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DRUG-RECEPTOR RECOGNITION: ELECTROSTATIC FIELD LINES AT THE RECEPTOR AND DIELECTRIC EFFECTS

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1 In this paper the directional component of the vector electrostatic field emanating from a drug receptor is analysed in the three orthogonal planes.

2 B-DNA with an alternating guanine-cytosine sequence was chosen for a receptor model and the lines of force constructed for two dielectric conditions, namely the cases where firstly the dielectric is homogeneous and a constant throughout the space surrounding the receptor, and secondly where the dielectric is inhomogeneous and is treated as a vector quantity.

3 Electric field line maps indicate marked differences in the local fields situated in the helical wide and narrow grooves for the different sequences of cytosine and guanine; these variations are enhanced when the dielectric is handled as a vector.

4 The significance of electric field lines in interpreting receptor-induced ligand orientation effects is discussed since the direction of the lines is related to the torque that a receptor would impose on an attacking drug molecule.

Introduction

The three-dimensional shapes of the electrostatic fields surrounding molecules are related directly to the spatial distribution of the imbalance of residual electronic charge; these fields are vector fields in three dimensions and their analysis is consequently difficult. Electrostatic fields round small drug molecules have been well characterized and more recently those in the vicinity of a drug receptor have been studied (Perahia & Pullman, 1978; 1979; Dean & Wakelin, 1979; 1980a,b). The perturbation of the resultant electrostatic field between a drug and its receptor site can be monitored to visualize the electrostatic component of the molecular interaction. It is generally believed that for a chemical reaction to proceed efficiently, the mechanism of information transfer must satisfy two criteria: speed, and the presentation of an optimal stereochemical configuration (Porschke & Eigen, 1971; Burgen, Roberts & Feeney, 1975). If drug molecules are to 'recognize' their binding sites, in a quantum-mechanical sense, then the electric fields which operate over large distances must contain information that can be decoded back to the molecular structure of the receptor site. This decoding can be achieved mathematically by determining the divergence of the field in a particular region, since the divergence is a property of vector fields that yields information about the source. Evidence in favour of this idea has recently been provided by topological analysis of the electrostatic fields generated by segments of B-DNA containing only alternating or homopolymer sequences of guanine-cytosine (Dean & Wakelin, 1980a). Different arrangements of the bases produce striking changes in field structure at considerable distances from the atoms. Let us assume that the criteria for efficient information transfer hold true at the drug receptor; in other words that a favourable orientation is a prerequisite for a collision that produces an effective complex. Thus it would appear that for a productive collision, recognition would be well under way, if not more or less complete, before molecular contact occurs. If this were the case, then long-range forces would be responsible for producing the desired orientation. The question that now arises is: can one interpret the function of the receptor electrostatic field in terms of a torque that it would impose on *any* incoming attacking drug molecule?

An electric field is said to exist in any region of space where a charged body experiences a force related to the charge it carries. For an assembly of N charges $q_1, q_2, q_3 \ldots q_n$ situated at the points $P_1, P_2, P_3 \ldots P_n$ with position vectors $r_1, r_2, r_3 \ldots r_n$, then if $P_{(r)}$ is a general point in space, the field E_1 at P_1 due to q_1 in a medium of dielectric constant D, is given by

$$E_1 = q_1(r-r_1)/(D|r-r_1|^3)$$

The total field due to all the charges is obtained by summation:

$$E = \sum_{n=1}^{N} q_n(r-r_n)/(D|r-r_n|^3)$$

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The direction of the field at any point can be determined by vector summation of the first partial derivatives with respect to the three coordinate axes and is normal to the equipotential surface. If the dielectric properties of the solvent are not constant near to the solute ion surface but are related to the position vector, then dielectric inhomogeneities may also be included by replacing the dielectric constant by a vector $D_{(r)}$. It can be readily shown that if a molecule having a permanent dipole were placed in a uniform electric field, the molecule would align itself with the dipole turned antiparallel to the line of force. If there were no net charge on the molecule, it would experience no translational force; however, if it were positively charged the molecule would move in the direction of a line of force.

