A Continuous Transition from A-DNA to B-DNA in the 1:1 Complex between Nogalamycin and the Hexamer dCCCGGG*

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The antibiotic nogalamycin, a drug with high specificity for TG and CG steps in double-stranded DNA, has been crystallized as a 1:1 complex with the hexamer d(CCCGGG). The antibiotic is inserted at the central CG step of the duplex, with the two sugars oriented in the same direction and with strong interactions with the DNA within the grooves. The amino-glucose residue makes an integral part of a well defined major groove hydration network with van der Waals contacts and several strong hydrogen bonds to the duplex. The nogalamycin resides in the minor groove, making primarily van der Waals contacts. The single site allows an accurate molecular description of the intercalation, without perturbations from end effects observed previously. The local unwinding induced by nogalamycin is completely relaxed 2 base pairs away from the intercalation site. The two strands of the DNA show a continuous deformation from the A to the B form: 1) the cytosines toward the 5′ end of the nogalamycin site in each strand have c3'-endo conformations while 5 guanosines toward the 3′ ends have c2'-endo conformations; 2) within each strand, the phosphate-phosphate distances increase in a continuous manner from 5.7 Å (A-form) to 7.1 Å (B-form).

The anthracycline antibiotic nogalamycin (Fig. 1) is biologically important for its anti-tumor activities and its ability to inhibit DNA-directed RNA synthesis in vivo (1). X-ray studies have shown how it binds to double-stranded DNA by intercalation at TpG, CpG, and Cpa steps (2, 3). The anthracycline ring is parallel to the base pairs with its long axis aligned perpendicular to the base pairs and with the hydrophobic nogalamycin and amino-glucose sugar moieties lying in the minor and major grooves, respectively. DNA footprinting studies have indicated preferential binding at d(CA)d(TG) steps (4). NMR studies using nuclear Overhauser effect data have confirmed that sequences containing more than one potential site can form two different intercalation complexes with roughly equal proportions (5). The x-ray structures of the DNA complexes with nogalamycin and with daunomycin and adriamycin, other anthracycline antibiotics with anti-tumor activities and similar sequence preferences, have utilized modifications to the hexameric sequence d(CGTACG). The initial structure of this sequence used in the high resolution x-ray structure of a complex with daunomycin (6, 7). Intercalated at steps 1 and 5. This structure and the related structures with d(CGATCG) or d(TGATCA) utilized the same 2:1 drug:DNA stoichiometry, crystallizing as an isostructural series in space group P43212 with 0.5 complex in the asymmetric unit. Later structures with nogalamycin in other space groups with either 0.5 or 1 complex per asymmetric unit have utilized *CGT_A*CG (6, 7) and TCGATC (8, 9), where *C indicates C or 5-MeC and where the phosphate group of the central step was replaced by a (R)-p-phosphorothioate to improve the crystallization characteristics.

The intercalation of nogalamycin into DNA presents a mechanistic problem, since the dumbbell shape of the intercalator with its bulky sugar residues presumably requires substantial deformation of the DNA to effect entry. These deformations may persist in the resultant complex structure where the highly hydrophobic nogalamycin sugar lying in the minor groove will displace water molecules from the DNA in order to utilize maximum Van der Waals interactions. In the structural studies where there are two drug molecules per hexamer at steps 1 and 5, the deformation required to effect the intercalation process may be reduced, because there is no longer a requirement to propagate deformation beyond the terminal steps of the DNA. Another consideration in all previous crystallographic work is that the orientation of the two drug molecules is 2-fold symmetric about the central step in the DNA with 4 base pairs separating the two intercalation steps.

We were interested in determining the structure of a nogalamycin:DNA complex containing a single drug molecule. Early work by Viswamitra and Salisbury1 established that crystalline complexes could be obtained with nogalamycin and the synthetic hexamer d(CCCGGG). However there were severe problems with the reproducibility of crystallization and the poor diffraction and thermal characteristics of the crystals, which optimally are unstable above 10 °C. However we were particularly interested in this sequence, which has the advantage of a single, centrally located, putative site and therefore the potential for observing a stepwise effect of intercalation on the DNA conformation.

