cis-trans-cis-Ammine(cyclohexylamine)diacacetatodichloroplatinum(IV) is an oral analog of the platinum anti-cancer drug cisplatin that is currently in phase III clinical trials. Its active form, \(\text{Pt(amine)(cyclohexylamine)}^{2+}\), binds to DNA similarly to cisplatin, forming intra- and interstrand cross-links between adjacent purine bases. Since \(\text{Pt(amine)(cyclohexylamine)}^{2+}\) contains two different ligands, it can form two isomeric 1,2-d(GpG) intrastrand cross-links. Here we report the 2.4 Å resolution x-ray crystal structure of the major adduct between \(\text{Pt(amine)(cyclohexylamine)}^{2+}\) and a DNA dodecamer, using the same sequence as previously reported for crystal structures of cisplatin-DNA (Takahara, P. M., Rosenzweig, A. C., Frederick, C. A., and Lippard, S. J. (1995) Nature 377, 649–652) and oxaliplatin-DNA (Spingler, B., Whittington, D. A., and Lippard, S. J. (2001) Inorg. Chem. 40, 5596–5602). Both duplexes in the asymmetric unit contain 1,2-intrastrand cross-links in which the cyclohexylamine ligand is directed toward the 3'-end of the platinated strand. The chair conformation of the cyclohexyl group is clearly resolved. Platination distorts the duplex, resulting in a global bend angle of about 38° and a dihedral angle between platinated guanine bases of ~31°. Both end-to-end and end-to-groove packing interactions occur in the crystal lattice, the latter positioned in the minor groove across from the site of the platinum cross-link. A high degree of homology observed between this structure and the previously reported platinum-DNA structures suggests that these platinum complexes distort the DNA duplex in a very similar manner. These results suggest that differences in activity between these drugs are unlikely to result from gross conformational distortions in DNA structure following platinum intrastrand cross-link formation.

cis-Diaminedichloroplatinum(II), cisplatin, is a paradigm for the treatment of testicular and other germ-cell cancers (1). The diminished activity of cisplatin against several other cancers, the acquired immunity developed by many tumors, and unpleasant side effects caused by the drug have led to a search for improved platinum chemotherapeutics (2). Since the 1970's, thousands of new platinum anti-cancer drug candidates have been developed and screened (3, 4). Fewer than 30 compounds have undergone clinical trials, however, and none has surpassed the efficacy of cisplatin for treating testicular cancer. In fact, only one drug, cis-diammine-1,1-cyclobutenedicarboxylatoplatinum(II) (carboplatin), has been approved for use in the United States. Cisplatin and several of its cytotoxic analogs are depicted in Fig. 1.

Platinum anti-cancer drugs bind to DNA, forming a variety of intrastrand and interstrand adducts, the most abundant of which are 1,2-intrastrand cross-links between the N7 atoms of two adjacent guanine bases (5). X-ray crystallographic studies of cisplatin bound to a dodecamer DNA duplex indicate that the \(\text{Pt(NH}_3\text{)}_{2}\text{(GpG)}^{2+}\) adduct induces the duplex to bend toward the major groove, resulting in significant widening of the minor groove (6, 7). An NMR solution structure shows appreciably more bending and a larger dihedral angle between the cross-linked guanine bases than observed in the crystal structure (8). In the complex formed between domain A of high-mobility group box protein 1 (HMGB1a) and a cisplatin-DNA d(GpG) cross-link, a phenylalanine residue of the protein intercalates into the widened minor groove at the site of platination (9). The binding of HMGB-domain proteins to cisplatin adducts potentiates the activity of the drug by shielding the DNA from nucleotide excision repair and possibly by diverting the protein from its native function (10–12).

cis-trans-cis-Ammine(cyclohexylamine)diacacetatodichloroplatinum(IV) is a platinum(IV) compound related to cisplatin. It can be administered orally and has been in phase III clinical trials (13, 14). The mechanism of action of this compound is similar to that of other mononuclear platinum anti-cancer drugs following conversion to cis-ammine(cyclohexylamine)dichloroplatinum(II) by intracellular reducing agents, such as glutathione. The activated species, \(\text{Pt(NH}_3\text{)}_{2}\text{(NH}_2\text{Cy)}^{2+}\) \((\text{NH}_2\text{Cy} \text{is cyclohexylamine)}\), forms two 1,2-d(GpG) adducts with DNA, termed 3' and 5' orientational isomers (15), according to the positioning of the substituted amine with respect to the direction of the platinated strand. These isomers occur in an ~2:1 ratio, the more abundant isomer having the cyclohexylamine ligand directed toward the 3'-end of the platinated strand (15).

