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The molecular electrostatic potential and steric accessibility of poly (dI.dC). Comparison with poly (dG.dC)

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ABSTRACT

The influence of the amino group of guanine on the molecular electrostatic potential and the accessibility to reactive sites of B-DNA is investigated by comparing the two model double helices poly (dI.dC) and poly (dG.dC). The calculations clarify the "disruptive" role of the guanine amino group on nucleic acid-polypeptide interactions.

INTRODUCTION

Recent studies from our laboratory have demonstrated the significance of the molecular electrostatic potential, in particular when considered in parallel with the accessibility to reactive sites, for the elucidation of various aspects of the biochemical behaviour of the nucleic acids (1, 2). For B-DNA, the form of DNA considered as the most relevant under physiological conditions, these studies have brought into evidence the differences between sequences of G-C and A-T base-pairs, from the point of view of the above properties and in particular of the electrostatic potential (3, 4). Thus, studies of complete turns of the model double helices of poly (dG.dC) and poly (dA.dT) have shown that while in the former the deepest potentials are associated with the major groove, they are associated with the minor groove in the later. This result has been abundantly correlated with the reactivity of nucleic acids, synthetic polynucleotides and their constituents towards a variety of reactants involving carcinogens, mutagens, antibiotics, chemotherapeutic agents and oligo- or polypeptides (5-10).

In order to elucidate the role played by the structural variations between the two types of the fundamental complementary base pairs, A-T and G-C, in the origin of the differences in the molecular electrostatic potentials and the accessibilities of sequences involving these pairs we have now extended our computations to a complete helical turn of a B-DNA model of poly

(dI.dC) which differs from poly (dG.dC) by the replacement of guanine by hypoxanthine (thus of guanosine by inosine). Hypoxanthine (inosine) differs from guanine (guanosine) by the absence of the 2-NH_2 group and as a result of this situation the I-C pairs contain only two hydrogen bonds, as do the A-T pairs. This investigation should thus bring straightforward information on the effect on the potential and the accessibility of B-DNA of the suppression of the third H-bond of the G-C pair, through the elimination of the guanine amino group. It should also throw light on the consequent modifications of biochemical reactivity.

METHOD

The model polynucleotides studied were in the B-DNA double helical conformation with the geometry due to Arnott and Hukins (11). A full turn of the helix was considered. Poly (dI.dC) was constructed from poly (dG.dC) simply by replacing guanine by hypoxanthine, the latter base having the same geometry as guanine with the exception of the replacement of the C2-NH_2 bond by a C2-H bond with the standard length of 1.08 Å.

The calculation of the molecular electrostatic potentials of these polynucleotides was performed as described in our previous publications (1, 2). The overall potential of the polymer was obtained as a superposition of the potentials of its subunits, the bases, sugars and phosphates. The potentials of these subunits were computed from multipole expansions of their electron density, derived from ab initio wavefunctions. This procedure has been shown to lead to a satisfactory reproduction of the electrostatic potential of the subunits down to a distance of 2 Å from their constituent atoms. Below this distance exact potentials were evaluated.

In the forthcoming discussion we shall use two representations of the results obtained. The first pertains to the potential minima associated with the most important nucleophilic sites on the bases (1, 2, 5). The second refers to surface envelopes surrounding the polynucleotides (2, 4, 12). These envelopes are formed by the intersection of spheres, centered on each atom of the macromolecule, with radii proportional to the van der Waals atomic radius (a proportionality factor of 1.7 was used here, as in our preceeding studies (2, 4)).The advantage of these envelopes is to give a clear view of the potential distribution over a large surface of the macromolecule and they are thus particularly useful in interpreting its interaction with other large molecular species, such as e.g. polyamines, oligo and polypeptides etc...

The static steric accessibilities of the base atoms of the polymers were calculated using the technique also described previously (2, 13). The results are presented as accessible areas in $\mathring{A}^2$ on the van der Waals sphere of the atoms concerned, towards a test sphere with the van der Waals radius of 1.2 $\mathring{A}$, representing water attacking through one of its hydrogens (4).