This paper is a development of previous work on electrostatic features of drug-receptor recognition (Dean & Wakelin, 1979; 1980a,b) and attempts to answer two questions. Firstly, can one interpret the electric field generated by the receptor in terms of a torque imposed on an attacking drug molecule? A novel method for analysing this problem has been developed by constructing the lines of force of the electric field round the receptor. Secondly, to what extent does dielectric inhomogeneity in the vicinity of the receptor modify the electric field near the molecular surface? The results indicate that treating the dielectric as a vector enhances the directional component of the lines of force. A subsequent paper is devoted to examining whether a correlation exists between the current findings and the computed orientation of a drug molecule approaching its receptor (Dean, 1981).

Methods

Atomic residual charges were obtained from a previous semi-empirical computation on B-DNA using the alternating guanosine-cytosine (G-C) sequence (Dean & Wakelin, 1980a). In the cases where dielectric inhomogeneity is introduced, the dielectric vector has been taken from the values given by Conway, Bockris & Ammar (1951) and plotted in Figure 1. Intermediate values were interpolated linearly. It can be seen that the dielectric value rapidly approaches the bulk constant with increasing distance from the ion. The reasons for, and problems associated with, accepting this type of dielectric behaviour are amplified in the discussion.

A right-hand cartesian coordinate system for the receptor was established so that the z-axis was coincident with the helix axis and the origin was positioned midway between the ends of the helix. The electric field lines (lines of force of the electric field) were computed, by vector addition from the three components, at 1 Å grid positions excluding those points that lay within 2 Å of the Van der Waals atomic surface. Field lines were plotted as arrows, to indicate the direction, in the x–y planes for different base-plane regions in order to distinguish differences between the sequences dCpdG (2-deoxy-D-ribose – cytosine – phosphate – 2-deoxy-D-ribose – guanosine) and dGpdC (2-deoxy-D-ribose – guanosine – phosphate – 2-deoxy-D-ribose – cytosine); the x–y and y–z planes were mapped to chart the field characteristics in the wide and narrow grooves. Atom positions were drawn on the field line maps by the routine NAMOD, modified from Beppu (1978), to relate the lines of force to molecular structure.

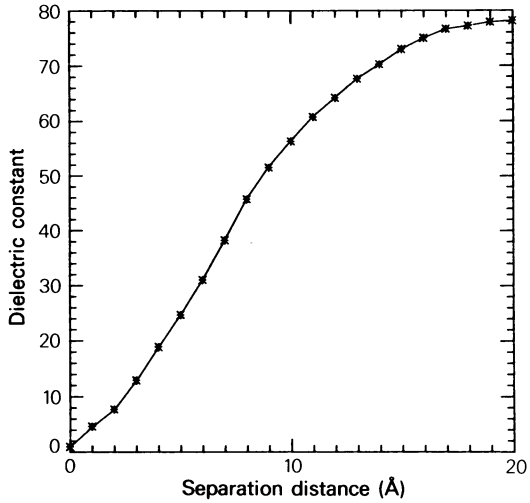

Figure 1 The relationship between distance from a point charge placed in water and the effective dielectric constant.

Results

Figures 2 and 3 show the electrical field lines in the x–y plane midway between the base pairs of a dinucleotide; Figure 4a is mapped in the x–z plane and 4b in the y–z plane. Two dinucleotide sequences, with very different coordinate characteristics, are provided by the alternating (G-C) polymer of B-DNA. This geometrical property of the nucleic acid structure therefore allows one to examine differences between the two dinucleotides dCpdG and dGpdC by observing the pattern of field lines found in the mid-planes between the triplet dCpdGpdC. A large segment of DNA, more than one turn, makes it possible to examine any field differences between terminal and central portions of the helix. Only quantitative variations in direction can be compared because the magnitudes of the vector fields are not

