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RAPID LOCATION OF THE PREFERRED INTERACTION SITES BETWEEN SMALL POLAR MOLECULES AND MACROMOLECULES. I. BINDING OF WATER TO THE COMPONENT UNITS OF NUCLEI ACIDS*

by

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ABSTRACT

An approximate procedure for the rapid detection of favoured sites for the location of bound water on macromolecules has been developed with the aid of accurate electrostatic energy calculations. The method also enables to picture the lability of the bound water molecules around the substrate. As an application the method has been used to study the interaction surface between nucleic acid components and a water molecule.

Key-words: Nucleic acid constituents; Quantum mechanical computations; Electrostatic interaction; Hydration.

I INTRODUCTION

In the last few years, a large number of theoretical studies have been concerned with solute-solvent interactions. Two theoretical approaches are widely employed in such studies: the continuum[1-4] and the discrete models[5-7]. Computer simulations based on Monte Carlo calculations using analytical potential functions for the potential energy have been also used[8-10].

A thorough investigation of the hydration scheme of numerous fundamental biomolecules using the *ab initio* SCF method (within the "supermolecule" procedure) has been carried out applying the discrete approach[11-16]. The majority of such works has been directed to the main component units of the essential biopolymers, i.e., nucleic acids (pyrimidine bases[11-13]; phosphate group[14]; sugar ring[15]) and proteins (amino acid side chains[16]).

However, the extension of these studies to larger molecular systems within the same framework is prohibitively expensive. Further investigations have shown, nevertheless, that a correct picture of the hydration scheme of a substrate mimicking *ab initio* SCF results, may be obtained with the help of an electrostatic approximation alone, with the right cautions about the closest distance between water and substrate[11].

In this work we extend the applicability of the electrostatic approach to comprise more complex systems. Our purpose is to develop a procedure by which the general features of the interaction between small dipolar molecules (in particular the very important water molecule) and macromolecules are promptly depicted. Of course, such a technique may not provide the same

precision as an *ab initio* SCF calculation. Nevertheless, one may expect to obtain significant informations on the affinity of biological macromolecules towards water molecule, for example the detection of their specific binding sites. During the development of this procedure it was proposed not only to establish the optimum position of the water molecule and its respective energy, but also to characterize its lability, by investigating throughly all possible orientations of water molecule at each binding site.

In part I of this work we illustrate the procedure to describe the surface interaction between the nucleic acid constituents (guanine, adenine, cytosine, thymine, uracil, phosphate group and sugar ring) and a water molecule. These results may then be used to deduce the effect of inserting these constituents into macromolecular biopolymers. Such effects are described in part II of this work. As a model compound, model segments of poly(dA).poly(dT) and poly(dG).poly(dC) were considered assuming them on the double helical structure of B-DNA.

II METHOD

In order to bring a water melecule into contact with the substrate, we use a technique similar to that previously developed for the calculation of steric accessibility[17]. This first consists of surrounding both species with envelopes formed by the juxtaposition of van der Waals spheres centered on all the constituent atoms. Two atoms in mutual contact are then considered: an atom of the molecule to be hydrated (receptor

sphere) and either the oxygen atom or one of hydrogen atoms of the water molecule (attacking sphere). We keep the interaction distance at $2A^{o}$ (see ref. 11) for this contact (the radius of receptor and attacking spheres in contact taken as 1 A^{o}). We impose the same approach limit to the neighbouring atoms of the receptor susceptible of forming hydrogen bonds with the water molecule, but between other atom pairs the approach limit is set as the sum of their van der Waals radii. In this way the binding of water to any atom belonging to the receptor molecule can be studied. The orientation of the water is varied by rolling the surface envelope of its attacking atom over the sphere of the receptor atom. Any position where the envelopes of the two species intersect is taken as inaccessible, while for every other position the electrostatic energy of interaction is calculated. The exact positions to be studied for a given atom pair contact are determined by placing a uniformly spaced grid of points defined by a Korobov distribution (see, e.g., ref. 18) on the surface of the receptor and attacking spheres, and restricting contacts to pairs of these points. For any such contact it is further necessary to consider a rotation of the water molecule around an axis joining the centers of the two atoms in contact. Tests have shown that 144 points on the receptor sphere combined with 89 points on the attacking sphere of water and 24 rotation steps lead to convergence of results both regarding the interaction energy and the most favourable positions for the bound water molecules.

The evaluation of the electrostatic interaction between the receptor and water molecules is made by using electron density distributions for both species. For water and for receptors of

normal molecular size this is obtained directly from *ab initio* wave functions. When the receptor is a macromolecule, a single wavefunction cannot be obtained directly and we employ an approximation developed previously for the study of intrinsic electrostatic properties of the nucleic acids[19-22]. This consists of subdividing the macromolecule into a number of subunits small enough to be studied quantum mechanically and chosen so as to minimize as much as possible the resulting electronic perturbations. Thus for the nucleic acids these subunits are the bases, sugar and phosphate, the points of subdivision being the backbone linkages $C_{3'}-O_{3'}$, $C_{5'}-O_{5'}$ and the glycosidic bonds. The electrostatic properties of the macromolecules are obtained by a superposition of properties of the subunits appropriately positioned in space.

The wavefunctions are obtained by *ab initio* SCF calculations using a 7s3p/3s Gaussian basis set (10s6pld for phosphorus) contracted to minimal basis[23]. The continuous electron density distributions of each subunit and of the water molecule are replaced by a discrete multicenter overlap multipole expansion, up to quadrupoles, termed OMTP[11,24-25] which has been shown to reproduce accurately the properties of the continuous distribution. The interaction energy between the receptor and water molecule is then calculated as the total interaction between the two sets of multipoles, using the classical electrostatic expressions of the charge-charge, charge-dipole, charge-quadrupole, dipole--dipole, dipole-quadrupole and quadrupole-quadrupole interactions (see, e.g., 26, 27).

