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Minor groove binding of a bis-quaternary ammonium compound: the crystal structure of SN 7167 bound to d(CGCGAATTCGCG)$_2$

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ABSTRACT

The X-ray crystal structure of the complex between the synthetic antitumour and antiviral DNA binding ligand SN 7167 and the DNA oligonucleotide d(CGCGAATTCGCG)$_2$ has been determined to an *R* factor of 18.3% at 2.6 Å resolution. The ligand is located within the minor groove and covers almost 6 bp with the 1-methylpyridinium ring extending as far as the C9-G16 base pair and the 1-methylquinolinium ring lying between the G4-C21 and A5-T20 base pairs. The ligand interacts only weakly with the DNA, as evidenced by long range contacts and shallow penetration into the groove. This structure is compared with that of the complex between the parent compound SN 6999 and the alkylated DNA sequence d(CGC[e^{6}G]AATTCGCG)$_2$. There are significant differences between the two structures in the extent of DNA bending, ligand conformation and groove binding.

INTRODUCTION

Compounds which bind reversibly in the minor groove of DNA possess a wide spectrum of biological behaviour, including antimicrobial, antiprotozoal and anticancer activities. It has been suggested that these effects are mediated by blocking transcription (1–4), by inhibition of polyamine biosynthesis (5) and/or by inhibition of topoisomerase I (6) or II (7,8). Minor groove binders also show excellent molecular recognition properties, binding with high specificity to AT-rich sequences up to 6 bp in length. For this reason, they are also of interest as carriers of intrinsically less specific functionalities, such as nitrogen mustard alkylating agents, and the resultant 'DNA-targeted' alkylators have been shown to possess significantly modified patterns of DNA adduct formation (9–12). The minor groove binding motif is also evident in several classes of highly cytotoxic natural products which are DNA sequence-specific alkylators, such as the anthramycins (13) and duocarmycins (14).

For these reasons there is wide interest in the molecular level interactions of reversible minor groove binders with DNA, to determine if there are common structural features for this binding mode. Particular attention has been paid to the polypyrrole and bisbenzimidazole series, with many NMR (15,16) and X-ray (17–19) studies of selected analogues with a range of oligodeoxynucleotides.

Another class of reversible minor groove binders which has received less attention in structural studies is that which can be loosely called the polybenzamides. An early example of this class, a terephthalanilide, was evaluated clinically as an anticancer drug (20). Later structure–activity studies on bis-quaternary salt analogues showed a positive correlation between experimental antileukaemic activity and selectivity of binding to AT-rich over GC-rich DNA sequences (21). The bis-quaternary cation 4-[4-[4-[4(*N*-methylquinolinium)amino]benzamido]anilino]-*N*-methylpyridinium (SN 6999, Fig. 1) and analogues have also been shown to be very potent inhibitors of multidrug-resistant strains of the malaria parasite *Plasmodium falciparum* (22). Recent work has shown that polybenzamides are suitable DNA targeting carriers for both DNA cleaving (23) and DNA alkylating (10,24) moieties.

Most structural work on polybenzamide DNA binding has been carried out on SN 6999 and an amino analogue SN 7167 (Fig. 1). NMR studies confirmed that SN 6999 binds preferentially to AT-rich oligonucleotides in the minor groove and showed the formation of specific hydrogen bonds (25–27). An X-ray crystallographic study (28) of SN 6999 complexed to the modified oligodeoxynucleotide d(CGC[e^{6}G]AATTCGCG)$_2$ showed the ligand bound in the minor groove, but offset from the centre of the 5′-AATT tract. The structure was unusual in that the DNA was very bent and in a manner not seen before, even in other groove binder–[e^{6}G] complexes. It appears that the semi-rigid nature of the SN 6999 molecule forced this extreme distortion of the DNA to occur, with the distortion being facilitated by the weaker hydrogen bonding in the [e^{6}G]·C base pair. The [e^{6}G] base contains an ethoxy substitution of O6. The hydrogen bond from O6 to cytosine N4 is eliminated by this substitution and the [e^{6}G] base therefore makes only two hydrogen bonds to cytosine, instead of the normal three.