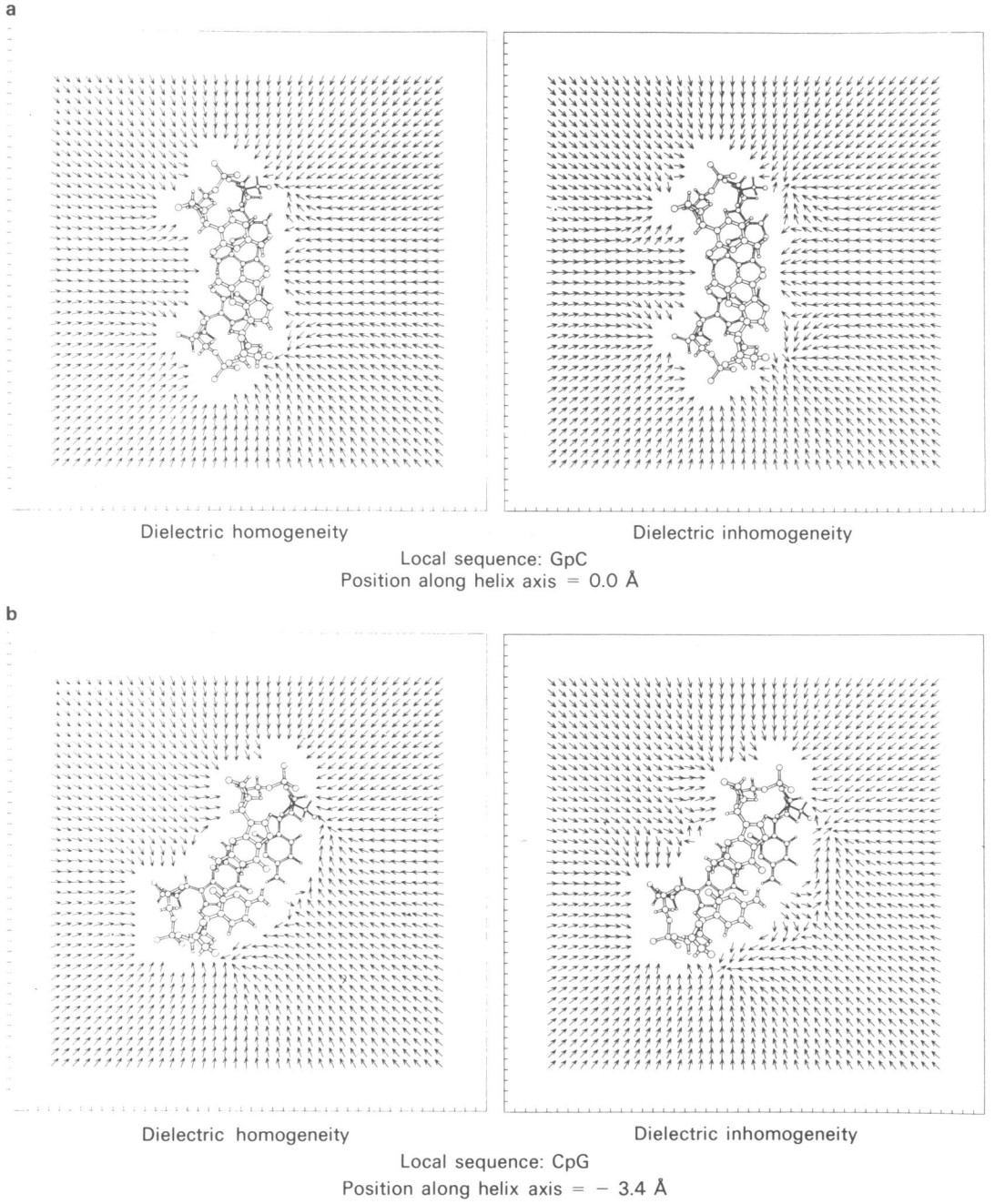

Figure 2 Electrostatic lines of force in the x–y plane surrounding a B-DNA receptor: (a) plane at 0.0Å;(b) plane at −3.4Å.

plotted on the lines of force (field strengths have been contoured previously, Dean & Wakelin, 1980a). In each figure the square has a side length of 36Å and the left-hand frame is computed with a homogeneous dielectric; computations for dielectric inhomogeneities in the electric field are illustrated in the right-hand frame.

