HMG-Domain Protein Recognition of Cisplatin 1,2-Intrastrand d(GpG) Cross-Links in Purine-Rich Sequence Contexts†

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ABSTRACT: HMG-domain proteins bind strongly to bent DNA structures, including cruciform and cisplatin-modified duplexes. Such protein-platinated DNA complexes, formed where the DNA is modified by the active cis but not the inactive trans isomer of diamminedichloroplatinum(II), are implicated in the cytotoxic mechanism of the drug. A series of oligonucleotide duplexes with deoxyguanosine nucleosides flanking a *cis*-[Pt(NH_3)$_2${d(GpG)-N7(1),-N7(2)}] cross-link have been synthesized. These probes were used to determine the flanking sequence dependence of the affinity of the individual HMG domains of HMG1 toward cisplatin-modified DNA. Nine related sequences, where N_1 and N_2 are not dG and G*G* is the 1,2-intrastrand cisplatin adduct in N_1G*G*N_2, were previously investigated [Dunham, S. U., and Lippard, S. J. (1997) *Biochemistry 36*, 11428−11436]. Three of the seven remaining possible sequences for which N_1 and/or N_2 was dG were prepared here by using normal deoxyguanosine, but the rest, where N_1 is dG and N_2 is dA, dC, T, or dG, could not be isolated in pure form. These sequences were accessed by using the synthetic bases 7-deazaadenine and 7-deazaguanine, which lack the nucleophilic N7 atom in the purine ring. Deaza nucleotides accurately mimic the properties of the natural bases, allowing the interaction of the HMG-domain proteins with cisplatin-modified DNA to be examined. These experiments reveal that the flexibility of A·T versus G·C flanking base pairs, rather than base-specific contacts, determines HMG1domA protein selectivity. This conclusion was supported by use of mutant HMG1domA and HMG1domB proteins, which exhibit identical flanking sequence selectivity. The methods and results obtained here not only improve our understanding of how proteins might mediate cisplatin genotoxicity but also should apply more generally in the investigation of how other proteins interact with damaged DNA.

The cytotoxicity of the anticancer drug cisplatin[1] [*cis*-DDP, *cis*-diamminedichloroplatinum(II)] can be potentiated by the binding of nuclear proteins to cisplatin−DNA 1,2-intrastrand cross-links (*1*). Such interactions inhibit replication (*2*, *3*), shield the adducts from nucleotide excision repair (*4*), and interfere with transcription by recruiting essential transcription factors from their native binding sites (*5*, *6*). Many proteins that bind to the bent structure of cisplatin-damaged DNA (*7*) contain a high-mobility group (HMG) domain as the DNA recognition motif (*8*). Recently, the structure of HMG1domA bound to a 16 bp oligonucleotide duplex containing a *cis*-diammineplatinum(II) 1,2-intrastrand cross-link was determined by X-ray crystallography (*9*). The choice of oligonucleotide sequence used in this experiment was based on previously derived data which indicated that the affinity of HMG1domA varied by several orders of magnitude depending on the base pairs immediately flanking the platinum adduct (*10*). Of the 16 possible N_1G*G*N_2 oligonucleotide sequences, where G*G* indicates the platinum-bound deoxyguanosines, only the nine where N_1 and N_2 are dA, dC, or T were examined in this study. Sequences containing flanking deoxyguanosines, where N_1 and N_2 are dG, were not investigated because of synthetic difficulties encountered when trying to prepare site-specifically modified oligonucleotides. For example, platination of an oligonucleotide with an embedded $(dG)_4$ sequence resulted in a mixture of products G*G*GG, GG*G*G, etc., that were difficult to separate and purify in sufficient quantities. This limitation prevented a conclusive determination of factors that influence protein binding affinity.

7-Deazaadenine and 7-deazaguanine are isosteric analogues of adenine and guanine, respectively, in which the aromatic N7 atom is replaced with a C-H unit (Figure 1). These modified bases have been used as probes to modulate the chemical reactivity of DNA (*11*). The purine N7 is the site where cisplatin preferentially reacts in DNA, and it was therefore expected that 7-deazapurine nucleosides would be

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[1] Abbreviations: EMSA, electrophoretic mobility shift assay; HMG, high-mobility group; HMG1domA, high-mobility group protein 1 domain A; HMG1domB, high-mobility group protein 1 domain B; PAGE, polyacrylamide gel electrophoresis; cisplatin, *cis*-diamminedichloroplatinum(II); ts, top strand; bs, bottom strand; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane; HPLC, high-performance liquid chromatography; BSA, bovine serum albumin; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; ESI-MS, electrospray ionization mass spectrometry; dA, 2′-deoxyadenosine; dC, 2′-deoxycytidine; dG, 2′-deoxyguanosine; T, thymidine.

