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The Molecular Electrostatic Potential of the B-DNA Helix

IV. C_8 and Amino Sites of Purines and the Binding of Carcinogenic Acetylaminofluorene to DNA

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The influence of a single and double helical DNA environment on the molecular electrostatic potentials at the C_8 and amino sites of guanine and adenine are studied. The results are employed in a tentative explanation of the variation in binding sites of N-acetoxy-N-2-acetylaminofluorene with DNA, following whether the nucleic acid is in a native or denatured state.

Key words: B-DNA helix – Acetylaminofluorene, carcinogenic, binding of ~ to DNA

1. Introduction

In recent publications from this laboratory [1, 2] the strong influence of the DNA double helical environment on the electrostatic potentials at reactive sites of the nucleic acid bases has been demonstrated. It has been shown that even the relative order of the local minima of the potential at the various nucleophilic sites of the bases can be changed as a result of the electrostatic fields of the phosphate-sugar backbones, of the paired base and, to a lesser extent, of the neighbouring stacked bases.

These calculations, which approximate the influence of the various units within the DNA macromolecule by their individual molecular electrostatic fields [3, 4], or at longer range, by the multipolar models of these fields [5–8], allow the units of the macromolecule to be treated in a simple additive way, essential for so large a system, while retaining a rather satisfactory representation of the electrostatic environment at the base reactive sites.

When reaction of electrophiles with DNA is considered this electrostatic field is a large component of the interaction energy and may be dominant in determining the

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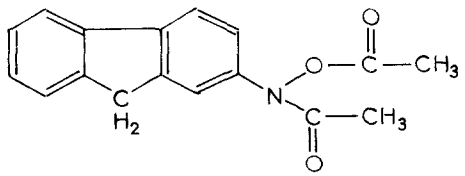

Fig. 1. N-acetoxy-N-2-acetylaminofluorene (N-Ac-AAF)

site of reaction of the electrophile [3], although it must be borne in mind that other factors which, besides the purely electrostatic one, contribute to interaction energies – the polarization, charge transfer and exchange components of the energies, the solvent effect or the particular steric effects associated with the nature of the reactant – may also have an important bearing on the site of the reaction [9, 10].

In spite of these reservations, the electrostatic effect, if only because of its magnitude, represents certainly a first-order view of the reactional potentialities of these sites, in particular of the relative reactivities of similar sites, and in fact its usefulness for the studies of such reactivities has been demonstrated in our previous work. In particular, the increased potential at the guanine amino group of the bases in a double helical DNA environment has been noted. This site is not normally thought of as important for electrophilic reactions but it has recently attracted attention as the binding site of carcinogenic hydrocarbon metabolites such as benzo[a]pyrene-7,8-diol-9,10-epoxide [11–13]. The consideration of the electrostatic molecular

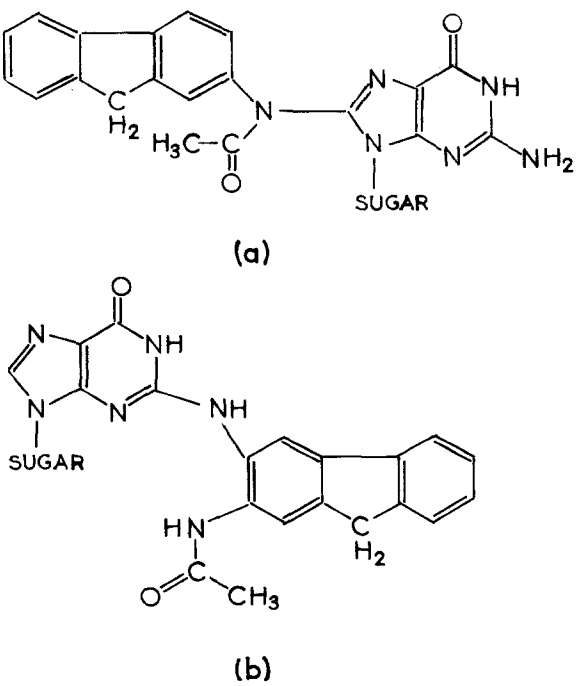

Fig. 2. (a) the binding of N-Ac-AAF at the C_8 site of guanine, (b) the binding of N-Ac-AAF at the N_2 site of guanine

potential enables one to account for the relative affinity of this metabolite for the amino groups of guanine, adenine and cytosine within DNA [1, 2].