RESULTS AND DISCUSSION

The calculated potentials associated with nucleophilic sites on the hypoxanthine and cytosine bases in the poly (dI.dC) model double helix are given in table I. They refer to the central base pair of the model. The deepest potentials are seen to be associated with the N3 and N7 sites of hypoxanthine (notation N3 (I) and N7 (I)), followed by O2 (C). Two of these sites (N3 (I) and O2 (C)) lie in the minor groove of the double helix, while N7 (I) and all the remaining sites lie in the major groove. The overall ordering of the sites (absolute values) is :

N3 (I) > N7 (I) > O2 (C) > O6 (I) > C8 (I) > N4 (C) > C5 (C)

This ordering is altogether rather similar to that calculated for the poly (dG.dC) double helix (NH_2(G) omitted, of course) (table 2), as may be seen in the graphical comparison in figure 1. The only changes in ordering concern O2 (C) which in poly (dI.dC) is more negative then O6 (G) while the reverse is true in poly (dG.dC) and N3 (I) which is the most negative site in poly (dI.dC),

TABLE I. Potentials (kcal/mole) and steric accessibilities ($\mathring{A}^2$) of the bases in poly (dI.dC).

BASE				
HYPOXANTHINE	N3	N7	C8	O6
POTENTIAL	- 673	- 672	- 634	- 646
ACCESSIBILITY	0.9	4.1	1.0	2.6
CYTOSINE	N4	C5	O2	
POTENTIAL	- 598	- 572	- 652	
ACCESSIBILITY	0.2	0.3	0.9	

although by a very small margin, while it is N7 (G) which represents the deepest minimum in poly (dG.dC). Apart from these changes we observe a slight decrease in all the site potentials, excepting C8 (I), in poly (dI.dC) compared to poly (dG.dC).

The accessibilities of the atoms of the bases of poly (dI.dC) and poly (dG.dC) are given in tables 1 and 2, respectively. A graphical comparison of the results is made in figure 2 from which it may be observed that the only sites affected by changing guanine to hypoxanthine are the N3 atoms of the purine and the O2 atoms of the pyrimidine which both, practically inaccessible in poly (dG.dC), become accessible by 0.9 $\mathring{A}^2$ in poly (dI.dC).

Complementary aspects of the distribution of the potentials are obtained from the calculation of their values on the surface envelopes of the oligomers. Figure 3 presents the molecular diagram of the poly (dI.dC) model double helix and figure 4 gives a graphical representation of the corresponding surface

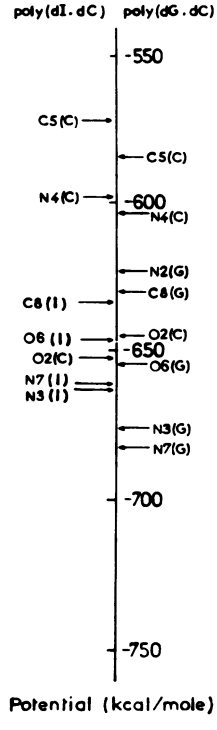


FIGURE 1. Comparison of base site potentials (kcal/mole)

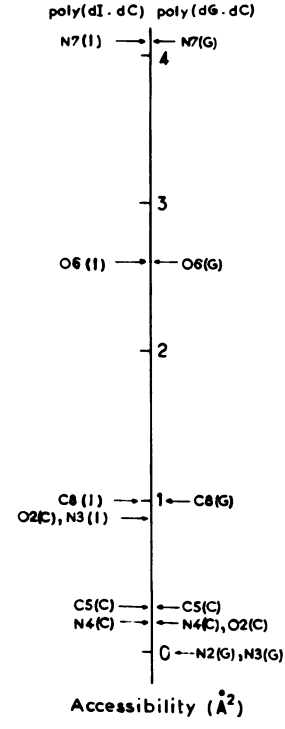
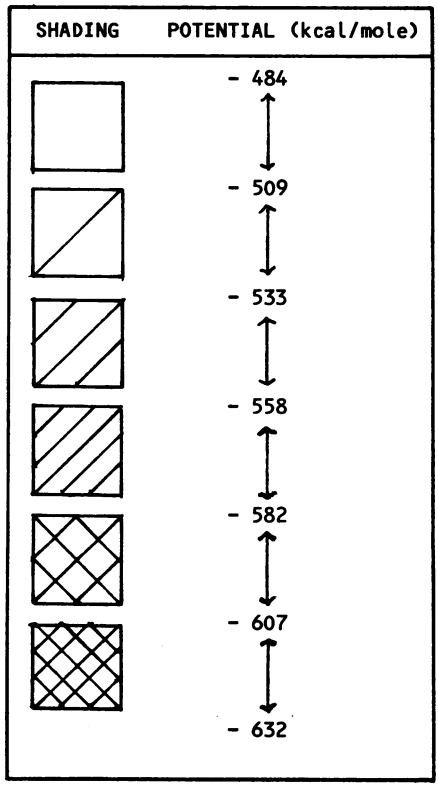