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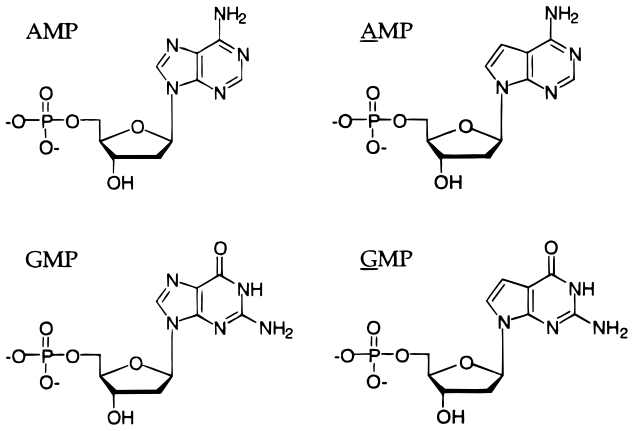

FIGURE 1: Structural diagram of deoxyadenosine monophosphate (AMP), deoxyguanosine monophosphate (GMP), 7-deaza-dA monophosphate (AMP), and 7-deaza-dG monophosphate (GMP).

incapable of forming platinum adducts. These commercially available materials provide a route to the preparation of cisplatin 1,2-intrastrand cross-links in a purine-rich sequence context, namely, N_1G*G*N_2, where N_1 and N_2 are A and G (where A is 7-deaza-dA, and G is 7-deaza-dG).

Here we report the preparation of seven oligonucleotide duplex probes of variable sequence context that contain at least three sequential guanine or deazaguanine bases. All of the probes were obtained by using 7-deaza-dG, and three were isolated by using native dG. Table 1 lists the sequences and their codes. Comparative binding studies were performed to evaluate whether the 7-deazapurines affect the affinity of HMG1domA and HMG1domB proteins for platinated DNA. Additional experiments with mutant HMG-domain proteins were carried out to determine the relative importance of DNA flexibility versus base-specific contacts in modulating protein−DNA selectivity. These studies provide insight into protein−DNA interactions implicated in the genotoxic mechanism of cisplatin. Moreover, investigating cisplatin adducts in dG-rich sequence contexts is an important step in elucidating the significance of such adducts in specialized regions of the genome such as gene promoters (*12*) and telomeres (*13*).

EXPERIMENTAL PROCEDURES

Proteins. HMG1domA and HMG1domB were prepared by E. R. Jamieson as previously described (*14*). The expression and purification of HMG1domAS41A, HMG1domAF37W, and HMG1domBI37F are reported elsewhere (*15*).

Oligonucleotide Probes. Oligonucleotide probes were synthesized (1.0 μmol scale) by using standard phosphoramidite methods on an Applied Biosystems 392 DNA/RNA synthesizer. All phosphoramidites were obtained from Glen Research. After automated synthesis, oligonucleotides were deprotected with ammonium hydroxide by incubating the crude reaction mixtures at 65 °C for 1 h. Purification was achieved on 12% denaturing polyacrylamide gels [7.5 M urea, 19:1 acrylamide:bisacrylamide ratio, 90 mM Tris-borate, and 1.0 mM EDTA (pH 8.3)] run at constant voltage (300 V) with cooling. Subsequent analysis by ion-exchange HPLC indicated single peaks for all oligonucleotides.

Activated cisplatin was prepared by mixing 2 equiv of silver nitrate with 1 equiv of cisplatin in water overnight. The mixture was protected from light and centrifuged to remove precipitated silver chloride. 7-Deaza-dA- and 7-deaza-dG-containing oligonucleotides with isolated GG sites were platinated with 2.0−2.2 equiv of activated cisplatin in a solution containing 10 mM sodium phosphate (pH 6.8). Reaction mixtures were incubated at 37 °C for 6 h, at which point ion-exchange HPLC analysis indicated the formation of one major product. Platination of tsAGGG, tsCGGG, and tsTGGG oligonucleotides with 1.1 equiv of activated cisplatin afforded two major products that were resolved by HPLC. All platinated oligonucleotides were purified by ion-exchange HPLC (Dionex NucleoPac PA-100, 9 mm × 250 mm, 10% acetonitrile, 25 mM ammonium acetate, linear gradient from 0.3 to 0.5 M NaCl over the course of 30 min). The purified material was desalted either by repeated concentration and dilution using a Centriprep 3 filter (3000 molecular weight cutoff, Millipore) or by dialysis against water using Slide-A-Lyzer cassettes (3500 molecular weight cutoff, Pierce). Platinum:oligonucleotide ratios were determined by a combination of UV−visible and atomic absorption spectroscopy. In addition, all platinated oligonucleotides were characterized by electrospray ionization mass spectrometry (ESI-MS, Supporting Information).