MATERIALS AND METHODS

Preparation and Crystallization—The hexanucleotide was synthesized by automated methods with an Applied Biosystem 391 synthesizer and phosphoramidite monomers obtained from Millipore with 4 × 1 μmol G-loaded CpG columns. The product was hydrolyzed with 3 M aqueous ammonia, washed with ether, and precipitated with ethanol. After lyophilization, the unpurified product was used to determine the optimum stoichiometry for crystallization. Since nogalamycin is almost insoluble in water at pH 7, the complex was formed by dissolving the nogalamycin in chloroform and extracting with a 2 mM solution of

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1 Viswamitra and S. Salisbury, personal communication.
Drug/DNA Interaction: a 1:1 Complex with Nogalamycin

Daunomycin

Nogalamycin

**Chemical formulae of daunomycin and nogalamycin with the numbering adopted in this study.** A polar amino-sugar is linked at the right side of the aglycone (C7) in daunomycin and at the left side (C1) in nogalamycin. In both drugs, the opposite side of the aglycone bears a hydrophobic residue: a small methoxy group for daunomycin and a bulky nogalose residue for nogalamycin. In spite of these differences, the two drugs have their aglycone inserted the same way in DNA.

DNA as described above. The initial droplet composition was: 0.3 mM spermine. The central reservoir in each group contained 200 μl of 0.1 M aqueous MPD. The plate was sealed, placed in an oven at 38°C for 6 h, and then transferred to a double-sealed insulated box containing water in two compartments, one inside the other. The complete system was set inside a cold room at 3–4°C and the plate removed from the bath after 24 h. Crystals appeared within 2 weeks growing to an maximum size of 0.25 × 0.25 × 0.15 mm over a period of a month with both [001] and [011] forms present. These crystals were stable for a period of about 4 months at 4°C.

**Data Collections—Diffraction data were recorded at the LURE synchrotron facility, in Orsay, at a wavelength of 0.9 Å using a MAR Research Image plate system connected to the monochromated W32 beam line (10). Two crystals were randomly oriented in capillaries without alignment of a crystallographic axis with the spindle. The temperature was maintained throughout the recordings below 4°C to limit decomposition. The crystal-to-film distance was set according to the processing. Afterwards, the two different data sets were merged together with a Rmerge = 5.2% at the maximum resolution of 2.25 Å, and a

**TABLE I**

Crystallographic details

| 1 | 2 |
|---|---|
| Space group | P4<sub>3</sub>2<sub>1</sub>2<sup>+</sup> |
| a = b = c | 45.84 58.08 |
| Crystal-to-plane | 240 2490 |
| Distance (mm) | 240 2490 |
| Rotation per frame | 100° 110° |
| Total rotation | 90° 110° |
| Exposure time | 40 s/degree 90 s/degree |
| Number of measured intensities | 8728 10,025 |
| Number of unique intensities | 2443 2490 |
| Rsym (°) | 8.3 7.2 |
| Total of merged and scaled reflections | 2492 |
| Rmerge (%) | 5.2 |

*The enantiomorph group, P4<sub>3</sub>2<sub>1</sub>2<sup>+</sup>, was rejected after the translational step in the molecular replacement.

**TABLE II**

Refinement parameters (NUCLESQ) and root mean square deviations from ideality. (the model comprises 296 atoms and 47 water molecules)

| Type of constraints | Root mean square (number of constraints) |
|---|---|
| Bond distances (Å) | 0.011 (290) |
| Angle distances (Å) | 0.023 (433) |
| Phosphate distances (Å) | 0.011 (101) |
| Phosphate angles (Å) | 0.033 (224) |
| Planes (Å) | 0.036 (13) |
| Chiral volumes (Å<sup>3</sup>) | 0.17 (40) |
| Single contact torsions (Å) | 0.08 (7) |
| Multiple contact torsions (Å) | 0.23 (46) |
| Hydrogen bonds (Å) | 0.18 (36) |
| Average B factors (Å<sup>2</sup>): | B (number of atoms) |
| Biases: | 18.3 (114) |
| Sugars: | 26.3 (72) |
| Phosphates: | 32.2 (54) |
| Nogalamycin: | 21.3 (56) |
| Water molecules: | 41.9 (48) |
| R factor: | 0.183 |
| Weights in R factor | 27.0–110 × (5–1/6) |
| Root mean square shifts in last cycle: | |
| On co-ordinates (Å): | 0.01 (9) |
| On B factors (Å<sup>2</sup>): | 0.9 (2) |
| Number of F<sub>res</sub> used: | 2365 |
| Resolution range | 18–2.4 Å |