This paper reports the crystal structure of the complex between the self-complementary dodecamer d(CGCGAATTCGCG)$_2$ and SN 7167. This work allows us to examine the binding of a member of the SN 6999 ligand series to unmodified DNA and also the effects on binding of the two additional exocyclic amines.

MATERIALS AND METHODS

Synthesis and crystallization

The DNA dodecamer d(CGCGAATTCGCG)$_2$ was purchased from Oligos Etc. Inc. and annealed before use. The drug SN 7167

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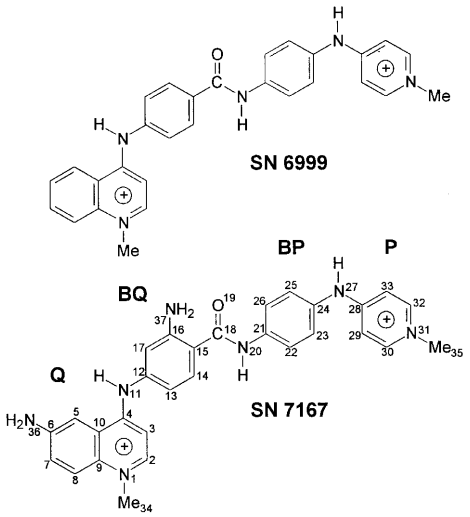

Figure 1. The cationic structures of SN 6999 and SN 7167.

was prepared as previously reported (29). Crystals of the complex (yellow prisms) were grown from hanging drops at room temperature. The crystal used for data collection was grown at room temperature from a drop containing 4.8 µl 50% 2-methyl-pentane-2,4-diol, 4 µl 30 mM sodium cacodylate, pH 6.5, 4.8 µl 5 mM SN 7167, 3 µl 200 mM $MgCl_2$, 2 µl 5 mM spermine hydrochloride and 4.8 µl 5 mM dodecamer. The droplet was equilibrated against a reservoir containing 1 ml 50% 2-methyl-2,4-pentanediol. An X-ray quality crystal was obtained after ~3 months from a gelatinous precipitate of the complex. The crystallization process was not highly reproducible because of formation of this precipitate.

Data collection

The single crystal used for data collection was of approximate dimensions 0.3 × 0.2 × 0.1 mm and was mounted in a 0.7 mm Lindemann quartz capillary with a small amount of mother liquor. Diffraction data were collected at 293 K using a Rigaku R-axis 2C diffractometer equipped with two image plate area detectors, a rotating anode X-ray generator and a graphite monochromator. Oscillation data was collected with a crystal to detector distance of 10 cm and an oscillation angle of 2°. Forty four frames were collected through a total angle of 88° of rotation to obtain data to a maximum resolution of 2.6 Å. The crystal showed no significant decay during data collection. Data processing was performed using DENZO (30). After merging, the data set comprised 1955 unique reflections (91.4% complete) with a merging *R* value of 9.5%.

