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The primary mode of binding of cisplatin to a B-DNA dodecamer: C-G-C-G-A-A-T-T-C-G-C-G

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When cisplatin [*cis*-diamminodichloroplatinum(II)] is diffused into pre-grown crystals of the B-DNA double-helical dodecamer C-G-C-G-A-A-T-T-C-G-C-G, it binds preferentially to the N7 positions of guanines, with what probably is an aquo bridge between Pt and the adjacent O6 atom of the same guanine. The entire guanine ring moves slightly toward the platinum site, into the major groove. Only three of the eight potential cisplatin binding sites on guanines actually are occupied, and this differential reactivity can be explained in terms of the relative freedom of motion of guanines toward the major groove. This shift of guanines upon ligation may weaken the glycosyl bond and assist in the depurination that leads to mismatch SOS repair and G.C to T.A transversion.
Key words: cisplatin/B-DNA/X-ray structure

Introduction

Cisplatin [*cis*-diamminodichloroplatinum(II)] is one of the most effective and extensively studied inorganic antitumor drugs. In contrast, for reasons that are not fully understood, its transplatin isomer (*trans*-$Pt(NH_3)_2Cl_2$) exhibits little antineoplastic activity (Rosenberg *et al.*, 1969; Prestayako *et al.*, 1980). Cisplatin is believed to interfere with DNA replication and transcription in a manner similar to that of alkylating agents (Harder and Rosenberg, 1970; Howle and Gale, 1970; Taylor *et al.*, 1976). In solution under normal physiological conditions, cisplatin binds most strongly to the N7 position of guanine, with lesser attraction for adenine and cytosine and none for thymine or uridine (Mansy *et al.*, 1973, 1978).

Single crystal X-ray structure analyses have been carried out previously for complexes of cisplatin or of *cis* $Pt(en)Cl_2$ (en = ethylenediamine) with inosine monophosphate or with guanosine (Goodgame *et al.*, 1975; Gellert and Bau, 1975; Cramer *et al.*, 1980). In each case the platinum atom bridges the N7 positions of two guanine rings, with *cis* ligation to Pt. This has led to the suggestion that cisplatin might act *in vivo* by making interstrand or intrastrand cross-links between guanines. Interstrand cross-linking (Horacek and Drobnik, 1971; Roberts and Pascoe, 1972; Zwelling *et al.*, 1979) would necessitate unwinding of the double helix, since a cisplatin group bound to guanine in the bottom of the major groove would be inaccessible to another strand from the same or a different helix, for the second bond. Intrastrand cross-linking could be achieved between adjacent guanines along a polynucleotide chain (Cohen *et al.*, 1980; Lippard, 1982), but only at the price of destacking the bases and tipping the N7 positions toward the metal site in the major groove.

Alkylating agents such as benzo[a]pyrene, 2-acetylaminofluorene and aflatoxin B1 have another important effect on DNA, in addition to simple alkylation and cross-linking. They are potent mutagens, introducing G.C to T.A transversions in a parallel fashion (Foster *et al.*, 1983). The common feature of these alkylation adducts seems to be a weakening of the glycosyl bond leading to depurination, followed by insertion of adenine opposite the depurinated site by the SOS repair system of the cell (Witkin, 1976, 1982). Miller (1983) has suggested that cisplatin might also mimic this property of alkylating agents, leading to mutagenesis with transversion as well as cross-linking. In support of this idea, Brouwer *et al.* (1981) have found that cisplatin strongly favors G.C to T.A transversions among 650 nonsense mutations produced in the *lacI* gene of *Escherichia coli*, supporting the idea that what is occurring is depurination followed by bypass repair. $Pt(dien)Cl^-$ appears to strengthen the glycosyl bond when it complexes with the N7 position of guanine (Johnson, 1982; Johnson *et al.*, 1982). However, as these authors themselves point out, 'The enhanced biological activities of *cis*-PDD compared with *trans*-PDD and [Pt(dien)Cl]Cl are a consequence of different platinum-DNA adducts formed by these compounds *in vivo*'. For example, cisplatin is a potent mutagen, with excision repair and daughter strand gap repair, and has clinically useful antitumor activity; $Pt(dien)Cl^-$, in contrast, is only a very weak mutagen, shows no evidence of repair, and has no antitumor activity. These observations provide strong circumstantial support for the idea that mutagen activity involves as an early step the weakening and rupture of the glycosyl bond, and subsequent depurination.