2 The abbreviations used are: MPD, methylpentanediol; HPLC, high performance liquid chromatography.
The final correlation coefficient was 59.6 and the 5\( R \) factor of 51%. To this fixed solution was added, in the translation search, the best rotation solution for the second, then the third spare part (models A and C). Only one solution with a correlation factor of 61 and an R factor of 47% emerged. The second peak was 45% in correlation. A graphic inspection showed that the three pieces were on top of each other, with correct orientation to build the missing pieces. The models were also built as alternative starting points.

The multi-body technique developed in the AMoRE program (14) was employed. Model B (nogalamycin plus the 2 flanking base pairs) was first oriented and translated according to the best solution (correlation factor \( C = 44 \) and R factor = 51%). To this fixed solution was added, in the translation search, the best rotation solution for the second, then the third spare part (models A and C). Only one solution with a correlation factor of 61 and an R factor of 47% emerged. The second peak was 45% in correlation. A graphic inspection showed that the three pieces were on top of each other, with correct orientation to build the missing phosphodiesters linkages between them. The model was then submitted to the fast rigid body refinement included in AMoRE, the FITING step (15). The final correlation coefficient was C = 64.9 and the R factor = 44.1% (resolution: 15–3 Å) in P4\(_2\)2\(_2\)2, with a good packing while the best solution in P4\(_2\)2\(_2\)2 gave C = 59.6 and R = 49.3 with important overlaps in the packing. Once the three parts were oriented and translated according to the best correlation factor, the model was further divided in seven parts (the six CG or GC base pairs plus the nogalamycin) and refined again with the FITING program. The whole model was reconstructed on a graphic display using 2\( F_o \) – \( F_c \) maps. The R factor was 33% at 3.1 Å resolution.

Refinements—The rigid body model was submitted to the annealing procedure described in XPLOR (16). The starting resolution of 3.1 Å was gradually increased to the maximum of 2.4 Å. A combination of crystallographic and molecular dynamic refinements were performed during which solvent atoms with good hydrogen bonding geometry were gradually added to the model following visual inspection of 2\( F_o \) – \( F_c \) and \( F_o \) – \( F_c \) maps on a graphic system. The maximum temperature of the molecular dynamics was gradually decreased from 900 to 300 K as the refinement stabilized following inclusion of well-defined solvent atoms. During this process, the conformation of the sugar residues changed toward their final values without manual intervention. Difference maps indicated that one of the methoxy groups of the nogalose residue had changed and needed manual repositioning. The coordination geometries around prominent solvent peaks were periodically examined to determine whether they would be better modeled as hydrated Na\(^+\) or Mg\(^{2+}\) ions. Solvent atoms were also discarded if during B factor evolution, individual values became greater than B = 60 Å\(^2\). The final stages of the refinement were performed with NUCLSQ (17) essentially to flatten the rings, but otherwise no constraints were placed on torsion angles or sugar conformations. The final geometric parameters and deviations from ideality are given in Table II.

RESULTS

Overall Molecular Structure

The refined structure is shown in Fig. 2 (stereo view) which gives a view of the unsolvated complex perpendicular to the long axis of the drug and the DNA helix axis. The residues are numbered from the 5' end C1 to G6 in chain 1 and C7 to G12 in chain 2. The DNA is oriented with the C1-G12 base pair at the top and the C7-G6 base pair at the bottom; the nogalamycin is intercalated between the C3:G10 and G4:C9 base pairs with the long axis of the chromophore perpendicular to the mean direction of the 2 flanking base pairs and with the nogalose and amino-glucose residues pointing toward the G6:C7 base pair at the bottom. The hydrophilic nogalose sugar residue is situated in the minor groove on the right-hand side of Fig. 2; it is associated with the duplex mainly by van der Waals interactions. The hydrophilic amino-glucose lies in the heavily solvated major groove on the left-hand side of the Fig. 2, it has several strong hydrogen bonds strengthening its association with the duplex. The overall shape of the DNA in the complex is intermediate between the A- and B-conformation. The sugar
the z axis and about 20 Å in length and 10 Å in width. This region of the structure is characterized by higher thermal parameters for both the nogalose (B = 28–31 Å²) and the duplex (B = 22–29 Å²) and is apparently void of well ordered solvent atoms.

