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DRUG-RECEPTOR RECOGNITION: MOLECULAR ORIENTATION AND DIELECTRIC EFFECTS

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1 The orientation of ethidium and its carboxyphenyl derivative was computed at various positions around one turn of the B-DNA helical receptor.

2 Dielectric effects of solvent were included using values for the bulk constant and a dielectric vector to simulate dielectric inhomogeneities.

3 From the electrostatic energy values for the orientations, the corresponding Boltzmann distributions were obtained to assess the orientation restriction to the drug imposed by the receptor.

4 A correlation was observed between the direction of the drug molecular dipole and the local line of force generated by the receptor vector field.

5 These findings are discussed with respect to the phenomenon of drug-receptor recognition.

Introduction

Drug-receptor recognition is often characterized by a stereospecific binding reaction between the two molecular species. The final event in the recognition process for a productive collision to occur is an accurate geometrical positioning of atoms in the drug molecule with respect to those of the receptor binding site. This spatial juxtaposition may be brought about by initial chance alignments of the two interacting coordinate systems, or orientation-dependent forces may operate to modify the alignment as the reaction proceeds. In a model study, taking a fragment of DNA as a receptor for the intercalative drug ethidium and its carboxy derivative, it has been shown that as the drug molecule approaches the nucleic acid its rotatory freedom is curtailed even though atomic collisions may not occur. Quantum mechanical analysis of this receptor-induced ligand orientation revealed that two components of the interaction energy are predominant, namely the electrostatic interaction between the two species and an induced electron delocalization in the drug molecules (Dean & Wakelin, 1979). Studies on the molecular electrostatic field of a larger DNA segment have shown that the field structure varies in magnitude near to the molecular surface and that variations are introduced by the base sequence (Perahia & Pullman, 1978; 1979; Dean & Wakelin, 1980). Two considerable problems arise when attempting to investigate in detail the receptor-induced ligand orientation event for a larger portion of receptor. Firstly, the extensive cost of performing calculations, even by approximate molecular orbital methods, prohibits a full study of rotations around the three coordinate axes; it is only feasible to work with small molecular fragments and do partial orientation computations. Secondly, there is no satisfactory quantum-mechanical method of including solvent dielectric effects in the calculations. Nevertheless, by using a less rigorous methodology both difficulties can be circumvented to give an idea of the extent of dielectric effects on the complete orientation problem. This procedure employs simple electrostatic theory based on point charges and neglects any electronic delocalization phenomena; it must be stressed from the outset that only the electrostatic term in the interaction is considered here.

In the preceding paper (Dean, 1981), a technique was developed for charting the direction of the torque at any point generated by the electrostatic vector field of the receptor. The objective of the present paper has been to compute the electrostatic energy for the orientation of a drug molecule in the vicinity of the receptor and to compare the minimum energy position with the torque imposed by the receptor field. Modifications of the interaction between ethidium, or its carboxy derivative, and B-DNA (12 base-pairs long) by the intervening dielectric have been examined. These dielectric conditions were: the *in vacuo* state with a dielectric value of unity; an homogeneous dielectric with a value equivalent to that of bulk water; and a case with dielectric inhomogeneity where the dielectric was taken to be a vector quantity intermediate between the extremes for a vacuum and bulk water. The statistical significance of the effect of the orientation energies on a population of drug molecules near the receptor is assessed and the correlation between the imposed torque and the preferred orientation of the drug is

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evaluated. These findings are discussed with respect to their possible importance in the mechanism of drug-receptor recognition.

Methods

Orientation relationships

The coordinate systems for both molecules, shown in Figure 1, are fixed on their respective centres of mass. The z-axis of B—DNA is coincident with the helix axis. At zero z value the positive x-axis lies in the wide groove and the negative x-axis is in the narrow groove. The heteroaromatic ring of ethidium is in the x-y plane and the C = N bond is at right angles to the y-axis. The orientation of the drug at any position with respect to the receptor can be computed by the orthogonal transformation of Fraga, Saxena & Torres (1978) with the translation shift of the centres of mass expressed in polar coordinates.

Calculation of the intermolecular interaction energy

The computational method was adopted from Caillet & Claverie (1975) where the interaction energy is considered as four contributions, namely, electrostatic, polarization, dispersion and repulsion energy terms. Dielectric modifications of the electrostatic term were examined under three conditions as before (Dean 1981); (a) the *in vacuo* state where the dielectric is homogeneous and has a value of 1; (b) an homogeneous state with a dielectric constant equal to that of bulk solvent; (c) the case of dielectric inhomogeneity where the dielectric is a vector and dependent on interatomic separation as described in the previous paper; data for the dielectric values were taken from Conway, Bockris & Ammar (1951). Residual atomic charges used in this work were previously calculated by the CNDO/2 procedure for DNA and PCILO for the drugs (zeroth order) (Dean & Wakelin, 1979; 1980). Directional components of the electric field were computed by methods outlined in the preceding paper.

Results

Orientation of drug molecules near the receptor

Preliminary computations were performed with the drug molecules to assess the freedom for rotation without collision between atoms of the drug and the receptor. Rotation was considered to be free when the nuclear repulsion energy contribution *in vacuo* was less than 1% of the total interaction energy. Free rotation occurred at all positions for a radius of 18 Å from the helix axis and this radius was chosen for all the orientation calculations. The four components of the total interaction energy for ethidium and its carboxylated derivative with the DNA receptor are given in Table 1. For each of the drugs the largest part is the electrostatic energy, although the polarization energy is significant. At this separation the nuclear repulsion and dispersion energies are very small. However, the difference between the minimum and maximum energies, corresponding to the most and least favourable orientation, is greatest for the electrostatic contribution with both drugs. Only the electrostatic component of the interaction was then considered in all further calculations to save computing time and minimize the cost. Orientations round the

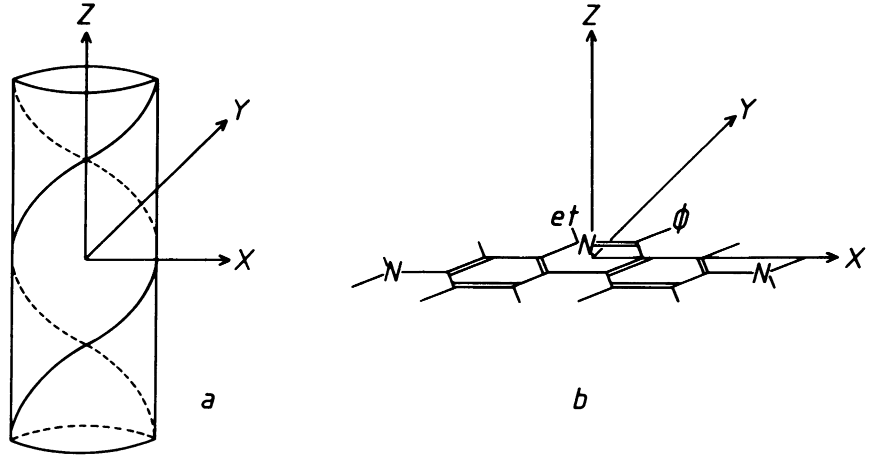

Figure 1 Definition of the coordinate systems (a) for the helical B—DNA receptor; (b) for the drug molecule.

