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DNA-drug interactions

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Abstract

To date some 30 DNA-drug complexes have been solved, using synthetic deoxyolignucleotides of length between 6 and 12 base pair units. The drugs divide into three main categories: molecules which bind in the minor grove of DNA, molecules which both intercalate between the base pairs and bind to the minor grove and bifunctional intercalators. There are determinations of the same drug binding to different base sequences, and the same base sequences interacting with drug molecules which have been modified chemically in small but significant ways. By comparing the different structures it is possible to determine changes in both the drug and the DNA molecules on complex formation, the types of forces which are involved in the interactions and possibly put forward some suggestions for modifying the drug molecules so as to make them more specific for certain base sequences. The paper gives a brief overview of the field.

INTRODUCTION

DNA is a natural product, indeed the natural product of the paramount importance in understanding the mechanism of genetic processes, of cell growth and diffrentiation, of ageing and senescence. It is also a logical target for chemotherapy. Binding of peptides, small organic and inorganic molecules to DNA can interfere with the numerous processes, including transcription and replication in which DNA participates. Such interference can retard or prevent cell growth. Extensive chemical and biochemical studies have characterised a variety of molecules of this type and numerous antiviral, antibiotical, antiprotozoal and antitumour agents have been identified. Some of these have clinical applications, others are under clinical trial.

The development of methods of deoxyoligonucleotide synthesis in the early 1970s has made it possible to investigate details of the structure of DNA at a molecular level by X-ray crystallographic and NMR studies. (for a review of X-ray structures see refs 1 and 2). The X-ray studies, carried out at resolutions of between 1.2 and 2.5 Å, gave a wealth of information about the conformational variability and flexibility of DNA, the influence of base sequence and the role of water and counterions. The challenge now is to obtain similar information about drug-DNA complexes. The hope is that such studies will lead to rules governing sequence specific binding and an insight into the correlation between DNA structure, sequence and activity. Such information is evidently important for rational drug design (ref. 3) and may well lead to the development of more potent antibiotics and antitumour agents. One must, however, beware of too simplistic a reliance on structural information and on molecular modelling studies. The delivery of a drug to the target site, and only the target site, is a complex problem which calls for solution studies, biological experiments and different types of insight into design requirements.

The X-ray structure analysis of DNA drug complexes made a relatively slow start. Up to 1984 the structure of only one complex was published, as a preliminary report (ref. 4), but increasingly more complexes have been solved. Progress has been particularly rapid in the past four years and currently some 30 complexes have been characterised. There have been studies of the same drug complexed to two different deoxyoligonucleotide sequences and the same deoxyoligonucleotide sequence complexed to a series of molecules obtained by subtle chemical modifications of a particular drug. Such comparative studies offer the best hope of understanding the principles of DNA drug recognition and the mutual influence of the two components on complexation.

EXPERIMENTAL CONSIDERATIONS

The X-ray investigations discussed in this paper all refer to single crystal X-ray analyses, which are critically dependent on the preparation of diffraction quality crystals. The starting materials are relatively readily obtained. The deoxyoligonucleotides are synthesised by standard solid phase techniques (ref. 5)

which allow the rapid synthesis of reasonably homogeneous deoxyoligomers up to 20-24 base pairs in length. Stringent purification appears to be an essential pre-requisite for successful crystallisation. It generally involves high pressure liquid chromatography on a strong anion exchange resin and a second HPLC run on C18-reverse phase material. The pure oligonucleotide is finally desalted by gel filtration and lyophilized to give typically 5-15 mgs of the ammonium salt of the target DNA. This technique has been used successfully for deoxyoligonucleotides up to 12 base pairs, the longest sequences which have been crystallised with drug complexes. The various drugs for complexation are generally readily available in much larger quantities.

The usual technique for the crystallisation is to diffuse a precipitating agent into a solution containing the deoxyoligonucleotide, the drug, an associated cation, a buffer, and in many cases, spermine. A wide range of conditions is commonly set up with variations in the oligonucleotide concentration, the nature and concentration of the counterions, pH, and temperature.

The success rate of crystallisation is, however, very low. Only about 10% of any random sequence of 15 base pairs or less can be crystallised. Most commonly only disordered crystal are formed or none at all. Even crystals with perfect optical order often do not diffract, probably because of rotational disorder of the cylindrical DNA helixes in the adjacent unit cells which form the crystal. The problem is further exacerbated by the extensive hydration of the crystals, which commonly contain about 50% solvent. Consequently the number of different types of deoxyoligomers which have been complexed with drugs is extremely limited.

