanine involved in base-pair interactions within DNA. Quantum chemical analysis provides improved understanding of the electronic structure of the cisplatin DNA adduct. We report on calculations in which a series of metal cations (M) is bonded to guanine (G) at the N7 position and alter the interactions between cytosine (C) and the guanine-metal ion (GM). Our finding of decreased stability for the complex CGM relative to CG, as the sum of increased H-bonding strength and a larger decrease in stacking stability, is consistent with recent experimental studies of decamer double helices containing cisplatin adducts (5, 6). A structure-activity relationship is identified for analogues of cisplatin that offers hope for design of less toxic antitumor drugs.

Methods

Ab initio SCF calculations were carried out with the Gaussian 82 program (7) at the STO-3G level using standard geometry for the bases and the H-bonded base-pairs (8). The stacking geometry, determined by quantum mechanical perturbation calculations (9), was a separation of 3.5 Å for the coplanar bases rotated by 36°. Fig. 1 presents the geometries used herein. The electronic energy (E) was calculated for GM with the cations Li^+, Na^+, B^+, Be^{2+}, Mg^{2+}, where the N7-M bond lengths, reported in Table 1, were predetermined by optimization of all bond lengths and angles in NH_3M. For G the bond was fixed to bisect the C8N7C5 angle, and reoptimization of the N7 – Li^+ bond of GLi^+ decreased the length by 5%. The energy of the complex, CGM, calculated for five cations at H-bonded and three cations at stacked geometries, is reported as the *relative* binding energy for the complex relative to the binding energy of CG, i.e., E(CGM) – E(CG). Calcultion of the binding energy as a difference between the energies of a supermolecule and its components is known to result in an overestimate of binding, especially when using a small basis set (10). The use of relative binding energies for a homologous series of molecular structures minimizes the basis set problem and provides useful qualitative information on electronic alteration of the CG interactions due to cation binding at the N7 position of G.

The dispersion energy contribution to molecular interactions is reportedly not included within the SCF method, whereas the induction energy is (9, 11–13). For the stacking interaction of adenine-adenine (AA), the dispersion correction was made by a second order perturbation method (13), whereas Forner *et al.* (14) used a semi-empirical London approximation method to add corrections for a series of base dimers which gave good agreement with the more rigorous perturbation method for AA. For CG they reported dispersion energies of −9 kcal/mol and −6 kcal/mol for H-bonded and stacked geometries, respectively (14). The semi-empirical method was used to determine the contributions of the metal ions to the dispersion energy of CGM and showed the largest correction, approximately 0.01 kcal/mol, to be insignificant due to the relatively small polarizabilities and large ionization potentials of the metal ions (15).

Results and Discussion

The calculated relative binding energies of the complex, CGM, for the series of cations reported in Table 1, show an

ABBREVIATIONS: G, guanine; C, cytosine; M, metal cations; GM, guanine-metal ion; CGM, complex of cytosine, guanine, and metal cation; CG, cytosine-guanine complex; AA, adenine-adenine.