Table 1 Interaction energy components for ethidium and carboxyphenylethidium at the minimum and maximum energy orientations *in vacuo* (kJ mol^{-1})

Energy	*Ethidium* *Minimum*	 *Maximum*	 *Difference*	*Carboxyphenylethidium* *Minimum*	 *Maximum*	 *Difference*
Electrostatic	−1528.62	−1450.87	77.75	−325.37	442.66	768.03
Polarization	−123.47	−113.02	10.45	−121.38	−136.52	15.14
Nuclear Repulsion	0.17196×10^{-6}	0.31876×10^{-8}	0.16870×10^{-6}	0.15027×10^{-5}	0.96056×10^{-6}	0.54220×10^{-6}
Dispersion	−0.9325	−0.7603	0.1722	−0.9835	−1.3702	0.3867
Total	−1653.19	−1564.57	88.62	−447.67	304.59	752.26

Centre of mass positions for the drug molecule are at Z = 0, R = 18 Å, Ø = 0°.

drug centre of mass were performed at 36° intervals thus producing 1000 orientations for each position. The centre of mass was then displaced by 30° steps for measurements round a hemicircular arc. Starting positions were adjusted so that the drug molecule was directly in the wide or narrow groove. Four planes for these arc positions were taken at 0, −3.4, −13.6 and −17 Å along the helix axis and correspond to the intercalative planes where the lines of force were charted in the previous paper (Dean, 1981). The stereoscopic illustrations, drawn by the program of Beppu (1978), in Figures 2, 3, 4 and 5 show the orientations of ethidium or carboxyethidium at plane positions Z = 0 or −17 Å for calculations performed with dielectric homo- or inhomogeneity. Only atoms of the related dinucleotide portion are drawn to simplify the view. These sketches show the minimum energy orientations for each centre of mass position; the corresponding energy values are graphed in Figures 7 and 8.

The stereoscopic illustrations for ethidium placed round the central dinucleotide region (Figure 2a) show that the major axis of the heteroaromatic ring is directed towards the helix axis and that the ethyl substituent is positioned nearer to the nucleic acid than the aryl group. This corresponds approximately to the positive end of the dipole of ethidium pointing to the helix axis. In both the wide and narrow grooves the minor axis of the chromophore is roughly parallel with the helix axis. Near the sugar phosphate region, the orientation is modified so that the plane of the aromatic ring lies in the base plane. When the alignment is computed in the plane of the terminal dinucleotide, the helical position is rotated by 1/2 a turn so that the narrow groove of Figure 2b is on the opposite side from that of Figure 2a. At Z = −17 Å (Figure 2b) the heteroaromatic portion is turned in both grooves to follow the direction of the screw of the right-handed helix. As with the central plane, the dipole is rotated with the positive end aimed at the helix axis. Tilting instead of planarity is found in the phosphate region. In all positions the ethyl moiety is directed to the centre of the helix.

The effect of dielectric inhomogeneities on the orientation of ethidium are shown steroscopically in Figure 3. In the central plane (Figure 3a) the preferred orientations in the wide and narrow grooves are similar to those observed with a homogeneous dielectric and with drug dipole positive end pointing towards the helix axis. Similarly at Z = −17 Å (Figure 3b) the plane of the heteroaromatic ring system is tilted in each groove to follow the helical turn of the receptor. However, at the position Ø = 90°, near the sugar phosphate backbone, the ring system is in the inspection plane contrary to that observed in Figure 2b. One may conclude that for ethidium there are marked orientation differences between positions at

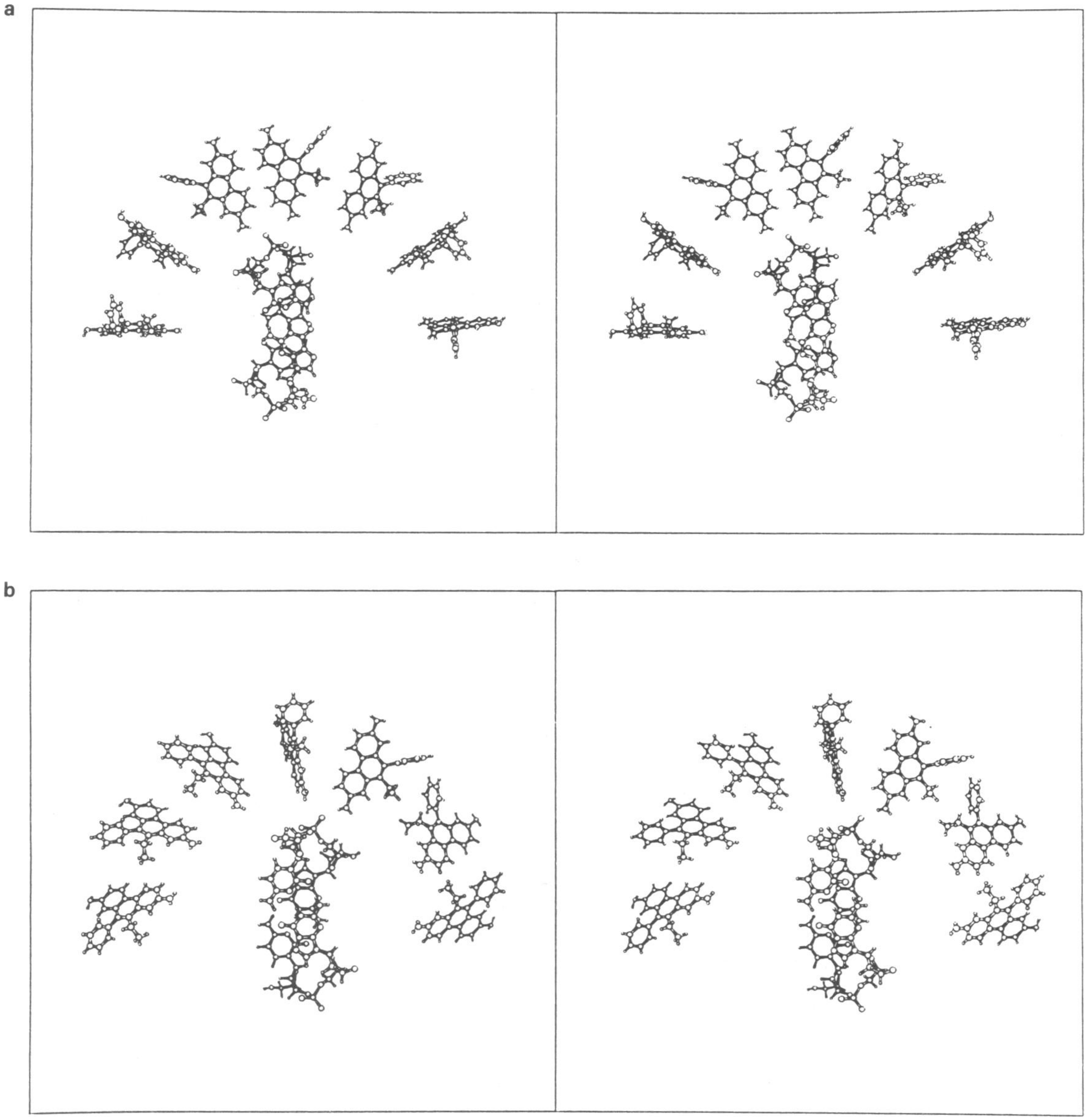

Figure 2 Stereoscopic view of the orientation of ethidium round B—DNA with an homogeneous dielectric: (a) position of inspection plane: Z = 0 Å; (b) position of inspection plane: Z = − 17 Å.

the centre and ends of the helix, whereas only slight changes are introduced by dielectric inhomogeneities in the computations.