Structure refinement

The unit cell dimensions of the crystal are $a = 24.56$, $b = 40.15$ and $c = 65.68$ Å, in the orthorhombic space group $P\,2_12_12_1$. This cell is close to that reported for related native dodecamers and groove binder complexes and the coordinates for the native structure (PDB entry 9BNA; 31) were used as the initial model for refinement. The refinement process was carried out using the program X-PLOR v.3.1 (32). Rigid body refinement of the whole duplex model was carried out with the resolution range of the data gradually increased from 8.0–4.0 Å (496 reflections) to 8.0–3.0 Å (1198 reflections). The DNA duplex was then divided into 24 rigid groups, comprising the 22 nucleotide and 2 nucleoside units, and the upper resolution limit gradually increased to the maximum of 2.6 Å (1888 reflections) during rigid body refinement. The *R* factor converged at 36.0%. Positional and temperature factor refinement further reduced the *R* factor to 29.3%. Electron density maps were calculated and displayed using the graphics package Alberta/Caltech TOM v.3.1. The DNA molecule showed a good fit to the electron density in all regions of the model and a continuous lobe of density located within the minor groove (Fig. 2) could be assigned to the SN 7167 molecule. Coordinates of the ligand SN 6999 were obtained from the published structure of the SN 6999–d(CGC[e^6G]AATTCGCG)$_2$ complex and were altered using the computer modelling package InsightII (33) to create a molecule of SN 7167. The electron density in the groove showed sufficient detail to allow the ligand to be placed in a unique direction and position in the groove. There was some uncertainty in the rotational conformation of the BQ-amido moiety and two possible ligand forms were refined further. The first rotamer has N37 pointing directly out of the groove (this can be called the 'amino out' conformation). The second rotamer has the same amino group facing the floor of the groove, the BQ-amido moiety having rotated 180° into the groove ('amino in'). Partial charges were calculated for the SN 7167 molecule by empirical calculations using the cvff (default) forcefield of InsightII. Planar restraints were applied to each individual aromatic ring system. Positional and temperature factor refinements were continued for both models with the SN 7167 molecules included in the calculations. Both 'amino in' and 'amino out' structures refined to the same *R* factor value. At this stage the ligand conformations and DNA binding were examined in more detail to try to elucidate the correct structure. The 'amino out' structure was chemically and stereochemically sensible and showed a good fit to the experimental electron density. The 'amino in' rotamer appeared to fit the electron density less well and the molecule looked strained into a distorted conformation. An unfavourable ligand conformation in itself is not enough evidence to say that this structure is not correct, as the nature of the DNA and the groove binding process invariably result in conformations that are different from the minimum energy conformations favoured in solution. However, the 'amino in' conformation was excluded from further refinement because of the presence of a repulsive contact of 2.6 Å between the ligand atom O19 and O2-T19. Modelling of this section of the structure using the Amber forcefield of InsightII indicated that this contact is strongly repulsive in nature and that these atoms would be forced apart to at least 3 Å. The resulting ligand conformation would not fit the experimental electron density well. Because of these considerations, the 'amino in' rotamer was discarded as a likely conformation and only the 'amino out' rotamer complex was refined further.

Water molecules were located in $2F_o - F_c$ electron density maps in likely hydrogen bonded positions and were refined by positional and temperature factor refinement protocols as above using the maximum resolution data. Criteria for the acceptance of water molecules were: proximity to the complex (within 5 Å); peak height >3σ in difference maps; potential hydrogen bonding partners (2.4–3.5 Å); thermal parameters not exceeding 70 Å^2. Water refinement was carried out using partitioned data sets; a working data set comprising 90% (randomly selected) of the full data set and a test set of the remaining 10% of the data for

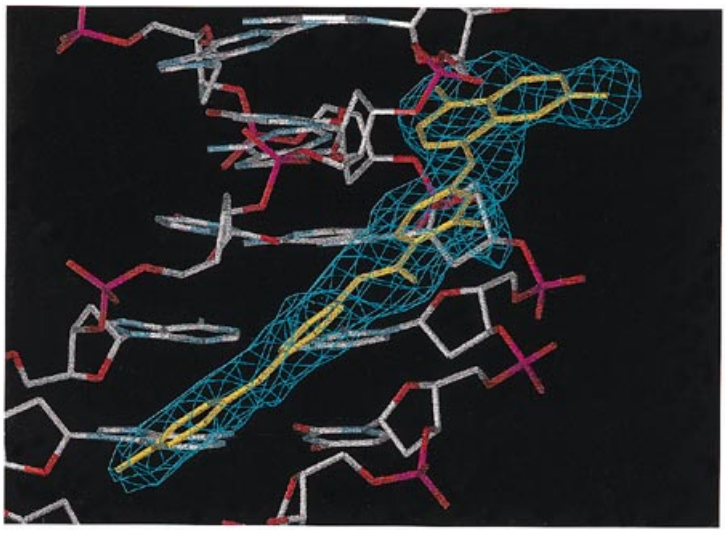

Figure 2. The SN 7167 molecule superimposed on the electron density calculated from a $F_o - F_c$ map, drawn at a contour level of 1σ using the program O (39). The SN 7167 molecule has been omitted from the map calculation.

calculation of a free *R* value (34). The free *R* value was used as an indicator to validate the water picking and refinement procedure and to guard against possible overfitting of the data.