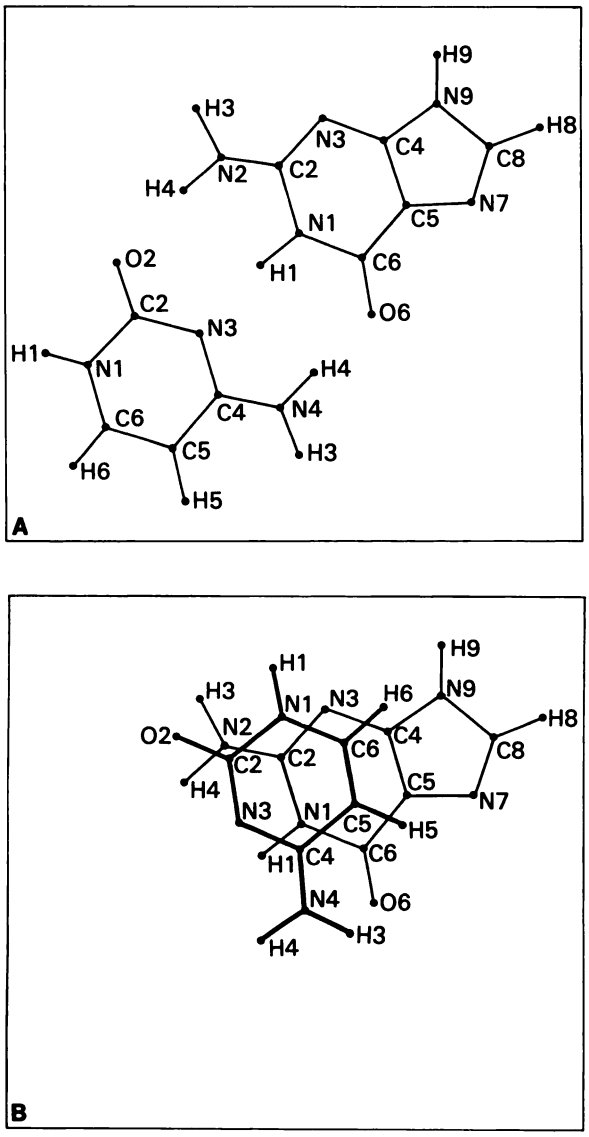

Fig. 1. Molecular structure for cytosine and guanine in H-bonded (A) and stacked (B) geometry.

TABLE 1

Calculated relative binding energies of the complex CGM for a series of cations

Complex (CGM)	N7-M (A)	H-bonding energy[a]	Stacked energy[b]	q/r (1/A)[c]
		kcal/mol		
CGH^+	(1.0)[d]	−5.51	3.78	
$CGLi^+$	1.90	−3.00	3.98	1.47
$CGNa^+$	2.13	−1.76	4.32	1.03
CGB^+	1.65	−5.25		2.86
$CGBe^{2+}$	1.63	−8.90		5.71
$CGMg^{2+}$	1.93	−5.75	7.72	3.03
$CGPt^{2+}$				2.50

[a] Total energy of CG in H-bonded geometry = −920.0199 atomic units.
[b] Total energy of CG in stacked geometry = −919.9747 atomic units.
[c] Ionic radii are taken from Ref. 26.
[d] Value from Ref. 8.

increase in H-bonding strength and decrease in stacking stability for all cations considered. Fig. 2 presents relative binding energies for the complex, CGM, as a function of the metal ion charge (q) divided by the ionic radius (r), where q/r is a measure

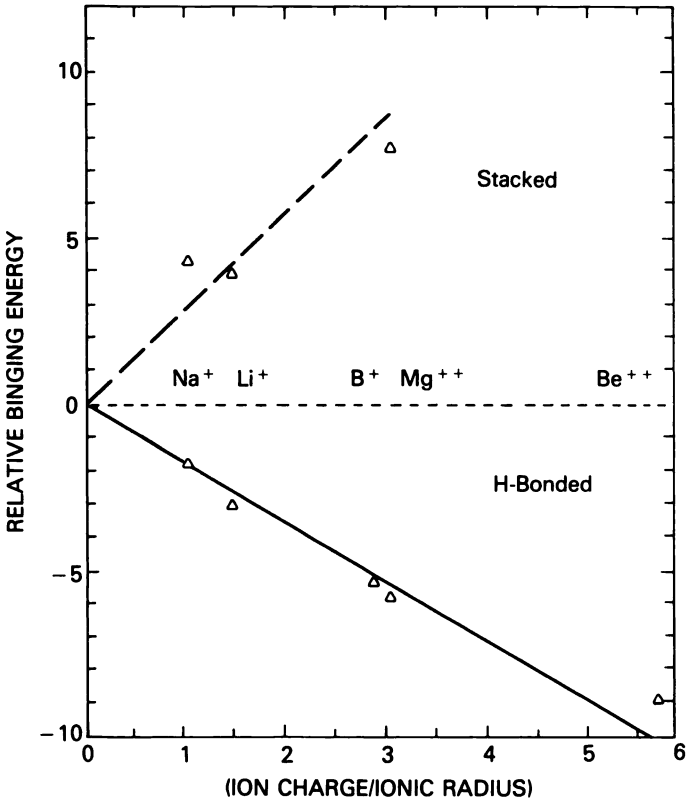

Fig. 2. Effects on the interactions of cytosine with guanine due to metal ion binding at N7 of guanine, for both H-bonded and stacked geometries, are given as a function of (ion charge/ionic radius) for the ions Na^+, Li^+, B^+, Mg^{2+}, Be^{2+}. Relative binding energies are given in kcal/mol, where negative values indicate attractive binding. The *points* are the calculated energies and the *lines* are the best fit linear regressions which were forced through the zero intercept.

of the cation charge density. The data points are the calculated relative binding energies and the lines are the best fit linear regressions which were forced through the zero intercept for CG with no cation bound. Relative to CG, all H-bonded complexes increase in stability (negative energies), and all stacked complexes decrease in stability with increasing q/r. Both types of interactions appear linear over a sufficient range that one can interpolate for known q/r values and estimate effects for other compounds.