FIGURE 2. Comparison of base atom accessibilities ($\mathring{A}^2$)

TABLE 2. Potentials (kcal/mole) and steric accessibilities ($\mathring{A}^2$) of the bases in poly (dG.dC).

BASE	SITES				
GUANINE	N2	N3	N7	C8	O6
POTENTIAL	- 623	- 677	- 683	- 630	- 654
ACCESSIBILITY	0.0	0.1	4.1	1.0	2.6
CYTOSINE	N4	C5	O2		
POTENTIAL	- 602	- 584	- 645		
ACCESSIBILITY	0.2	0.3	0.2		

TABLE 3. Shadings used for surface envelope potentials.



potentials, more negative potentials being indicated by darker shadings (for details see table 3). These results are to be compared with those for poly (dG.dC) (molecular diagram in figure 5 and surface potentials in figure 6).

One of the most striking features common to both polynucleotides is the concentration of the most negative potentials in the grooves of the double helices. This interesting finding has already been remarked in our previous publications (1, 2, 12, 14) and was considered as an important factor in explaining the observed binding of polypeptides, polyamines and a number of antibiotics in the grooves of double stranded nucleic acids.

Looking at the envelope potentials in more detail we observe further that in poly (dI.dC) the potentials appear to be equally negative in the minor groove (upper half of figure 4) and in the major groove (lower half of figure 4) and this result is confirmed by calculating the local minimum potentials on the surface envelope in each groove (marked by the letters M in figure 4). The results, given in table 4, differ by less than 1 kcal/mole. This is significantly different from the situation in poly (dG.dC), as may be seen from the shadings in figure 6 and the numerical results in table 4. For this polynucleotide the minimum in the major groove is almost 30 kcal/mole more negative than in the minor groove. The cause of this situation can be easily related to the effect of the 2-amino protons of guanine which render less negative and "disrupt" the potentials in the minor groove. Their effect is particularly visible in the surface potentials (figure 6) where they produce more lightly shaded zones, regularly spaced along the minor groove (in the upper half of this figure). This effect disappears when hypoxanthine replaces guanine (Fig. 4).

Finally, for the sake of the forthcoming discussion we also give in table 4 the potential minima of the grooves for a poly (dA.dT) model B-DNA double helix, which has been shown previously (2, 4, 14) to have considerably more negative potentials in the minor groove than in the major one. Thus considering the three base sequences of table 4, poly (dI.dC) is seen to be a rather intermediate case between poly (dG.dC) and poly (dA.dT).

As an illustration of the practical significance of our results we may consider the interpretation of the experimental findings concerning the binding of the antibiotics netropsin (I) and distamycin A (II) to double stranded DNAs. These molecules are found to bind non-intercalatively on the surface of B-DNA and show a marked preference for the minor groove of AT-rich base pair sequences (15 - 19). They also bind to the synthetic polynucleotides poly (dA.dT) and poly (dI.dC) but not to poly (dG.dC) (15, 16).