The x–y mapping plane of Figure 2a is midway between the central dinucleotide and the local sequence, reading in the direction 5′→3′, is dGpdC.

Scale marks at 1Å intervals are traced along the sides of the frame. At the edges of the map the field lines point towards the helix axis for both dielectric conditions. The field in the wide groove (right-hand half of the frame) can be split conveniently into three regions. A central band, about 9Å wide, points into the wide groove and the field lines converge in the region where the guanine oxygen atoms show overlap. On either side of this band the lines diverge away from the wide groove and turn towards the phosphate groups. Strong similarities are observed when the computations are carried out for both dielectric conditions, directional effects are perhaps slightly more pronounced where dielectric inhomogeneities are considered. In the narrow groove, left-hand half of the frame, three similar regions are again observed. A central portion 5Å wide has the field lines pointing into the groove. On either side of this band the fields near the van der Waals surface are aligned towards two positions centred on the phosphate group and the deoxyribose-ring oxygen atom. Again in the narrow groove the maps for the two dielectric conditions are very similar.

The mapping plane in Figure 2b is displaced by −3.4Å down the z-axis so that the local sequence is dCpdG. Here the pattern of the lines of force is very different from Figure 2a. With an homogeneous dielectric only two major regions are observed and the lines point approximately in the direction of the phosphates as the molecular surface is approached. The central converging band of field lines observed in the wide groove with the dGpdC sequences is lost as the field lines diverge sharply away from the base-pair region. This difference is even more marked with dielectric inhomogeneity; the zone where the cytosine amino groups come close together shows a local repulsive field as the lines of force are reversed in direction and point away from the helix axis for about 5Å from the molecular surface. Similarly in the narrow groove the field lines also diverge for both dielectric conditions. Field perturbations near the deoxyribose-ring oxygens and the phosphates are like those observed with the dGpdC sequence.

Electric field lines between the dGpdC sequence located at −13.6Å along the helix axis (Figure 3a) are essentially very similar to those computed for the central dGpdC dinucleotide; the only small difference is that the field lines of the central band in each groove do not point symmetrically to a locus but are slightly oblique. This vectorial drift is much more pronounced when the terminal dinucleotide is considered (Figure 3b) where the sequence is dCpdG. In the wide groove (Figure 3b) there is a predominant pointing of the lines of force towards the phosphate uppermost in the picture. Similarly, in the narrow groove the field lines are generally directed towards the phosphate at the lower part of the map. Furthermore, where there is dielectric inhomogeneity this asymmetric field divergence is more noticeable. Once again, near the wide groove cytosine amino groups a repulsive region extends about 5Å from the molecular surface; in all other respects the field patterns are similar to the analogous central sequence, shown in Figure 2b). Field line asymmetry for the terminal dinucleotide may be attributed to the positioning of adjacent phosphate groups on the sugar phosphate backbone. These groups would be located approximately 36° in the anticlockwise direction and above those shown in the figure. No phosphates are found below the bottom base-pair to balance out the helical twist imparted to the field lines in the terminal region.

Electric field line maps shown in Figure 4 are constructed along the helix axis and in orthogonal planes for one turn of alternating (G-C) B-DNA. When interpreting these figures, care must be taken in relating atomic positions to the field lines since only a few atoms lie near the mapping plane. The x−z component of the field lines is given in Figure 4a. The fields are dominated by the phosphate groups and in these anionic regions different dielectric conditions do not exert a noticeably dissimilar effect. However, when the section plane cuts across a groove, then dielectric distinctions become significant. For example, in the wide groove of the lowest dinucleotide, Figure 4a, the field lines point away from the groove where there is dielectric inhomogeneity and are comparable with the results charted in Figure 3b. Similarly for the central dinucleotide, the field lines point directly into both grooves as they do in Figure 2a.

