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The crystal structure of the DNA-binding drug berenil: molecular modelling studies of berenil–DNA complexes

Laurence H.Pearl, Jane V.Skelly, Brian D.Hudson and Stephen Neidle*

Cancer Research Campaign Biomolecular Structure Unit, Institute of Cancer Research, Clifton Avenue, Sutton, Surrey SM2 5PX, UK

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ABSTRACT

The crystal structure of the DNA minor-groove DNA-binding drug berenil has been determined. Molecular-modelling techniques have been used to establish plausible binding modes of the structure to A-T sequences. These have shown that specific hydrogen bonds are possible between the amidine groups of the drug molecule and O2 atoms of thymine, although global energy minimisations tended to emphasise electrostatic interactions with phosphate groups rather than these hydrogen bonds with bases.

INTRODUCTION

Berenil (Fig 1) is one of a class of diarylamidine compounds, distinguished by a wide spectrum of anti-microbial and anti-parasitic properties (1,2), berenil itself being an effective trypanocide with modest cytotoxic activity. It has been shown to bind to DNA (3,4), but does not cause unwinding of closed circular DNA (5), suggesting that the compound does not intercalate, but rather binds, like netropsin and distamycin, in the minor groove of the double helix (6,8). Berenil exhibits sequence specificity in its binding, showing a five-fold preference for poly-d(AT) over poly-d(GC), as judged by its ability to displace ethidium bromide (4). Saturation binding studies suggest that berenil occupies four base pairs on double helical DNA (9).
In this work we present the crystal structure of berenil itself. This structure has been used in modelling of its binding in the minor groove of B-DNA at A/T base pairs, by molecular graphics and molecular mechanics methods.

EXPERIMENTAL

Crystal Structure of Berenil

Crystals of berenil, as the N-acetylglycine salt (Sigma) were grown by vapour diffusion from aqueous methane-penta-1,2-diol solution. They were thin fragile monoclinic plates of poor diffracting quality. Unit cell dimensions were: a=14.434(3)Å, b=12.857(2)Å, c=8.690(2)Å and $\beta = 98.95(2)^{o}$. The structure

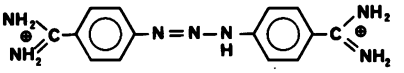

Fig.1 Berenil

was solved and refined satisfactorily in space group P2/c. Other space groups that were possible on the basis of systematic absences, did not give a solution.

Intensity data were collected on an Enraf-Nonius CAD4 diffractometer using Cu-K_α radiation and an ω-2θ scan technique, for 2376 reflections measured to a limit of 60^0, only 715 of which were of statistically significant intensity. The structure was solved by direct methods, and refined by full-matrix least-squares to a final R factor of 0.105. Table 1 gives atomic coordinates for the berenil and N-acetylglycine atoms.

Molecular Modelling

Initial cartesian coordinates for berenil were from the crystal structure described above. Coordinates for DNA oligomer duplexes were generated by the application of B-helix repeat parameters (S Arnott, personal communication) on coordinates for individual base-pairs. Docking of the berenil and the various

Table 1 Atomic positional and isotropic thermal parameters for the asymmetric unit of berenil.