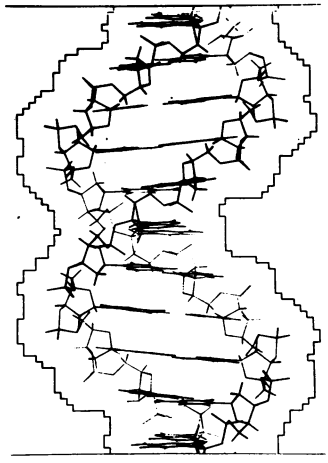


FIGURE 3. Molecular graphic of model poly (dI.dC) double helix

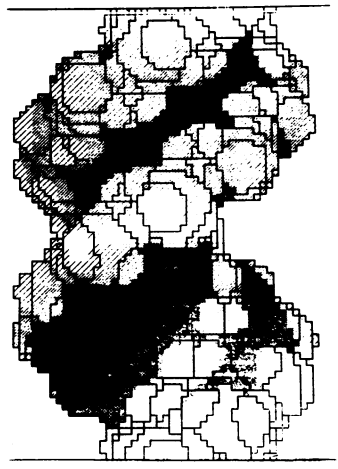


FIGURE 4. Surface potentials of model poly (dI.dC) double helix (for significance of shading see table 3).

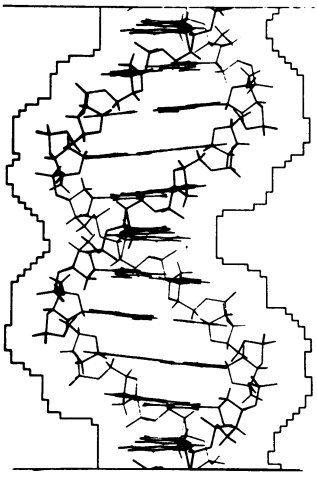


FIGURE 5. Molecular graphic of model poly (dG.dC) double helix.

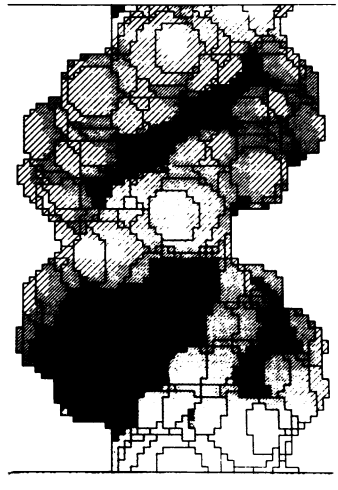
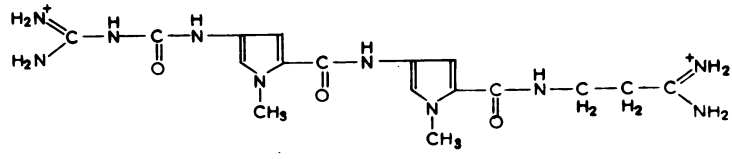


FIGURE 6. Surface potentials of model poly (dG.dC) double helix (for significance of shading see table 3).

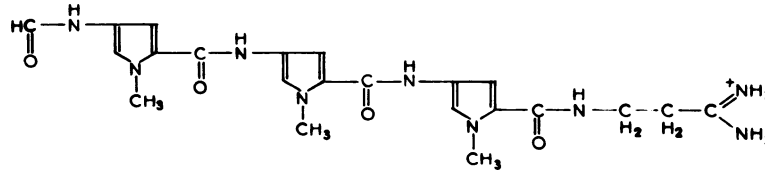
TABLE 4. Local minima on surface envelopes for various base sequences in B-DNA.

BASE SEQUENCE	ENVELOPE LOCAL MINIMUM (kcal/mole)	
	MINOR GROOVE	MAJOR GROOVE
poly (dI.dC)	- 622.7	- 622.1
poly (dG.dC)	- 602.9	- 631.6
poly (dA.dT)	- 625.3	- 598.4

In the proposed representation of the binding to the B-DNA double helix (15, 16), hydrogen bonds are considered to be formed between the NH groups of the peptide links of the antibiotics and O2 of thymines and N3 of adenines and also between the positively charged propionamidine group(s) of the antibiotics and the phosphate oxygens of the double helix.