In Figure 4b the wide groove for the bottom dinucleotide faces the observer and the field lines are directed very strongly towards the phosphate groups. The 3rd, 4th and 5th base pairs up from the bottom of the diagram give the reading sequence dCpdGpdC and the fields in the wide groove are viewed on the right-hand side. It can readily be observed that in the y−z plane there are differences between the sequences dCpdG and dGpdC; with dCpdG the field lines point away from the groove but in the dGpdC sequence the lines are aligned towards the helix axis. Figure 4a and b shows that where the wide groove repulsive fields are encountered in the dCpdG sequence, the z-component of the vector is in a direction towards the centre of the helix.

Discussion

The purpose of this paper has been to chart the electrostatic field lines round a drug receptor and to examine the nature of dielectric differences on the overall pattern; the following paper links these observations to the orientation imposed on a drug

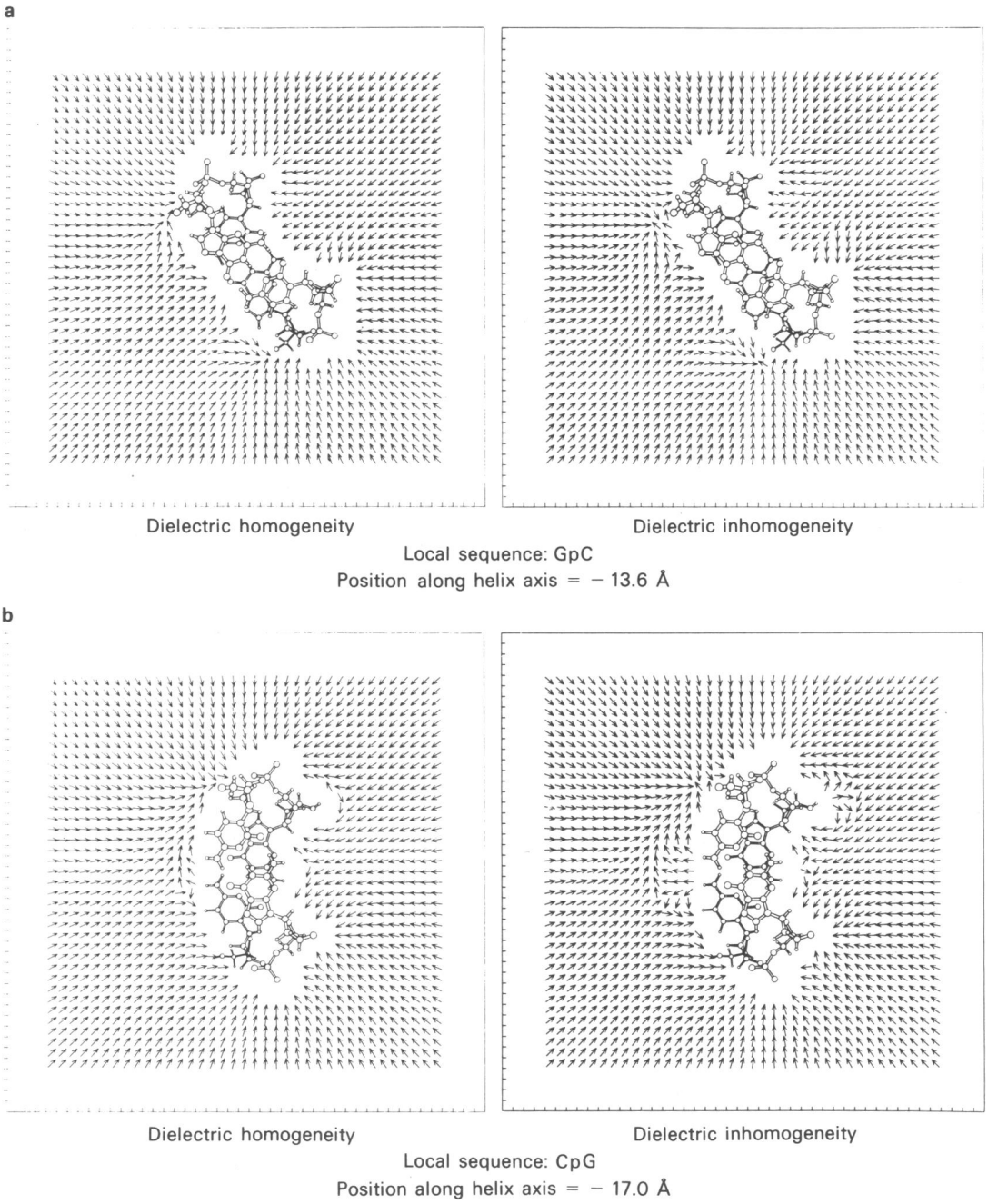

Figure 3 Electrostatic lines of force in the x–y plane surrounding a B-DNA receptor: (a) plane at −13.6Å; (b) plane at −17Å.

molecule in the vicinity of its receptor. The rationale for using DNA as a model for a drug receptor has been given previously (Dean & Wakelin, 1979) and this work is part of a continuing study of the action of long-range forces, originating from the receptor, on the interaction of an approaching drug molecule. The computational procedure developed here provides a general method for observing the electrostatic lines of force for any receptor whose geometrical structure is known and for which the atomic charges can be