On the grounds of this success, we now present an extension of these studies to include the C_8 site of the purine bases, guanine and adenine, which again, although not normally considered important as a reactive site, is, in the case of guanine, involved in binding metabolites of carcinogenic amines, notably, N-acetoxy-N-2-acetylaminofluorene, (N-Ac-AAF) [14–16] (Fig. 1) and the related molecules, N-acetoxy-N-4-acetylaminobiphenyl [17, 18] and N-acetoxy-N-2-acetylamino phenanthrene [19–20]. We are particularly interested in attempting to find an explanation for the shift of the percentage of N-Ac-AAF binding at the C_8 and amino sites of guanine when a comparison is made between attack on DNA in its native and denatured states. Thus, when the reaction takes place with denatured DNA, binding of N-Ac-AAF occurs nearly exclusively (97%) at the C_8 site of guanine. However, when the reaction takes place with native DNA only 80–85% of binding is to this latter site while 15–20% of the reactant is bound at the guanine amino group, N_2. The two types of product obtained are illustrated in Figs. 2a and 2b, respectively.

2. Method

The model of B-DNA chosen is, as described in our earlier publications [1, 2], a mini-helix represented in Fig. 3, composed of three complementary base pairs and the associated sugar-phosphate backbone, which enables us to study the influence of this backbone and of the adjacent base pairs placed on both sides of the central one, on the potential in the vicinity of this central pair. In Fig. 3 this is a G–C pair but our computations considered also the A–T pair in that position. As a matter of fact, in the calculations carried out here the effects of the neighbouring base-pairs were omitted as they have previously been shown [1] to be very small compared to the influence of the backbone. The backbone geometries were taken from the work of Arnott [21] and the base geometries are those employed in previous studies carried out in this laboratory [22].

The representation of the denatured DNA raises a difficult problem which cannot be solved exactly at present. It requires a representation of the electrostatic potential at the base sites in a single stranded DNA, the difficulty being due to the flexibility of the nucleic acid backbone and the fact that no fixed secondary structure can be assigned. In the present study, in view of the complexity of the problem we

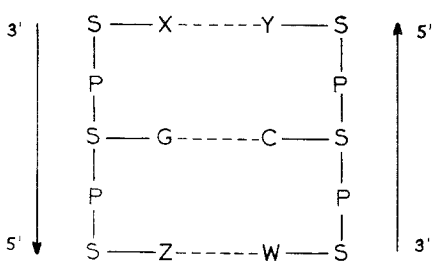

Fig. 3. Schematic representation of the B-DNA model (illustrated for a central guanine-cystosine base pair)

have as a first and easiest approximation chosen simply to retain the B-DNA helical structure for a single strand DNA. Such a representation should translate to some extent the features of the distribution of the potential in a single strand and the variation with respect to the double helix.

Exact electrostatic potentials were used for all units of the model when any one of the constituent atoms was approached to a distance of less than 2.2 Å. Beyond this limit the multipolar approximation to the exact electrostatic potential has been shown to yield the same results [23] and was consequently employed. All potentials were calculated from wavefunctions generated with the atomic basis defined in Ref. [24].

For the amino groups of guanine and adenine, minima can be located in the electrostatic potential above and below the plane of the base and close to the nitrogen, for both the free bases, base pairs, the bases in the B-DNA model [1, 2] and for single stranded DNA. A similar minimum has been located in a plane perpendicular to the plane of the base near the C_8 atom of guanine, in the free base, while adenine showed no minimum in that region [25]. We have now extended the calculations to the bases in single and double stranded DNA in which a similar situation prevails. In the following, the minimum value for guanine at a distance of 2 Å from the base plane, along a line through C_8, perpendicular to the base plane and the corresponding value of the potential at the same plane for adenine, will be used to represent the potential in that region in the different systems considered.

3. Results and Discussion

In Table 1 the minimum potentials at the amino groups and the chosen potentials at the C_8 atoms of guanine and adenine are compared for the free bases and for single stranded and double stranded DNA models.

It can be seen immediately as a general result that the potentials at all sites increase markedly due to the presence of sugar-phosphate backbones. However, we shall essentially limit our interest to the relative differences of the potential between the

Table 1. Electrostatic potentials at the C_8 and amino groups of the purine bases (kcal/mole)

Base	Site	Free base potential	Single helix B-DNA potential	Double-helix B-DNA potential
Guanine	C_8(3′ side)	−6.6	−124.7	−176.1
	C_8(5′ side)	−6.6	−124.2	−176.3
	N_2(3′ side)	+0.6	−77.7	−171.5
	N_2(5′ side)	+0.6	−74.1	−170.9
Adenine	C_8(3′ side)	+3.8	−114.1	−162.8
	C_8(5′ side)	+3.8	−113.6	−162.9
	N_6(3′ side)	−15.6	−88.3	−158.2
	N_6(5′ side)	−15.6	−87.2	−157.9

bases and between the sites within a given base when passing from the free bases to one of the DNA models we have studied. (It can also be seen that differences in potential between the 3′ and 5′ sides of a given base site in either single or double stranded DNA are rather small. Consequently, as there is no available data on the route of attack by electrophilic species, each site will be referred to by a single value, whichever is the more negative of the 3′ and 5′ results.)