The orientations of carboxyphenylethidium calculated with a homogeneous dielectric are illustrated in Figure 4, and are different from those adopted by ethidium in Figure 2. In the central plane (Figure 4a) the major axis of the chromophore is tilted against the helical turn in the wide and narrow grooves. The carboxyphenyl group points directly away from the helix axis for all positions, suggesting that the molecule is strongly aligned with the dipole positive end aimed at the centre of the helix. At the terminal region (Figure 4b) the major axis of the chromophore is parallel to the helix axis. However, in the sugar phosphate region the molecule is turned so that the minor axis is parallel to the helix axis and a phenanthridine amino group is towards the phosphate anionic charge. At all positions the carboxyphenyl group points away from the centre of the helix. When

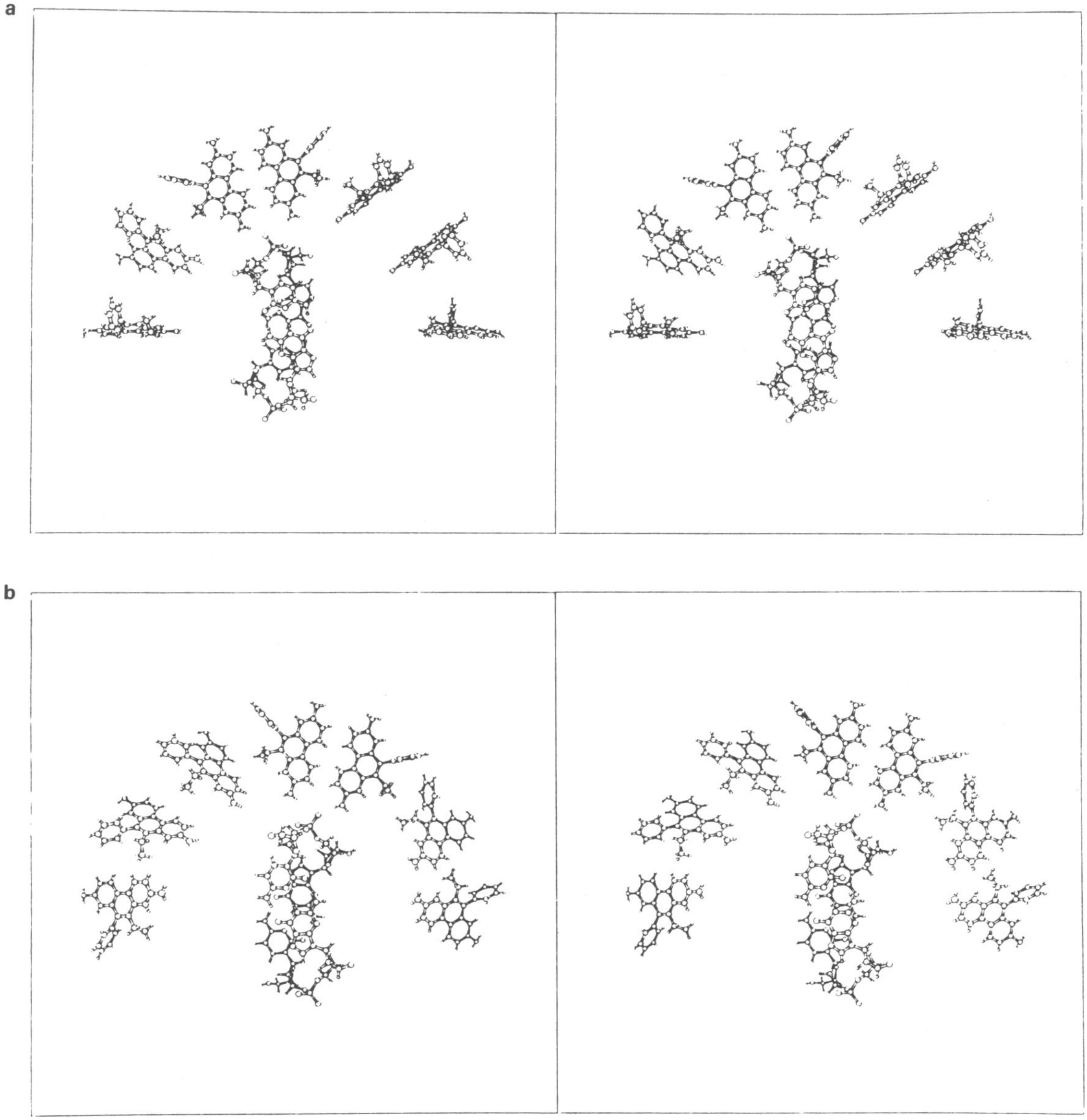

Figure 3 Stereoscopic view of the orientation of ethidium round B—DNA with an inhomogeneous dielectric: (a) position of inspection plane: Z = 0 Å; (b) position of inspection plane: Z = −17 Å.

dielectric inhomogeneity is included in the calculations, positional differences are clearly observed in both planes particularly near the sugar phosphate backbone (Figure 5a and b). In each case the heteroaromatic ring is turned into the inspection plane with possible interaction between the phenanthridinium amino groups and the anionic charge. Orientations in the grooves are similar for the two dielectric conditions.

The electrostatic interaction energy between ethidium and B—DNA is illustrated in Figure 6 for both dielectric states; the line of attack is directly into the wide groove of the central plane. A strong attractive interaction is observed as the drug molecule approaches the receptor. Greater attraction is found where dielectric inhomogeneity is present over a distance of 40 Å and at 13 Å is about $-6\,\mathrm{kJ.mol^{-1}}$ more pronounced. *In vacuo* values are given for comparison on the right ordinate scale of Figure 6 for the curve depicting the homogeneous dielectric.