There remains a technical problem to be solved before these calculations can be made. The choice described above of

144 receptor points, 89 attacking points and 24 rotation steps implies that roughly 310.000 configurations should be tested for a water molecule in contact with only a single atom of the receptor molecule. Although this number is considerably reduced by forbidding the intersection of the two envelopes, there remain nevertheless between 50000 and 100000 configurations to be studied for a relatively accessible atom. This number is much too high to envisage calculating the full electrostatic interaction energy in all cases, even with our OMTP expansions. The problem is overcome by studying the accessible configurations in several steps with increasing levels of accuracy. The first step consists of representing the water molecule simply by a single dipole and a quadrupole, both calculated from the water SCF wavefunction and placed at the barycenter of the molecule. The energy of a given configuration is then approximated by the product of the dipole and the local electrostatic field vector of the receptor added to the product of the quadrupole and the local field gradient. In this step, moreover, the field and the field gradient are not recalculated for each water position, but rather calculated once for a grid of points surrounding the receptor atom, the point closest to the water molecule barycenter being used in the energy calculation. By using points along radial vectors originating from the center of the receptor atom and passing through accessible points in its Korobov grids, it is possible to ensure that there is always a point within a few tenths of an Angström of the water molecule barycenter. At this level, all the accessible configurations are analyzed, ordered and the best 500 are selected. The second level of approximation reconsiders these selected configurations, keeping the single

center multipole model of water but employing the full OMTP representation of the receptor molecule to calculate the interaction energies. From the resulting energies the best 200 configurations are selected. Finally, for these latter configurations the interaction energy are recomputed, using now the full OMTP representations both for the water molecule and for the receptor. A final reordering yields the best possible water position at this site. Tests showed that this three-step procedure can obtain at least the first 150 water positions correctly, while saving a great deal of computer time. These calculations give not only the optimal energy configuration of the bound water at a given substrate site, but also a whole range of configurations with closely related energies. In this way a view of the lability of the bound water may be obtained: we employ a simple graphic representation of the water positions around the receptor molecule in which we include all the configurations at a given site having energies within 1 Kcal/mole of the optimum energy. With this energy limit between roughly 10 and 150 water configuration were found at each site.

III RESULTS AND DISCUSSIONS

The results obtained by the present technique for each of the subunits studied are displayed in figures 1,3-8. We give also as an example the electrostatic field on the surface envelope of guanine (figure 2). The remaining results and the detailed technique of calculating the field on surface envelopes are described in earlier works (see, e.g., ref. 28).

For the nucleic acid bases the hydration schemes have a central diagram showing the interaction zones in the plane of the base, each of the distinct spatial zones being numbered in order of decreasing binding energy for the optimum configuration belonging to the zone. In order to simplify the hydration schemes we have drawn the water molecule only at its optimal energy position for each zone and for the other water configurations we only indicate with a point the oxygen atom position. Note that we do not show the results of water binding in all weak sites where the optimum interaction energy is less than-3 Kcal/mole. These diagrams were drawn using a graphic plotter connected to a microcomputer system.

The results are reported in table I where we give the interaction energies, the receptor atoms of the subunit involved with each zone and the number NC of water configurations having energies within 1 Kcal/mole of the optimum energy.

Out-of-plane views of the water interaction zones with the bases (figures 1,3-6) are given in two diagrams: at the top the upper half of the central in-plane diagram and at the bottom the lower half of the central diagram. The corresponding zones in the different diagrams are easily associated with one another by their numbering, which remains unchanged. The separation of the out-of-plane views into two diagrams avoids confusion due to the superposition of the interaction zones on opposite sides of the bases. In order to simplify the comparison, the surface field envelopes for guanine (figure 2) are similarly disposed: at the center, a view on the base plane, at the top a view of the upper edge of the base and, at the bottom, a view of its lower edge.

On these envelopes we show both the intensities of the fields, indicated by various degrees of shading (Table II) and their vectorial directions indicated by one of three symbols. These symbols are: a) an arrow ↑ for vectors which point within 30^{o} of the local envelope tangent; b) a triangle Δ for vectors pointing outwards from the envelope by more than 30^{o}; c) a distorted cross ⧖ for vectors pointing inwards by more than 30^{o}. In addition, each of these symbols indicate the direction of the component of the field tangential to the surface envelope. (In the case of the triangle or the distorted cross this direction is from the broader end of the symbol towards its narrower end)[28].

The phosphate subunit hydration is treated in a similar manner to that of the nucleic bases; but in this case the "plane" of this unit is taken to be the one containing the phosphorus atom together with two anionic oxygens. For the sugar subunit the plane is taken to be that containing the atoms $C_{1'}$, $C_{2'}$ and $C_{3'}$. In this case only one other view of its hydration perpendicular to the plane was neccessary, as only a single water binding site was envolved. It should be noted that because of the subdivision scheme we adopt for DNA, the sugar subunit lacks its hydroxyl groups at $C_{3'}$ and $C_{5'}$. It is thus not a complete deoxyribose and it will be used for comparison with the polymer results.