Assuming the relation holds for Pt^{2+}, the effect of cisplatin on CG can be considered. A detailed quantum mechanical SCF calculation by Miller *et al.* (16) of cisplatin, $(Pt(NH_3)_2)^{2+}$, bound to N7 of G was found to be predominantly an electrostatic interaction such that replacing cisplatin by a simple point charge of value 1.25 at the Pt site in the SCF calculation reproduced the valence-electron bonding energies. For cisplatin with a q/r value for Pt^{2+} of 2.50, an estimate from Fig. 2 suggests that cisplatin adducts strengthen CG H-bonding by approximately 4 kcal/mol and destabilize CG stacking by 7 kcal/mol. For Pt^{2+} involved in a cisplatin bridge bond with a stacked GG sequence, the q/r value would be 1.25.

The decreased stacking stability suggests a lowered melting temperature for DNA which is consistent with the 15° decrease reported by den Hartog *et al.* (5) for a decamer double helix with cisplatin, which was synthesized to form a Pt^{2+} bridge bond to each N7 of the GG sequence in one strand. They also observed a strong NMR chemical shift of the N1—H imine

protons of the GG sequence (*G—H1* of Fig. 1A), which indicates a change in the electronic environment of these protons which are involved in H-bonding. Our molecular orbital wave functions show a change in electronic charge for both H1 and H4 protons of G as relative electronic charges on the protons, determined by Mulliken population analysis, increase in positive charge with increasing q/r. In Fig. 3, the relative change in electron density for the H-bonds is shown. For the C—O2 H4—G (Fig. 3A) and C—N3 H1—G (Fig. 3B) H-bonds, the electrostatic interaction becomes stronger with increasing charge density, q/r, for the metal cation bound to G—N7, but for C—H4 O6—G (Fig. 3C), no such simple relation was seen. The density on the C—H4 proton increases with increasing q/r which would be expected to weaken the C—H4 O6—G H-bond. However, the change in electronic density on the G imine protons is an indirect effect of a cation-altered electronic valence shell, whereas G—O6 is positioned for a direct interaction with the cation as well. The electron density of G—O6 decreases with

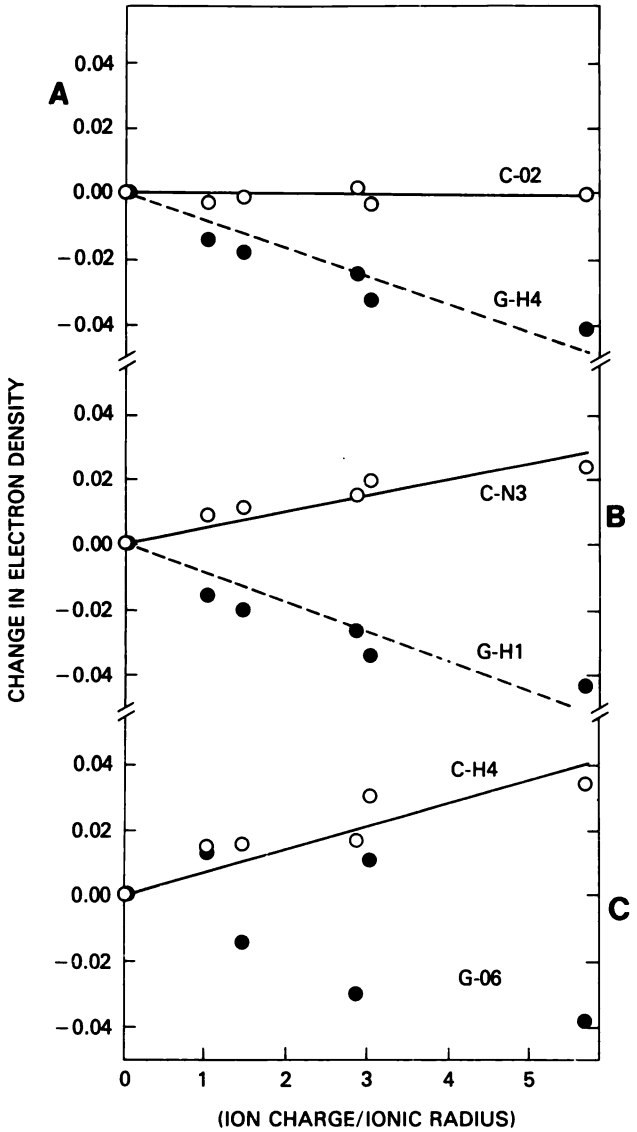

Fig. 3. Change in electron density on atom sites involved in hydrogen bonds between cytosine (*C*) and guanine (*G*), using notation from Fig. 1, for the three bonds C—O2 H4—G (A), C—N3 H1—G (B), and C—H4 O6—G (C).