Regardless of whether the ultimate outcome is cross-linking or mutagenesis, the initial biological interaction of cisplatin with double-helical DNA almost certainly is binding to guanine N7 with loss of one of the chloro (or rather, aquo) ligands. As Lippard (1982) has pointed out, aqueous solution of cisplatin is followed by displacement of chlorides by water molecules. Displacement of one water and ligation to N7 then follows, but it is unclear whether a second interaction occurs with the adjacent O6 under physiologically reasonable conditions (Chu *et al.*, 1978). The distance between Pt and O6 is too long for a direct Pt-O bond, but too short to accommodate an intermediate bridging water molecule unless the square planar Pt complex is rotated about the Pt-N7 bond, and out of the plane of the guanine rings (Goodgame, 1975).

In lieu of co-crystallized complexes of cisplatin with G-G-containing DNA oligomers, thus far unattainable, we have solved the crystal structure of the diffusion complex of cisplatin with the B-DNA double-helical dodecamer of sequence C-G-C-G-A-A-T-T-C-G-C-G. Attempts to achieve complete substitution or 100% binding to the major site led to destruction of crystal order as reflected in degradation of the X-ray pattern, an observation that may support the hypothesis that cisplatin binding ultimately deforms and

Table I. Binding of cisplatin to C-G-C-G-A-A-T-T-C-G-C-G as a function of concentration

Data set		Native	Pt1	Pt2	Pt3
Cell dimensions (Å)	*a*	24.87	24.36	24.33	24.16
	b	40.39	40.05	40.08	39.93
	c	66.20	66.13	66.26	66.12
Resolution, *d* (Å)		1.9	2.5	2.2	2.6
Residual error, R.	All data:	23.9%	20.0%	27.0%	16.6%
	Two-sigma data:	17.8%	–	–	11.2%
Percent site occupancy	G16	–	20%	38%	61%
	G4	–	10%	17%	30%
	G10	–	–	13%	22%
Pt-N7 bond length, G16 (Å)		–	2.51	2.43	2.16
Apparent guanine shift (Å)		–	0.51	0.43	0.16
Occupancy ratios:	G16/G4		2.0	2.24	2.03
	G16/G10		–	2.92	2.77

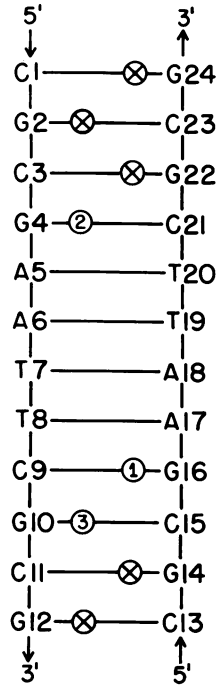

Fig. 1. Unrolled ladder diagram of the major groove of the B-DNA C-G-C-G-A-A-T-T-C-G-C-G dodecamer, showing the eight potential cisplatin binding N7 sites as circles. Numbers 1–3 indicate observed binding sites in order of decreasing occupancy by cisplatin complexes. No binding was observed at the five positions marked by circled X.

destroys the double helix. However, lower levels of substitution yield a clear indication of the cisplatin binding sites, and a pattern of selective binding to guanines that provides information about the primary binding steps in the interaction of cisplatin with double-helical DNA. The most highly substituted data set has been refined to completion, and two other sets have been refined to the point where meaningful structure comparisons can be made with the primary set.