The Nogalamycin in the Complex

In the Minor Groove (Fig. 4)—The nogalose residue sits in the minor groove of the duplex reaching almost 2 bases after the C-G site and oriented toward the same end of the duplex as does the amino-glucose. The minor groove is shallower and wider than the prototype B-DNA structure observed (18) for AT-rich structures and is closer toward a B-DNA structures containing CG-rich stretches, where there is enough width to allow a double chain or zig-zag pattern of water molecules (19–21). This characterization is appropriate in that the nogalose is in van der Waals contact with chain 1 (G4 to G6), but less so with chain 2 (Fig. 4). There is an apparently empty channel between the nogalose residue and the other bank of the minor groove. (residues C7, C8, and C9).

In the Major Groove—The amino-glucose residue resides in the major groove extending from the central C3-G4 site toward the 3' end of chain 1. The amino-glucose spans the major groove edge of the G:C pair G4:C9 but with only one direct hydrogen bond between the amino-glucose and the duplex. This is between O2G and N7 of G4 (2.84 Å). On the other side of the edge, the interaction between O4G and N4 of C9 is mediated by a bridging water molecule W2 (O4G-(2.79 Å)-W2-(2.89 Å)-C9N4). In this respect the interactions between the amino-glucose and the duplex are close to previous structures. There is an interaction between the amino-glucose and the duplex 2 base pairs after the intercalation site. This involves the dimethylamino group and the amino group C8N4 of the G5:C8 base pair and a bridging solvent molecule W28. This hydrogen bonding interaction is strong as indicated by the hydrogen bonding distances: (N3G-(2.60 Å)-W28-(2.81 Å)-C8N4).

The orientation of the dimethylamino group is probably influenced to some extent by solvation effects where the amino-glucose partakes in the intricate network of hydrogen bonds corresponding to the “crossover” contact. There is a direct hydrogen bond (3.08 Å) between O4G and C8N4. This is bridged by W28, which participates in the strong intermolecular interaction between O2G and N7 of G4 (2.84 Å). On the other side of the edge, the interaction between O2G and N7 of G4 (2.84 Å). On the other side of the edge, the interaction between O4G and N4 of C9 is mediated by a bridging water molecule W2 (O4G-(2.79 Å)-W2-(2.89 Å)-C9N4). In this respect the interactions between the amino-glucose and the duplex are close to previous structures. There is an interaction between the amino-glucose and the duplex 2 base pairs after the intercalation site. This involves the dimethylamino group and the amino group C8N4 of the G5:C8 base pair and a bridging solvent molecule W28. This hydrogen bonding interaction is strong as indicated by the hydrogen bonding distances: (N3G-(2.60 Å)-W28-(2.81 Å)-C8N4).

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The Axial Carbomethoxy Group—This group is situated within the minor groove of the duplex interacting one step...
before the C-G insertion site. There is a hydrogen bond between the carbonyl oxygen O14 of the aglycone and G10N2 (3.15 Å). There are two additional polar contacts through water molecules toward the furanose oxygen atoms of residues C1 and C2. The geometry of these interactions suggests that alteration to this carbomethoxy group (i.e., to a free carboxylate) could affect both the solubility and the binding affinity of the drug by strengthening the hydrogen bonding in this region.

**Distribution of Solvent**

The hydration on the major groove side of the duplex is ordered with readily visible water molecules and an abundance of significant electron density. This contains the more polar of the two sugar residues, the amino glucose. On the minor groove side there are very few ordered water molecules. The way in which the nogalose sits in the minor groove leaves a space which should be filled with solvent/MPD (Fig. 3); however, the electron density is too low to say what the fill is. One groove is more hydrated than the other, and low B values are observed.

About 47 water molecules were localized with temperature B factors ranging from 12 to 60 Å². They form in the major groove a complicated hydrogen bonded network with many tetra-coordinated molecules. The most important, already described above, are W28 which connect the O4G of the amino-glucose to O2P of C2*, the N4 of C8, and W20 (2.9 Å, this water molecule is one having the lowest B temperature factors, B = 12 Å²). W20 also connects O2P of C3 (2.65 Å, symmetry-related) and two other water molecules: W15 (2.80 Å) and W30 (2.79 Å). Finally, W30 is connected to W9 (3.03 Å), N4 of C7 (2.81 Å), and W34 (2.99 Å). The water molecules in this region represent a remarkable arrangement of five tetra-coordinated water molecules, that builds a strong zig-zag spine, not analogous to the one observed in the minor groove of the B form of DNA (19, 21), but equivalent in characteristics.