After the final refinement cycles the *R* factor was 18.3% for all data in the range 8.0–2.6 Å [1888 reflections with $F > 2\sigma(F)$]. The free *R* factor converged at 23.8% for 10% of the data, with an *R* factor of 17.8% for the remaining 90% of the data. The final structure contains one dodecamer duplex, the SN 7167 ligand and 76 water molecules. Root mean square (r.m.s.) deviations from target values were 0.022 Å and 1.61° for bond lengths and angles respectively. Mean thermal parameters for the structure are 33.8 Å^2 for phosphates, 27.7 Å^2 for sugars, 23.5 Å^2 for bases, 38.7 Å^2 for the SN 7167 ligand and 40.0 Å^2 for the waters. Final refined coordinates for this SN 7167–d(CGCGAATTCGCG)$_2$ crystal structure, together with the observed structure factors, have been deposited in the Nucleic Acid Database with identity code GDL053.

RESULTS AND DISCUSSION

Structure of the complex

The DNA duplex adopts a B-DNA conformation, with the SN 7167 ligand lying in the narrow central region of the minor groove but offset towards one end (Fig. 3). The overall crystal packing is analogous to that observed for the native sequence and the various groove binder–dodecamer complexes. In all these structures the two terminal GC base pairs of each helix interact with a neighbouring duplex with the minor grooves slotting into each other and being involved in inter-duplex hydrogen bonding. The ligand binds in the minor groove over the 5′-AATTC site with the quinolinium ring system (Q) lying towards the 5′-end of the sequence. The quinolinium extends beyond the 5′-A site towards the adjacent guanine and the pyridinium (P) extends to the C9 base at the 3′-end, and so the SN 7167 molecule binds over a site ~5½ bp in length. There is no evidence of disorder or mobility along the sequence and the ligand adopts a unique position and orientation in the groove.

Ligand–DNA interactions

The nitrogen of the SN 7167 amido linker faces the floor of the minor groove and hydrogen bonds to O2-T19. The hydrogen bonding distance at 3.4 Å is probably too long to be considered a true hydrogen bond but it does indicate a weak interaction. The two amine linkers of the ligand have their hydrogens pointing directly out of the groove and do not hydrogen bond to the DNA. There are 15 contacts between the ligand and the minor groove which are shorter than 3.5 Å (Table 1) and all are long range at 3.1–3.4 Å. The ligand does not show a strong preference to interact with one of the DNA strands over the other. The shortest contacts involve the two rings on the ends of the molecule, although the pyridinium ring (P) has only one close contact with the DNA. There is also a possible charge-induced dipolar interaction between the quinolinium atom N1 and O4′-A6, but with a distance of 3.3 Å this interaction can also be considered weak.

The SN 7167 molecule is approximately planar overall with relatively small dihedral angles between ring planes (Table 2). The largest dihedral angle found, between the two central rings (BQ and BP) at 17°, is interesting in that co-planarity of the two ring systems linked by the *trans* amide would normally be favoured. The other two ring systems at the ends of the molecule have more free rotation about their amine linkers but require relatively small twists in order to follow the groove. Average temperature factors for the individual ring systems of the SN 7167 molecule show that the BQ ring is held somewhat more rigidly in place than the remainder of the molecule. The BQ ring has an average atomic temperature factor of 24 Å^2, while the remaining systems have values of 43, 41 and 51 Å^2 for the Q, BP and P rings respectively. This low value of 24 Å^2 could be indicative of a lack of rotational mobility for the BQ ring because of the exocyclic amino group substitution.