Table 1: Codes and Sequences for 15 bp Probes Containing 7-Deaza-dA, 7-Deaza-dG, and dG-Rich Sequences

Code[a]	Sequence[a]
CGGA	5'-CCTCTCCGGATCTTC-3'
	3'-GGAGAGGCCTAGAAG-5'
AGGC	5'-CCTCTCAGGCTCTTC-3'
	3'-GGAGAGGCCGAGAAG-5'
AGGA	5'-CCTCTCAGGATCTTC-3'
	3'-GGAGAGTCCTAGAAG-5'
GGGA	5'-CCTCTCGGGATCTTC-3'
	3'-GGAGAGCCCTAGAAG-5'
GGGC	5'-CCTCTCGGGCTCTTC-3'
	3'-GGAGAGCCCGAGAAG-5'
GGGT	5'-CCTCTCGGGTTCTTC-3'
	3'-GGAGAGCCCAAGAAG-5'
GGGG	5'-CCTCTCGGGGTCTTC-3'
	3'-GGAGAGCCCCAGAAG-5'
AGGG	5'-CCTCTCAGGGTCTTC-3'
	3'-GGAGAGTCCCAGAAG-5'
CGGG	5'-CCTCTCCGGGTCTTC-3'
	3'-GGAGAGGCCCAGAAG-5'
TGGG	5'-CCTCTCTGGGTCTTC-3'
	3'-GGAGAGACCCAGAAG-5'
AGGG	5'-CCTCTCAGGGTCTTC-3'
	3'-GGAGAGTCCCAGAAG-5'
CGGG	5'-CCTCTCCGGGTCTTC-3'
	3'-GGAGAGGCCCAGAAG-5'
TGGG	5'-CCTCTCTGGGTCTTC-3'
	3'-GGAGAGACCCAGAAG-5'

[a] A represents 7-deaza-dA, and G represents 7-deaza-dG.

Platinated oligonucleotides were combined with 3 equiv of the corresponding bottom strand and annealed by heating to 90 °C for 4 min followed by cooling to 4 °C over several hours in a solution containing 10 mM Tris, 10 mM $MgCl_2$, and 50 mM NaCl. The platinated, double-stranded probes were purified by ion-exchange HPLC using the same gradient as employed for the platinated, single-stranded oligonucleotides. HPLC purification was followed by dialysis against water using Slide-A-Lyzer cassettes (3500 molecular weight cutoff, Pierce).

Platinated oligonucleotides were radiolabeled in a solution (20 μL) containing 70 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 5 mM DTT, and 20 μCi of γ-^{32}P-labeled dATP (Dupont/NEN). The oligonucleotide solutions were mixed with 10 units of T4 polynucleotide kinase (New England Biolabs) and incubated at 37 °C. After 45 min, an additional 10 units of T4 polynucleotide kinase was added, and the mixtures were incubated at 37 °C for an additional 45 min. The reaction mixtures were spin dialyzed through G-25 Sephadex Quickspin columns (Boehringer-Mannheim). The effluents were diluted with water to a total volume of 100 μL and extracted with 2 × 100 μL of a 25:24:1 phenol/$CHCl_3$/isoamyl alcohol mixture. Finally, the DNA was isolated by ethanol precipitation.

Sequencing of dG-Rich Oligonucleotides. Maxam−Gilbert sequencing of the platinated dG-rich oligonucleotides was performed by using a modified version of the standard methodology (*16*, *17*). Briefly, base-specific reactions, the G reaction with dimethyl sulfate (DMS), the A+G reaction with formic acid, the C reaction with hydrazine-acetic acid, and the C+T reaction with hydrazine, were performed on 5′-^{32}P-labeled single-stranded oligonucleotides according to standard methods, followed by ethanol precipitation. For platinum-containing oligonucleotides, the platinum was removed by incubation in 0.2 M NaCN at 37 °C overnight followed by a second ethanol precipitation. The platinum-free oligonucleotides were treated with 1.0 M piperidine at 90 °C for 30 min. Piperidine was removed by repeated lyophilization−water addition cycles. The sequencing products were resolved by electrophoresis on 20% denaturing polyacrylamide gels prepared with 1× TBE. The gels were dried and exposed to a molecular imaging plate overnight. Images were visualized by using a Bio-Rad GS-525 molecular imager.