It is immediately seen that the change from free bases, to a single stranded helix and then to the double helical DNA is accompanied by drastic displacements of the potential minima and modifications of their relative values between the C_8 and N_{amino} sites.

In the free bases the potential is negative (attractive for electrophiles) at the N_{amino} group of adenine and C_8 of guanine. In absolute value it is greater at the former (-15.6 kcal/mole) than at the latter (-6.6 kcal/mole). The potential at the other two sites, N_{amino} of guanine and C_8 of adenine is repulsive (positive).

In the single stranded helix all the potentials become negative, due obviously to the additive effect of the strongly negative potential of the phosphate group. Moreover for this strand the potentials at C_8 are very substantially more negative than those at the N_{amino} groups for both purines. The deepest potential minimum is associated with C_8 of guanine. It is separated by about 10 kcal/mole from that of C_8 of adenine.

All the potentials deepen, in absolute value, in the double helical DNA. Moreover, both the C_8 and N_{amino} potentials of guanine have now become significantly deeper than the two corresponding potentials of adenine. The deepest potential is associated with C_8 of guanine (-176 kcal/mole), followed, however, closely (-171 kcal/mole) by that of its N_{amino} group.

Although as stated before the correlation of these results of molecular electrostatic potential calculations with experimental reactivities must be made with caution due to the number of factors, beyond the electrostatic environment, which may be involved and to the approximations used in the models employed, it is gratifying to observe that the computations are able to account for some of the most prominent features of the observed binding of N-Ac-AAF to DNA in vitro [14, 15]. Thus, for the single stranded DNA, the guanine C_8 potential minimum is very considerably more attractive than all the other sites and the observed practically exclusive binding of N-Ac-AAF at this site is therefore explicable. For the double helical DNA the guanine C_8 potential still remains the deepest one but is, however, followed closely (a difference of 5 kcal/mole) by that of its N_{amino} group. The occurrence of a fraction of the binding at this latter site seems therefore reasonably accounted for. The absence of reactivity of adenine, whether in the denatured or native DNA is also satisfactorily accounted for by the computations. It may also be noted that the distribution of the potentials in the free bases is quite different from that in either single or double strand DNA models and could not be used to explain the observed binding data of N-Ac-AAF.

In spite of these satisfactory correlations some difficulties remain. Thus our results which indicate that the potentials are more negative for double stranded than for

single stranded DNA, would suggest more reaction overall with native than with denatured DNA. The reverse is said to be observed experimentally in the very case of N-Ac-AAF [14–16]. On the other hand, the predicted situation (smaller reactivity with denatured than with native DNA) is observed in binding to DNA (mostly at C_8 of guanine) of the related carcinogen N-hydroxy-N-2-aminofluorène [26]. A certain variability of the results may also occur as a function of the nature of the attacking ligand. Thus our theoretical results account for the competitivity between C_8 and N_{amino} of guanine not only for N-Ac-AAF but also for N-acetoxy-N-4-acetylaminobiphenyl, which qualitatively seems to behave similarly (the latter compound being somewhat less exclusive for C_8 in denatured (86%) and helical (55%) DNA [27], but showing the same increase in amino binding when passing from the former to the latter). On the other hand, N-acetoxy-N-2-acetylaminophenanthrene [27, 28] seems to bind nearly equivalently to the C_8 and N_{amino} sites both in denatured and helical DNA. This example indicates the possible significance of the specificity of the ligand.

4. Conclusions

The C_8 site of the purine bases has been included in the previously presented study of the electrostatic potentials of the free nucleic acid bases and of these bases within a B-DNA environment.

By adopting a simple model of denatured DNA and by focusing on electrostatic potential effects we have been able to propose an explanation for the observation that while N-Ac-AAF binds nearly exclusively at C_8 of guanine in denatured DNA, the attack on native DNA leads to a 20% binding at the guanine amino group. In view of the complexity of the problem, which requires the computations to be carried out for fragments of DNA, this is an encouraging result and an incentive for a deeper exploration of the problem.

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