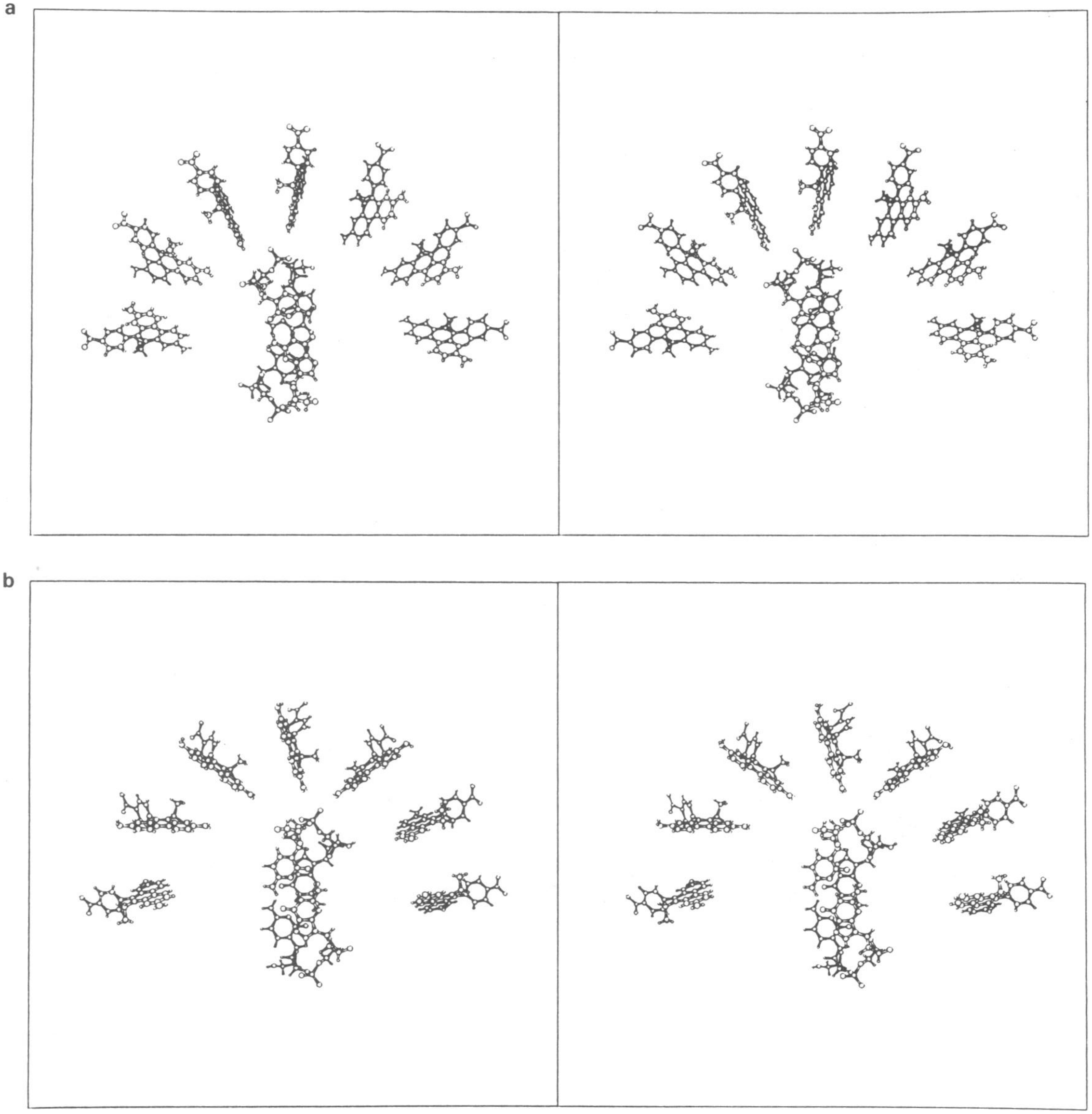

Figure 4 Stereoscopic view of the orientation of carboxyphenylethidium with an homogeneous dielectric: (a) position of inspection plane: Z = 0Å; (b) position of inspection plane: Z = −17Å.

Orientation energy minima and maxima for ethidium and carboxyphenylethidium are plotted respectively in Figures 7 and 8 against position defined by the polar coordinate angle Ø. For conditions of homogeneous dielectric the *in vacuo* values are on the right ordinate scale. The abscissae are plotted in reverse order to make comparison with the stereo pictures easier; the wide groove location of Figure 2a is 0° with respect to the x-axis of the DNA coordinate system; all angles Ø are positive anticlockwise with respect to this point. Minimum energy curves marked A, B, C, D are respectively in the inspection planes, 0, −3.4, −13.6 and −17Å; the double letters signify the maximum orientation energy curves. The radial minimum energy position round the helix in the plane Z = 0Å of Figure 7a occurs in the region 120–150° and is close to the narrow groove side of the phosphate. The narrow groove is electrostatically more attractive to ethidium than the wide groove by about $-0.5\,\mathrm{kJ\,mol^{-1}}$ ($-40\,\mathrm{kJ\,mol^{-1}}$ *in vacuo*); this obser-

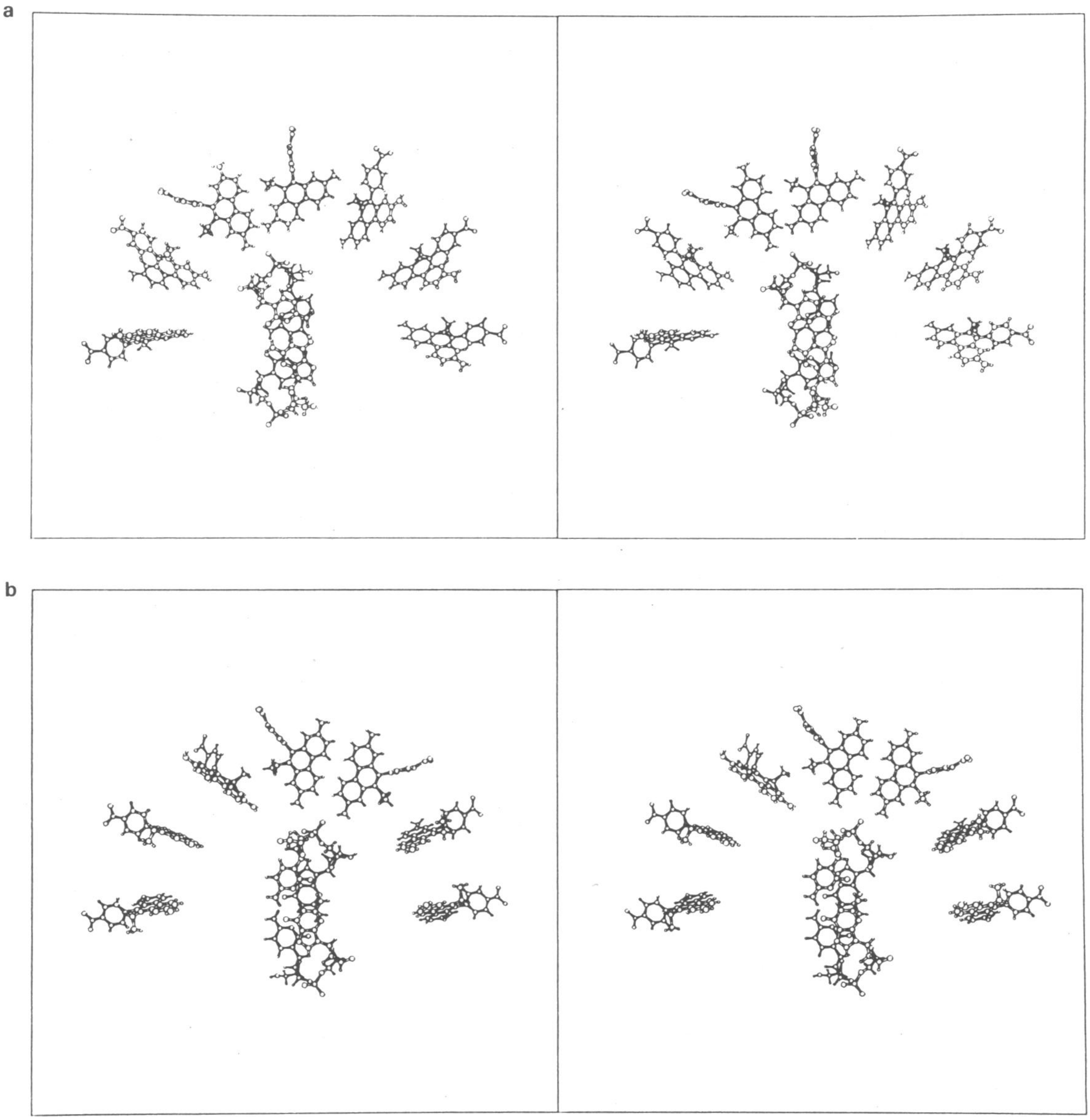

Figure 5 Stereoscopic view of the orientation of carboxyphenylethidium with an inhomogeneous dielectric: (a) position of inspection plane: Z = 0 Å; (b) position of inspection plane: Z = −17 Å.

vation is qualitatively consistent for all planes investigated. Orientation maxima are about 1 kJ mol^{-1} higher (80 kJ mol^{-1} *in vacuo*) for all positions. At Z = −3.4 Å, curve B of Figure 7a, the angles are displaced by 36° rotation of the base pairs, otherwise the shapes and values of the curve are similar to curve B. Towards the end of the helix the energy minima and maxima exhibit greater variation, curves C and D. For curve D the minimum occurs at 90° and is 3 kJ mol^{-1} (240 kJ mol^{-1} *in vacuo*) greater than the global minima in curve A, signifying less electrostatic attraction by the receptor at the end of the helix.