The differences in activity among platinum drugs may involve variations in their DNA binding properties. Very few studies have been performed to explore this possibility. Recent
work has demonstrated that both the nature of the ligands on the platinum atom and the bases immediately flanking the d(GpG) adduct can affect the binding of HMGB1 to platinated DNA (16). These studies indicated that HMGB1a differentially recognizes the adducts of platinum complexes containing different carrier ligands and that HMGB1a binds to (Pt(NH₃)₂(NH₂Cy))²⁺ adducts in TG*G*A and AG*G*C sequences about half as well as cisplatin adducts. It has also been demonstrated that (Pt(NH₃)₂(NH₂Cy))²⁺ adducts are more effective than those of oxaliplatin and cisplatin in blocking translesion synthesis past the site of damage (17). This property may be one reason for its success in early clinical trials.

Structural studies have provided important information about the interactions between platinum compounds and DNA. The crystal structures of cisplatin (6, 7) and oxaliplatin (18) bound to homologous DNA sequences reveal several interesting differences and provide some insight into the effects of carrier ligands on the structure of platinum-DNA adducts. The most significant feature of the oxaliplatin-DNA structure that differentiates it from that of the cisplatin-DNA adduct is the presence of a hydrogen bond between the O6 atom of G7 and an amino group of the coordinated 1,2-diaminocyclohexane (dach) ligand.

In the present article we report the first x-ray crystal structure of an asymmetric platinum complex, (Pt(NH₃)₂(NH₂Cy))²⁺, bound to a DNA duplex. For comparison purposes we have employed the same DNA sequence as used for the previous two structures (Fig. 2). The major orientational isomer of this platinum complex was investigated. This work significantly extends the structural information available on platinum-DNA adducts and offers insights into the differences and common features between a variety of platinated DNA structures.

EXPERIMENTAL PROCEDURES

Materials—The compound cis-[Pt(NH₃)₂(NH₂Cy)Cl₂] was prepared by standard methods (19, 20). Crystallization reagents were obtained from Aldrich, Fluka, and Sigma. Phosphoramidites and reagents for DNA synthesis were purchased from Glen Research.

High-performance liquid chromatography (HPLC) was carried out on a Waters 600E system controller using a Waters 486 detector (λ, 260 nm) for analytical runs. Aldrich, Fluka, and Sigma. Phosphoramidites and reagents for DNA synthesis were purchased from Glen Research.

Deoxyoligonucleotide Synthesis and Purification—Top and bottom strands of the 12-bp deoxyoligonucleotide (top strand sequence 5'-d(CCTCTGGTGTCCTC)) were prepared (10-μmol scale, 2 syntheses for top strand) by using standard phosphoramidite methods on an Applied Biosystems 392 RNA/DNA synthesizer. After automated synthesis, the oligonucleotides were deprotected with ammonium hydroxide by incubating the crude reaction mixtures at 65 °C for 2 h.

The bottom strand was purified by ion-exchange HPLC (Dionex NucleoPac PA-100, 9 mm × 250 mm, 10% acetonitrile, 25 mM ammonium acetate, linear gradient from 0.2 to 0.4 M NaCl over 30 min). The product was desalted by dialysis against water using Slide-A-Lyzer cassettes (Pierce). The resulting product was analytically pure as judged by both ion exchange and C18 reverse-phase HPLC.

The top strand was platinated with (Pt(NH₃)₆(NH₂Cy)Cl₄)²⁺. Activated (Pt(NH₃)₆(NH₂Cy)Cl₄)²⁺ was prepared by mixing 2 equivalents of silver nitrate with 1 equivalent of cis-[Pt(NH₃)₂(NH₂Cy)Cl₂] in 1.0 ml of water for 6 h. The mixture was protected from light and centrifuged to remove precipitated silver chloride. The top strand was platinated with 2.2 equivalents of the activated platinum complex in a solution containing 10 mM sodium phosphate (pH 6.8) at 37 °C for 22 h. The product was purified by ion-exchange HPLC (Dionex NucleoPac PA-100, 9 mm × 250 mm, 10% acetonitrile, 25 mM ammonium acetate, linear gradient from 0.2 to 0.4 M NaCl over 30 min). The product was desalted by dialysis against water using Slide-A-Lyzer cassettes (Pierce). The resulting product was analytically pure as judged by both ion exchange and C18 reverse-phase HPLC.
Structure of a cis-(Pt(NH₃)(C₆H₁₁NH₂))₂⁺-modified DNA Dodecamer