Results

The three data sets, labeled Pt1, Pt2 and Pt3 in order of increasing cisplatin substitution, are listed in Table I. In set Pt2 the emphasis was on resolution of the data; in Pt3, on maximal substitution. The Pt3 set was chosen for complete refinement (see Materials and methods), to a final zero-sigma residual error or R factor of 16.6%, and a two-sigma R factor of only 11.2%, the lowest values obtained in any of our oligonucleotide refinements. The double-helical B-DNA dodecamer C-G-C-G-A-A-T-T-C-G-C-G has eight guanines that could serve as binding sites for cisplatin, as indicated by circles on the rungs of the DNA ladder in Figure 1. No more than three of these were occupied by cisplatin even in the most highly substituted crystal, and always in the order of decreasing affinity: G16, G4, G10. As Table I indicates, in the most highly substituted Pt3 set, site G16 exhibits 61% occupancy, with 30% at G4 and 22% at G10. Such incomplete site occupancy is not without precedent in other crystal structure analyses of complexes of cisplatin with organic bases. Goodgame (1975) reacted cisplatin with inosine monophosphate prior to crystallization, but still obtained a non-stoichiometric compound with only 0.56 platinum atoms per two inosine bases. The relative site occupancies in the cisplatin complex of C-G-C-G-A-A-T-T-C-G-C-G listed in Table I can be given a straightforward structural explanation, as described below.

Figure 2 shows a minor groove stereo pair drawing of the refined Pt3 structure, with cisplatin groups depicted at sites G4, G16 and G10 from top to bottom. The three ligands drawn around each platinum atom were located from difference electron density maps and then refined as described in Materials and methods. No restraints were applied to tie the ligands to the platinums during refinement, and the fact that the PtL_3 complexes remained intact, with reasonable Pt-L bond lengths of ~2.0 Å as listed in Table II, indicates that even at less than complete substitution, real information about the structures of the complexes is being obtained. Platinum positions may be regarded as secure; ligand positions are reasonable and suggestive.

A close up view of sites G16 and G10 in the bottom half of the helix is provided by the stereo drawings of Figure 3. Bearing in mind the provisional nature of the ligand sites, it appears that the platinum atom is indeed bridged by a ligand, probably water, to the adjacent O6 atom on the same guanine ring, and that the square plane of Pt ligands is rotated out of