One large class of compounds, the groove binders described below, have been complexed with the so called "Dickerson-Drew" dodecamer d(CGCGAATTCGCG) (ref. 6) or a variant of this sequence. These complexes are isomorphous with the native, well studied DNA fragment. Like the native crystals they diffract to a resolution of only around 2.0-2.5 Å, possibly in consequence of both solvent and DNA disorder. The analysis of these complexes is relatively straightforward, using molecular replacement for the structure solution and some form of constrained refinement to derive details of the conformation of both the drug and DNA. To avoid bias it is important to locate the drug from so called "omit maps". These are electron density maps of the structure, which were prepared with the contribution of the drug omitted from the calculations. The solvent molecules, and in some cases the spermine molecules are located by difference Fourier techniques. At this resolution, while it is possible to deduce the broad structural features care must be taken in the interpretation of the position of the drug, which might possibly have disordered occupancy. The finer details of drug binding and hydrogen bonding must also be interpreted very cautiously, particularly when water molecules are involved.

The majority of the second class of drug molecules discussed in this paper, the monofunctional and bifunctional intercalators, are generally crystallised with the hexamer d(CGTACG) or a variant of this sequence. The native sequence has never been crystallised and it was only a fortuitous experiment by Rich and co-workers, who included daunomycin in the crystallisation mixture (ref. 4), which revealed the potential of this sequence for crystallising drug complexes. It is interesting that in some cases chemical modification, such as the introduction of a phophorothioate linkage (refs T17, T20, T25, T26) induced or improved crystallisation, a phenomenon first observed in crystallising a B-DNA hexamer (ref. 7).

The intercalating complexes diffract to a resolution of between 1.2-2.0 Å. These structure analyses are thus more detailed and precise than those of the groove binders.

It is evident that the maps based on experimental data at 1.0 Å give atomic resolution, and thus reliable molecular dimensions. At a resolution of 2.0 Å it is more an overall shape fit between the model structure and the electron density. This will give reliable information about conformation and torsion angles if interpreted cautiously. Figure 1 illustrates the electron density obtainable at different resolution.

In case of the intercalator complexes, since the native structure could not be studied it is possibly more difficult to evaluate distortion of the DNA. However, comparison of the many different relatively high resolution studies does allow reliable deductions both about detailed binding interaction and mutual deformations of the drug and the DNA double helix. It is only very recently that some new sequences have been successfully crystallised, notably those with actinomycin (refs T29, T30) and chromomycin (ref. T28). There were formidable difficulties in solving the structures.

This rather detailed description of the experimental procedures is included here to give the general reader an insight in the problems encountered in the preparation of the crystals and the evaluation of results in this area of crystallography which falls midway between techniques and difficulties of small molecule and protein crystallography.

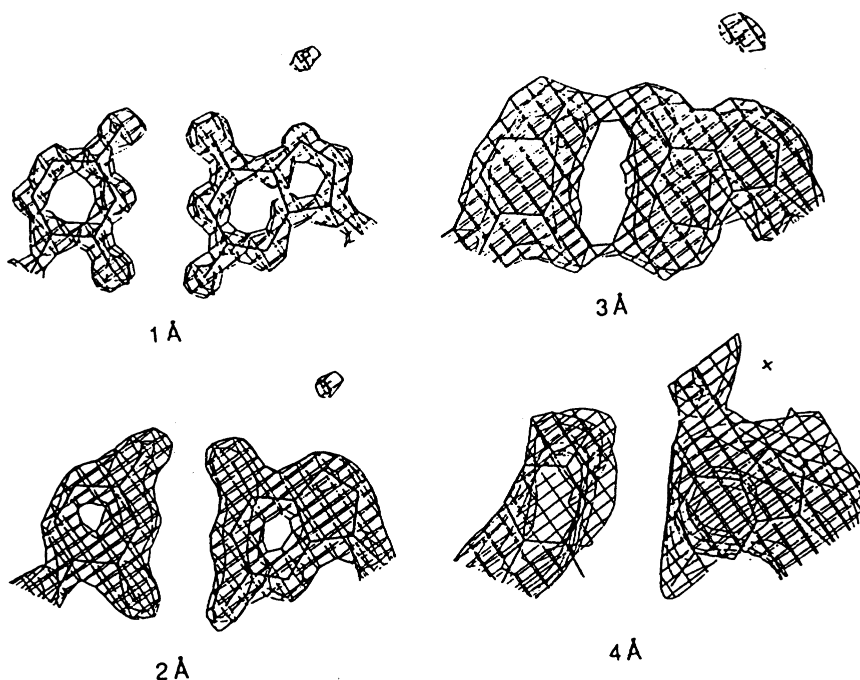

Fig. 1 Electron density maps at various resolutions.