RESULTS

Unit Cell Composition and Crystal Packing—The unit cell is isomorphous with that of the previously reported cisplatin-DNA structure (6, 7) and similarly contains two DNA duplexes in the asymmetric unit. These duplexes are labeled molecule A (Fig. 3) and molecule B. Two types of hydrophobic packing interactions occur between duplexes in the crystal lattice, end-to-end and end-to-minor groove. These packing interactions are mediated by hydrogen bonds between individual duplexes. The cisplatin-DNA and oxaliplatin-DNA structures exhibit similar packing interactions.

In the hydrophobic end-to-minor groove packing, depicted in Fig. 4, the end of one molecule packs against the minor groove of the other molecule. The packing interactions occur between duplexes in the crystal lattice, end-to-end and end-to-minor groove. These packing interactions are mediated by hydrogen bonds between individual duplexes. The cisplatin-DNA and oxaliplatin-DNA structures exhibit similar packing interactions.

In the hydrophobic end-to-minor groove packing, depicted in Fig. 4, the end of one molecule packs against the minor groove of the other molecule. The packing interactions occur between duplexes in the crystal lattice, end-to-end and end-to-minor groove. These packing interactions are mediated by hydrogen bonds between individual duplexes. The cisplatin-DNA and oxaliplatin-DNA structures exhibit similar packing interactions.
The dihedral angle between the G6 and G7 nucleobases is 32° between the platinated G-C base pairs remain intact (Fig. 6). Despite the large positive roll caused by the platinum bind-

| Base parameters for molecules A and B | A | B | A | B | A | B | A | B |
|-------------------------------------|---|---|---|---|---|---|---|---|
| C1 - G24                            | 16.6 | 11.9 | 0.01 | -1.26 | -6.88 | -3.58 | 0.65 | 0.70 | -0.24 | -0.18 | -0.20 | 0.02 |
| C2 - G23                            | 4.38 | 9.17 | 1.03 | 4.78 | -2.90 | -2.73 | 0.57 | 0.50 | -0.12 | -0.12 | -0.21 | 0.25 |
| T3 - A221                           | 3.63 | 4.86 | 14.4 | 2.89 | 12.5 | -5.52 | -0.04 | 0.22 | 0.06 | -0.22 | 0.17 | -0.20 |
| C4 - G21                            | -3.73 | -3.10 | 2.68 | 2.58 | -1.04 | -5.05 | 0.39 | 0.07 | 0.06 | 0.35 | 0.34 | 0.14 |
| T5 - A20                            | 2.83 | 4.28 | -1.50 | -0.96 | -5.20 | -5.20 | -0.09 | -0.14 | -0.29 | -0.19 | 0.26 | 0.17 |
| G6 - C19                            | 19.5 | 21.7 | -0.99 | 4.03 | -15.0 | -16.4 | -0.34 | -0.26 | 0.02 | 0.12 | 0.18 | 0.51 |
| G7 - C18                            | -6.91 | -4.49 | 3.51 | 0.47 | -13.2 | -16.8 | -0.18 | -0.14 | 0.06 | 0.03 | 0.03 | 0.27 |
| T6 - A17                            | -0.66 | -11.8 | 4.44 | 2.02 | -21.8 | -21.8 | 0.21 | -0.06 | 0.05 | -0.13 | 0.66 | -0.25 |
| C9 - G16                            | -2.69 | -10.9 | 1.01 | 1.47 | -13.4 | -6.41 | 0.25 | -0.14 | -0.35 | -0.31 | 0.11 | 0.00 |
| T10 - A15                           | 2.77 | -7.26 | -5.15 | 0.13 | -8.94 | -2.73 | -0.69 | -0.66 | -0.28 | -0.07 | 0.13 | 0.47 |
| C11 - G14                           | 0.42 | 0.36 | -6.23 | -4.54 | -11.4 | -11.6 | -0.05 | -0.03 | -0.17 | -0.01 | 0.03 | 0.27 |
| C12 - G13                           | -6.66 | -10.1 | 2.77 | 7.51 | -3.74 | -0.63 | 0.64 | 1.08 | -0.10 | -0.30 | 0.13 | 0.34 |
| A-DNA                               | 0.00 | 0.00 | -0.85 | -0.85 | 11.4 | 11.4 | 0.00 | 0.00 | -0.11 | -0.11 | 0.15 | 0.15 |
| B-DNA                               | 0.00 | 0.00 | -0.38 | -0.38 | -1.29 | -1.29 | 0.00 | 0.00 | 0.01 | 0.01 | -0.02 | -0.02 |