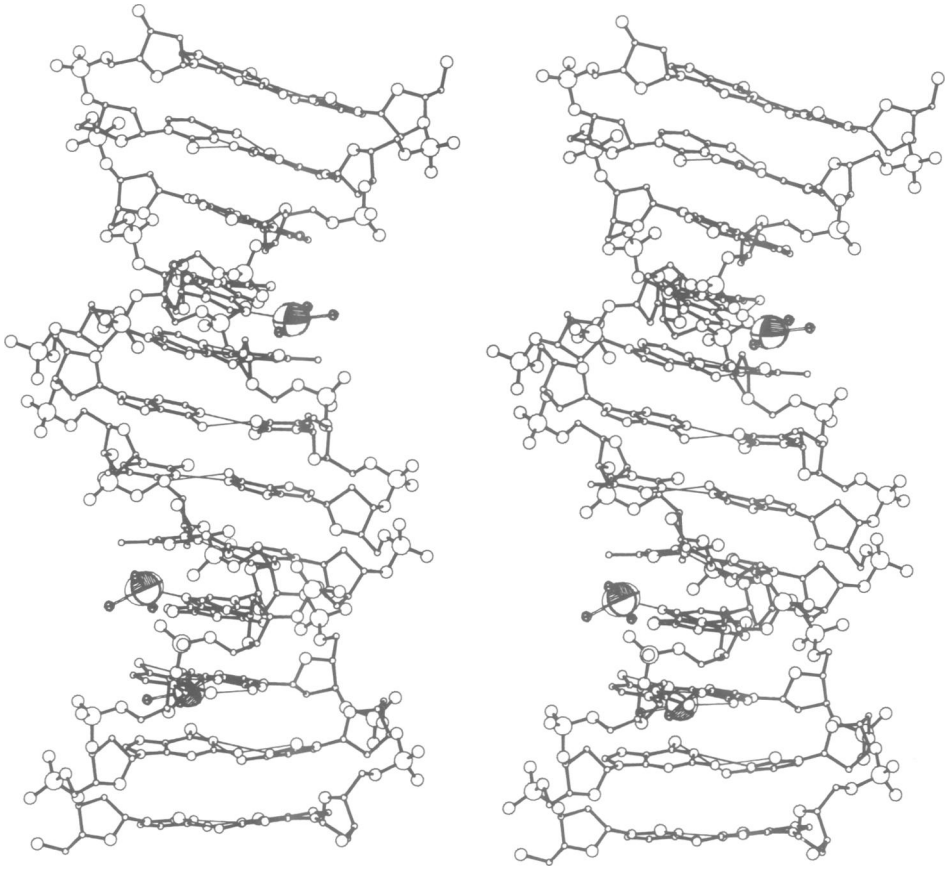

Fig. 2. Major groove view of the cisplatin complex of C-G-C-G-A-A-T-T-C-G-C-G as refined from data set Pt3. Base pair C1.G24 is at the top, and G12.C13 is at the bottom. Pt atoms at guanines G4, G16 and G10 (from top to bottom) are represented by their anisotropic thermal ellipsoids. The three smaller crossed spheres around each Pt are ligand sites obtained as described in Materials and methods. Other atoms are P, O, N and C in order of descending size.

Table II. Cisplatin-DNA complex geometry

	G16	G4	G10
Percent occupancy	61%	30%	22%
Bond lengths (Å)			
Pt-N7	2.16	2.31	2.23
Pt-L1	2.02	1.75	1.83
Pt-L2	1.81	2.25	2.10
Pt-L3	2.00	2.23	2.13
Bond angles (°)			
L1-Pt-L2	166	175	173
L1-Pt-L3	98	95	105
L2-Pt-L3	79	85	82

the guanine plane as in Figure 4. So far this only confirms expectations from previous crystal structure analyses with bases and nucleotides. However, Figure 3 also indicates that base pairs in the cisplatin complex are shifted relative to their positions in the native structure. At the most highly substituted G16 site, the guanine ring moves towards the platinum, bringing the hydrogen-bonded cytosine with it. Cytosine C15 just below it in Figure 3a moves in the same direction, but adenine A17 just above it moves in a different direction along the long axis of the base pair, indicating that what is being observed is not a rigid-body shift of the entire helix, but a local deformation. At the less highly substituted G10 site (top rear of Figure 3a), the guanine appears to pivot so the five-membered ring again moves toward its bound Pt site.

Discussion

An X-ray crystal structure analysis gives the averaged molecular structure over the entire crystal. If substitution were complete, one would observe an image of the pure cisplatin/DNA complex. If there were no substitution, one would see the image of the parent DNA molecule. For partial substitution, what is observed is a composite image of both structures, with the cisplatin-bound image at each site weighted according to the degree of substitution at that site. If the binding of cisplatin involves a displacement of the guanine ring, then for partial occupancy sites the apparent position of the ring will be intermediate between that of the parent helix and that of the 1:1 cisplatin complex. This seems the best explanation of a curious observation that arises when the three structures with different levels of cisplatin substitution are compared: Pt1, Pt2, and Pt3 (Table I). The apparent Pt-N7 bond length of 2.51 Å in the low-substitution Pt1 structure is far too long, but the bond becomes shorter in Pt2 and Pt3 as the degree of substitution increases. Linear extrapolation of a plot of bond length *versus* percent substitution leads to a Pt-N7 distance of 1.8 Å at 100% substitution, overshooting slightly the expected 1.97 – 2.02 Å (Goodgame *et al.*, 1975; Gellert and Bau, 1975; Cramer *et al.*, 1980), but