Electrophoretic Mobility Shift Assays (EMSAs). EMSAs were performed as previously described (*10*). Protein and probe DNA (10 000 cpm/μL, 3.5−7.5 nM) were incubated on ice for 1 h in a solution containing 10 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 50 mM LiCl, 100 mM NaCl, 1 mM spermidine, 0.2 mg/mL BSA, and 0.05% Nonidet P40 (15 μL reaction mixtures). The reaction mixtures were loaded onto 10% native polyacrylamide gels prepared with 0.5× TBE (prerun for >1 h at 300 V and 4 °C). Gels were run at 300 V and 4 °C for 1.5 h. The gels were dried under vacuum at 80 °C and exposed to a molecular imaging plate overnight. Images were recorded by using a Bio-Rad GS-525 molecular imager and quantified by using the Multi-analyst software package (Bio-Rad).

RESULTS

Synthesis of 7-Deazapurine-Containing Probes. 15-mer oligonucleotides containing either 7-deaza-dA or 7-deaza-dG were prepared under standard conditions by using an automated synthesizer. The products were purified by 12% denaturing PAGE to afford pure material as judged by ion-exchange HPLC. Reaction of these oligonucleotides with activated cisplatin gave only one major product. The platinated oligonucleotides were purified by ion-exchange HPLC and characterized by ESI-MS (Supporting Information). Use of the 7-deazapurines provided a facile route to all of the desired purine-rich, platinated oligonucleotides.

Synthesis of dG-Rich Probes. Seven 15-mer oligonucleotide analogues of all of the 7-deaza-dG probes were prepared on an automated synthesizer. Reaction of these oligonucleotides with activated cisplatin produced mixtures of products. In the case of the oligonucleotides tsAGGG, tsCGGG, and tsTGGG, two major products were resolved by ion-exchange HPLC. For tsAGGG and tsCGGG, the two products were obtained in an approximate 10:1 ratio, whereas for tsTGGG, the ratio was approximately 2:1. ESI-MS indicated that for tsTGGG both platination products had the same molecular weight, corresponding to the correct 15-mer oligonucleotide with one platinum atom and two NH_3 ligands (Supporting Information). Maxam−Gilbert sequencing was used to identify sites of platination, because platinum binding to the N7 position of adenine or guanine protects the bases from protonation or alkylation under the reaction conditions. Such protection results in missing bands in the sequencing gel that indicate the sites of platinum modification (*18*). Sequencing of the products isolated from platination reactions of the tsN_1GGG oligonucleotides indicated that the major products to be the desired were tsN_1G*G*G and the minor products to be the undesired were tsN_1GG*G*. Figure 2 shows a sequencing gel for the oligonucleotides tsAGGG and tsAG*G*G. The adduct is clearly identified as the desired isomer by the missing bands in gel (lanes 2 and 4). For the sequences $tsGGGN_2$, where N_2 is dC, T, or dG, the platination reactions gave mixtures of products that could not be separated. In the case of tsGGGA, one major product was isolated, which upon sequencing was identified as the undesired $tsG*G*GN_2$ product (data not shown). The desired $tsGG*G*N_2$ products may be present in the reaction mixtures, but they could not be isolated under the HPLC conditions that were employed. In summary, three of the seven possible sequences containing deoxyguanosines in the flanking positions could be prepared with the use of normal nucleotides.

Comparison of HMG-Domain Binding to 7-Deaza-Containing and Natural Oligonucleotide Duplexes. To determine whether 7-deaza-dA and 7-deaza-dG effectively substitute for the normal nucleosides, a series of bandshift experiments were performed. Oligonucleotides having the same flanking sequence containing either natural or 7-deazapurines were allowed to react with HMG-domain proteins at a given concentration. For flanking deoxyadenosines, the sequences AG*G*C, CG*G*A, and AG*G*A were compared with AG*G*C, CG*G*A, and AG*G*A. To compare deoxyguanosine-containing sequences, the probes AG*G*G, CG*G*G, and TG*G*G were evaluated in parallel with AG*G*G, CG*G*G, and TG*G*G. As shown in Figure 3, HMG-domain proteins cannot distinguish between probes containing 7-deaza (striped bars) and native (white bars) purines. In the case of HMG1domB binding to the N_1G*G*G probes, the 7-deaza-dG probes appeared to form slightly more