The hydration zones around guanine are shown in figure 1. Five binding zones are seen, of which the strongest (zone 1) is situated between the N_1-hydrogen and O_6. The configuration distribution of the water molecule in this region seems to be concentrated in the plane of the base; actually, as shown in the

upper diagram of figure 1, it covers a wide zone on both sides of the base plane. Thus, this strongly bound water molecule (-11.9 Kcal/mole) can be displaced out of the base plane very easily. The same remarks apply to the second most favourable site of guanine which falls between N_7 and O_6 (-11.3 Kcal/mole). The third site is on the opposite side of the base, associated with N_3, the N_9-hydrogen and one of the N_2 amino group hydrogen. This interaction zone is rather interesting because it is split into two distinct bands, one on each side of N_3. The fourth binding site falls between the N_1-hydrogen and the remaining N_2-hydrogen, the water configurations having a radial distribution around the former atom. As Table I shows, this zone includes 94 configurations. These configurations, as seen in this region, are divided in four distinct groups: for each group the oxygen atoms are in the same position and the hydrogen atoms in different ones. This division is a consequence of the restriction of the method, for we make use of a discret number of points on the spheres of the attacking and receptor atoms. Then, it may be expected that these configurations will be displayed in a continuous way in space when the number of points tends to infinite. We verify the same radial distribution for other examples in the present study. In a general way, they do not correspond to water molecules strongly bound to the bases. The remaining hydration site (zone 5) which has a rather weaker binding energy (-7.0 Kcal/mole) is associated with the C_8-hydrogen.

In the right hand diagrams of figure 2 we show the electrostatic field on guanine surface. With the aid of these diagrams we can see that the four strongest hydration zones can be well correlated with the regions of strongest field. In figure 2a

we can see that three particularly strong field regions are manifest and correspond nicely to the hydration zones 1, 2 and 4. The third group corresponds to the strong field seen in figure 2c and it should be noted that the strong field is divided into two vertical zones in exactly the same way as the hydration zone. The binding site number 5 corresponds to a zone of intermediate field strenght. The directions of the field vectors on the envelope of guanine are shown in the left hand diagram of figure 2. The water molecule may be expected to align its dipoles with these field vectors. This helps to explain quite subtle effects, such as the orientation of the water in the two regions forming zone 3 at receptor atom N_3. Figure 2f shows a rapid change in field direction on each side of N_3 due to the opposing influences of this high electron density atom and of the hydrogens at both sides of it, one on N_9 and the second attached to N_2. These oppositions, which lead to rapid potential changes, also explain why the field (and thus the hydration at this receptor) is separated in two strong interaction zones, instead of, for example, in a single zone along the external bisector of the angle C_2-N_3-C_4. Note also that the radial distribution found for zone 4 correlates very well with the field directions in this region of guanine (figure 2d).

The strongest binding site on adenine is close to N_3 (figure 3). Note that there is no division into two regions at this site as is the case for the corresponding position on guanine. Another binding site can be seen between N_3 and the C_2-hydrogen (zone 5), but this has a much lower optimum energy (only -5.7 Kcal/mole compared to -12.3 Kcal/mole for zone 1). The second strongest binding is between N_7 and one of the N_6 amino

hydrogens. The resulting configuration presents an important change with respect to the situation in guanine. The water molecule in this region of guanine tends to remain close to the base plane; in adenine, the attraction of N_7 results in the rotation of the water plane so as to turn its hydrogens towards this atom. In the third interaction zone (-10.8 Kcal/mole) the association involves N_1 and the remaining hydrogen of N_6. The zone 4 of adenine (-6.0 Kcal/mole) involves the C_8-hydrogen and presents a similar situation to zone 5 in guanine.

The overall comparison of the two purine bases should make clear that the nature of hydration around any given functional group is by no means transferable. The neighbouring atoms always play an important role and often entirely modify the nature of the water binding configurations.

The hydration zones of cytosine are shown in figure 4. Three of these are associated with strong binding energies. The first is shared between N_3 and one of the N_4 amino group hydrogens (-12.7 Kcal/mole); the second lies between O_2 and the N_1-hydrogen (-11.8 Kcal/mole) and the third between O_2 and N_3 (-11.2 Kcal/mole). The binding site associated to zone 4 (-6.4 Kcal/mole) is located between the remaining amino group hydrogen and the C_5-hydrogen, this distribution sweeping a great deal of space around the base.

The hydration scheme of thymine (figure 3) shows two relatively strong interactions zones. For the strongest binding, the water molecule is shared between O_2 and the N_1-hydrogen (-10.8 Kcal/mole). In the case of the second zone (-9.4 Kcal/mole) it is interesting to remark that the binding site is located on each side of N_3 and implies that the water molecule in this region can move very easily around the N_3-hydrogen so as to interact with

O_2 or O_4. The third zone, as shown in figure 5, is situated between the hydrogens of C_6 and N_1. The methyl group interaction with water involves two distinct regions. The first one is associated with the interaction with the methyl hydrogen closest to O_4 and gains a supplementary interaction with this atom (zone 4). The second region is associated with the remaining two hydrogens (zone 5) and has a very weak interaction energy (-3.0 Kcal/mole). However, it is interesting to note that water molecule configurations are radially distributed around the atoms and show one of the largest zones investigated in these studies. It be should be noted that this hydration zone results show a very good correlation with those found by Langlet *et al.*[29] using simplified formulas to study the hydration of the dimethylphosphate anion (DMP^-); a spherical distribution of weakly bound water molecules was observed around the methyl group of this molecular system.

The hydration of uracil (fig. 6) is very similar to that of thymine. The water molecule configurations around the carbonyl oxygens O_2 and O_4 are distributed in the same way, as well as those for the water molecule between the N_1- and C_6 hydrogens. The only significant change occurs at C_5 where the absence of the methyl group allows the bound water to approach the base more closely and leads to a slight gain in interaction energy with the adjacent center O_4 (-6.5 Kcal/mole for uracil zone 4 against -6.2 Kcal/mole for thymine zone 4).

The results of our study of the phosphate group $H_2PO_4^-$ in its geometry from B-DNA show high lability associated with strongly bound water molecules. In fact, the phosphate water binding energies are roughly 50% stronger than those of the best among the base sites. The hydration scheme for the phosphate

(figure 7) shows three distincts zones, in which the most strongly associated binding, zone number 1, the water lies between the O_1 and O_2 atoms (-19.0 Kcal/mole). As indicated in Table I zones 2 and 3 are associated with water binding between one anionic and one esteric oxygen (O_1-O_3, and O_2-O_5,, respectively), the binding energy of the former group being somewhat favoured by the position of the hydrogen bound to O_3,.