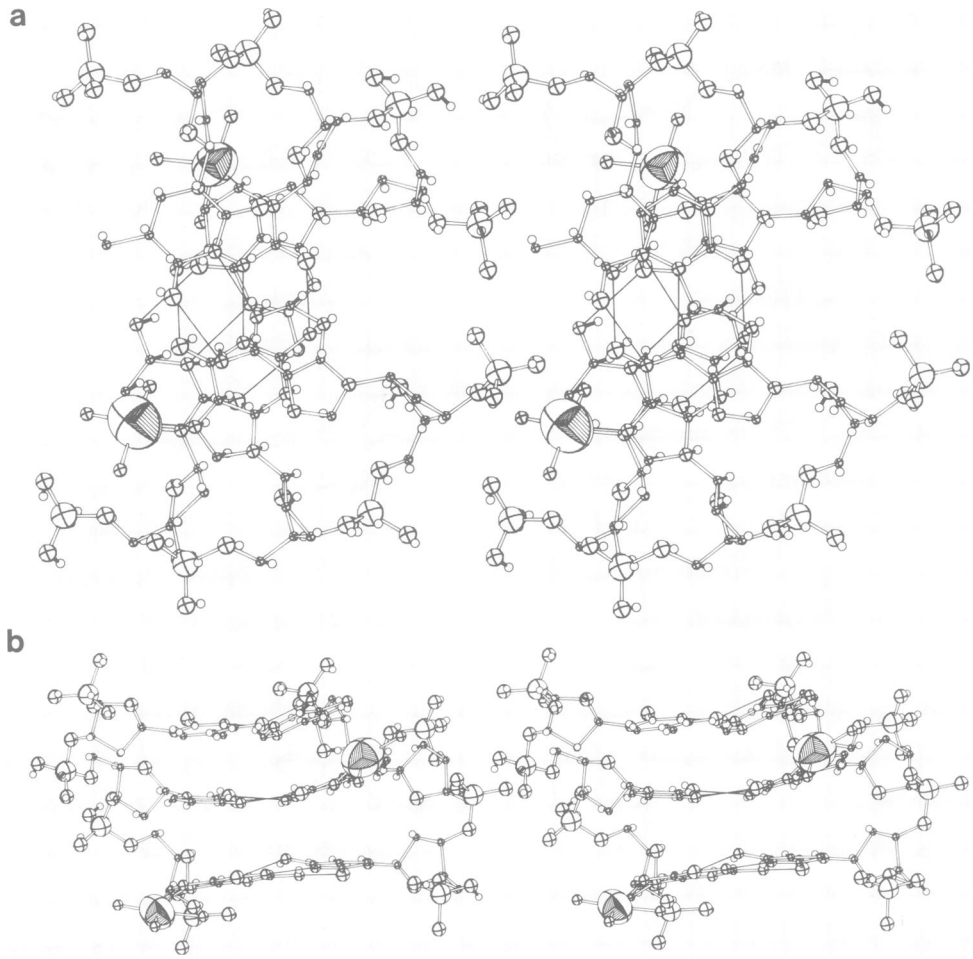

Fig. 3. Plan view (**a**) and major groove edge view (**b**) of cisplatin sites G16 and G10 and their immediate surroundings. Base pair T8.A17 is nearest the viewer in (**a**) and at the top in (**b**). Base pair G10.C15 is farthest from the viewer in (**a**) and at the bottom in (**b**). Platinum and ligand atoms are displayed as in Figure 2, and other crossed spheres are P, O, N and C in order of descending size. The small, uncrossed sphere beside each atom of the DNA helix indicates the location of the equivalent atom in the native dodecamer structure without cisplatin. (**a**) Shows particularly clearly that binding of cisplatin to the N7 of guanine G16, the most highly substituted site, pulls the entire base pair into the major groove in the direction of the Pt atom. A similar but smaller effect is visible at the more weakly substituted G10 site.