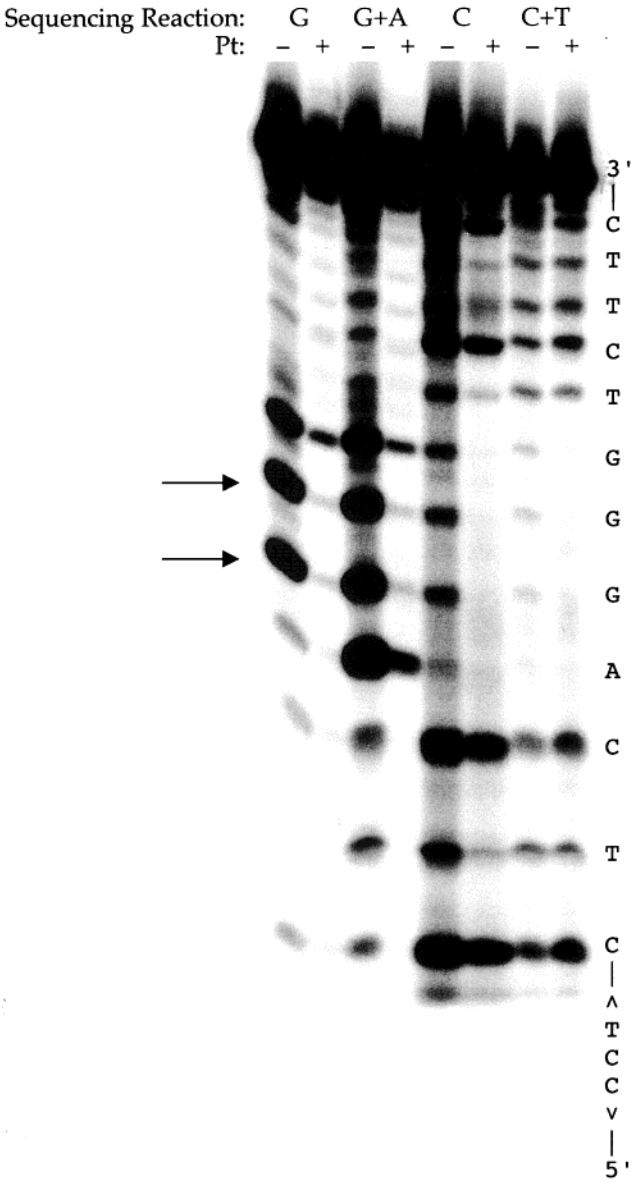

FIGURE 2: Sequencing gel analysis of the tsAGGG and tsAG*G*G oligonucleotides. The base assignments are given on the right-hand side of the gel. The arrows indicate the positions of the missing bands that verify the site of platinum modification.

complex than the natural probes, but the differences are within the error of the measurement (Figure 3). Overall, for both 7-deaza-dA- and 7-deaza-dG-containing oligonucleotides, the HMG-domain proteins shift equivalent amounts of the platinated probes and display the same sequence selectivity.

Sequence Context Dependence of HMG-Domain Protein Binding to Platinated DNA. To determine their flanking sequence selectivity, HMG-domain proteins HMG1domA and HMG1domB were allowed to react with all of the probes. Figure 4 allows direct visual inspection of a gel that clearly indicates sequence preferences. The graph shows the fraction of bound DNA with 7.6 nM HMG1domA. HMG1domA forms a more stable complex when the N_1 position is dA rather than dG, dC, or T. HMG1domA also prefers dA in the N_2 position, followed by T > dG > dC. HMG1domB shows smaller modulations in binding ability based on flanking sequences (Supporting Information). Nevertheless, HMG1domB also selects for dA in the N_1