increasing q/r for Li^+, B^+, Be^{2+}, but for Na^+ and Mg^{2+}, which have the electronic configuration $1s^22p^6$, there is an increase in electron density of G—O6 relative to that in CG. Cisplatin, with diffuse extended *p* and *d* orbitals on Pt^{2+}, probably results in a weaker C—H4 O6—G H-bond relative to CG. In the case of a mono-aquo cisplatin complex with G, the possibility of an intramolecular H-bond to O6 exists (17, 18) which would also be expected to weaken the intermolecular C—H4 O6—G H-bond. The electronic density on the metal ion site of the CGM complex always shows a net charge less than *q*, which is due to alteration of the valence shell electron density to accommodate the coordinate N7—M link.

X-ray structural data for experiments in which cisplatin was diffused into a pregrown crystal show considerable alteration of the DNA structure (6, 19). At the Pt^{2+} binding sites, G is moved slightly out of the base-pair stack of the double helix, and H-bonding is sufficiently strong to move the complementary C along with it. The shift is non-rigid, however, as bases stacked above the site appear to pivot away from the G. It would seem that strengthening the H-bonds coupled with the greatly decreased stacking stability will distort the double helix but not necessarily rupture it. den Hartog *et al.* (5) suggest that cisplatin gives rise to considerable added H-bond stability relative to other base-pairs in the double helix, whereas Wing *et al.* (6) suggest that a local distortion of DNA could be reasonably anticipated at the platination sites. This could be a relatively small localized denaturation that may be attributed to the observed shrinking of platinated DNA (20).

On the basis of the relationship observed between relative binding energies and cation charge densities (Fig. 2), a hypothesis for a structure-activity relationship of cisplatin analogues is presented. It is assumed that activity for cisplatin analogues is related to the charge density at the Pt site. Greater effects of stronger H-bonds and destabilized stacking are expected for analogues in which the ligands bonded to Pt decrease the electronic density at the Pt site, thus increasing the magnitude of q/r. As an example, comparison of cisplatin analogues for which the amine ligands are, respectively, NH_3, CH_3NH_2, *n*-$C_3H_7NH_2$, *i*-$C_3H_7NH_2$ show an increasing LD_{50}, i.e., decreasing toxicity, in mice bearing ADJ/PC6 tumors (21, 22). By the electronic induction effect, one expects an increasing electron density on the amine N-atom for the series which results in a decreasing positive charge on the Pt site of the drug, thus yielding the decreasing pharmacologic activity.

In support of the hypothesis, Fig. 4 shows the log-linear relationship for cisplatin and 17 analogues between experimental LD_{50} values and calculated energies for the interaction of a point charge placed above the Pt site of the analogue (23). The energies, calculated by a quantum chemical semi-empirical INDO method (23, 24), give a measure of the electronic density near the Pt site due to the polarizability of the cisplatin analogue. The energies are plotted as relative values by normalizing the cisplatin interaction energy to 1.00. A least squares fit to the data in Fig. 4 demonstrates a significant correlation, yielding parameters (± standard deviations) that give a zero intercept (0.036 ± 0.116) and positive slope (7.08 ± 3.03) for the 18 cisplatin type compounds ($r = 0.61$).

The correlation is surprisingly strong when one considers the variability inherent in the pharmacologic data. Intraperitoneal administration of a drug generally results in variation as to the fraction of the dose that is actually absorbed into the systemic