DRUG CATEGORIES

A variety of compounds interact with DNA, ranging from complex natural product such as the antibiotic triostin A to inorganic compounds like tris-platin. All of them bind to double stranded DNA

Prior to describing the results of the X-ray analyses it may be useful to recall the structural features of the DNA duplex which may be important in sequence specific recognition by other molecules be they large or small. These features include the negatively charged phosphate backbone, the hydrogen accepting and donating functional groups in the major and minor grooves, the phosphate oxygen atoms and aromatic, hydrophobic components able to promote van der Waals interactions. There are a number of additional, structural, aspects. As is well known, DNA is polymorphic, and has been observed in several different conformations. The A, B and Z forms have been characterised initially by fibre diffraction and then by single crystal X-ray studies. The geometry of the double helix, including the depth and width of the minor and major grooves are different in the different conformations (ref. 8). The groove shapes are also, to some extent, sequence dependent within a particular conformation. The hydration patterns also vary and are again related to sequence. Finally, the structural flexibility of the double helix has been strikingly demonstrated both in the single crystal analyses of DNA fragments and DNA-protein complexes. All these factors contribute to the formation of a DNA-drug complex.

Intercalators

The phenomena of intercalation involves the aromatic portion of a drug molecule positioning itself between base-pairs. Intercalation increases the separation of adjacent base pairs and the resultant helix distortion is compensated by adjustments in the sugar-phosphate backbone and an unwinding of the duplex. Aromatic stacking interactions between the bases and the intercalating molecule are a major stabilising feature of the complexes formed.

Simple mono-intercalators like acridine have only been examined crystallographically in dinucleotides, which are too short for reliable extrapolation to intercalation of the double helix. More complex mono-functional intercalators such as daunomycin and related anthracycline drugs (Fig. 2), have been successfully co-crystallised with various hexamers principally d(CGTACG), d(CGATCG) d(TGTACA) d(TGATCA) and variants of d(CGTACG) with methylated cytosine and/or a phosphorothioate linkage. Table 1 (Appendix) categorise the various complexes analysed to date and gives the relevant references.

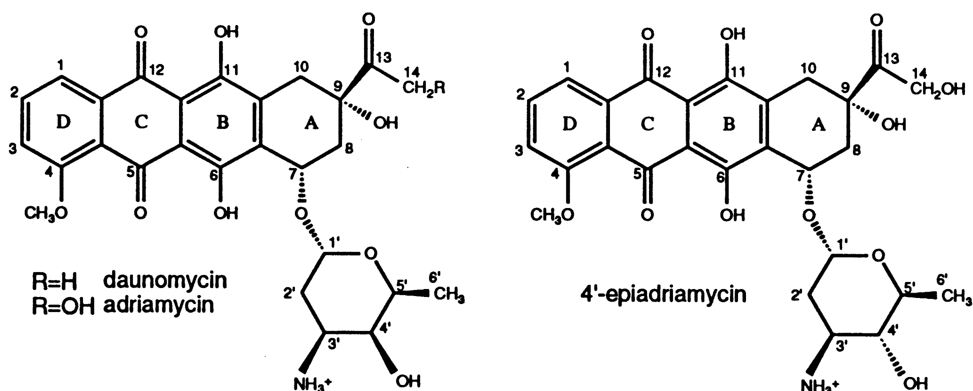

Fig. 2 Chemical formulae for some monofuctional intercalators of the anthracyclin family.

All these compounds contain a planar chromophore group of four fused six membered rings variously substituted, and an amino sugar residue. Slight changes in the substituents, including stereochemical changes, significantly change the biological activity. Daunomycin, for example, is most effective in the treatment of leukemias, while adriamycin is more effective in the treatment of solid tumours.(refs 9, 10).

The different complexes crystallised have a 2:1 ratio of drug to DNA. They are also, with the exception of the 11 deoxydaunomycin complex, crystallographically isomorphous. As illustrated in Fig. 3, two drug molecules are intercalated in the double helix, one at each NpG step. The chromophores are inserted in a "head-on" fashion with the long axis of the aglyclone moiety oriented at right angles to the long axis of adjacent base pairs and with the cyclohexane ring A protruding into the minor groove. The amino sugar lies in the minor groove. Figure 3 shows daunomycin complexed with the hexamer d(CGATCG) (ref. T16) viewed (a) from the minor groove side of the complex and (b) perpendicular to the chromophore.