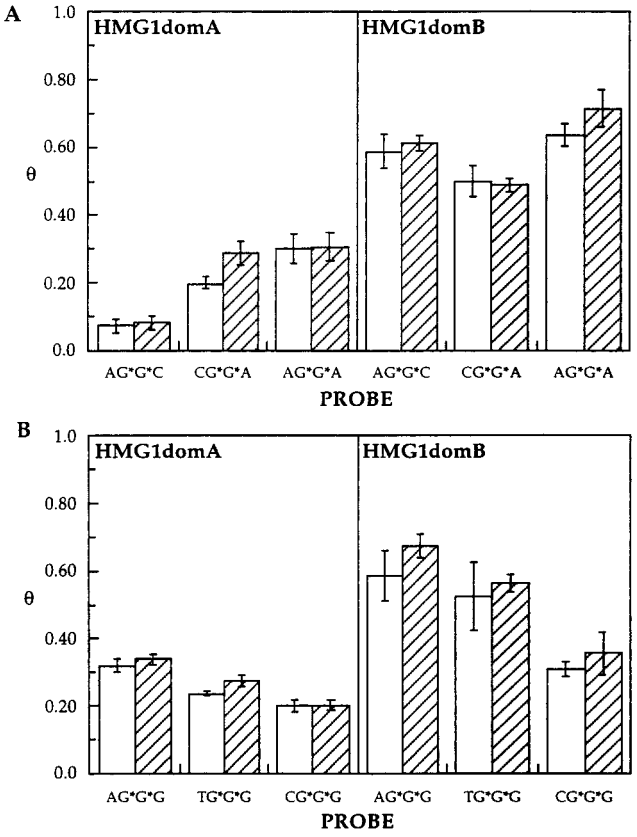

FIGURE 3: Bandshift experiments indicating that 7-deazapurines and natural purines cannot be distinguished by HMG-domain proteins. The graphs show the fraction of bound probe DNA over the total amount of probe DNA (θ). White bars represent probes containing natural bases, and striped bars represent probes containing 7-deazapurines. Panel A shows that normal and 7-deaza-dA probes are bound to the same extent by HMG-domain proteins. Panel B shows the same trend for normal and 7-deaza-dG. Error bars indicate one standard deviation derived from at least three independent experiments. [HMG1domA] = 7.6 nM; [HMG1domB] = 285 nM, and [probe] = 5.0 nM.

position and prefers N_2 of T ≈ dG > dA > dC.

Sequence Context Dependence of HMG-Domain Mutant Protein Binding to Platinated DNA. Mutant proteins HMG1domAS41A, HMG1domAF37W, and HMG1domBI37F were allowed to react with DNA probes having several different flanking sequences. HMG1domAS41A and HMG1domAF37W both bind to cisplatin-modified DNA with lower affinity than wild-type HMG1domA (*15*). HMG1domAS41A (white bars) and HMG1domAF37W (striped bars) both have the same sequence selectivity as wild-type HMG1domA (Figure 5). HMG1domBI37F has a higher affinity for cisplatin-modified DNA than wild-type HMG1domB (*15*). Bandshift results with probes of varying flanking sequence indicate that HMG1domBI37F also retains the same sequence selectivity as the wild-type protein (data not shown).

DISCUSSION

Successful Use of 7-Deazapurine Probes. Recently, the use of nucleoside isosteres has provided a wealth of biophysical and biochemical information about the stability of duplex DNA and its interactions with enzymes (*19*). The 7-deazapurines are considered reasonably good isosteres of the natural purines, although substitution of a C-H group for a nitrogen atom results in ~0.7 Å of additional steric