Table 1. Intermolecular contacts (Å) between SN 7167 and d(CGCGAATTCGCG)$_2$ (<3.5 Å)

Ligand atom	DNA atom	Distance
N1	O4′-A6	3.3
C2	N3-A5	3.4
C2	O4′-A6	3.2
C3	N3-A6	3.4
C3	O2-T20	3.1
C4	C4′-C21	3.4
C4	O4′-C21	3.4
C34	O4′-A6	3.4
C13	N3-A6	3.4
N20	O2-T19	3.4[a]
C22	C2-A18	3.4
C22	O2-T19	3.4
C23	O2-T8	3.2
N27	C4′-C9	3.4
C30	O4′-A18	3.1

[a]Hydrogen bond.

Table 2. Angles between normals to calculated ring planes (°) for SN 7167 and SN 6999 (from crystal structure results)

Ring systems	SN 7167	SN 6999
Q-BQ	13	50
BQ-BP	17	6
BP-P	8	59

Table 3. Local inter-base pair roll (°) from selected crystal structure results

Base pair	SN 7167	Native (31)	Hoechst 33258 (35)
G4/A5	1.5	2.1	1.2
A5/A6	**6.4**	1.3	5.7
A6/T7	–1.3	–2.3	2.3
T7/T8	–2.2	–3.4	2.0
T8/C9	–5.2	–0.7	4.7
C9/G10	**5.9**	5.6	5.3
G10/C11	–11	–14	–12

DNA structure

The overall DNA structure is similar to those in the native and other minor groove binder–dodecamer crystals. The dominant features of the helix that distinguish it from the native structure are the helical bending and groove widening, much of the bending a result of the groove widening to accommodate the SN 7167 molecule. The DNA bends away from the binding site of the ligand, compressing the major groove in a similar way to that observed for a number of groove binder–DNA structures. This bend of 8° is close to that found for Hoechst 33258 bound to d(CGCGAATTCGCG)$_2$ (35). Helical parameters were calculated using the program CURVES v.5.0 (36). Some values of inter-base pair roll are listed in Table 3. Two high values of roll

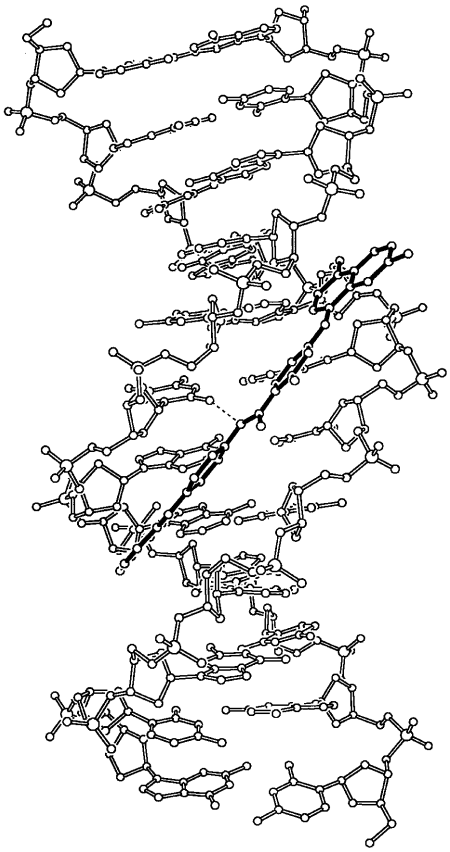

Figure 3. Molecular structure of the SN 7167–DNA complex. The SN 7167 molecule is drawn with shaded bonds and the hydrogen bond is represented by a dashed line.

at the A5/A6 and C9/G10 steps show the locations of the bending. The minor groove width (Fig. 4) shows a widening of the minor groove above that of the native DNA. Groove widening occurs over the binding site of the SN 7167 molecule and is most apparent over the T7–C9 bases in both the C1′ and P plots. The relatively rigid BQ-amido-BP moiety binds over this approximate site and this groove widening again illustrates the shallow binding and poor fit to the minor groove of the ligand over this region.