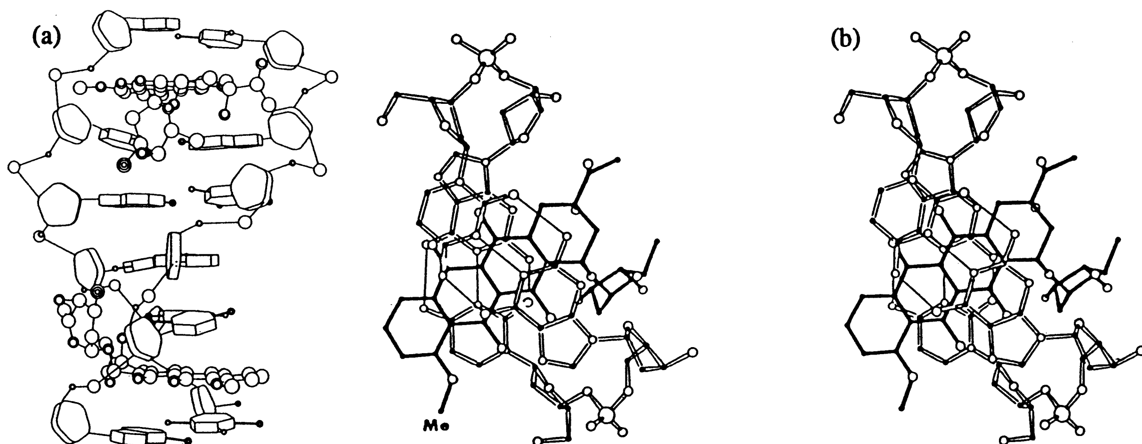

Fig. 3 (a) Stylised diagram of daunomycin complexed with the hexamer d(CGATCG) viewed from the minor groove side of the complex. Atoms of daunomycin are shown as open circles. The sugar and base components of the nucleotides are represented as disks. Program for diagram courtesy of Dr A. Lesk.

Fig. 3(b) Stereoview of the daunomycin d(CGATCG) complex perpendicular to the chromophore of the drug, which is depicted as dark bonds. The diagram illustrates the "head on" intercalation.

Within this general pattern of complexation the individual contacts between the drug and the DNA depend both on the sequence of the bases adjacent to the intercalation site and on the chemical modifications of the intercalating drug. The studies already reported give us an opportunity of evaluating the various factors since we have, as indicated in Table 1 (Appendix) examples of complexes between different drugs and the same sequence, such as, for example, the sequence d(CGATCG) complexed to daunomycin, adriamycin and 4'-epiadriamycin and the same drug analysed in different sequence environments, e.g. daunomycin complexed to d(CGTACG), d(CGATCG) d(TGATCA) and d(TGTACA). We even have two independent

determinations of the same complex, daunomycin, to the hexamer d(CGATCG) (refs T14, T16). It is reassuring that the two determinations led to the same structure within the relatively small experimental errors expected at the resolution of around 1·5 Å.

Comparison between two daunomycin structures complexed to d(CGATCG) (ref. T16) and d(CGTACG) (ref. T13) illustrates the type of information which can be obtained from such studies. Fig. 4 a and b is a schematic representation of the hydrogen bonding between the drug and the DNA in the two complexes and shows the flexibility of the hydrogen bonding arrangements. There are direct hydrogen bonds between the functional groups of the drug and the DNA as for example between the hydroxyl group of ring A, which is essential for drug action, and the functional groups N2 and N3 of guanine at the intercalation site. There is also hydrogen bond interaction between another essential feature of the drug, the NH_3^+ group, and O2 of cytidine. The inversion of the base sequence next to the intercalation site is accommodated by having a water mediated rather than a direct hydrogen bonded interaction of the appropriate base with the NH_3^+ group. The use of water mediated contacts between the drug and DNA is general phenomenon which needs to be considered in all modelling studies.

Daunomycin also interacts with DNA in the major groove with water or with Na^+ mediated interactions involving O4 and O5 of the chromophore substituents. Further stabilisation is provided by a number of van der Waals contacts of less than 3.4 Å (not shown in the figure). These contacts are more numerous in the d(CGATCG) complex than in the d(CGTACG) complex. A possible explanation is that these contacts compensate for the somewhat weaker hydrogen bond in the d(CGATCG) complex, which is mediated by water instead of the NH_3^+. In neither complex were any electrostatic interactions observed.