Finally, concerning the sugar subunit, we recall that the studied molecule is not a complete deoxyribose and thus its interest is principally limited to comparisons with our next polymer studies. In consequence there is only one interesting hydration site to be studied, namely, around the ring oxygen O_1,. As figure 8 shows the water bound at this site has a high lability, but the interaction energy is rather low (-5.7 Kcal/mole). The sugar out-of-plane view shows that the water positions are distributed more or less uniformly around the external bisector of the C_1,-O_1,-C_4, angle.

IV CONCLUSIONS

The main purpose of the present work is to develop a method which enables, with relatively moderate effort and cost, the description of the interaction surface between macromolecules and small, neutral, dipolar molecules, specifically water molecules. This approach has been applied to describe the interaction surface between the nucleic acid components and a water molecule. The results satisfactorily reproduce the salient features of hydration schemes of these systems brought to light by *ab initio* SCF

computations: pyrimidine bases[11-13], phosphate group[14] and sugar rings[15].

Despite of the great number of theoretical works concerning hydration of nucleic acid components the present study reveals interesting aspects of this process such as the visualization of the water molecule lability; this is shown by employing simple graphical representations of the water positions around the receptor molecule. It has been found that many bound water molecules can be displaced over relatively large regions around their optimum binding position with only very small weakening of their interactions energies. Important spatial zones associated with weakly bound water molecules were observed, for instance, that situated between one of the N_4 amino group hydrogen and the C_5-hydrogen of cytosine, as well as that associated with the methyl group of thymine. On the other hand, relatively large spatial zones associated with strongly bound water molecules were also observed; for instance, the interaction zone determined around the phosphate group oxygens.

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FIGURE CAPTIONS

FIG. 1 - Interaction zones of a water molecule with guanine (the out-of-plane distributions for sites on the upper edge of the central base are show above and those on the lower edge are show below. For the corresponding interaction energies see Table I. These remarks also apply to figures 3, 4, 5 and 6).

FIG. 2 - Surface field intensities of guanine (the views of the base are disposed as in the preceeding figure. For the definition of the shading se Table II).

FIG. 3 - Interaction zones of a water molecule with adenine.

FIG. 4 - Interaction zones of a water molecule with cytosine.

FIG. 5 - Interaction zones of a water molecule with thymine.

FIG. 6 - Interaction zones of a water molecule with uracil.

FIG. 7 - Interaction zones of a water molecule with phosphate.

FIG. 8 - Interaction zones of a water molecule with subunit.

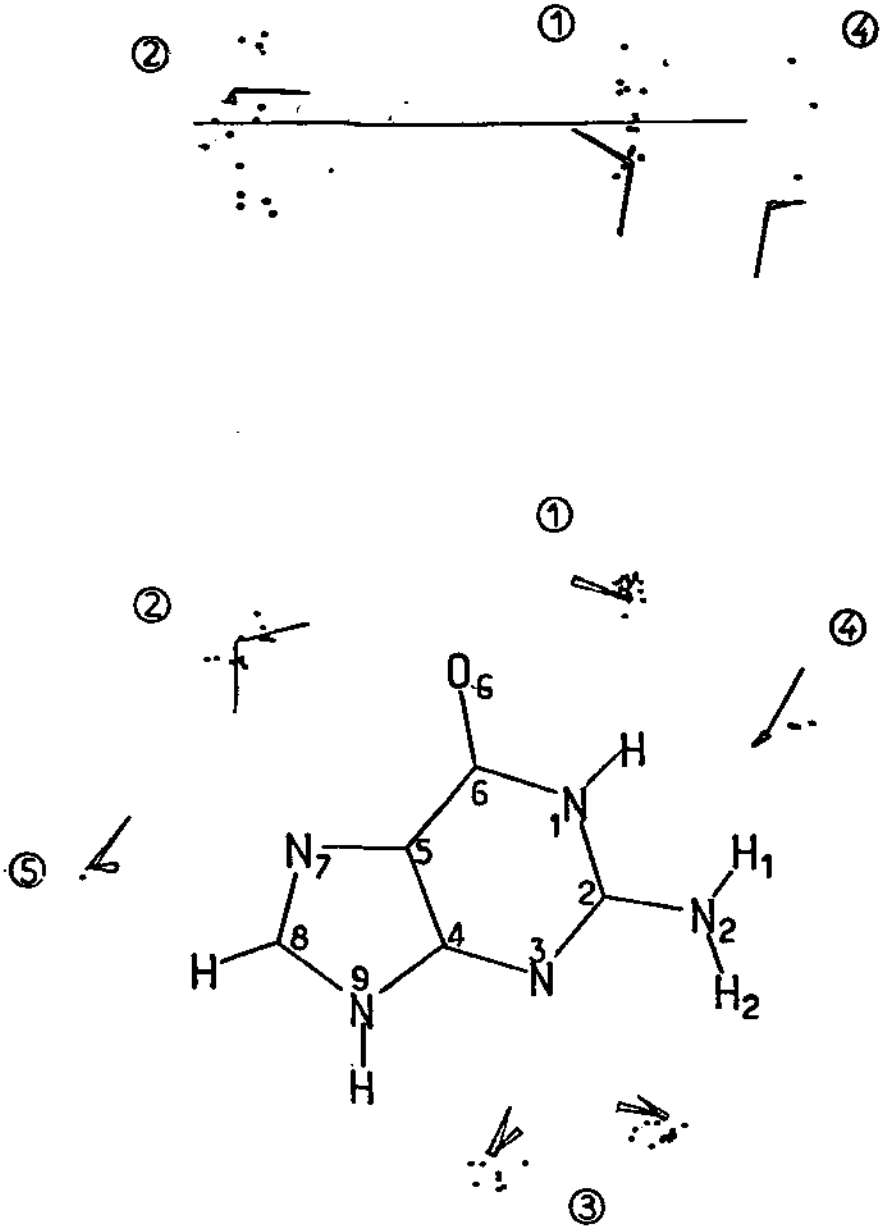
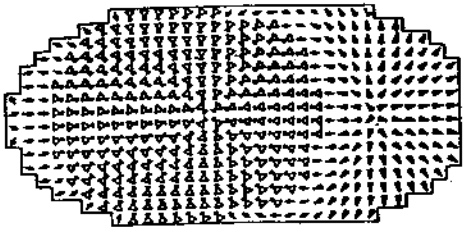