Minor groove water structure

There are a number of water molecules located in the minor groove. They are mostly found hydrogen bonded to the DNA and fulfilling a space filling role in the regions of the groove which are not occupied by the ligand. Water 28 (W28) effectively bridges the ligand atoms O19 (amido carbonyl) and N37 by hydrogen bonding. No other waters are found hydrogen bonded to O19, N37 or to W28, although there is residual electron density near this triplet of atoms which cannot cleanly be assigned as water molecules. The SN 7167 atom N36, the exocyclic amine of the quinolinium ring, does not hydrogen bond to any water, perhaps because of the proximity of O2P-G4 (4.3 Å) of a symmetry-related helix. The symmetry-related atom lies directly in front of N36 and there is not enough room for water molecules to bind near this site. This of course does not preclude water molecules

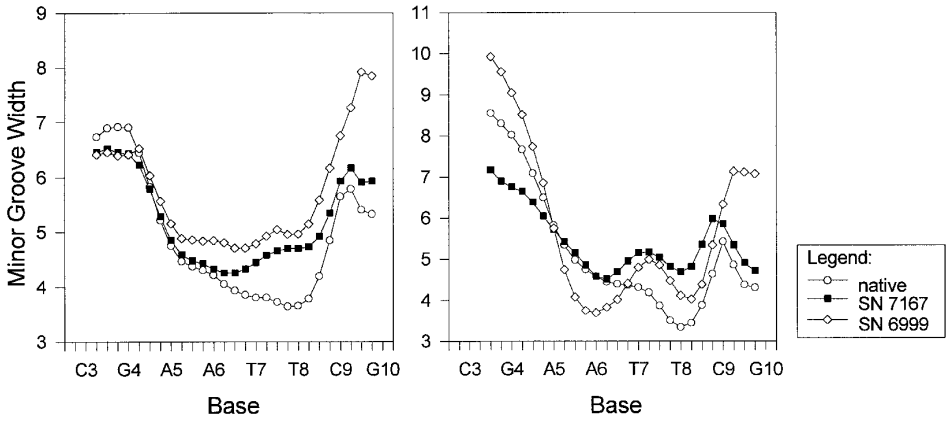

Figure 4. Plots of minor groove width based on C1′ and P atoms for the SN 7167, SN 6999/[e^{6}G] and native dodecamer structures. Groove width is measured in Å.

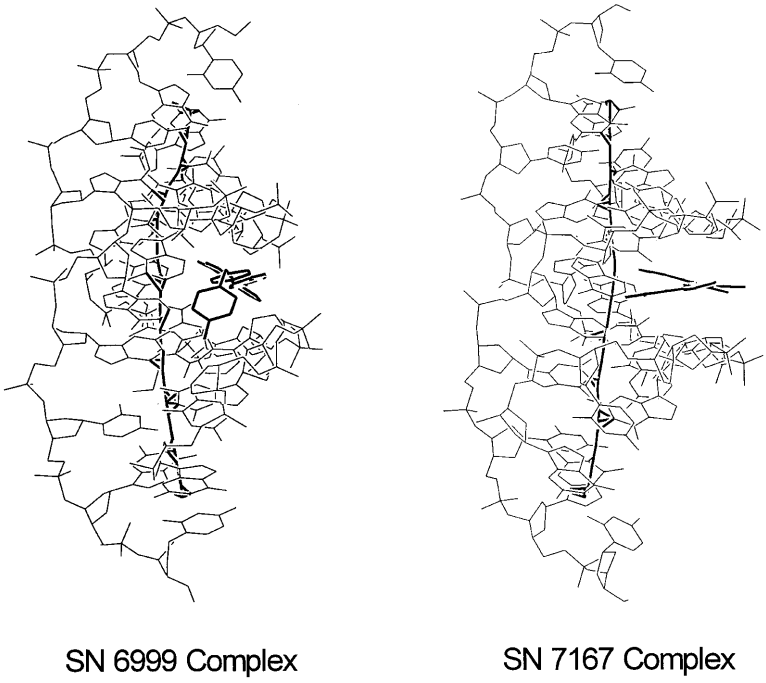

Figure 5. Views of SN 7167–DNA and SN 6999–DNA complexes down the minor groove. The ligands and the helical axes (as calculated by CURVES) are highlighted in bold and show clearly the differences in the DNA bending between the two complexes.

approaching from either side, but none are observed in the experimental data. Overall, the water structure of the groove does not appear to play an important role in stabilizing groove binding of the SN 7167 molecule other than filling the minor groove hydrogen bonding network where the ligand is not sited.