In the same region a hydration pentagon is observed in the major groove, probably related (22) to the A-like conformations of the cytosines, connecting the O1P atom of C2 and the nogalamycin O5G atom (Fig. 7). These water molecules have low B values, in the range 13-20 Å².

Some phosphate groups are bridged by water molecules like W11 or W10. These molecules are located on the first strand C1-G6. The second strand is less hydrated. The minor groove contains six water molecules (with <B> ≥ 45 Å²), while more than 25 water molecules are present in the major groove (Fig. 8).

**Helical Properties**

The helical parameters of the duplex and the torsion angles of the sugar-phosphate backbone were calculated with the program NEWHEL (23) and are given in Tables III and IV. In order to make enough room for the nogalamycin to enter between the central C3:G10 and G4:C9 base pairs (step 3), the helix has been unwound, allowing a gap. This separation is accompanied by some changes in the torsion angles along the sugar-phosphate backbone, including changeover in sugar conformation from c3endo to c2endo at the intercalation site for chain 1 and one step further along from the intercalation site the 3' end of chain 2.

In this structure, it is possible to see how the deformation is propagated in a diminished fashion two steps above and below the intercalation site. At the central step the helical twist angle is reduced from 36° (for B-DNA) to 20.0° corresponding to an unwinding of 16.0°. The rise parameter h, corresponding to the vertical displacement of the C1'-C1' vectors parallel to the helix axis, is 5.9 Å compared with a value of 3.4 Å for B-DNA. The cavity in which the nogalamycin aglycone sits is not flattened across top and bottom but is buckled upwards from the centre by −14° for the C3:G10 base pair and downwards by +11° for the G4:C9 base pair. The propeller twist angles for the C3:G10 and G4:C9 base pairs are reduced to values of −3° and +3°, respectively. The diamond-shaped cavity is similar to that observed in previous structures containing nogalamycin, where...
the changes associated with buckling and reduced propellor twist were explained in terms of optimization of the van der Waals contacts with the surface of the aglycone.

If we now consider the intermediate steps 2 and 4, the values for helical twist and rise are still smaller than standard values for B-DNA. The helical twist angles are 30.7° and 31.9° for steps 2 and 4, respectively, corresponding to small unwinding values of 5.3 and 4.1°. The sugar-phosphate backbone is still somewhat stretched with rise values of 4.4 and 3.9 Å.

The terminal steps 1 and 5 have values of 37.0 and 36.9° for helical twist and 3.4 and 3.5 Å for rise, which are more characteristic of B-DNA, and Table III shows how the propellor twist and buckle angles change from special values at the intercalation site to more standard for the terminal base pairs. The only base pair away from the cavity that shows an appreciable buckling angle is the intermediate base pair C2:G11, which has a value of 17.0°. This corresponds to a change of +21° from the C3:G10 base pair. It is probably correlated with the observation that the sugar-pucker changes from c3'endo for G10 to c2'-endo for G11.

The manner in which the individual base pairs stack on top of each other can be seen in Fig. 9. There is an overall similarity to the stacking observed in d(G4C4) (24) and other C- and G-rich structures (25) that crystallize in the A-conformation. There is partial overlap between guanine residues of successive base pairs but the overlap between cytosine bases is poor. The large values of −17.0° and −13.7° for the propellor twist angles of base pairs C1:G12 and G6:C7 are probably indicative of structural adjustments associated with optimal stacking of adjacent complexes along the x and y axes.

The adjustments along the sugar-phosphate backbone are both complex and regular with individual backbone torsion angles falling near to the average values for A-DNA or B-DNA depending on the sugar conformation of the particular residue. The sugar conformations belong to the c3'-endo family for each cytosine residue and to the c2'-endo family for each guanosine residue except for G10 which is c2'-endo. In terms of pseudo-rotation parameters (26, 27), depicted in Fig. 10, the cytosine

![Image](B)

![Image](A)

**FIG. 5.** The least squares superimposition of the nogalamycin in this work (bold tracing) with: (A) (lower) the free state, light tracing, coordinates from Ref. 13, and (B) (upper) in the 2:1 mCG-TAmCG complex, light tracing, coordinates from (6).