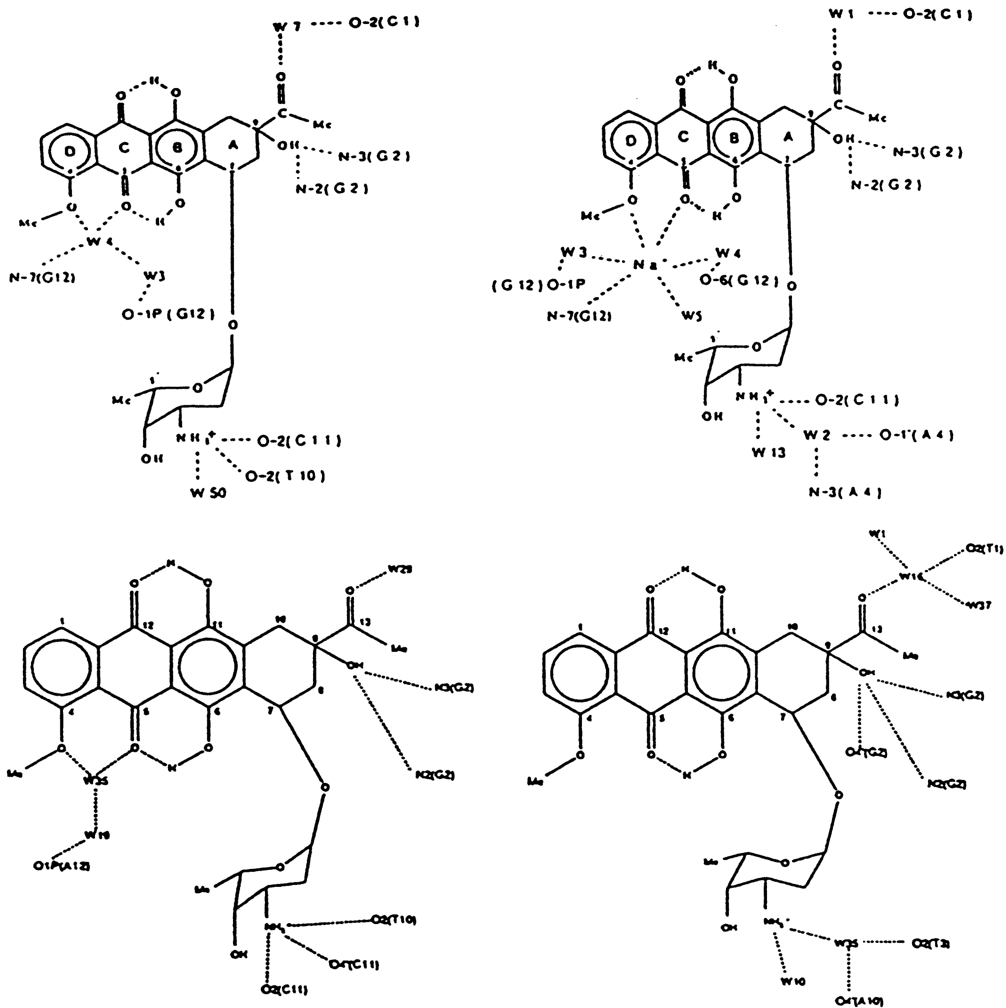

Fig. 4 Two dimensional schematic diagrams to show the hydrogen bonding to daunomycin in:
(a) d(CGATCG) (b) d(CGTACG)
(c) d(TGATCA) (d) d(TGTACA)

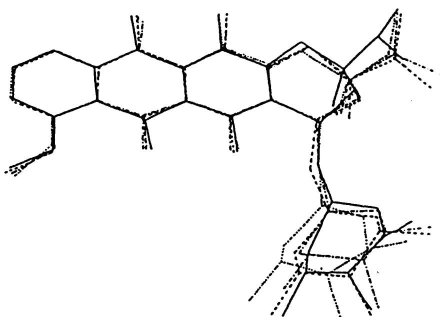

Fig. 5 Superposition of the daunomycin molecule as determined in the four complexes d(CGATCG), d(CGTACG), d(TGATCA), and d(TGTACA), as the best molecular fit with the uncomplexed daunomycin.

The two structure determinations show clearly why CpG is the preferred intercalation site for this drug and suggest that only the d(TpG) step could provide a similar hydrogen bonding environment on intercalation. The crystal structure of daunomycin complexed with d(TGATCA) and d(TGTACA) has recently been determined (ref. T15). Fig. 4 (c) and (d) show the corresponding hydrogen bonded interactions.

Two other aspects of these high resolution studies are worthy of mention; changes in the drug structure and the distortion of the DNA double helix. Daunomycin and related molecules are relatively rigid but there are nevertheless some changes from the free drug molecule (ref. 11). Figure 5 is a superposition of the daunomycin molecule as determined in the four complexes as best molecular fits with the uncomplexed daunomycin.

The distortion of the DNA can only be estimated indirectly since the structure of the uncomplexed hexamer is not known. There are, however, marked asymmetric distortions of the double helix and it is possible to estimate the total unwinding angle which is approximately 8° per daunomycin molecule.

The principal features of the DNA drug structures discussed above are substantiated and extended in studies of complexes with the bifunctional intercalators triostin A and echinomycin (refs T21, T22, T23)

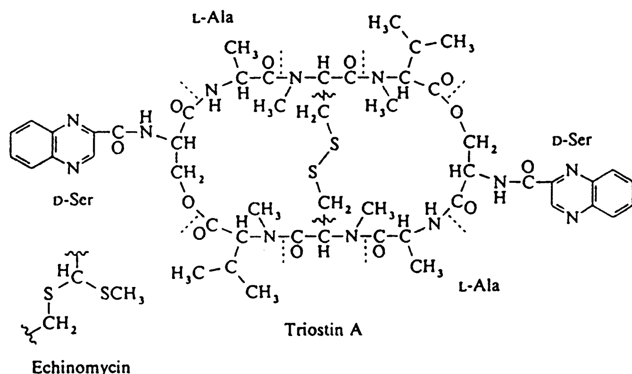

Fig. 6 Chemical formulae of triostin and echinomycin.