Fig. 1



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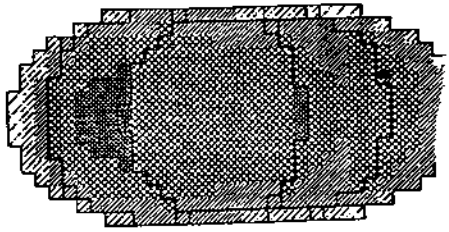

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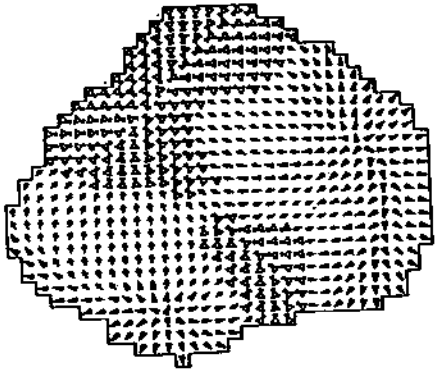

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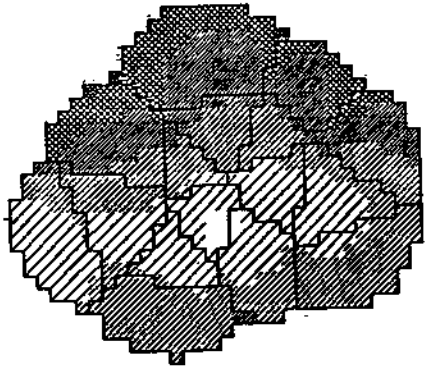

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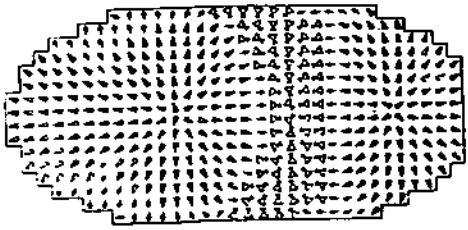

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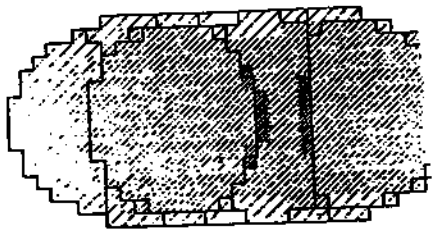


(c)