Structures of SN 6999 and SN 7167 complexes

The SN 6999–d(CGC[e^{6}G]AATTCGCG)$_2$ crystal structure is very different from the present SN 7167 structure, with a r.m.s. deviation in DNA coordinates of 1.76 Å. This SN 6999 complex is also significantly different from other [e^{6}G] minor groove complexes, with a r.m.s. deviation of 1.35 Å in coordinates between itself and a [e^{6}G] netropsin complex (37). When other [e^{6}G] complexes of netropsin, Hoechst 33258 and Hoechst 33342 (38) are compared amongst themselves, the r.m.s. deviations are in the range 0.77–0.89 Å. These complexes all have a 19° bend in the helical axis while the SN 6999 complex bends some 25° and in a different manner. These differences are due to the binding and nature of the SN 6999 molecule. The relatively straight and rigid SN 6999 ligand forces one end of the complex to tilt significantly and results in a new crystal lattice not previously seen. The helical distortion in this case is facilitated by the relatively weak [e^{6}G]·C base pairs on the duplex (Fig. 5). The d(CGCGAATTCGCG)$_2$ duplex of the present structure does not contain alkylated bases pairs and thus does not distort readily and the helix is found to be much less bent than in the SN 6999–[e^{6}G] complex (Fig. 5).

The SN 6999 molecule binds over the 5′-C[e^{6}G]AATT site and has the reverse orientation to the SN 7167 molecule in the groove.

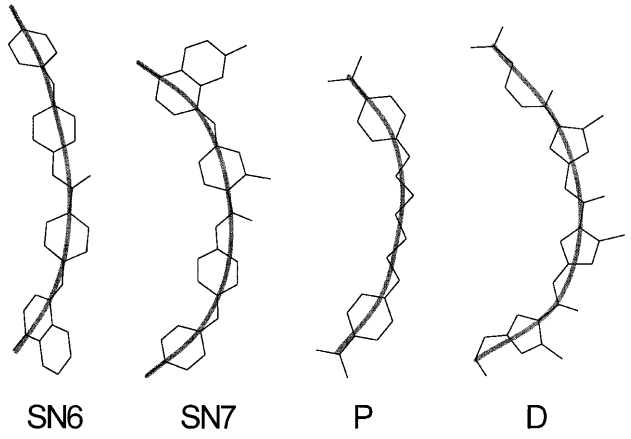

Figure 6. DNA-bound conformation (from crystal structures) of various minor groove binding ligands: SN 6999 (SN6), SN 7167 (SN7) pentamidine (P) (40) and distamycin (D) (41). The heavy lines emphasize the ligand curvatures.

Both molecules have the quinolinium moiety located near the centre of the helix with the pyridinium group towards one end. In solution both ends of the duplex are indistinguishable from each other and the two binding sites would be approximately equivalent. The binding interactions and ligand conformations that result for each of the two complexes are very different. While the SN 7167 ligand has very few close contacts with the floor of the groove, the SN 6999 molecule binds deeply into the groove and with 46 non-hydrogen close contacts in the range 2.5–3.5 Å. The central amido NH of SN 6999 is deep enough in the groove to form a reasonably strong hydrogen bond to O2-T20 (3.1 Å) and both quaternary nitrogen atoms are located close to sugar O4′ atoms, possibly involved in charge-induced dipole interactions (3.2 and 3.1 Å for the Q and P rings respectively). The two central aromatic rings also show stacking interactions with sugar O4′ atoms at distances of 2.8 Å between ring centroids and the sugar oxygen in both cases. The depth of binding is clearly seen in Figure 5. Here the ligand binds deeply and the groove walls wrap around the ligand. This results in groove widening for the inner groove walls (near the DNA sugars), but the groove narrows at the outer edge of the groove wall (near the phosphates). This trend can be seen clearly in the plots of minor groove width for the C1′–C1′ and P–P distances (Fig. 4). The SN 7167 complex on the other hand has a more regular groove width from the floor to the groove entrance, with no wrapping of the groove around the molecule.