These molecules, illustrated in Fig. 6 have two planar quinoxaline groups separated by a fairly rigid cyclic depsipeptide system. The X-ray structure of the uncomplexed triostin was determined some years ago (ref. 12) and numerous theoretical studies were published on the docking and binding of bifunctional intercalators. None of these predicted the binding mode found in the high resolution X-ray structures of triostin and echinomycin complexed with the hexanucleotide d(CGTACG) and in the triostin A d(CGCTATCG) complex.

Two views of the octamer complex are shown in Fig. 7. This Figure shows clearly the intercalation of the optimally aligned chromophores between two adjacent base pairs from the minor groove side of the B-DNA helix. The resultant unwinding of the double helix, however, is so drastic that the DNA resembles a ladder more closely than a double helix. There are specific hydrogen bonds between the functional groups of the drug and the nucleotide bases at the intercalation site, which account for the drug specificity. The most novel and surprising feature of the complex is the disruption of the Watson-Crick hydrogen bonding of the base pairs adjacent to the intercalation site.

In the octamer illustrated in Figure 7 it is clear that both the central A.T base pair and the G.C pairs at each end adopt the Hoogstein geometry. In the hexamer structures only the central A.T pair is of the Hoogstein type. These changes allow the drug to approach the DNA more closely and results in extensive van der Waals interactions. The importance of these weak interactions was also not anticipated but they appear to be the dominant forces in the stabilisation of these DNA drug complexes.

The mono and bifunctional intercalators as well as the groove binders described in the next section all interact with DNA in the minor groove whereas most DNA binding proteins interact in the major groove. There has been speculation (ref. 2) that these antibiotics have evolved through natural selection by microbes developing secondary metabolites, which attack in the relatively unprotected minor groove. Recently the

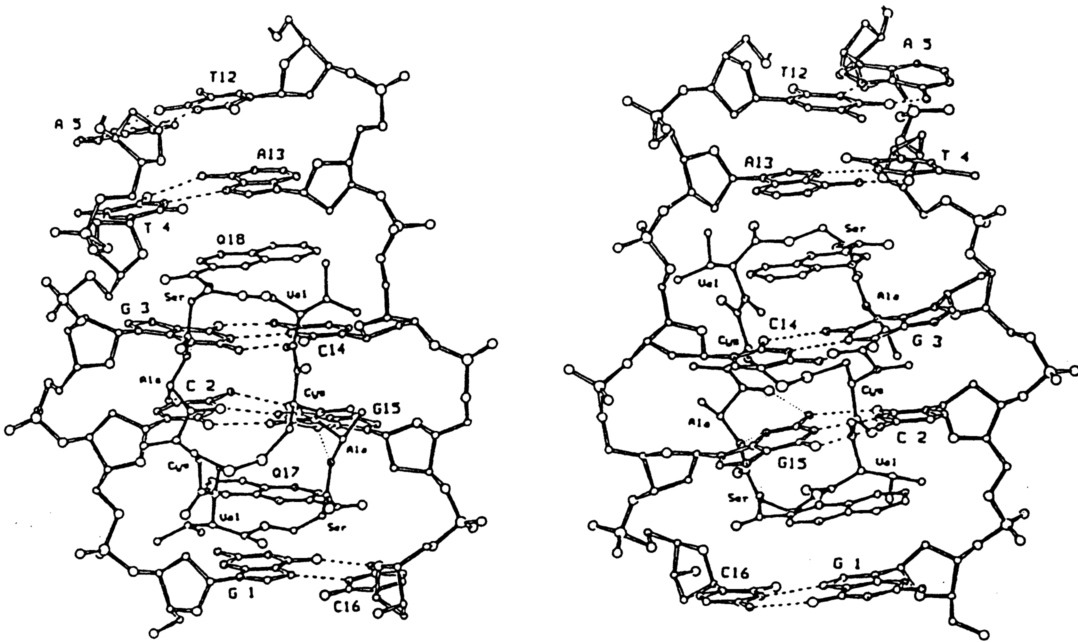

Fig. 7. The crystal structure of the complex between triostin A and d(GCGTACGC) (ref. T23) viewed fom the minor groove. The hydrogen bonds between the drug and the nucleotide are indicated by dotted lines. Note that the G1.C16 and T4.A13 base pairs are in the Hoogstein conformation.

crystal structures of two nogalamycin hexamer complexes were published (refs T25, T26, T27). This antitumour drug has two bulky sugar groups on either end of the chromophore and binds in both grooves simultaneously. It is thus an interesting candidate for the design of novel antitumour agents. The crystal structure also raises the question of how the bulky sugars are threaded into the double helix and postulates the transient opening of the base pairs.