Atom	x	y	z	B(A^2)
C1	0.370(1)	0.452(1)	0.564(2)	4.7(4)
C2	0.298(1)	0.401(1)	0.471(2)	6.3(5)
C3	0.225(1)	0.451(1)	0.377(2)	6.2(5)
C4	0.231(1)	0.554(1)	0.364(2)	4.3(4)
C5	0.303(1)	0.611(1)	0.450(2)	5.9(5)
C6	0.370(1)	0.564(1)	0.547(2)	6.4(5)
C7	0.155(1)	0.609(1)	0.253(2)	5.1(4)
*C8	-0.047(1)	0.741(1)	-0.057(2)	4.4(4)
*C9	-0.1397(9)	0.9029(9)	-0.137(2)	2.6(3)
*C10	-0.197(1)	0.933(1)	-0.045(2)	5.5(5)
*C11	-0.211(1)	1.051(1)	-0.035(2)	6.9(5)
N1	0.5000	0.453(1)	0.7500	5.1(5)
N2	0.4407(9)	0.3982(9)	0.663(2)	5.4(4)
N3	0.0950(9)	0.554(1)	0.160(2)	5.7(4)
N4	0.1497(8)	0.711(1)	0.251(2)	5.1(3)
*N5	-0.127(1)	0.794(1)	-0.164(2)	9.5(5)
*01	0.0072(7)	0.8005(8)	0.034(1)	5.5(3)
*02	-0.0425(7)	0.6456(8)	-0.063(1)	6.5(3)
*03	-0.2422(7)	0.8719(8)	0.029(1)	6.3(3)
*04	0.000	0.955(1)	0.250	5.4(4)

* Denotes N-acetylglycine atoms.
Atom N1 of berenil is on the crystallographic two-fold axis, as is atom 04 of the N-acetylglycine molecule.

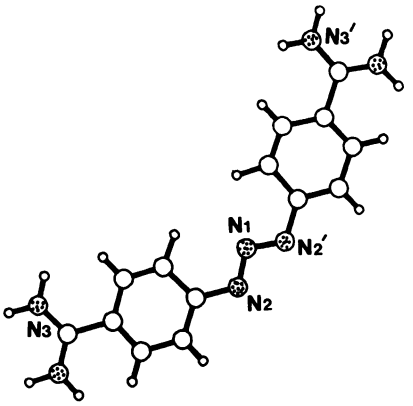

Fig.2 Computer-drawn plot of the crystal structure of berenil.

oligomers was performed using the interactive molecular graphics program MIDAS (10), implemented on a Silicon Graphics IRIS 2400 workstation. Atomic partial charges for berenil were calculated using the CNDO/2 procedure; partial charges and internal geometry parameters for nucleotides, and all Lennard-Jones parameters were from reference (11). Geometric parameters for the triazene linking group and the aryl amidine groups in berenil, not provided in this force field, were taken from the crystal structure. The torsional barriers for the triazene and amidine groups, which are not simply derivable from the crystal structure were estimated as being comparable to those of a peptide. Although the molecule in the crystal structure has a statistically perfect diad symmetry in which atoms N2 and N2' are equivalent for the charge and molecular mechanics calculations, a single hydrogen was localised on the N2 atom of the berenil, and the N2 and N2' atoms were considered to be of different force-field atom types in subsequent calculations. All hydrogen atoms on berenil and the various oligonucleotides were explicitly included in the molecular mechanics calculations. Energy minimisations were performed using the program EMPMDS (12), implemented on a DEC VAX 11/750, and were considered to have converged when the rms value of the first derivative was 0.25 $kcal^{-1}$ $mol^{-1}$$A^{-1}$. A distance-dependent dielectric constant was used.

RESULTS AND DISCUSSION

Crystal Structure of Berenil

The berenil molecule sits on a crystallographic two-fold axis, hence the asymmetric unit is a half molecule, and the hydrogen atom associated with the

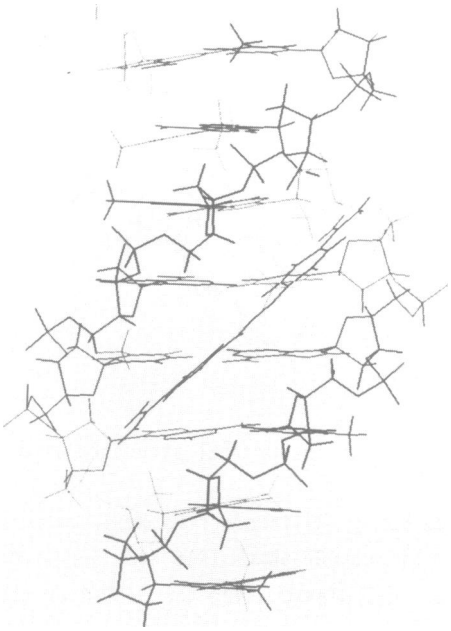

Fig.3 Computer-drawn view of the berenil-d(AT)$_4$.d(AT)$_4$ complex, with the helix axis vertical.