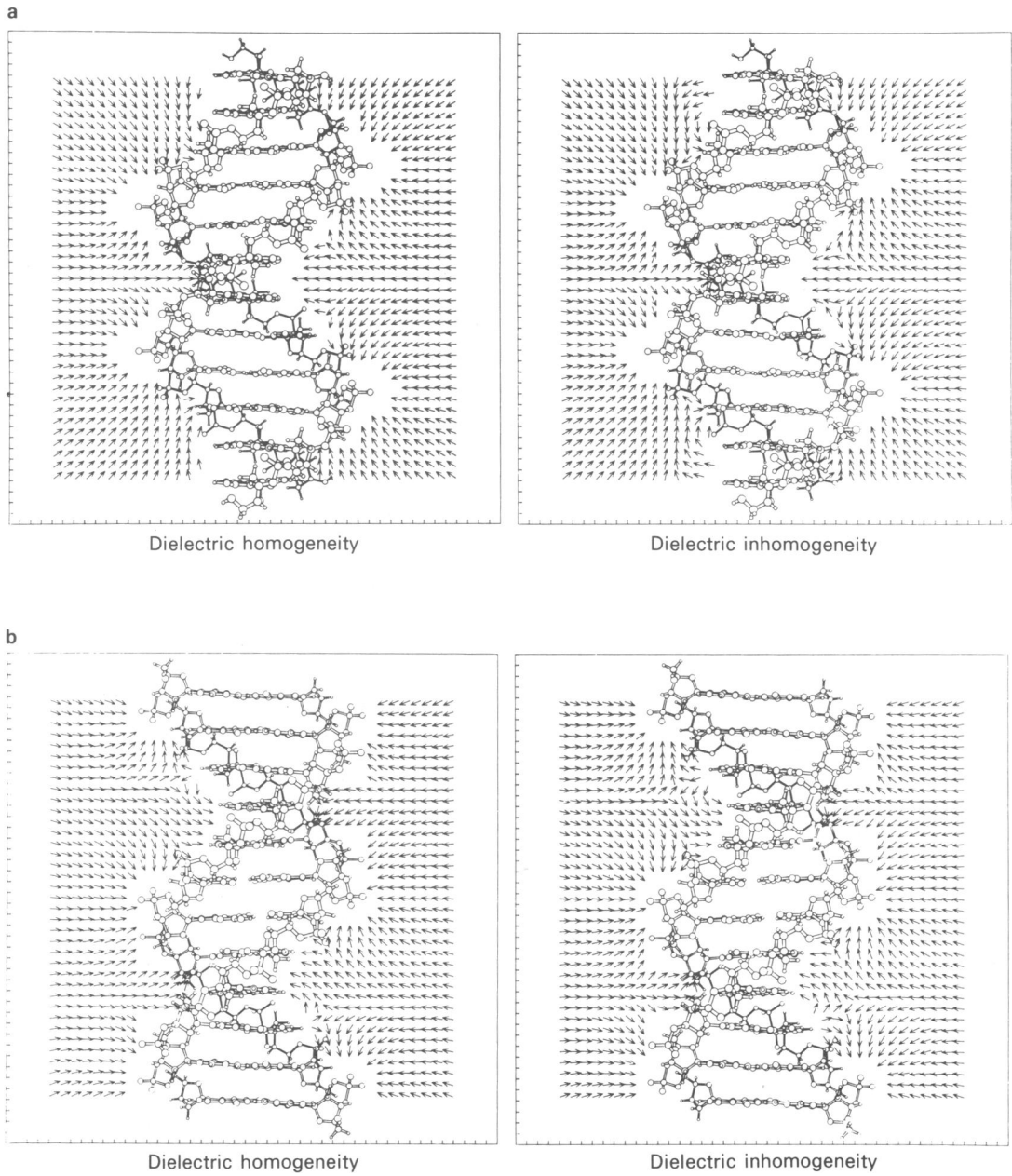

Figure 4 Electrostatic lines of force along the helix axis for 1 turn of B-DNA: (a) the x–z plane; (b) the y–z plane.

calculated. This information is important because the electrostatic field surrounding a molecule is a vector and not a simple scalar quantity. Analytical methods that handle the magnitude component only, are inadequate to describe the function of the field, but once one has both components one can begin to assess the operational characteristics of the receptor field at any position. The lines of force are, therefore, an integral part of the recognition process occurring between a drug molecule and its receptor. Before dealing with the implications of these findings it is necessary to consider the approximations used in the computations.

Dielectric effects arise from the shielding of charge

by solvent atoms; however, the behaviour of solvent dielectric near to a macromolecular skeleton is little understood. Close to the molecular surface, where the number of intervening solvent molecules is small, it is generally believed that the dielectric approaches unity, whereas, at large distances the dielectric is taken to be that associated with the bulk solvent. It is the variation at intermediate distances that is least understood. Hopfinger (1973) has proposed a linear relationship in this range based on an effective separation of charges. The method adopted in this paper has been to take the dielectric values tabulated by Conway *et al.