DNA in the B form may not be the only target for drugs, and other conformations, such as A or Z may have a role in specific stretches of DNA. The structure determination of chromomycin with the novel sequence d(TBrUGGCCAA) (ref. T28) shows it binding to the wide and shallow groove of A-DNA. The possibility of stretches of A-DNA occurring *in vivo* was raised when the X-ray structure of a nonamer representing the essential binding site of the transcription factor TFIIIA of *Xenopus* indicated that this sequence adopts the A conformation (ref. 13). The same conformation was found in solution by Rhodes and co-workers (ref. 14) not only for the 9-mer fragment, but also for the complete TFIIIA fragment of 54 base pairs. Subsequently the coexistence of A and B conformations was discovered in the crystal structure of d(GGBrUABrUACC) (ref. 15). This heavily hydrated structure is built up of A-DNA helices, arranged about the crystallographic screw axis. The helices enclose a large central channel which was shown to contain B-DNA helices. Similar observations have been reported in some other crystal structure. The possibility that certain groups of sequences may *in vivo* adopt conformations other than B-DNA under the influence of environmental factors needs to be considered in any modelling of drugs binding to DNA.

Minor groove binder

A number of studies have been reported on the structure of drugs which bind to the minor groove of DNA These include netropsin, distamycin, Hoeschts 33258, DAPI, berenyl and, most recently, spermine, all of them complexed to either to the Dickerson Drew dodecamer d(CGCGAATTCGCG) or to one of its variants The chemical structures of some of the groove binders analysed are given in Figure 8 and the list of references in Table 1 (Appendix).

The structure of the Dickerson-Drew dodecamer has been extensively studied and the structures of the uncomplexed drugs are mostly also known. It is possible therefore, to gain a detailed idea of the effect of binding on both the drug and the DNA.

In all the complexes the drug displaces the so called "spine of hydration" and fits snugly into the minor groove. It might thus be expected that there is relatively little change in the DNA itself on complexation. However, the results from one of these structure determinations, of Hoescht 3325 complexed with d(CGCATATCGCG) (ref. T11), indicates that this is not the case. In this structure the

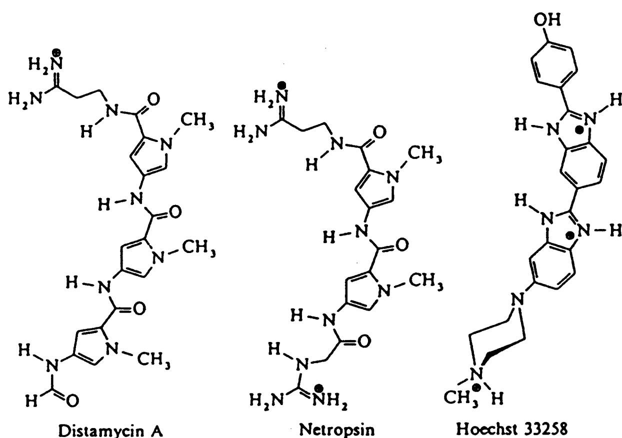

Fig. 8 Chemical formulae of some selected groove binders. The diagrams are arranged with the left side as the one which interacts with the minor groove side of the bases.

alternation of the helical twist at the ApT and the TpA steps is reversed from that commonly observed for the uncomplexed alternating DNA (ref. 16). An additional change involves the rotation of one of the bases to allow for hydrogen bonding to the drug. It may well be that while in some complexes the drug is a passive replacement for the ordered water structure in the narrow minor groove, in other sequences the narrow minor groove is not a feature of the native solvated sequences but is induced through an active interaction with the appropriate drug.

Hydrogen bonding, mediated in some cases like berenil (ref. T6) and fluorochrome 4'-6-diamidine-2-phenyl indole (DAPI) (ref. T7) by a water molecule between the drug molecules and the DNA, is the major stabilising force. Van der Waals contacts also play an important role. Figure 9 shows a view of the DAP-dodecamer complex and a schematic view of contacts between DAPI nitrogens and the bases.

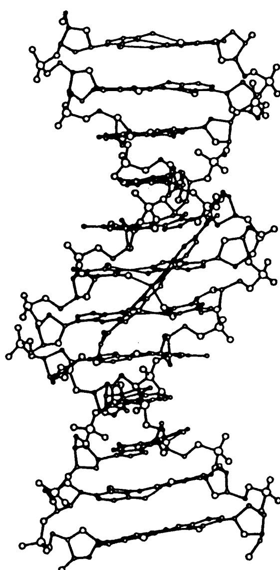

Fig. 9 (a). Schematic drawing of the DAPI complex.