central triazene system is therefore of statistical half- occupancy, shared between atom N2 and its two-fold-related mate. The molecule as a whole is closely coplanar, with only a small twist between the phenyl rings, of 9.6^o (Fig 2). One molecule of N-acetylglycine is also found per asymmetric unit, and it is therefore assumed that the amidine group is protonated, and that the whole berenil molecule carries a net charge of +2.

Molecular Modelling

Initial docking of berenil with the various A-T oligonucleotide duplexes was in accordance with the observation that binding occupies four base pairs (9), and the molecule was positioned in the minor groove so that the triazene link was aligned with the space between the two innermost base pairs of four, with the amidine groups symmetrically aligned with the spaces between the outermost base pairs and the corresponding inner base pairs (Fig 3). The diad of the berenil was close to the pseudo-diad of the double helix in this configuration. The position of the berenil molecule was adjusted interactively to optimise hydrogen bonding contacts, and to minimise overlap of its van der Waals surface with that of the oligonucleotide. The amidine N3 hydrogen atom-

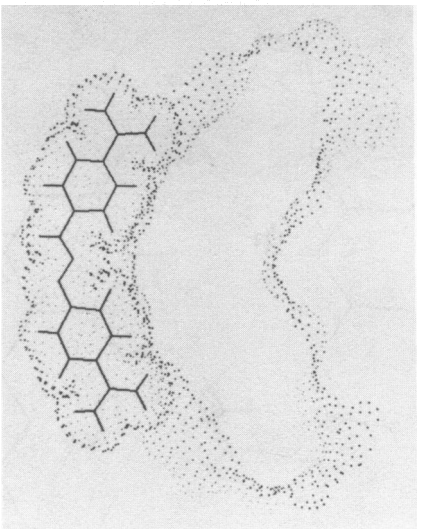

Fig.4 A view into the minor groove of the berenil-$d(AT)_4.d(AT)_4$ complex showing the complementarily of the molecular surfaces between drug and DNA.

thymine O2 atom distances are 2.03Å and 1.98Å with standard hydrogen-bond geometry. Neither the conformation of the oligonucleotide duplex, nor that of the berenil needed alteration in order to produce a good fit in the minor groove, the curvature of the berenil molecule very closely matching the curvature of the floor of the minor groove along its helical track (Fig 4). When docked in this position in the $d(AT)_4.d(AT)_4$ oligomer, two hydrogen bonds with acceptable geometry could be formed between the berenil amidine groups and the ring O2 atoms of the diad-related central thymines (Fig. 5). A similar arrangement could be formed in the $d(TA)_4.d(TA)_4$ oligomer with the N3 atoms of the central adenines acting as hydrogen bond acceptors, and an asymmetric arrangement with one thymine O2 and one adenine N3 accepting hydrogen bonds in the $dA_8.dT_8$ oligomer. Formation of the hydrogen bonds to the adenine N3 atoms was hindered by the adenine H2, which came into close contact with the phenyl hydrogens of the berenil. It is expected that this might result in a preference for binding at 5'-AT-3' sequences rather than 5'-TA-3' sequences, although this would be slight. The presence of an amino group at the 2 position, as in guanine, would completely occlude the N3 atom and prevent any favourable hydrogen bonding interaction between berenil and a G/C base pair.