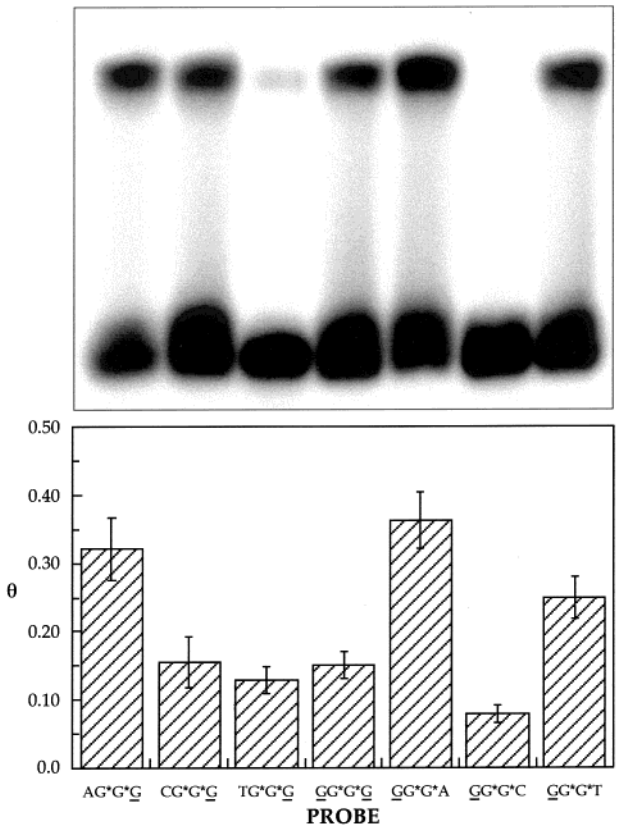

FIGURE 4: Bandshift experiments showing the flanking sequence preference for dG-rich oligonucleotides. Native 10% polyacrylamide gel of HMG1domA (top) with the 7-deaza-dG-containing probes (from left to right): AG*G*G, CG*G*G, TG*G*G, GG*G*G, GG*G*A, GG*G*C, and GG*G*T. The sequence dependence is clearly visible in the gels. The graph (bottom) shows the fraction of bound probe DNA (θ) and the sequence selectivity for HMG1domA. Error bars indicate one standard deviation derived from at least three independent experiments. [HMG1domA] = 7.6 nM, and [probe] = 5.0 nM.

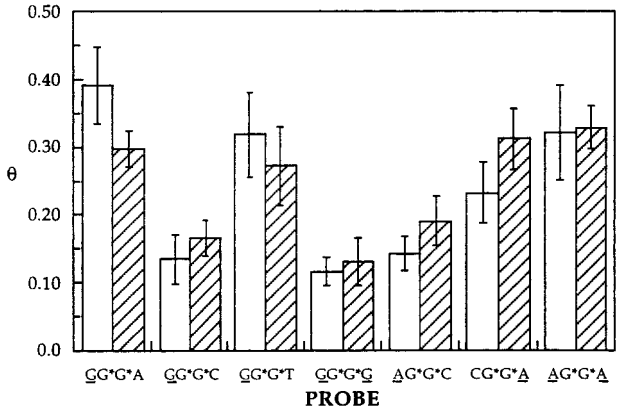

FIGURE 5: Bandshift experiments showing the flanking sequence preferences of mutant HMG-domain proteins. The graph shows the fraction of bound probe DNA (θ) and the sequence selectivity for HMG1domAS41A (white bars) and HMG1domAF37W (striped bars). Both mutant proteins display the same sequence selectivity as wild-type HMG1domA. Error bars indicate one standard deviation derived from at least three independent experiments. [HMG1domAS41A] = 148 nM; [HMG1domAF37W] = 203 nM, and [probe] = 5.0 nM.

bulk. Deaza bases have also found utility in modulating DNA structure, because of the removal of the hydrogen bond acceptor N7 (*11*).

In the present study, we have employed 7-deaza-dG to facilitate the synthesis of site-specific 1,2-intrastrand cisplatin adducts in dG-rich oligonucleotide sequences. Three of the oligonucleotides studied could also be prepared with normal dG, providing a basis for direct comparison. Of the seven possible combinations of nearest-neighbor flanking sequences, only N_1G*G*G probes could be prepared with the 1,2-intrastrand cross-link in the desired position. The facile synthesis of these probes is consistent with previous investigations showing that G_1 and G_2 in a $G_1G_2G_3$ sequence are more susceptible to chemical oxidation (*20*). Also, platination studies of oligonucleotides indicate that a monofunctional adduct preferentially forms at the G_2 position followed by the more favorable ring closure in the 5′ direction to afford the G_1*G_2* adduct (*21*−*23*). This previous work suggested that GG*G*N_2 oligonucleotides could not be readily prepared, as we have confirmed. Data indicate, however, that the reactivity of poly-dG sequences toward platinum reagents can be modulated by tertiary structure (*24*), flanking sequence (*20*, *25*), and the nature of the reactive platinum species (*24*). The reactivity of the deoxyguanosines in a $N_0G_1G_2G_3N_2$ sequence is modulated by the base at the N_0 position (*20*, *24*). If N_0 is dC, then G_1 is more reactive than G_2 or G_3, but if N_0 is T, then G_2 is more reactive than G_1 or G_3. In the oligonucleotides studied here, where G_1 represents the N_1 position in N_1GGN_2 (Table 1), N_0 is dC, which makes G_1 most reactive, consistent with tsG*G*GA being the major platination product of tsGGGA (vide supra). As suggested by these considerations, the N_0GG*G*N_2 cross-links are accessible in similar oligonucleotides where N_0 is T (*26*).