**TABLE III**

| Helical parameters | Vertical displacement Å |
|--------------------|------------------------|
| C(1) --------------- | G(12) 36.9° 3.4Å |
| C(2) --------------- | G(11) 30.7° 4.4Å |
| C(3) --------------- | G(10) 20.0° 5.9Å |
| C(9) --------------- | C(8) 31.9° 3.9Å |
| G(4) --------------- | C(7) 37.0° 3.5Å |
| G(5) --------------- | C(6) 20.0° 2.0Å |
| G(6) --------------- | C(5) 23.5° 3.2Å |

| "Propeller twist" | "Buckle" |
|-------------------|----------|
| C(1) -17° G(12) 2° |
| C(2) -9° G(11) 7° |
| C(3) -14° G(10) 1° |
| G(4) +11° C(9) 1° |
| G(5) +1° C(8) 1° |
| G(6) -3° C(7) 1° |

![Image](Fig. 6)

**FIG. 6.** Electron density maps of the central base pair G4:C9, flanked by the two protruding sugars of the drug (A) and the aglycone of nogalamycin (B). Resolution is 2.4 Å (2Fo − Fo maps, contouring at 2σ, where σ = mean root mean square value of the map).
residues and G10 are clustered in the region \(^3E\) (Northern) and the remaining guanosine residues are spread over region \(^2E\) to \(^3E\), situated on the other side (Southern) in the diagram. For chain 1 the conformation is A-like for the first three nucleotides changing at the intercalation step to a B-like conformation for the last three residues. For chain 2 the conformation is A-like for the first four residues, changing to B-like for the last two residues. The transition from A to B shows up in the \(P_i - P_{i+1}\) distances along each chain. One interesting feature of these values is that for the intercalation step, the distances, 6.36 Å from P3 to P4 in chain 1 and 6.14 Å from P9 to P10 in chain 2, are intermediate between the standard values of 5.9 Å for A-DNA and 7.0 Å for B-DNA (Table V).

The backbone torsion angles in Table IV are in general close to values associated with A-DNA and B-DNA. The only unusual changes from normal A-DNA and B-DNA values are \(\beta = 202^\circ\), \(\zeta = 212^\circ\), and \((\epsilon - \zeta) = -1^\circ\) for residue G4. Changing \(\beta\) from a “normal” value around 162–202° for residue G4 allows the bases C3 and G4 to open. On the other chain, where the sugar conformations of residues C9 and G10 are c3-endo, the value \(\beta = 174^\circ\) for G10 is normal, but a small change to higher values would have a substantial effect on the opening of bases C9 and G10. The other unusual value for \(\zeta\) and \(\epsilon - \zeta\) for residue G4 is associated with a swinging of the phosphate group of P4 toward the major groove. In this structure, the phosphate oxygen atom OP4 is now able to form a hydrogen-bonded interaction via the bridging water molecule W17 to O12 and O1 of the aglycone.

**DISCUSSION**

Intercalation is one of the most important ways the drugs interact with DNA. This normally leads to perturbation of the double helix, altering the cell metabolism and very often leading to the end of cell growth (28). A large number of such chemicals are known, some are synthetic (9-aminoacridine, acridine orange, ethidium bromide) some are naturally produced by plants (ellipticine) or fungi or bacteria (daunomycin, 

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**FIG. 7.** A pentagonal hydration pattern in the major groove (C1-C2-C3 region) which connects the OSG oxygen of nogalamycin to the phosphate backbone.

**FIG. 8.** The complete hydration network and interactions of the amino-glucose in the major groove (stereo view).
actinomycin, nogalamycin...). They all have strong biochemical activities and are evaluated in medicine as antibiotics, antimitotics, carcinogens... The mechanism of drug intercalation has been extensively investigated (29). Thermodynamic and kinetic studies by spectroscopic techniques are in favor of a two-step mechanism (30, 31), while a close description of the intercalation geometry has been determined by x-raydiffraction on a number of short dinucleotide complexes as well as some longer sequences (hexamers). But up to now, all the crystallized complexes have the drug inserted in the first/last step of the DNA which limits the description because of termination effects.