Fig. 2

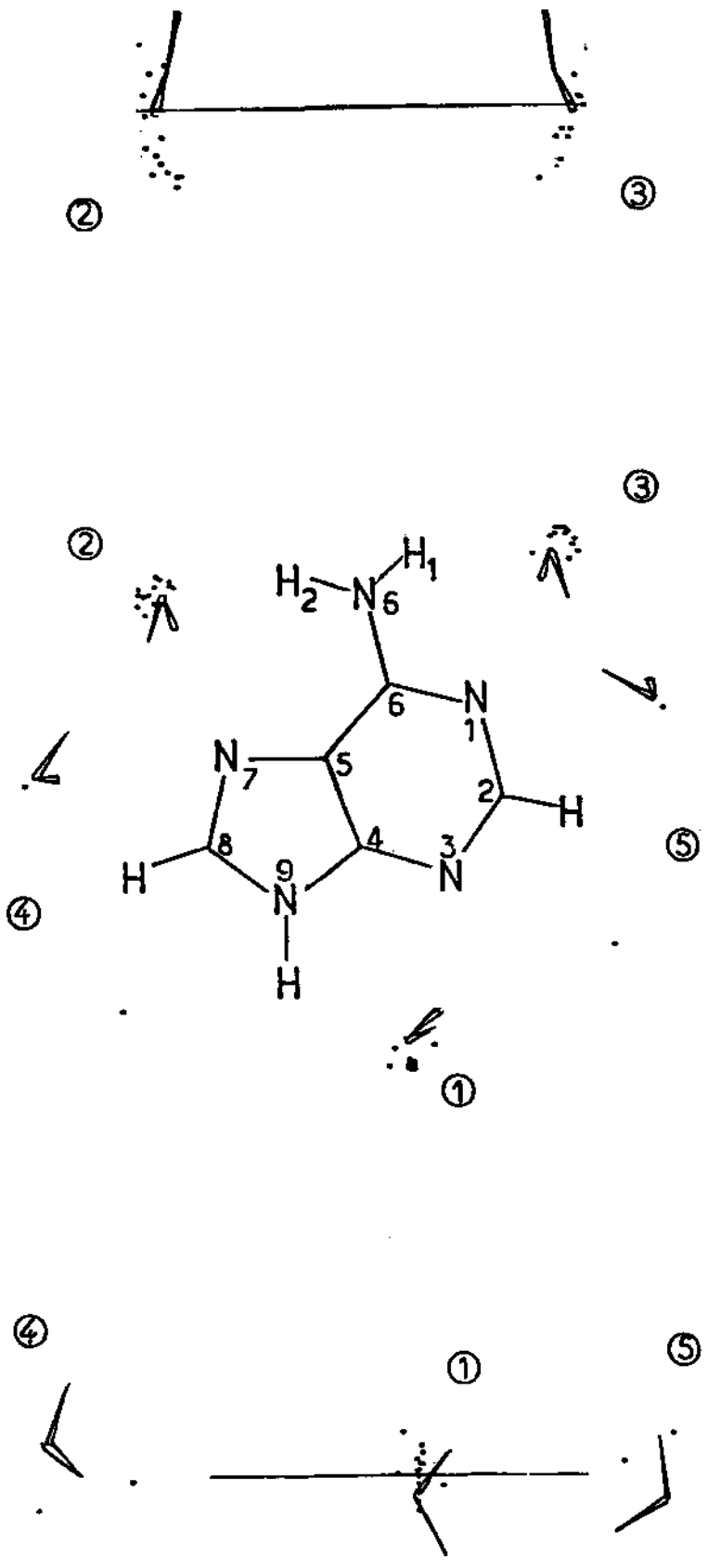
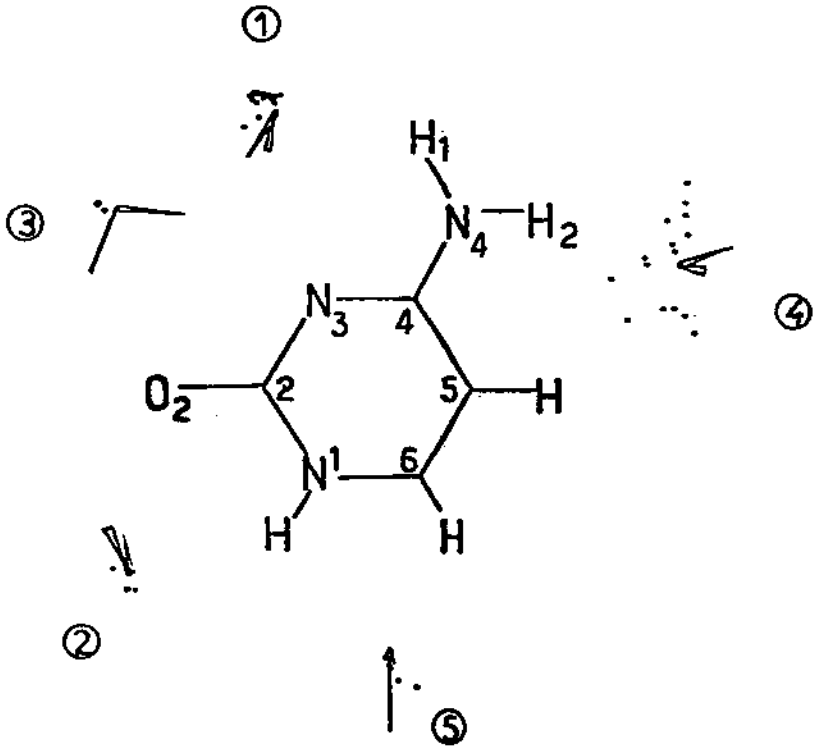