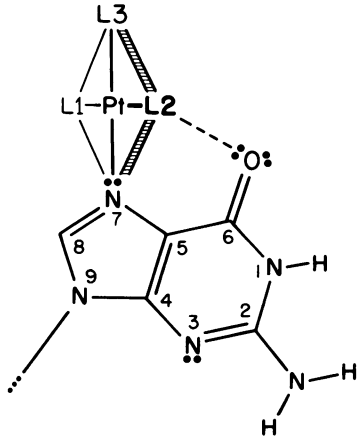

Fig. 4. Ligation geometry suggested by the Pt3 structure analysis. The N7 of guanine supplies one of the four square planar Pt ligands. A second ligand L2, most probably a water molecule, bridges the Pt and the guanine O6. Ligands L1 and L3 probably are the ammonia molecules of the original cisplatin complex. Since the Pt atom is symmetrically located relative to the geometry of the five-membered ring of guanine, the separation between Pt and O6 is too short to permit a bridging ligand unless the square planar Pt complex is rotated out of the plane of the guanine. This same geometry was suggested by Goodgame *et al.* (1975) as the initial mode of binding of cisplatin to double-stranded DNA.

indicating a substitution-dependent effect on apparent bond length.

This observation and the atom shift evidence in Figure 3 both indicate that when cisplatin binds, the guanine ring moves out of the base pair stack of the double helix into the major groove. Since the apparent shift in the composition image is 0.5 Å for 61% occupancy, one might expect a full-occupancy real shift of nearly an Ångstrom. This could be enough to destabilize the helix, disrupt the crystal, and perhaps even to weaken and break the glycosyl bond that attaches the guanine ring to the deoxyribose. Hence the present structure analysis, even though it is concerned directly only with the initial steps of cisplatin-DNA interaction, also provides support for the idea that depurination and mutagenesis are involved in the overall process.

The radically different degrees of substitution at the eight guanine sites along the double helix at first are surprising, but they have a simple explanation in terms of local guanine environment. The occupancy trends in Table I can be factored into two components: (i) increased reactivity of guanines the farther they are from the ends of the helix, and (ii) greater reactivity in the bottom half of the molecule than the top. The occupancy ratio between equivalent sites four base pairs in from each end, G16/G4, is 2.0–2.2 in all three data sets of Table I, and that between the sites four and three base pairs in

from the same end, G16/G10, is 2.8–2.9. An occupancy ratio of 2.3 corresponds at 298 K to a free energy difference of 0.5 kcal/mol. Why should symmetrically equivalent positions on the two helix strands exhibit a difference in binding energy of a little less than 0.5 kcal, and why should the binding energy at the fourth base pair from the end differ from that at the third base pair by a slightly greater amount?

The increased reactivity of guanines the farther they are from the ends of the helix, probably is explained by intermolecular interactions in the crystal. As can be seen from Figure 3 of Wing *et al.* (1980), the crystals contain columns of helices with overlapping ends. The minor grooves of two successive helices are interlocked, with the first two base pairs of one helix hydrogen-bonded to the first two base pairs of the next helix via N3...H-N2 and N2-H...N3 bonds between guanines. Hence the first two base pairs in from each end of the helix are immobilized on the minor groove side, and cannot shift toward the major groove to accommodate cisplatin binding. The third base pair is somewhat less constrained, and the fourth pair is freest of all. The reactivity of a particular guanine appears to be related directly to its freedom from constraints, and its ability to move toward the potential cisplatin site.

Why should the extent of cisplatin binding, at the same distance in from the end of the helix, differ between the two ends? The molecules after crystallization have an overall 19° bend in helix axis, and the sharpest bending occurs at the upper end of the molecule in Figure 2, where a spermine molecule bridges the major groove in the parent DNA structure. The molecule appears to close down its major groove slightly around the spermine. In the cisplatin complex the spermine molecule is displaced by the diffused-in cisplatin, but the asymmetric bending at the two ends of the molecule remains. In effect, the top four base pairs are inclined to the left in Figure 2 by rolling the fourth base pair against its neighbors along its long axis (Dickerson *et al.*, 1983). This constricts the major groove, and probably makes it difficult for guanines G22 and G4 to slip toward the major groove, hence decreasing their affinity for cisplatin.