* (1951) for a point charge immersed in water and then to assign a value for the dielectric proportional to the position vector between the atom and the point at which the field is to be calculated. It is assumed that the 22 anionic charges of the DNA dominate the dielectric properties of the solvent, therefore all atoms have been treated equally, irrespective of their charge and position in the helix. The effect on the dielectric of organizing solvent molecules into hydration shells around the helix has been ignored. Of course, in practice the position vector often passes through a substantial atom conglomerate of the receptor with a different dielectric behaviour from that of the solvent; for computational purposes such a pathway is treated as one through pure solvent. Furthermore, the finite size of the atoms has been neglected when measuring the separation distance. These simplifications are necessary to make the computing costs reasonable and are consistent with the procedure used in the following paper where large-scale iterative methods are employed. The desire has been to illustrate differences that arise when homogeneous and heterogeneous dielectric conditions are taken into account; the results are, therefore, only qualitative reflections of an empirical approach to the dielectric problem.

The use of a dielectric vector to take into account inhomogeneities rather than the use of a homogeneous dielectric results in a more pronounced effect on the field line maps and is most clearly visible near the base-pair region and close to the molecular surface. This observation is to be expected from the distance-dielectric relationship used in Figure 1. Further out, that is greater than 5Å, the force vectors are little affected by how the dielectric is calculated, although the magnitude of the molecular interaction energy is considerably different (Dean, 1981).