The effect of dielectric inhomogeneities on the orientation energies for ethidium are shown in Figure 7b. The shapes of the minimum energy curves are quite dissimilar from analogous curves in Figure 7a although the graphs of the maxima are much the same. Sharp minima are formed for each plane investigated. In the central plane, the global minimum is found at 120° and is −4.5 kJ mol^{-1} less than the wide

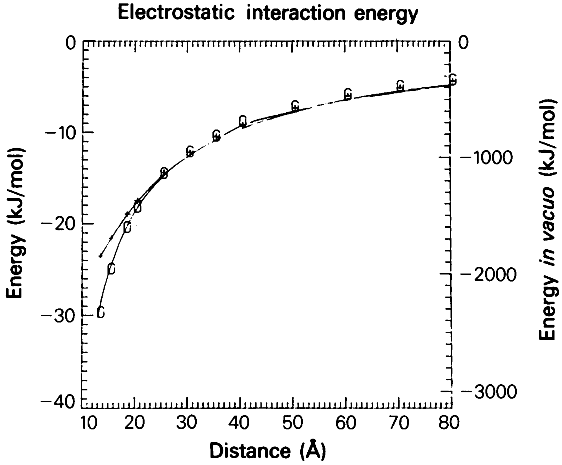

Figure 6 Relationship between interaction energy and separation distance for ethidium and B—DNA. The right ordinate scale gives the values *in vacuo* for the curve marked (+); the left ordinate scale gives the values for homogeneous dielectric (+) and inhomogeneous dielectric (0).

groove minimum energy position. In all planes the minimum is near to the phosphate region. There is about a $-4\,\mathrm{kJ\,mol^{-1}}$ drop in energy between the end and middle of the helix.

Orientation restrictions

The orientation energy minima and maxima for the zwitterion carboxyphenylethidium with homogeneous dielectric conditions are displayed in Figure 8a. All minimum energy positions have an attractive interaction energy of between -3 to $-5\,\mathrm{kJ\,mol^{-1}}$ (-240 to $-400\,\mathrm{kJ\,mol^{-1}}$ *in vacuo*), and where the orientation is unfavourable the energy values are between $+5$ and $+9\,\mathrm{kJ\,mol^{-1}}$ (400 to 720 $\mathrm{kJ\,mol^{-1}}$ *in vacuo*). Most favourable energy locations are found near the phosphates and the least unfavourable ones in the grooves of the helix. Dielectric inhomogeneity is taken into account in Figure 8b. Electrostatic attraction is increased significantly to $-9\,\mathrm{kJ\,mol^{-1}}$ for the most favourable position although the curves for each inspection plane are relatively flat compared to those obtained for the worst orientations. Dielectric inhomogeneity has a very striking effect on the unfavourable interactions. The energies span a range of $+10$ to $+73\,\mathrm{kJ\,mol^{-1}}$ with the major difference occurring in the sugar phosphate backbone region; the least unfavourable energy positions are found in the grooves of the DNA helix.

Orientation energy barriers for both drug molecules have been observed in the vicinity of the receptor. Do these barriers represent a significant restriction to rotation within a population of drug molecules? For a rotamer population where the phase-space has dimensions of the rotation angles, α, β, γ, the probability P_i of finding a molecule with a particular energy E_i is given by the Boltzmann expression:

$$P_i = \frac{n_i}{N} = [\exp(-E_i/kT)]/Z$$

where k is the Boltzmann constant, T is the tempera-

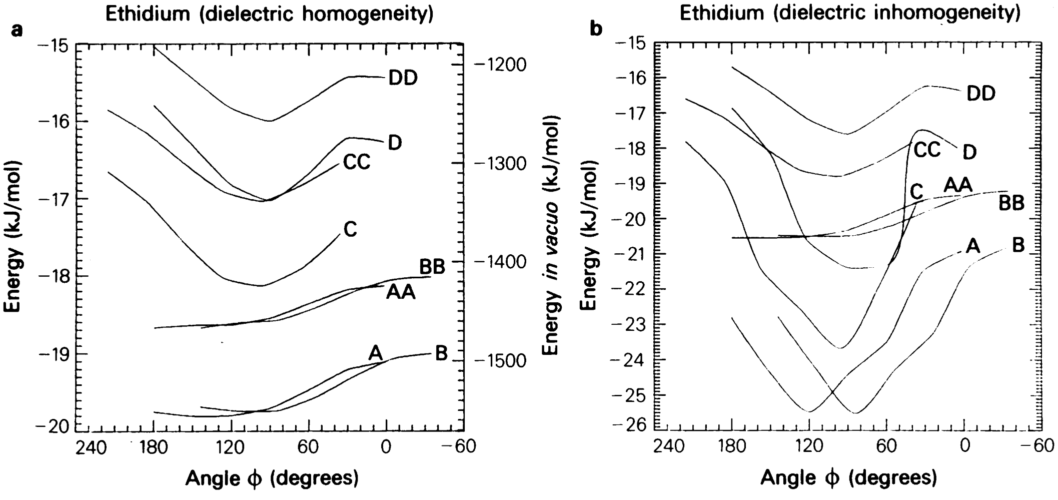

Figure 7 Minimum and maximum orientation energies for ethidium at various positions round the receptor: (a) homogeneous dielectric; (b) inhomogeneous dielectric. Single letters denote curves for the minimum energy, double letters are for the maximum energy. A = 0 Å, B = −3.4 Å, C = −13.6 Å, D = −17 Å for inspection planes down the z-axis.