* Base parameters are defined as follows: κ, buckle; σ, opening; ω, propeller twist; Sx, shear; Sy, stretch; Sz, stagger.

The Platinated DNA Duplexes—The crystal structure reveals the asymmetric attachment of the cis-[Pt(NH₃)₂(H₂NCy)]²⁺ fragment to the d(GpG) unit in both molecules A and B. Both maintain full Watson-Crick base pairing despite the distortion of the base steps. Their global bend angles and base step parameters differ slightly as a result of minor differences in local base pair and base step parameters (Tables II and III). Both helices start with A-DNA conformations at the 5’ end of the platinated strand and gradually become more B-DNA-like, as determined by the slide and sugar puckering parameters (Table IV and Fig. 5).

Despite the large positive roll caused by the platinum binding to the N7 atoms of G6 and G7, even the hydrogen bonds between the platinated G-C base pairs remain intact (Fig. 6). The minor groove is widened, and the major groove is narrowed significantly. The platinum-nitrogen distances are 2.0 ± 0.1 Å. The dihedral angle between the G6 and G7 nucleobases is 32° in molecule A and 29° in molecule B. These values are slightly greater than those from the previously reported platinated-DNA structures. In molecule A, the platinum atom is displaced 1.2 Å from the plane of the 5' guanine and 0.61 Å from the plane of the 3' guanine. The corresponding displacements in molecule B are 1.1 and 0.93 Å, respectively. The platinum atoms are 0.05 and 0.11 Å from the least-squares plane containing the four coordinated nitrogen atoms in molecules A and B, respectively.

The cyclohexylamine ligand is well resolved at the 1σ level in an omit map, shown in Fig. 7, and is directed toward the 3’-end of the platinated strand. It adopts a chair conformation and points away from the major groove. The amino group of the NH₂Cy ligand forms a hydrogen bond with the O6 atom on G6. The N–O distances is 2.6 Å for molecule A and 2.9 Å for molecule B. The ammine ligand forms a weak hydrogen bonding interaction with the O2P atom on G7, at a distance of 3.1 Å for molecule A and 3.4 Å for molecule B. The ammine ligand is about 4.7 Å from the O-6 atom of G7 in both duplexes, too far to form a hydrogen bond.

**DISCUSSION**

The five platinated duplexes contained in the three platinum-DNA structures determined in our laboratory possess considerable homology. The root-mean-square deviations (RMSD) between comparable atoms in the various duplexes and platinum binding sites are listed in Table V. All RMSD values are less than 1.0 Å, indicating a great deal of similarity in both the bent duplexes and in the sites of platination. A comparison of selected distances and angles (Table VI) similarly reveals only small variations.

The crystal packing in the platinum-DNA duplex structures has an effect on the observed symmetry as originally indicated by the homology between the cisplatin and oxaliplatin structures (7, 18). The present results support this idea. The common end-to-minor groove packing significantly affects the ge-
structures (8). This difference suggests that the solution form of platinated-DNA differs from that in the crystal lattice.

The present structure supports our previous assignment of the major product of DNA platination by \( \text{Pt(NH}_3\text{)}_2\text{C}_6\text{H}_11\text{NH}_2\text{)}^2+ \) as the isomer with the cyclohexylamine oriented toward the 3'-end of the platinated strand (15). The hydrogen bonds observed between the platinum ligands and the DNA strand may contribute to this preference. One hydrogen bond is formed between the NH\_3 and the 5' O2P atom, and another occurs between the NH\_3 group of the cyclohexylamine ligand and O6 of the 3' guanine base. In the cisplatin-DNA structure, the distance between the 3'-directed ammine ligand and O6 of the 3' guanine is 3.5 Å. This distance is too long for significant hydrogen bonding, suggesting that such a bond may not form in the minor orientational isomer of the \( \text{Pt(NH}_3\text{)}_2\text{C}_6\text{H}_11\text{NH}_2\text{)}_2\text{(GpG)}^{2+}\) cross-link. Moreover, it is unlikely that the cyclohexylamine amino group would form a hydrogen bond with the 5' guanine O6 atom in the minor isomer, since the NH\_3-O6 distance in the major isomer is 4.7 Å.