Why Did We Use This Hexameric Sequence?—The original purpose of this work was to get a more detailed description for the intercalation process for a single site and in particular determine the extent to which the helical parameters are changed without the complication of termination effects. The sequence d(C₃G₃) was chosen it was a self-complimentary sequence, containing a single CpG site. This avoided potential difficulties arising in the case of a hexamer with a central TpG site where the crystallization process would require some kind of discrimination between two different chains. There is an additional problem with nogalamycin as the intercalant. The DNA must at least partially melt at the CpG site to allow nogalamycin with its bulky nogalose and amino-glucose side groupsto enter. In addition, there may be some conformational changes needed such as reorientation of the sugar residues with respect to the aglycone to permit entry. Also there may a preliminary transition intermediate defining specificity in the direction of approach.

Our crystallization problems seem to concur with these requirements. First we observed that the crude hexamer readily formed a crystalline complex but that addition of salt, whether intentionally or as an artifact of HPLC purification, hinders solubilization of the drug in the nucleotide solution. High salt conditions tend to stabilize the duplex. Second, we found that the ability to form well diffracting crystals involves annealing at 38° and slow cooling to 4°C. The instability of the crystals above 4°C is probably directly related to the weak interactions between the extended solvent channels onto which the minor

![FIG. 9. Stacking of adjacent base pairs (steps) viewed along the helical axis. A, the intermolecular stacking C1-G12 over the symmetry-related G6*-C7*; B, the first step, C1-G12 over C2-G11; and C, the second step, C2-G11 over C3-G10. D, represents the central step where the nogalamycin has been removed to illustrate the unwinding at the intercalation site. The overlapping surfaces of each step are blackened. The most efficient stacking is the intermolecular step (A).](http://www.jbc.org/Downloaded from)

### Table IV

| Residue | a | β | γ | δ | ε | ζ | ξ | x |
|---------|---|---|---|---|---|---|---|---|
| C1 | -71 | 161 | 57 | 86 | -161 | -67 | -157 | 15565 |
| C2 | -67 | 161 | 55 | 76 | -147 | -83 | -157 | 15565 |
| C3 | -68 | 202 | 66 | 146 | -149 | -148 | -103 | 15565 |
| C4 | -60 | 158 | 51 | 140 | -181 | -105 | -118 | 15565 |
| C5 | -60 | 205 | 61 | 144 | -103 | 15565 |
| C6 | -77 | 174 | 59 | 86 | -150 | -80 | -148 | 15565 |
| C7 | -72 | 170 | 57 | 91 | -165 | -86 | -133 | 15565 |
| C8 | -60 | 174 | 62 | 85 | -186 | -75 | -143 | 15565 |
| C9 | -62 | 181 | 58 | 153 | -175 | -145 | -110 | 15565 |
| C10 | 57 | 156 | -51 | 165 | -105 | 15565 |

Mean values for A and B DNA (36): A: -73 173 64 78 -151 -77 -165 B: -65 167 51 129 -157 -120 -103

### Table V

| Strand | P2-P3 | P3-P4 | P4-P5 | P5-P6 | P6-P7 | P7-P8 | P8-P9 | P9-P10 | P10-P11 | P11-P12 |
|--------|-------|-------|-------|-------|-------|-------|-------|-------|---------|---------|
| 1      | 5.61  | 6.36  | 6.73  | 7.07  | 5.78  | 6.14  | 6.35  | 7.11  |         |         |

*Mean values for A-DNA: 5.9; B-DNA: 7.0.
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monophosphates where opening up is observed by variation of good agreement with crystalline complexes of dinucleoside whereas here the variation is well defined. Although there is around intercalation site in earlier 2:1 complexes (8, 9), and hydration. There are a variety of sugar conformations ther with the sugar conformations and/or the crystal packing This suggests some distinct stabilizing property associated ei-

Fig. 10. Pseudo-rotation diagram of the sugar residues (\(P, r_m\)).

\(E\) = envelope; \(T\) = twist conformations.

groove of the complex and the nogalose face. Re-annealing followed by slow-cooling probably helps purify the complex in terms of improving conformational homogeneity of the sugar residues thus reducing the tendency for crystal defects.