The field line maps for the alternating (G-C) B-DNA receptor, in a helically stacked and unopened state, show marked differences for adjacent base-pairs. The dGpdC sequence has a broad band attractive field in both grooves, whereas the dCpdG sequence has a strong repulsive field in the wide groove and a divergent field in the narrow grooves. Attractive regions in the wide groove occur when the guanine oxygen atoms are nearly stacked and repulsive zones prevail when cytosine amino groups are near together. This arrangement of bases in B-DNA would produce in the wide groove alternating repulsive and attractive fields with a period of 6.8Å along the helix axis. These observations complement the findings of previous calculations of field magnitude in the grooves of B-DNA (Dean & Wakelin, 1980a). Directional effects are imparted to the field by: the phosphate groups, the relative positions of the guanine oxygen atoms, the cytosine amino groups, and the ring oxygen of deoxyribose. At the end of the helix the field structure is perturbed significantly from that encountered centrally and this difference can be attributed to the asymmetrically arranged contributions from the charged phosphates.

In the following paper the orientation energies of two drugs, ethidium and its carboxyphenyl derivative, have been calculated for different positions in the receptor field, mapped in this work, where rotational freedom is unhindered by atom collisions (Dean, 1981). A good correlation was found between molecular orientation and the direction of the receptor field when the drug dipole was aligned approximately anti-parallel to the field line. Furthermore, in the case of carboxyphenylethidium, the energy associated with the torque generated by the receptor electrostatic field was considerable and could perturb the orientation of a significant proportion of drug molecules in solution. Therefore, computation of electrostatic field lines can be used satisfactorily to produce partial orientation maps in two dimensions for polar molecules near a receptor site provided that discontinuities in the field are not encountered; however, no statement is provided by the charts about rotation round the dipole axis. Analysis of the field lines yields, at a glance, very useful information about the mechanism of long-range recognition, at different positions, between a drug and its receptor.

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References

BEPPU, Y. (1978). NAMOD: A computer program for drawing perspective diagrams of molecules. *Q.C.P.E.*, **11**, No. 370.

BURGEN, A.S.V., ROBERTS, G.C.K. & FEENEY, J. (1975). Binding of flexible ligands to macromolecules. *Nature, Lond.*, **253**, 753–755.

CONWAY, B.E., BOCKRIS, J. O'M. & AMMAR, I.A. (1951). The dielectric constant of the solution in the diffuse and Helmholtz double layers at a charged interface in aqueous solution. *Trans. Faraday Soc.*, **47,** 756–766.

DEAN, P.M. (1981). Drug-receptor recognition: molecular orientation and dielectric effects. *Br. J. Pharmac.* **74,** 47–60.

DEAN, P.M. & WAKELIN, L.P.G. (1979). The docking manoeuvre at a drug receptor: a quantum mechanical study of intercalative attack of ethidium and its carboxylated derivative on a DNA fragment. *Phil. Trans. R. Soc.*, B, **287,** 571–604.

DEAN, P.M. & WAKELIN, L.P.G. (1980a). Electrostatic components of drug-receptor recognition: 1, Structural and sequence analogues of DNA polynucleotides. *Proc. R. Soc.* B, **209,** 463–471.

DEAN, P.M. & WAKELIN, L.P.G. (1980b). Electrostatic components of drug-receptor recognition: 2, The DNA-binding antibiotic actinomycin. *Proc. R. Soc.* B, **209,** 473–481.

HOPFINGER, A.J. (1973). *Conformational Properties of Macromolecules.* New York: Academic Press.

PERAHIA, D. & PULLMAN, A. (1978). The molecular electrostatic potentials of the complementary base pairs of DNA. *Theoret. Chim. Acta (Berl.),* **48,** 263–266.

PERAHIA, D. & PULLMAN, A. (1979). The molecular electrostatic potential of the B-DNA Helix. II. The region of the adenine-thymine base pair. *Theoret. Chim. Acta (Berl.),* **50,** 351–354.

PORSCHKE, D. & EIGEN, M. (1971). Cooperative non-enzymic base recognition. 3. Kinetics of the helix-coil transition of the oligoribouridylic·oligoriboadenylic acid system and of oligoriboadenylic acid alone at acidic pH. *J. mol. Biol.*, **62,** 361–381.

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