Energy minimisations were performed in order to test the feasibility of

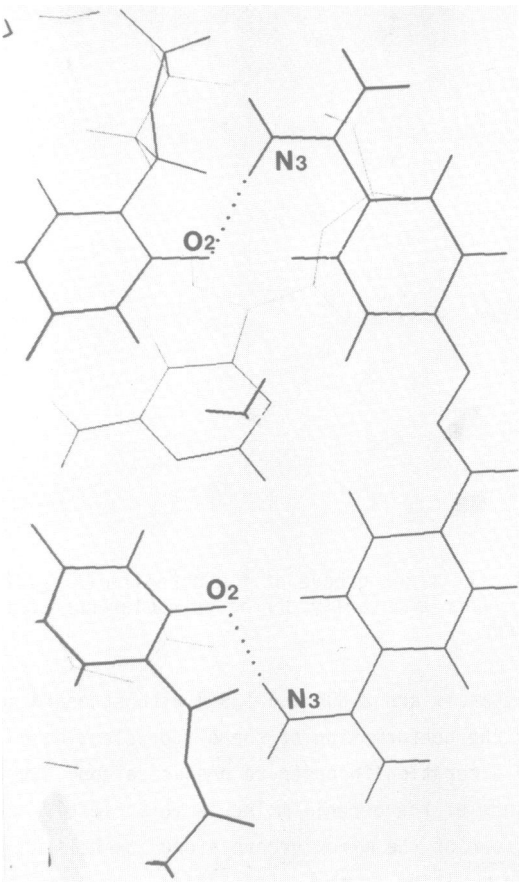

Fig.5 A computer-drawn view of the berenil-d(AT)$_4$.d(AT)$_4$ complex, showing the drug-DNA hydrogen bonds.

the modelled complexes described above. Minimisations were performed for each of the three oligomer sequences alone, and for the complexes of each with berenil (Table 2). All of the uncomplexed oligomers showed marked changes from their starting standard B-DNA conformations, particularly at the ends of the helix, where the base pairs tended to buckle. The disruption to the parallel base pair stacking mainly effected the outer two base pairs on each end of the helix, with the inner four base pairs remaining largely undisturbed in the d(AT)$_4$.d(AT)$_4$ and d(TA)$_4$ .d(TA)$_4$ structures. In the dA$_8$.dT$_8$ structure, in addition to these end effects, there was a tendency for the thymine bases to become tilted relative to the helix axis similar to A-DNA, while the adenine

TABLE 2 Results of the energy minimisations (in kcal $mole^{-1}$) for the three sequences and their berenil complexes.

	Van der Waals interaction energy	Electrostatic interaction energy	Total non-bonded interaction energy	Root-mean-square mismatch between structures before and after minimisation (Å)
$d(A)_8.d(T)_8$	-381	-859	-1240	1.65
$d(A)_8.d(T)_8$ + berenil.	-437	-863	-1300	1.82
$d(TA)_4.d(TA)_4$	-379	-858	-1237	1.08
$d(TA)_4.d(TA)_4$ + berenil.	-432	-856	-1288	1.55
$d(AT)_4.d(AT)_4$	-385	-843	-1228	1.06
$d(AT)_4.d(AT)_4$ + berenil.	-439	-853	-1292	1.58

bases remained perpendicular to the helix axis in the normal B-DNA conformation. The minor groove in the resultant structure was generally narrower than for the two hetero-oligomers. The sugar puckers in all oligomers were unchanged from the C2'-endo conformation, normal for B-DNA. The A-like thymine strand and the B-like adenine strand resemble the heteronomous structure found in recent molecular mechanics studies of uncomplexed dA.dT homopolymers (13), rather than the conclusion from X-ray fibre diffraction analysis of an A-DNA adenine strand and a B-DNA thymine strand (14).