The refinement of the structure from the initial model is interesting because the oligonucleotide pieces A and C were initially built as B-DNA models. The conversion of the sugar conformations to \(c^2\)-endo for cytosine occurred after the addition of the prominent solvent atoms and subsequent annealing at 900 K with XPLOR. We finished the refinement using NULCSQ, because we suspected that the nonplanarity of the aromatic core of the aglycone was an artifact coming from the dictionary. In our final model, the aromatic core of the anthra-cycline ring system was still slightly nonplanar and similar to the previous nogalamycin complexes (6-9). In NULCSQ, the option of constraining the sugar puckers was not used and the pseudo-rotation parameters were not restricted to specific A- or B-DNA target values but left to refine freely.

How Does the Drug Enter?—In the case of nogalamycin with its two bulky side groups, there is a problem with complex formation, which requires some kind of melting or opening up of the base pairs at the intercalation step. This can be achieved either by melting the duplex and/or by local A- to B-DNA interconversion. The A/B-DNA interconversion is a known process that occurs in solution, it may even be trapped during the crystallization process (32).

From a thermodynamical viewpoint, changing the conforma-
tion of the sugar residues from \(c^2\)-endo and \(c^3\)-endo or vice versa is not a problem at 4°C, since both conformations have been observed together in the same crystal. However the reg-
ularity of the sugar conformations in these two domains seems inconsistent with the low melting temperature for the crystals. This suggests some distinct stabilizing property associated ei-

\(\beta\) and \(\gamma\) torsion angles at the intercalation step (33, 34), we have now a better description because our structure is extended 2 base pairs on each side of the intercalation step.

Based on This Structure, Is There a Sequence Specificity for Nogalamycin?—Nogalamycin has been described as having a high affinity for TpA or CpG or TpG steps. Comparison with daunomycin is interesting because the orientation of the polar and hydrophobic groups in the two molecules are reversed (Fig. 1). First, the intercalation of daunomycin would require less energy than nogalamycin. Second the insertion of the aglycone in the DNA follows the same orientation in the two molecules: either the hydrophobic nogalose, in the case of nogalamycin, or the polar amino-sugar, in the case of daunomycin, is located in the minor groove (6, 7), a strong indication that the hydropho-

bicity/hydrophilicity parameter is not the principal driving force for the insertion. On the other side of the duplex, nogalamycin fills the major groove with its bulky amino-glucose, while daunomycin has only a small methoxy group. The only group that shares similar orientation and interactions between the two drugs is the carbonyl group (methylketone in dauno-

mycin, methyl ester in nogalamycin) at C9. In all of the structures, it interacts with the G11 residue one step before either directly or mediated by a water molecule, despite a different configuration (axial in nogalamycin, equatorial in daunomycin).

Surprisingly, nogalamycin does not interact strongly by di-

rect bonding to the CG step where it sits. In our structure only one direct interaction is observed in the major groove, O2G \(\rightarrow\) N7 of G4, \(d = 2.84\) Å. In the 2:1 complexes already described it is the O4G of the drug which interacts directly to N4 of C9: \(d = 3.10\) Å (in the present work the corresponding distance is 3.56 Å, mediated by the bridging water W2). In contrast nogalamycin forms a number of important hy-

drogen bonds between the three donor/acceptor groups (O2G, N3G, and O4G) of the amino-glucose residue and the G4:C9 base pair which is spanned by the amino-glucose. This must be compared with previous structures and is certainly at the origin of the high specificity of nogalamycin. However the com-

parison with other structures is not straightforward as they all contain m5C-G steps instead of pure C-G steps (The present structure is the first of this kind).

As in previous 2:1 complexes the dimethyl-amino group N3G is strongly involved in the hydrogen bond network, but here, due to a rotation, there is a different hydration. It interacts through the tetra co-ordinated solvent residue W28 to N4 of residue C8. W28 is part of a strongly defined network, including a symmetry-related phosphate backbone (Fig. 8). In this respect, nogalamycin displays a completely different type of interaction network from that observed in daunomycin complexes which may well explain why its specificity is increased.

Finally, the parameters of the duplex at the two ends go back to “normal” values, indicating that the DNA deformation is rapidly compensated, at least \(\pm\) two steps before and after the intercalation site.

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