Both SN 7167 and SN 6999 molecules have approximately co-planar central rings (BQ and BP) held rigidly by a *trans* amide linker. The SN 6999 BQ and BP rings have a significantly smaller twist between them than the analogous SN 7167 rings. The two outer SN 6999 ring systems Q and P are twisted sharply relative to the plane of the BQ-BP rings. The same rings of the SN 7167 molecule are very much more co-planar with the rest of the molecule (Fig. 5 and Table 2). Overall, the SN 6999 ligand shows significantly less curvature than the SN 7167 molecule, which has a similar curvature to other minor groove binding ligands (Fig. 6).

The SN 7167 and SN 6999 ligands bind preferentially in the narrow central AATT region of the minor groove of the DNA. In the case of the SN 6999–[e^{6}G] complex the constrained ligand conformation and low curvature of the ligand force the DNA duplex to bend. This deformation straightens the minor groove curvature to maximize isohelical binding of the ligand. This bending is facilitated by the inherent weakness of the [e^{6}G].C base pair. The d(CGCGAATTCGCG)$_2$ duplex, without the destabilizing effect of alkylated bases, is not readily deformable and so in the SN 7167 complex it is the ligand that is forced to adopt a more isohelical conformation. The minor groove in this case is more curved and thus is less able to accommodate the co-planar BQ and BP rings. As a result these two systems twist away from co-planarity into a less favourable electronic conformation. However, the induced twist at only 17° is not very large when compared with other groove binding molecules, such as Hoechst 33258 (35), and the ligand still produces a poor fit to the groove topology. The ligand curvature is enhanced by the more co-planar conformation that the Q and P ring systems adopt. This increased isohelical nature allows more favourable groove binding to occur in the complex. The low twist between BQ and BP, the amido linker geometry and the co-planarity of Q and P with the rest of the molecule all contribute to the shallow binding with relatively few interactions with the groove floor.

Conclusions

The present structure highlights the inherent variability of binding of minor groove binders to DNA. In one case (SN 6999) the DNA target molecule distorts considerably to accommodate its ligand; in the other (SN 7167) the target remains relatively rigid with almost all the conformational change being imposed on the ligand. However, detailed comparisons are difficult because of the differing nature of the DNA substrates. It is very important to consider the exact nature of the target molecule, in this case alkylated and non-alkylated DNA, when designing a ligand to recognize and bind to this target. It is difficult to say from these X-ray structures whether the SN 6999/7167-type ligands would bind preferentially to alkylated DNA over unaltered DNA. Certainly, the alkylated DNA in the case looked at allows deeper binding in the groove, although the addition of exocyclic amino groups on the SN 7167 molecule complicates this argument. The overall binding of SN 7167 and SN 6999 to DNA (as measured by other techniques; 42) is similar and differences in the biological activities of SN 6999 and SN 7167 could merely be due to the hydrophilic properties of the amino groups or other such factors. The ‘amino out’ conformation of the present crystal structure also allows for the possibility of the amines acting as protein recognition sites, as previously postulated (28). Even if the amine on BQ were hydrogen bonded to the minor groove floor, the amine on the Q ring system still remains as a possible recognition site. The symmetry-related O2P atom close to the amine of the quinolinium ring could be considered a very crude model for such an interaction, although its approach to the amine is only as close as 4.3Å. A complementary shaped protein segment would have no problem making a closer approach or interacting via water molecules. The SN 7167 ligand appears to be a poor groove binding molecule for native DNA, yet it is still a biologically active molecule. Further work will need to be done to answer the questions raised by the present X-ray structure and other polybenzamide crystal structures using native DNA would be useful in the investigation of the applicability of these systems for groove binding. Of particular interest would be the examination of a potential ‘amino in’ conformation, which could have important implications for the activity of the SN 7167 molecule

and in the design of other polybenzamide systems for DNA minor groove binding.

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