Minimisation of the berenil-DNA complexes, showed similar changes in the structures of the oligomers from the starting conformation. Indeed the energy-minimised structures of the complexed oligomers and the corresponding minimised structures of the uncomplexed oligomers could be far more exactly superimposed upon each other than either could be superimposed upon the original unminimised B-DNA structure. The berenil in the complexes retained the general planarity for the crystal structure, but showed some bending and twisting, particularly at the triazene and the amidine groups. The berenil complexed with the $dA_8.dT_8$ oligomer showed the largest distortion, due to the different radii

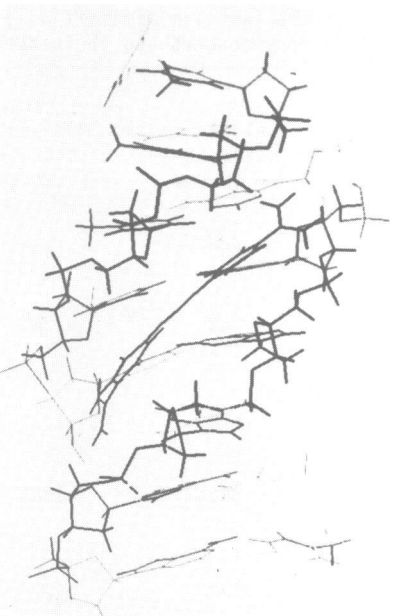

Fig.6 A view of the berenil-d$(A)_8$.d$(T)_8$ complex, after energy refinement.

of curvature of the thymine and adenine 'walls' of the minor groove in that structure (Fig. 6). It is notable that the energy minimisations indicate that the three AT sequences examined, all have equivalent energies, within the limits of the methodology. The hydrogen bonds located in the modelled complexes, were only retained in the minimised berenil-d$(AT)_4$.d$(AT)_4$ structure, and were weakened in this case. In all three cases, the amidine groups had rotated to make hydrogen bond contacts with oxygen atoms of the phosphate backbone, disrupting the bonds with the base pairs. The berenil molecules as a whole, while still remaining tightly bound in the minor groove, tended to move so as to put one amidine group in close proximity to a backbone phosphate. While an electrostatic component is surely involved in binding of berenil to DNA, the behaviour observed in the energy minimisation, is probably due to the very high charge density given to phosphates in the Kollman force field and the absence of any counterions or solvent in the modelling. The tendency of the aryl amidine groups to rotate out of the plane may be due to the inadequacy of the torsional parameters employed for these groups in the minimisations.

DISCUSSION

Modelling of various A-T polynucleotide sequences with berenil has explained this drug's ability to bind in the minor groove of B-DNA with specificity for A-T rich sequences. The drug itself has a good isohelical fit (15) with the flat surface of the A-T minor groove, which would be perturbed by the presence of guanine N2 amino groups. While binding to A-form DNA cannot be entirely discounted, its wide shallow minor groove affords few of the favourable van der Waals contacts between berenil and the sugar-phosphate backbone available in the minor groove of B-DNA. Previous molecular- modelling studies on berenil-DNA complexes (16,17) are broadly in agreement with these conclusions although they did not involve the geometry optimisations performed here. These studies also postulated a number of hydrogen-bonded contacts between drug and DNA, only some of which concur with the present work. Recent DNAase I footprinting studies with berenil (Portugal and Waring, to be published), have independently of the present work, shown that the drug has a degree of preference for 5'-AT-3', rather than 5'-TA-3' sites, in accord with the present findings. An appropriate X-ray crystallographic analysis of a berenil-oligomer complex would provide further clarification to these points.

The movements upon refinement of the charged amidine groups in becoming closer to phosphate groups, that we observe here, are paralleled by similar findings in an analogous molecular mechanics study of netropsin-DNA interactions (18); it is hoped that X-ray crystallographic analyses of these minor groove complexes will provide relevant data on solvent and counter-ion structure so that they can be included in future modelling calculations, in order to realistically dampen out these over-emphasised electrostatic interactions. If indeed they are present, the specific hydrogen-bonded contacts made by berenil could be exploited in the development of DNA-binding drugs with high sequence specificity.

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*To whom correspondence should be addressed

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