To confirm that 7-deaza-dA and 7-deaza-dG could substitute for the native base without affecting the affinity of an HMG-domain protein for platinated DNA, we performed bandshift experiments to evaluate sequences that differed only at the flanking natural or 7-deazapurine nucleotides. Quantitation of the amount of protein−DNA complex formed at a fixed protein concentration indicates that HMG1domA and HMG1domB bind equally well to both substrates, regardless of whether a natural or a 7-deaza base was present (Figure 3). This observation was confirmed for both 7-deaza-dA and 7-deaza-dG. This result also demonstrates that HMG-domain proteins display the same sequence selectivity in the presence of 7-deazapurines.

Sequence Selectivity of HMG-Domain Proteins for a Cisplatin 1,2-Intrastrand Cross-Link. Access to seven additional platinated probes allowed for completion of a prior investigation in which the protein affinity for platinated GG sites was significantly modulated by the flanking sequence (*10*). For HMG1domA, two trends are observed (Figure 4). The first and most dominant is selectivity for the base in the N_2 position with the following preferences: dA > T > dG > dC. The second trend, observed for weakly binding probes where N_2 is dG or dC, is that N_1 has a strong preference: dA > dC ≈ dG ≥ T (*10*). The presence of 7-deaza-dG produces a low-affinity target, and Figure 4 clearly supports the expected trend in N_1 with dA strongly preferred over T and dC. The results also show that dA is strongly preferred over dG in the N_1 position. The data reveal that HMG1domA has an overall preference for A·T base pairs over G·C base pairs in the positions flanking the 1,2-intrastrand adduct. This preference may be due to the increased flexibility of the double helix caused by A·T base pairs (*27*). The data suggest that HMG1domA has a pref-

erence for purine bases on the same strand as the cross-link.

HMG1domB demonstrates less pronounced preferences for flanking sequences than HMG1domA (Supporting Information). The data from all 16 possible flanking sequences indicate the following preferences for N_1 and N_2: dA > dG > T ≈ dC and T ≈ dG > dA ≥ dC, respectively. The trends for HMG1domB are not easy to decipher because of the low selectivity and the lack of structural data for this ternary complex. The strongest preferences exist when N_1 is dA and N_1 and N_2 are not dC. This result is somewhat indicative of stronger binding for more flexible sequences, but the interpretation is not as clear as for HMG1domA.

Sequence Selectivity of Mutant HMG-Domain Proteins. HMG1 mutant proteins provide a means by which to probe the importance of base-specific contacts in dictating sequence selectivity. The molecular structure of HMG1domA complexed with cisplatin-modified DNA reveals a single base-specific hydrogen bond contact between S41 and N3 of the neighboring adenine base located to the 3′ side of the G*G* adduct (*9*). Mutation of this residue to alanine removes the possibility of hydrogen bond formation and results in a decreased affinity for cisplatin-modified DNA (*15*). Surprisingly, elimination of this interaction did not alter the flanking sequence selectivity (Figure 5). Mutation of the intercalating residue from phenylalanine to tryptophan decreased the extent of binding, but caused no change in sequence preference. These findings support the hypothesis that sequence selectivity for HMG1domA is dominated by the presence of A·T base pairs that result in a more flexible DNA structure. The preference for flexibility is similar to that observed for the TATA-binding protein, which shows an increased affinity for consensus sequences having this property (*27*, *28*). A mutant of the HMG1domB protein, HMG1domBI37F, binds with higher affinity to cisplatin-modified DNA than the wild-type protein (*15*). This mutant also failed to show significantly different sequence selectivity (data not shown).

CONCLUDING REMARKS

The 7-deaza-dA and 7-deaza-dG bases substituted for natural bases in purine-rich oligonucleotides afford isosteres that allow for facile preparation of site-specifically platinated sequences that could not otherwise be obtained. The binding of HMG-domain proteins to deaza-containing oligonucleotides was identical to that for oligonucleotides containing normal dA or dG. This finding suggests that platinum adducts can be placed in every possible DNA sequence context while maintaining relevant biochemical properties. The interaction of mutant HMG-domain proteins with the platinum cross-link probes was investigated in several different flanking sequences. Base-specific contacts are not important for sequence specificity; instead, duplex flexibility appears to dictate the selectivity of the interaction. Access to all possible N_1G*G*N_2 sequences will be important for probing the effects of sequence context on other platinated DNA processing proteins, including transcription factors and repair enzymes.

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SUPPORTING INFORMATION AVAILABLE

Gels for CG*G*G, TG*G*G, and TGG*G* sequences, EMSAs for HMG1domB and HMG1domAS41A with oligonucleotide probes, and a table of mass spectrometric data for platinated oligonucleotides. This material is available free of charge via the Internet at http://pub.acs.org.

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