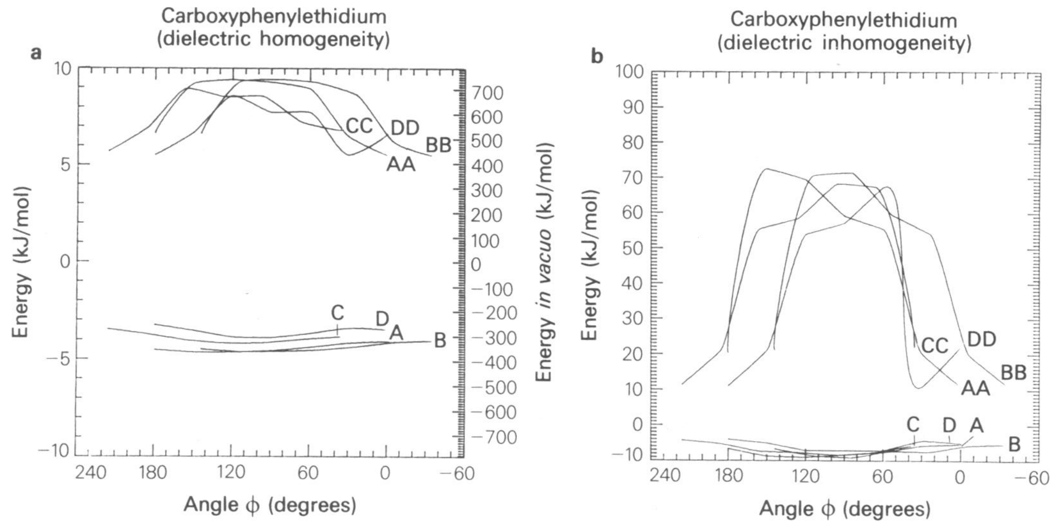

Figure 8 Minimum and maximum orientation energies for carboxyphenylethidium at various positions round the receptor: (a) homogeneous dielectric; (b) inhomogeneous dielectric. Positions defined in the legend of Figure 7.

ture, n_i is the number of molecules with energy E_i out of a population of N molecules and Z is the normalizing constant for the phase-space,

$$Z = \iiint_{0}^{2\pi} \exp(-E_{(\alpha,\beta,\gamma)}/kT)\, d\alpha d\beta d\gamma.$$

If there is a complete set of energy values throughout the phase-space, the fraction of molecules with orientation energies less than, or greater than, a particular value can be obtained by integration. Thus, within a population the statistical significance of an energy barrier to orientation can be assessed. Furthermore, if an arbitrary value is taken for the fractional number of molecules, e.g. 0.9, with energies less than a certain associated level, it is possible to find by integration, the equivalent fraction of phase-space restricted to this proportion of the population. Computations of this type were performed for orientations at each of the 7 positions in the 4 inspection planes for ethidium and carboxyethidium *in vacuo* and with both dielectric conditions.

In Figure 9a the orientation restriction placed on ethidium *in vacuo* at 18Å radius from the helix axis is

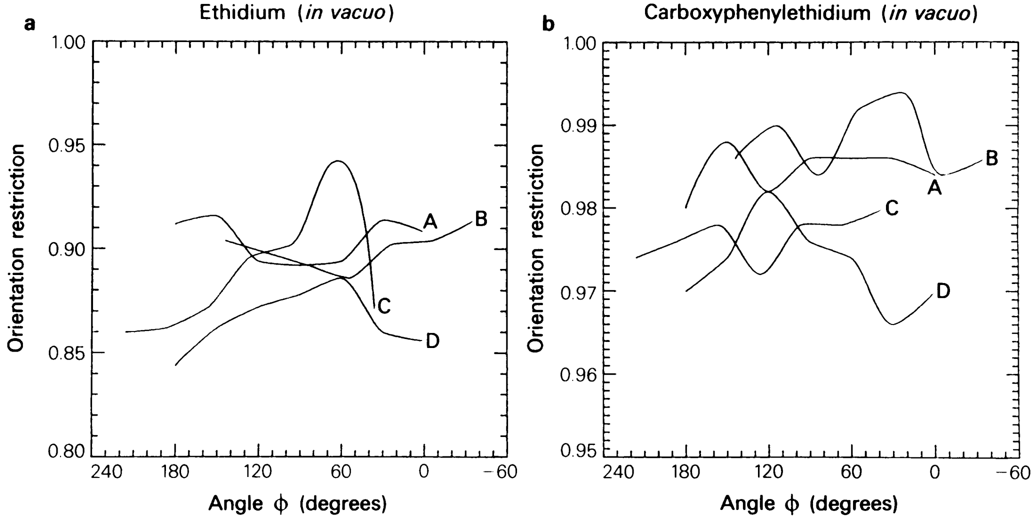

Figure 9 Orientation restrictions *in vacuo*: (a) ethidium; (b) carboxyphenylethidium. Positions defined in the legend of Figure 7.

plotted against polar angle Ø. It can be seen that 90% of the molecular population shows a large restriction of the orientation phase-space of between 0.85 to 0.95 (where the total possible phase-space is 1). If this constraint were to be apportioned equally between the three rotation axes, a rotational freedom of only 6° to 18° would be allowed out of the total possible 360° around each axis. With carboxyphenylethidium (Figure 9b) the orientation restriction *in vacuo* is even greater with a limitation of 0.96–0.99 of the phase-space. This corresponds to a small rotational freedom of 1°–4° around each axis. In contrast to the results *in vacuo*, the inclusion of dielectric homogeneity or inhomogeneity did not produce any orientation restriction for ethidium at a probability level of 0.9. However, with carboxyphenylethidium restriction is apparent but it is not as great as the *in vacuo* situation. For a homogeneous dielectric, Figure 10a, the orientation restriction lies between 0.04–0.17, equivalent to a rotational restraint of 123°–200° around each axis. Near the centre of the helix the orientation restriction is greatest in the narrow groove, whereas, towards the terminal region, it is largest near the sugar phosphate backbone; in the two grooves, restriction is least in the wide groove. The inclusion of dielectric inhomogeneity, Figure 10b, increases the restriction on the rotation of carboxyphenylethidium near the receptor to 0.15–0.37, amounting to 200°–260° around each axis. For all four planes the narrow groove is more constrained than the wide groove and towards the end of the helix the drug orientation restriction is greater near the sugar-phosphate region. These orientation restrictions are imposed electrostatically and do not include nuclear repulsion effects.

Drug orientation and receptor field lines

Both ethidium and its carboxyphenyl derivative show orientational preferences, mediated by electrostatic forces, at various locations surrounding the DNA helical receptor. It is possible to resolve the mutual electrostatic interaction separately into drug and receptor components by comparing the direction of two associated vectors at the minimum energy orientation. In Figures 11 and 12 the direction of the positive end of the drug dipole, calculated from the residual charges and originating from the centre of mass, is compared with the direction of the field line passing through the same position (Dean, 1981); the angular difference between the directions is termed the angular discrepancy and is plotted as a scattergram against polar position round the receptor. A maximum discrepancy of 180° would occur if the field line and positive end of the dipole pointed in opposite directions and a chance arrangement would be expected to give a mean angular discrepancy of 90°. Ethidium (Figure 11a) shows a small angular discrepancy of 20.6° ± 0.9° (s.e.mean for each plane) with a homogeneous dielectric, whereas with dielectric inhomogeneity the discrepancy is increased significantly to 52.5° ± 1.9° (Figure 11b). The results with carboxyphenylethidium are qualitatively similar but with a smaller angular discrepancy, 13.3° ± 3.1° (Figure 12a) and 19.9° ± 1.6° (Figure 12b) respectively for the homogeneous and inhomogeneous dielectric conditions. A larger discrepancy is noticeable in the