Recent work by the Marzilli group (34, 35) suggested that hydrogen bonding between platinated guanine bases and their associated phosphate groups with the ammine or amine ligands in a \( \text{PtL}_2\text{)}^2+ \) complex (\( \text{L}_2 \) is two amines or a diamine) is extremely weak. In the present 3' orientational isomer, the hydrogen bond with O-6 of the 3' guanine is quite substantial and, in our previous \( \text{Pt(dach)}\text{)}^2+ \) complex, there was a similarly strong hydrogen bond on the 3'-end involving the amino group of dach and the 3'-phosphate. Crystal packing (see above) may contribute to these effects, or perhaps the conclusion about weak hydrogen bonds in these platinated duplexes is premature. The very small differences observed in the G6-G7 base pair geometry for the cisplatin-, oxaliplatin-, and \( \text{Pt(NH}_3\text{)}_2\text{C}_6\text{H}_11\text{NH}_2\text{)}_2\text{(GpG)}^{2+}\)-DNA structures may signal a common geometric feature that modulates their recognition by minor groove binding proteins. These results also support the hypothesis that the flanking sequence dependence of proteins binding to platinum-DNA adducts is most likely an inherent property of sequence-dependent protein-DNA contacts, not differential distortion around the platination site (16, 36).

Most proteins that bind platinum-DNA adducts, such as HMGB1 and TBP, interact with the minor groove. The crystallographic studies from our laboratory and computational studies by Marzilli and co-workers (34, 35) have demonstrated that platinum-DNA adducts are nearly structurally homologous, even in the minor groove where most protein-DNA interactions occur. Yet these proteins are able to recognize differentially adducts that contain differing amine ligands (16), and platinum complexes with different spectator ligands inhibit transcription to varying degrees (37). This result suggests that hydrogen bonding, hydrophobic, and other major groove interactions between protein, DNA, and the ligands on the platinum may affect drug activity. The bulky dach ligand in oxaliplatin

![Fig. 5. Roll, slide, and zp parameters across all base steps in three reported platinum-DNA structures. Values typical for A-DNA and B-DNA (generated with 3-DNA) are also shown. The increasing slide is consistent with the switch from A-DNA to B-DNA along the duplex.](image)

**Table IV**

| Step | Sugar pucker (Mol. A) | DNA type | Sugar pucker (Mol. B) | DNA type |
|------|----------------------|----------|----------------------|----------|
| C1   | C2-‘exo              | A        | C3-‘exo              | A        |
| C2   | C3-‘endo             | A        | C3-‘endo             | A        |
| T3   | C3-‘endo             | A        | C3-‘endo             | A        |
| C4   | C3-‘endo             | A        | C3-‘endo             | A        |
| T5   | C3-‘endo             | A        | C3-‘endo             | A        |
| G6   | C3-‘endo             | A        | C3-‘endo             | A        |
| G7   | C3-‘endo             | A        | C3-‘endo             | A        |
| T8   | C3-‘endo             | A        | C3-‘endo             | A        |
| C9   | C4-‘exo              | A/B      | C4-‘exo              | A/B      |
| T10  | C1-‘exo              | A/B      | C1-‘exo              | A/B      |
| C11  | C1-‘exo              | B        | C1-‘exo              | A/B      |
| C12  | O1-‘endo             | B        | C4-‘exo              | A/B      |

![Fig. 6. Close-up view of the platinum binding site in molecule A. Significant hydrogen bonding occurs between the NH\_3 and the 5' phosphate groups, and between the NH\_3 and the 5' guanine-O6 units.](image)
and the cyclohexylamine ligand in $\text{cis-}\{\text{Pt(NH}_3\text{)(N}_2\text{Cy)}\}_2\text{H}_2$ ad-
ducts fill and increase the hydrophobicity of the major groove in the vicinity of the adduct. These major groove effects may fine tune platinum drug activity by interfering with transcription.

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