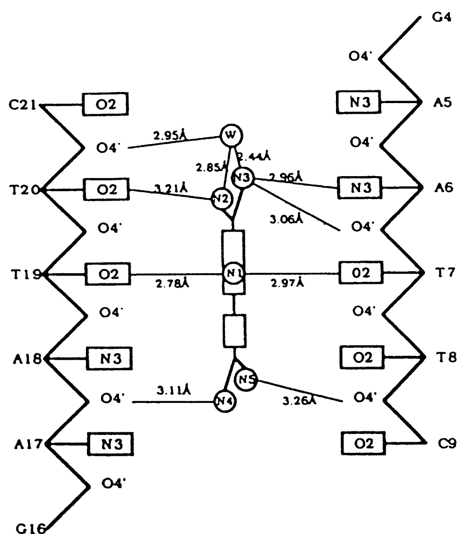

Fig. 9 (b). Schematic drawing of the interactions between some of the nitrogen atoms of DAPI, the bound water molecule (W) and the minor groove of the the Dickerson-Drew dodecamer.

Changes induced in the conformation of some of the groove binding drugs themselves are surprisingly large. They include changes in the dihedral angles between the four rigid units of the Hoescht 33258 compound (ref. T11).

SUMMARY

The DNA drug complexes so far examined have all interacted principally in the minor groove of B-DNA but have to some extent perturbed the major groove also. No direct electrostatic interactions have so far been observed between any charged portions of a drug and the negatively charged sugar phosphate backbone. Interactions between the drug and DNA generally involve hydrogen bonds linking the functional groups of the two components. These may be direct hydrogen bonds or bonds mediated by water molecules or by counterions. which thus introduce an additional degree of flexibility. Van der Waals forces contribute significantly to the stabilisation of the complexes and may lead to serious perturbations of the DNA helix

from the normal Watson-Crick geometry. At its most extreme such perturbations can lead to the formation of Hoogstein base pairs instead of the normal Watson-Crick pairs. Interaction with the DNA also induces changes in the conformation of the drugs themselves.

Intercalation at specific base steps can be rationalised in terms of the hydrogen bond interactions. The preference of minor groove binders for A.T rich regions seems to be related to the need to maximise van der Waals contacts. In some sequences the minor grooves in this region is intrinsically narrow because of the high propeller twists of the A.T base pairs, which is usually stabilised by solvent molecules. In other sequences the groove binding drug itself induces the narrowing of the minor groove in the A.T rich region to allow for close van der Waals contacts. It would, however, be much more difficult to induce a narrowing of a groove in a region containing guanines since the N2 group of guanine would hinder the entry of the drug into the minor groove (ref. 17).

The general picture which emerges from the X-ray studies is the extreme mutual adaptation of both the DNA and the drug on binding. The adaptation is such as to maximise the various interactions which stabilise the resulting complex. These interactions range from highly directional hydrogen bonds to non-specific van der Waals forces. With so many variables it is difficult to design new drugs with selected base specificity. We need to understand much more about the general principles of molecular interaction and also about the biological activity of DNA binding drugs before rational design of new DNA binding drugs becomes a real possibility.

Acknowledgements

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APPENDIX

Table 1: DNA complexed with drugs

Reference	Complex [a]
T1	d(CGCGAATTCGCG)/cisplatin
T2	d(CGCGAATTBrCGCG)/netropsin
T3	d(CGCGATATCGCG)/netropsin
T4	d(CGCAAATTTGCG)/netropsin
T5	d(CCCAAATTTGCG)/distamycin
T6	d(CGCGAATTCGCG)/berenil
T7	d(CGCGAATTCGCG)/DAPI
T8, T9, T10	d(CGCGAATTCGCG)/Hoechst 33258
T11	d(CGCGATATCGCG)/Hoechst 33258
T12	d(CGCGAATTCGCG)/pentamidine
T13	d(CGTACG)/daunomycin
T14	d(CGATCG)/adriamycin
T15	d(TGATCA)/daunomycin
T15	d(TGTACA)/daunomycin
T14, T16	d(CGATCG)/daunomycin
T17	d(CGTpsACG)/11-deoxydaunomycin
T18	d(CGATCG)/4'-epiadriamycin
T19	d(CGATCG)/4Odemethyldeoxyrubicin
T20	d(CGATCG)/idarubicin
T20	d(mCGTpsAmCG)/U 58872
T21	d(CGTACG)/triostin A
T22	d(CGTACG)/echinomycin
T23	d(GCGTACGC)/triostin A
T24	d(BrCGBrCG)/proflavin
T25, T26	d(CGTpsACG)/nogalamycin
T27	d(mCGTpsAmCG)/nogalamycin
T28	d(TBrUGGCCAA)/chromomycin
T29	d(ATGCATATGCAT)/actinomycin D
T30	d(GAAGCTTC)/actinomycin D
T31	d(CCAACGTTGG)/anthramycin

[a] ps = Rp-phosphorothioate, mC = 5-methylcytosine, BrC = 5-bromocytosine, BrU = 5-bromouracil.

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