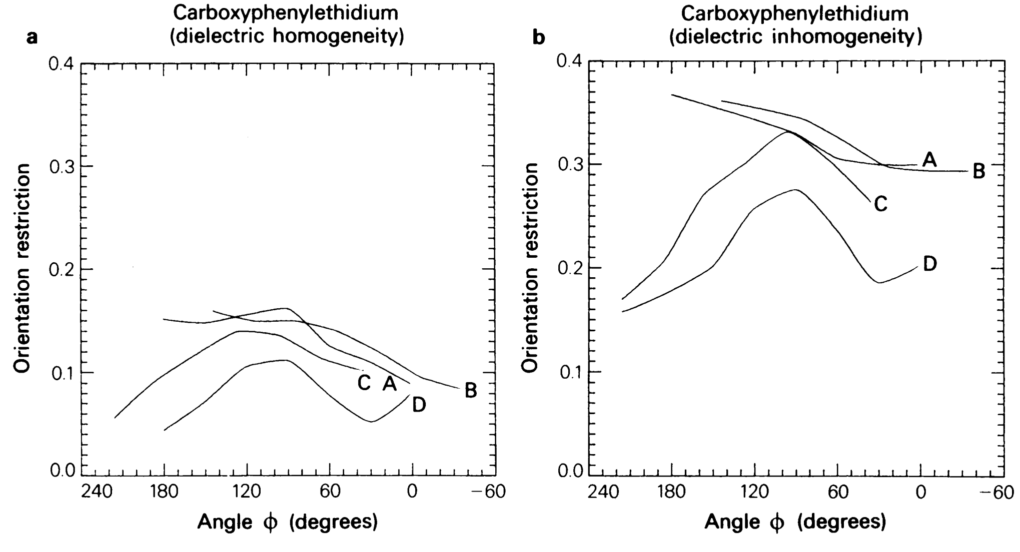

Figure 10 Orientation restriction for carboxyphenylethidium: (a) homogeneous dielectric; (b) inhomogeneous dielectric. Positions defined in the legend of Figure 7.

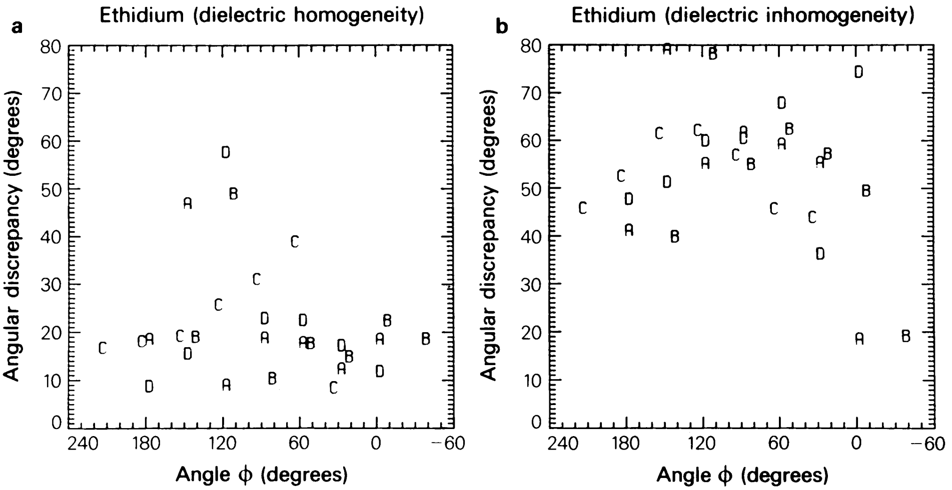

Figure 11 Scattergram of the angular discrepancy between the field line and the dipole direction for ethidium: (a) homogeneous dielectric; (b) inhomogeneous dielectric. Positions defined in the legend of Figure 7.

sugar phosphate region compared with positions in the wide or narrow grooves but there is no difference in discrepancy between the four inspection planes. At the minimum energy orientation the two vectors show a tendency to be aligned so that the dipole is antiparallel to the field line.

Discussion

Receptor-induced ligand orientation, occurring *in vacuo* before molecular contact, is deducible from quantum mechanical calculations. This finding implies that some form of drug-receptor recognition process is operating prior to collision (Dean & Wakelin, 1979). Little is understood about the relationship between the various intermolecular forces that participate in the pattern-matching process. The current work seeks to interpret the role of one of these forces, the long-range electrostatic component, in the orientation of two related polar drug molecules in the vicinity of a large segment of their highly polar drug receptor: modifications to this manoeuvre by different dielectric environments have been included. Electrostatic theory can be used to elucidate some features of the recognition mechanism and it would

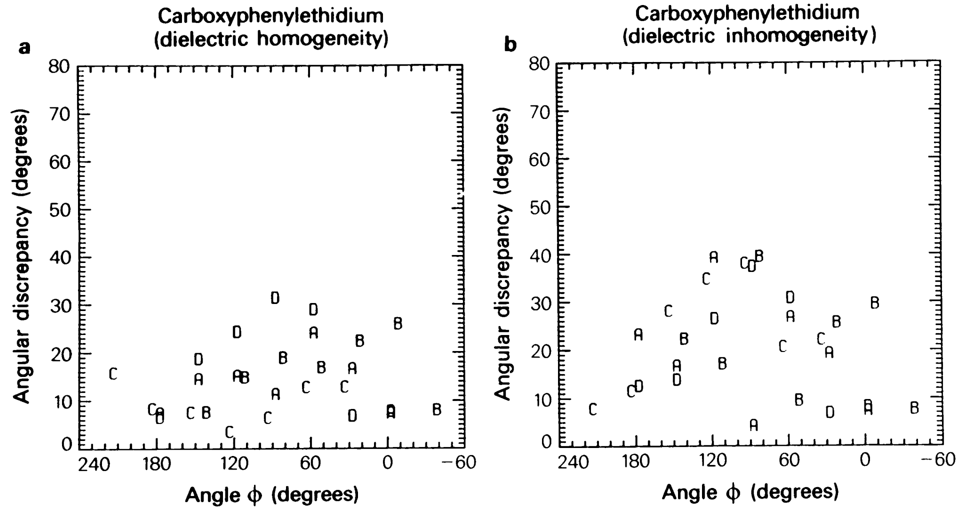

Figure 12 Scattergram of the angular discrepancy between the field line and the dipole direction for carboxyphenylethidium: (a) homogeneous dielectric; (b) inhomogeneous dielectric. Positions defined in the legend of Figure 7.

appear that the contribution to the interaction from the electrostatic element is really quite simple to understand. The electric field generated by a receptor, from the constituent residual atomic charges, is a vector field in three dimensions and the resultant direction and magnitude at any point can be calculated from components of the three unit vectors. Likewise, the drug molecular dipole is an electrostatic vector, derived from an assembly of charges, with direction passing through the centre of mass. The torque imposed by the receptor field on a drug molecule placed at a particular position can be correlated with the preferred alignment of the dipole: this suggests that the orientation is controlled by the interplay of the two vectors. Furthermore, the statistical significance of receptor-induced ligand orientation within a molecular population can be assessed together with a measure of the orientation restriction applied to the drug at different locations around its receptor.