Fig. 3



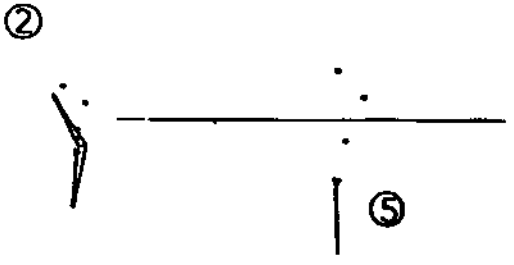


Fig. 4

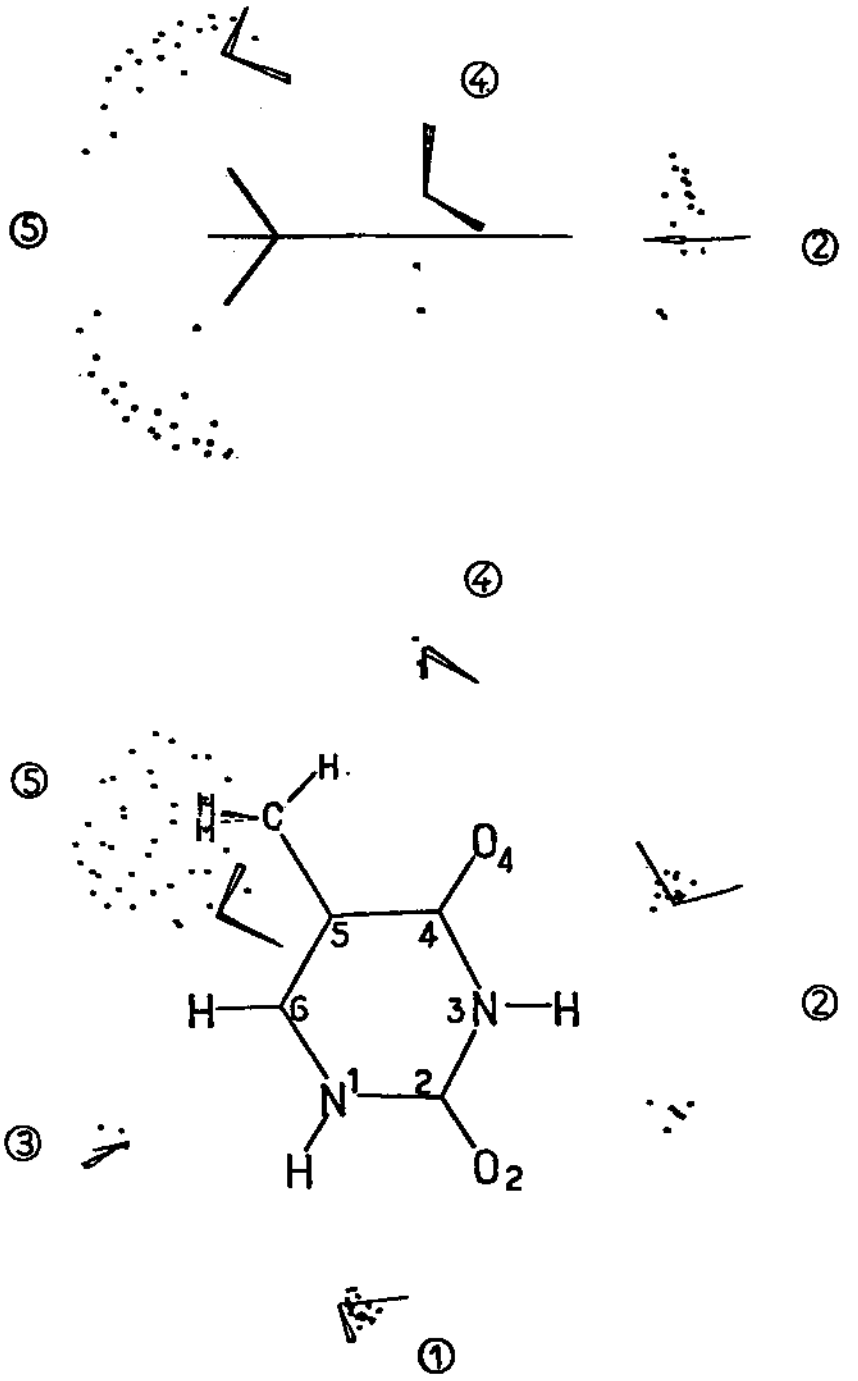
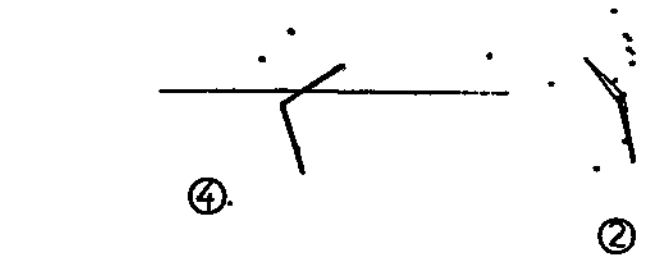
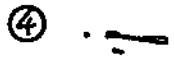
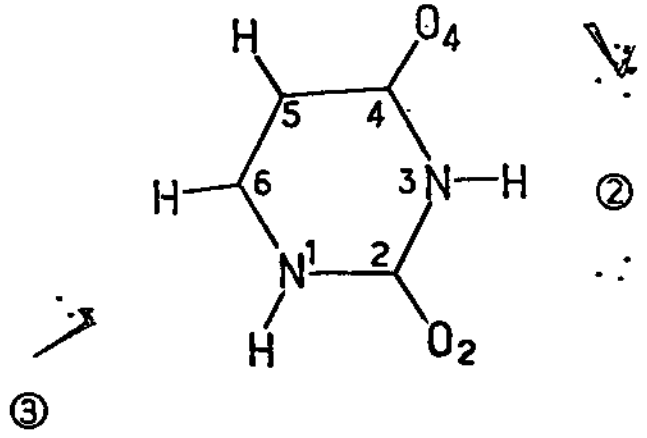