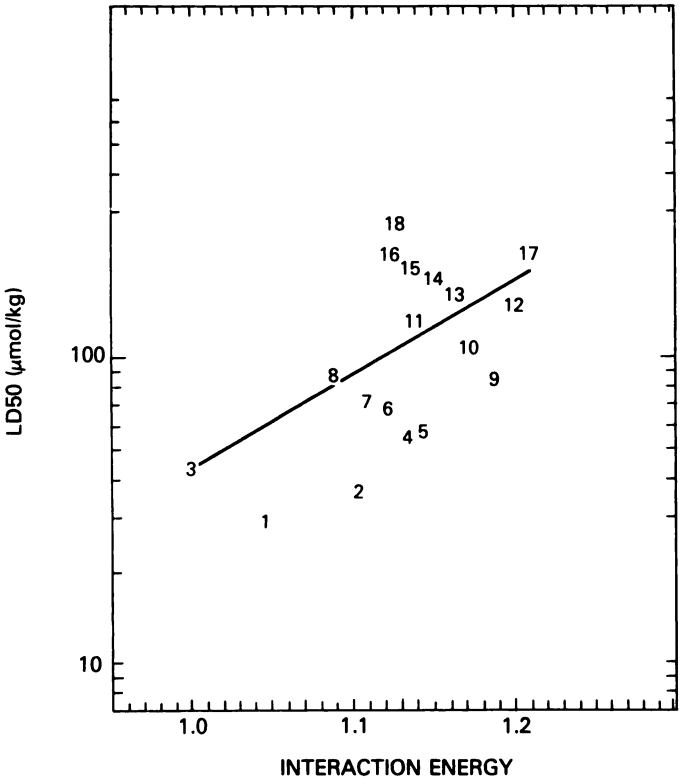

Fig. 4. The log-linear relationship between LD_{50} and relative interaction energies for cisplatin analogues with the following amine substituents, where the number in parentheses identifies the position of the compound on the graph: NH3 + cyclopentylamine (*1*), 1,2-diaminocyclohexane (*2*), cisplatin (*3*), methylamine (*4*), 3,4-diaminotoluene (*5*), *n*-propylamine (*6*), ethylamine (*7*), NH3 + ethyleneimine (*8*), isopropylamine (*9*), β-chloroethylamine (*10*), *o*-phenylenediamine (*11*), cyclopropylamine (*12*), ethyleneimine (*13*), cyclohexylmethylamine (*14*), isobutylamine (*15*), *n*-pentylamine (*16*), cyclobutylamine (*17*), *n*-butylamine (*18*). The interaction energies are all normalized relative to a value of 1.00 for cisplatin.

circulation. The rather broad range of compounds administered also introduces a distributive pharmacokinetic component to the expected variability. Earlier unsuccessful efforts to correlate activity with structure for this group of compounds considered lipophilicity as a variable. This appears to be a reason for some of the scatter in Fig. 4 as a counter-trend is suggested from the analogues *n*-butylamine, *n*-pentylamine, isobutylamine, and cyclohexylmethylamine. The analogues in Fig. 4 include a mixture of mono- and bidentate amine ligands bound to the central Pt^{2+} ion that generally appear to follow the proposed (q/r)-versus-activity relationship within amine classes. This structure-activity relationship extends beyond the listed compounds as well. For example, the relatively nontoxic cyclohexylamine is made considerably more toxic by introducing a hydroxyl group into the ring; the activity of *o*-phenylenediamine is greatly enhanced by introducing a methyl group in the 4-position, while a 4-carboxy substituent decreases the activity and 4,5-dimethyl-*o*-phenylenediamine is much less active.

The supporting data demonstrated in Fig. 4 are based on a combined therapeutic-toxicity protocol (25) which gives a global LD_{50} whereby specific mechanisms for the pathway(s) involved in the lethality cannot be separately identified. The data are also specific to the bioassay used in the original study. However, the data in Fig. 2 showing the effect of decreased stability of CG as a function of increasing charge density of the cation bound to G—N7 is a general observation. Whether the structure-activity hypothesis based on this observation will be found valid in general depends on further experimental studies.

Conclusion

A hypothesis has been advanced that is directly amenable to experimental verification. When the amine ligands of cisplatin analogues are chemically altered to increase the availability of the amine nitrogen lone-pair electrons, which should lead to an increased electronic density at the Pt site of the analogue, there should be a decrease in pharmacologic activity. This relationship between the structure and activity of cisplatin analogues bound to DNA should hold within amine classes, for bidentate as well as for homogeneous and heterogeneous monodentate amines, and may permit the design of less toxic antitumor drugs by alteration of the electronic characteristics of the DNA adduct.

It would be better to consider pharmacologic activity data for the more specific ID_{50} for tumor regression, but within this data base a structure-activity relationship is not readily apparent. The ID_{90} is a specific observable endpoint of a complex biochemical pharmacologic process which has been shown to depend on both drug stereospecificity and DNA base sequences. It is clear that a detailed understanding of the electronic structure of DNA adducts, with both antitumor drugs and carcinogens, will contribute to the understanding of the carcinogenesis process and lead to the design of more effective chemotherapeutic antitumor agents.

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