Classical electrostatic theory has been employed to analyse the electrostatic component of the orientation energies from charges previously determined by semi-empirical molecular orbital methods. The use of this simplification, instead of a quantum mechanical procedure, makes it practicable to examine large molecular interactions. The method of Caillet & Claverie (1975) for calculating intermolecular energies has been strikingly successful in predicting molecular orientation in the crystal cell. However, there are a number of assumptions implicit in the approximation. Firstly, the charge distribution in the two molecules is kept static whereas it is known that *in vacuo* charge delocalization in the drug molecule does occur and may shift the dipole and thus alter the orientation vectors (Weinstein, Osman, Edwards & Green, 1978; Dean & Wakelin, 1979). Secondly, the extent to which the electron distribution may be modified in the presence of solvent dielectric is not understood. Thirdly, the influence of polarization energy, which in the case of ethidium may amount to 12% of the total interaction, has been neglected. Fourthly, dispersion and nuclear repulsion terms have been omitted, although this would seem justifiable for a large separation distance since they amount to a very small contribution to the total energy. Two methods to account for the solvent dielectric effect have been used; the simplest assumes that the dielectric is homogeneous and equal to that of the bulk solvent; the second procedure considers dielectric inhomogeneities near the molecular surface and the dielectric value is dependent on the inter-atomic distance used in the pair-wise additive calculations. Both techniques are of the continuum type and are unsophisticated procedures since they do not include discrete solvent effects associated with localized charged regions. The dielectric inclusion therefore provides only a rough guide to the expected effect on the electrostatic interaction energy. Problems associated with dielectric phenomena in drug-receptor recognition have been discussed previously (Dean & Wakelin, 1979, 1980; Dean, 1981).

Electrostatic orientation energy

The influence of dielectric inhomogeneity on the interaction of ethidium with DNA occurs significantly at 40Å and increases as the separation distance diminishes. This finding suggests that dielectric inhomogeneity in the solvent may play a major modifying role in the interaction process and therefore should be taken into account when considering drug-receptor recognition. The effect of inhomogeneities, of the type dealt with here, would be to magnify electrostatic contributions from nearby atoms when compared with a constant reduction by the homogeneous dielectric state.

There are differences in the attractive interaction energy for ethidium in the two grooves of the helix; the energy is greater in the narrow groove than in the wide groove. This is due to the electric field strength being greater in the former region (Dean & Wakelin, 1980) and can be related to the shorter interatomic distance between phosphates on adjacent helical strands. Similarly there is an energy gradient of about 220 kJ mol^{-1} (*in vacuo*) from the end of the helix towards the centre, suggesting that this could produce a motive force for translation of a cation along the receptor surface. The stereoscopic illustrations of the minimum energy positions show differences in the orientation for molecules located in a plane central to the helix compared with those studied near the end. It would appear that in the sugar-phosphate backbone region a terminal amino group of ethidium interacts with the anionic charge. An often noticed feature with ethidium is the tilt of the molecule with the ethyl moiety pointing towards the helix axis when placed near the grooves. This observation may be related to the electron distribution since 22% of the cationic charge is found in the ethyl group, with only 50% of the total located in the phenanthridinium ring itself (Dean & Wakelin, 1979). The orientation energy difference between the best and least favoured alignment for ethidium at any position is small (about 4 kJ at the most in the case of an inhomogeneous dielectric) and is far outweighed by the overall attractive electrostatic energy.

Carboxyphenylethidium, at 18 Å radius from the helix axis, exhibits a very different orientation compared with ethidium and a much larger variation in orientation energy about a particular position. For example, with a homogeneous solvent dielectric the repulsive interaction is about 9 kJ mol^{-1} and magnified 7 fold if dielectric inhomogeneities are included. In contrast the range of favourable orientations with a homogeneous dielectric is small, -3 to $-5\,\mathrm{kJ\,mol^{-1}}$, and only doubled by dielectric inhomogeneity. This variation is almost entirely due to the repulsive interaction that occurs when the ionized carboxyl group is directed towards the helix. The negative charge is highly localized in the carboxyl terminal whilst the cationic charge is well spread throughout the rest of the molecule (Dean & Wakelin, 1979).

Orientation populations

The statistical significance of any orientation restriction associated with a particular interaction energy is a difficult problem to resolve in a three-dimensional phase-space. A partial analysis touched upon previously (Dean & Wakelin, 1979) has been expanded in this paper to a full set of orientations for the drug molecule. It is a formidable task to illustrate the probability levels for all orientation positions. Thus the procedure adopted here has been to select an arbitrary probability value, from the Boltzmann distribution, of 0.9 for orientations impeded by the receptor field and then to find the corresponding fraction of orientation space that is restricted. This method generates a single gross value for the restriction with an equivalent set of angles. The drawback of the technique is that the angles calculated may not represent latitude in the orientations about the global minimum energy because other minima may exist which cause a significant limitation elsewhere. The choice of a suitable probability level for restriction needs careful assessment; it may be that the value of 0.9 used in this work is too severe to reveal significant restrictive effects of the receptor field, for example in the case where solvent dielectric is included in the ethidium orientations. Carboxyethidium, on the other hand, does exhibit strong orientation restrictions with a dielectric equal to bulk solvent; this constraint is increased when dielectric inhomogeneities are added to the calculations.

Field lines and molecular alignment

The direction of maximum torque generated by the receptor field at a particular point can be resolved by vector analysis. Moreover, the position of a drug dipole centred on that point for the minimum energy value correlates best with an anti-parallel alignment of the field line. The increase in angular discrepancy when dielectric inhomogeneity is included in the calculations may be related to the magnification of local interactions between atoms in the drug and receptor molecules that are absent with a homogeneous dielectric. The angular discrepancy between the line of force and the direction of the dipole may not in practice be as large as the measured value because the rotation was performed at 36° steps. Detailed calculations with smaller angles would be necessary to determine the proportion of the discrepancy that could be attributed to the step size. The rotation of small molecular dipoles within an electric field is well understood and it would appear that the theory holds true for larger drug molecules at sufficient separation distances, even in conditions of dielectric inhomogeneity. This method of predicting orientation, because of its computational rapidity, may be useful in forecasting the alignment of drugs in the vicinity of their receptor site simply by calculating the direction of the receptor field line; the only weakness is that the most favourable rotation round the dipole axis cannot be gleaned by inspection. Indeed, a further point of interest to examine would be the energy differences for rotation round the drug dipole vector and to compare the values of this limited set with the full set for rotation round three coordinate axes. The field surrounding the DNA segment exhibits a considerable degree of symmetry which can be ascribed directly to the helical repeating structure of the receptor. This molecular geometry gives rise to lines of force which are, at 18 Å, almost radial from the helix axis, so that at these comparatively large distances the field is more or less uniform (Dean, 1981). However, the field structure resulting largely from 22 anionic charges in a segment of DNA is an artificial situation and would be expected to be perturbed substantially by bound counterions in solution. It would be fascinating to investigate an electric field emanating from a receptor structure lacking this symmetry and to explore the orientation energies in regions where discontinuities in the local field are encountered: non-uniformity in field composition may be anticipated with many biological receptors.

Drug-receptor recognition is only one example of a general recognition mechanism that appears to occur between many biologically active molecules (Phillips & Radda, 1975). The operative principles of this process can be understood by considering the various interaction energy components separately. Before molecular contact, the primary event in recognition must be an exchange of information over a relatively long range which may, perhaps, be mediated by electrostatic forces. A function can be allocated to the three-dimensional vector electrostatic field generated by the receptor molecule, namely one of exerting a turning moment on the dipole vector of the drug. This holding force may be strong enough temporarily to overcome random thermal motion and therefore initially position the drug molecule in a specific alignment at the start of a reaction pathway. As the interaction proceeds, other intermolecular forces would increase in importance to induce further orientation refinements to discriminate between three-dimensional shapes until a matched fit, in the case of complementary molecules, would lead to the formation of the complex. The analysis of long-range alignment mechanisms developed here may have a wider application in resolving the recognition processes intrinsic to the self-assembly of macromolecules: it also opens up the possibility of examining substrate chanelling in terms of translocation by electric vector fields across oligomeric enzyme interfaces.

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