Fig. 5







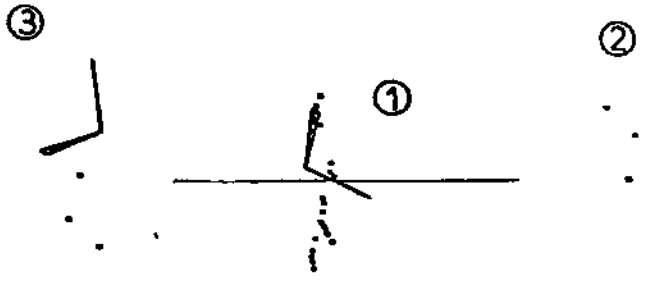


Fig. 6

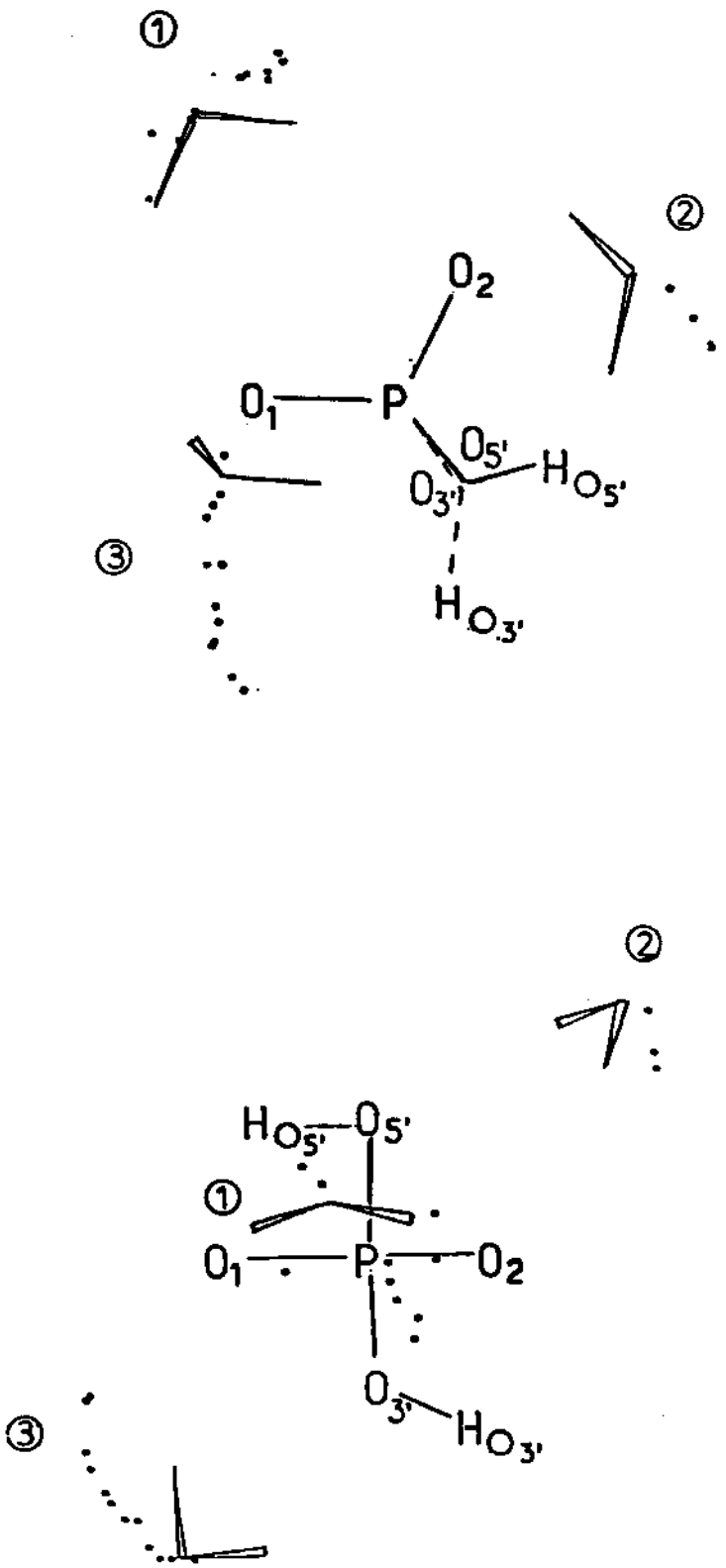
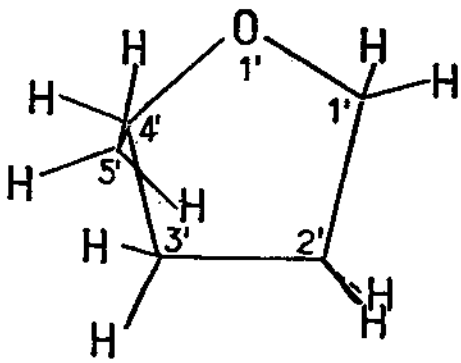


Fig. 7



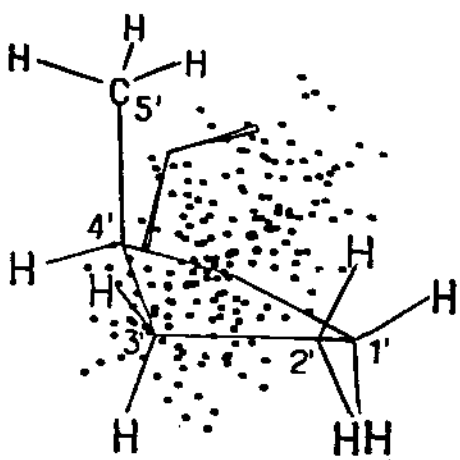


Fig. 8

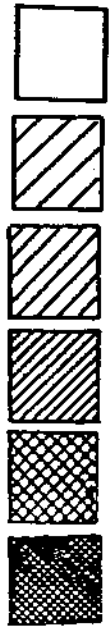
TABLE I

Energies (Kcal/mole) and interaction zones of a water molecule with the nucleic acid components. N_c indicates the number of configurations having energies within 1 Kcal/mole of the optimum energy.

Subunit	Zone	Optimum energy	Receptor atoms involved	N_c
Guanine	1	-11.9	$O_6, H(N_1)$	24
	2	-11.3	N_7, O_6	13
	3	-11.3	$H(N_9), N_3, H_2(N_2)$	22
	4	-10.1	$H(N_1), H_1(N_2)$	94
	5	- 7.0	$H(C_8)$	28
Adenine	1	-12.3	$H(N_9), N_3$	25
	2	-11.4	$N_7, H_2(N_6)$	21
	3	-10.8	$N_1, H_1(N_6)$	22
	4	- 6.0	$H(C_8)$	54
	5	- 5.7	$H(C_2)$	16
Cytosine	1	-12.7	$N_3, H_1(N_4)$	19
	2	-11.8	$H(N_1), O_2$	17
	3	-11.2	N_3, O_2	6
	4	- 6.4	$H_2(N_4), H(C_5)$	349
	5	- 5.9	$H(C_6)$	183
Thymine	1	-10.8	$H(N_1), O_2$	27
	2	- 9.4	$H(N_3), O_4, O_2$	90
	3	- 6.5	$H(C_6)$	90
	4	- 6.2	$H_1(CH_3)$	13
	5	- 3.0	$H_2(CH_3), H_3(CH_3)$	400
Uracil	1	-11.1	$H(N_1), O_2$	48
	2	- 9.1	$H(N_3), O_4, O_2$	54
	3	- 6.7	$H(C_6)$	155
	4	- 6.5	$H(C_5)$	18
Phosphate	1	-19.0	O_1, O_2	12
	2	-17.0	O_2, O_5'	4
	3	-16.0	O_1, O_3'	13
Sugar	1	- 5.7	O_1'	194

TABLE II

Shading used for the surface field intensities (volt/A^{o}) of guanine.

Shading	Field
	0.0
	0.11
	0.22
	0.32
	0.43
	0.54
	0.65

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