I. Netropsin



II. Distamycin A

The examination of these findings from the point of view of our theoretical results enables their rational interpretation. In poly (dA.dT) the potentials are considerably more negative on the surface of the minor groove than on that of its major groove and both N3 (A) and O2 (T) are accessible (the calculated accessible areas are 0.7 and 0.9 $\mathring{A}^2$ respectively (2, 4). In poly (dI.dC) the minor groove surface potentials are similar to those of poly (dA.dT) and are competitive with those of the major groove of this double helix. Again the purine N3 and pyrimidine O2 atoms are accessible. On the other hand, in

poly (dG.dC) it is the potentials in the major groove that dominate and N3 (G) and O2 (C) are virtually inaccessible.

This situation suggests that the affinity of the different types of double stranded polymers towards netropsin and distamycin A may be correlated with the existence of a deep electrostatic potential in the minor groove of these double helices and the simultaneous accessibility of the "anchoring" sites of this preferred groove, N3 of the purines and O2 of the pyrimidines.

A confirmation of our view as to the significance for this binding of the potential in the minor groove is substantiated by a recent extension of our work to A-DNA (20). Computations on this conformer of DNA, using again as models the complementary polymers poly (dA.dT) and poly (dG.dC) (coordinates from 11) indicate that in both cases the major groove presents by far a deeper potential zone than the minor groove. On the other hand, because of the displacement of the base pairs in A-DNA away from the helical axis towards the minor groove, this groove becomes extremely shallow (while the major groove is narrow and deep) and corresponds virtually to a flat face. Consequently the accessibilities of the N3 atoms of its purines and of the O2 atoms of its pyrimidines become quite large, larger that in B-DNA. Experimental results indicate that the binding of netropsin and distamycin A to DNA is progressively and strongly diminished when DNA undergoes a B→A transition (16, 19). This example clearly shows the importance of the existence of the deepest potential in the minor groove of the receptor double helix in order for the interaction to occur.

The above studied cases of the binding of netropsin and distamycin A to nucleic acids are examples in the vast field of polypeptide-polynucleotide interactions. In the search for a "code" for these interactions - the "second biological code" as it has been termed by Gursky et al. (15, 17) - much attention has been given to the possibilities of hydrogen bonding between the side chains or amide linkages of the proteins and the bases of the nucleic acids, to the electrostatic attractions between the charged groups on the nucleic acids (phosphates) and on the proteins (ammonium, guanidinium) and to a lesser extent to hydrophobic interactions (15, 21, 29). It appears from this investigation that attention should also be given to the electrostatic molecular potential in the grooves of the nucleic acids. From that point of view, the evolution of the situation with respect to the potential (and accessibility) in the minor groove of B-DNA upon replacing guanine by hypoxanthine, as demonstrated here, may be considered as a manifestation of the "disruptive", "repulsive" effect of guanine for the "second" code, advocated by Ivanov (23). This, of course, in no way means that the NH_2 of guanine has a general negative

effect on all interactions involving the nucleic acids. That this is absolutely not the case and that this group represents a particularly important center of attack in DNA is illustrated by the numerous recent results pointing to it as the principal target for many carcinogens, mutagens, chemotherapeutic agents, antibiotics etc ... (24 - 31).We have discussed the mechanisms involved in this reactivity and the role of the potential at the amino group previously (2, 6, 7, 32).

CONCLUSIONS

Comparison of the molecular electrostatic potentials and of the steric accessibilities associated with poly (dI.dC) and poly (dG.dC) has enabled us to quantify the effect of the guanine 2-amino group on these properties. The replacement of guanine by hypoxanthine is found to increase the accessibility of the N3 atom of the purine and of the O2 atom of the base paired pyrimidine. Parallel, it deepens the negative potentials associated with these atoms and renders the surface potential of the minor groove equivalent to that of the major groove in poly (dI.dC), in contrast to the situation in poly (dG.dC) where the potential is considerably deeper in the major groove. These factors make poly (dI.dC) appear more similar to poly (dA.dT) than to poly (dG.dC) and this finding may be related to the binding of the antibiotics netropsin and distamycin A to model nucleic acids and to B-DNA.

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