In summary, the initial steps of binding of cisplatin to this B-DNA double helix involve ligation to the N7 position of those guanines that are most free to move slightly into the major groove. A second, stabilizing interaction appears to form between the platinum and the adjacent O6 atom on the same guanine, most probably involving a water molecule as a bridge. This may be the basis for the difference in reactivity of the *cis* and *trans* isomers. The destruction of crystal order that always accompanies high substitution makes it clear that this is not the entire story; the binding that we see here is only the preface to a more serious rearrangement. This may involve distortion of the helix during formation of Pt cross-links between pairs of guanines. The shift of guanine positions upon interaction with Pt suggests that it may also involve straining the glycosy bond and depurination, whch could lead to mutagenesis during the repair process. What is needed at present is a structure analysis of a cisplatin complex containing the G-G sequence, in which the complex is formed in solution, purified, and crystallized. Efforts in this direction are continuing.

Materials and methods

Native C-G-C-G-A-A-T-T-C-G-C-G crystals grown by vapor diffusion as described previously (Wing *et al.*, 1980; Drew *et al.*, 1981) were equilibrated with solid cisplatin or with 25–55% saturated solutions of cisplatin in 40:60 water/MPD (2-methyl-2,4-pentanediol) at 4°C for periods of a few days to several weeks. The derivatized crystals then were back-soaked in fresh platinum-free MPD/water solution for 4–24 h to remove any non-covalently bound or interstitial platinum. Attempts to obtain high occupancy of platinum binding sites, e.g., a Pt to DNA site ratio close to 1:1, invariably led to destruction of the X-ray pattern from the outside inward, and to increased sensitivity of the crystals to X-ray damage. Although backsoaking was found to aid the situation, all cisplatin-substituted crystals remained very sensitive to X-ray irradiation; the useful data collection lifetime fell from ~200 h for the native dodecamer to 25 h for the highest level of Pt substitution.

Three data sets were collected at different levels of substitution, as listed in Table I. Pt1, the least substituted but most ideally isomorphous set, was used for phase analysis of the parent or native structure. The higher substituted of the remaining sets, Pt3, was selected for complete Jack-Levitt restrained energy refinement as described by Fratini *et al.* (1982), leading to a final residual error or crystallographic R factor of 16.6% for all data or 11.2% for those reflections above the two-sigma confidence level. 128 solvent peaks were added gradually during refinement, using the strategy described by Drew and Dickerson (1981) and by Kopka *et al.* (1983).

Initial ligand positions around the Pt sites were obtained from difference maps in which the DNA, ordered solvent, and Pt atoms were subtracted out. These ligands, roughly in a square planar array around each Pt site, then were refined in the Jack-Levitt procedure without restraints, i.e., independently of any specified connection to the Pt atoms. The fact that the Pt-ligand positions remained around 2.0 Å (Table II) is evidence that the sites are real and that the refinement is meaningful. At the very end of 61 cycles of Jack-Levitt refinement, one more test of ligand geometry was made: the DNA atoms were held fixed, ligand atoms were eliminated, and Pt atoms were subjected to full-matrix, anisotropic least squares refinement. New ligand positions then were obtained from difference maps, and refined isotropically along with the anisotropic Pt. This refinement was well-behaved for the G16 cisplatin complex with 61% occupancy, but ligand positions wandered away from the Pt positions for the two less-substituted sites. The final coordinate set, which has been deposited with the Brookhaven Protein Data Bank for general distribution, is a composite set: anistropic data for the three Pt atoms along with their isotropic equivalents, full-matrix refined positions for the ligands of site G16, and best Jack-Levitt refined positions for the ligands of G4 and G10, and for the DNA. The original X-ray diffraction data also have been deposited.

The Pt2 data set with intermediate substitution was refined only to the point where no further motion of atoms in the DNA was occurring, and it was judged that no further comparative structure information would result by continuing. The main value of this was its Pt-N apparent bond distance.

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Note added in proof

Since submission of this manuscript, Rubin, Sabat and Sundaralingam (*Nucleic Acids Res.*, **11**, 6571-6586, 1983) have published an account of the very same binding of cisplatin to guanines 15 and 18 of $tRNA^{Phe}$: direct platinum ligation to N7, and ligand bridging to O6.