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# A Theoretical Study of the Relative Affinities of an Aliphatic and an Aromatic Bisguanylhydrazone for the Minor Groove of Double-Stranded $(dA\text{-}dT)_n$ Oligomers

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The nonintercalative binding of an aliphatic and an aromatic bisguanylhydrazone (BGH) to the minor groove of double-stranded $(dA\text{-}dT)_n$ oligomers is investigated by means of theoretical computations. The preferred binding arrangements of both BGHs are stabilized by a number of H-bonding interactions with sites $O_2(T)$, $N_3(A)$ and $O_1'$ on the two strands, and require limited conformational rearrangements of the BGHs around their C—C single bonds. The intermolecular interaction energy is larger with the aliphatic BGH than with the aromatic one. The energy difference is, however, considerably reduced when the oligomer is lengthened: it passes from 16.1 kcal/mole at the heptamer level, to 7.9 kcal/mole at the undecamer level and to 4.6 kcal/mole when each strand of the undecamer is flanked with a complementary complete helical turn of phosphates, on both the 3′ and 5′ termini.

The interaction energies of the BGHs with water molecules in the first hydration shell are, however, also larger with the aliphatic BGH, than with the aromatic BGH. This energy difference is further enhanced when one considers also the water molecules in the second shell. It becomes greater than the difference in the interaction energy of the two BGHs with $(dA\text{-}dT)_n$ for large values of $n$. When the dehydration energy of BGHs is taken into account the overall energy balance is then more favorable for the interaction of the aromatic than of the aliphatic BGH with the polynucleotide. This last conclusion is in agreement with experimental results.

**Key words:** Bisguanylhydrazones—Nonintercalative binding—Minor groove—$(dA\text{-}dT)_n$ oligomers.

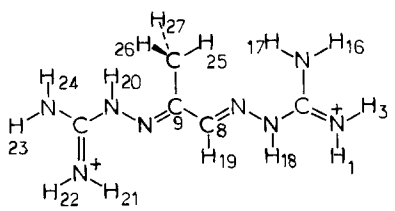
## 1. Introduction

Bisguanylhydrazones (BGH) are a class of planar, dicationic molecules, shown to bind nonintercalatively to DNA [1, 2]. They are built of two guanidinium fragments, separated by the moiety —N=X=N—, in which X can be either an aliphatic or an aromatic fragment, the structure of which can be varied by chemical synthesis. These compounds are generally endowed with antileukemic properties. In the case of the aromatic BGHs, a correlation was established between the antileukemic potency, the ability to inhibit DNA polymerase in L1210 leukemic cells and the binding affinity to either DNA [3, 4] or the synthetic poly (dA-dT) polymer [5].

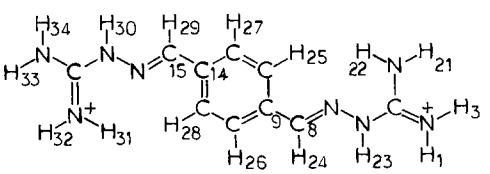
It was shown that the binding site of bisguanylhydrazones [1, 2, 5] to DNA is in its minor groove. These compounds behave thus like a large number of nonintercalative species, including netropsin and distamycin [6], bisquaternary ammonium heterocycles [7], terephtalanilides [1, 2], etc. A preferential binding to A—T rich sequences rather than to G—C rich sequences was further evidenced [5].

Whereas the affinity of the aliphatic BGHs is distinctly lower than that of the aromatic compounds, binding to DNA of methylglyoxalbisguanylhydrazone was nevertheless evidenced [4, 8].

As a first step towards an understanding of the structural and energetical aspects involved in BGH binding to DNA, we have compared in the present study the binding affinities towards the minor groove of $(dA\text{-}dT)_n$ oligomers, of a representative of the aliphatic BGHs, namely methylglyoxalbisguanylhydrazone (MGGH) and of a representative of the aromatic BGHs, namely parabenzylbisguanylhydrazone (PBGH). The structural formulae of the investigated compounds are given in Fig. 1, together with the atom numbering. The theoretical studies will be performed on the doubly protonated forms of the BGHs, which




(a)




(b)

**Fig. 1a, b.** Structural formulae and atom numbering for MGGH and PBGH

are the predominant forms in solution. It will imply among others the analysis of the extent to which will the interaction energy difference between the aliphatic and the aromatic BGHs change as a function of the length of the oligomer, or of countercation binding to the phosphates; an examination of the variation of the intramolecular energy expenditure required to generate conformational rearrangements around the single bond $C_8$—$C_9$ of MGGH and the two single bonds $C_8$—$C_9$ and $C_{14}$—$C_{15}$ of PBGH, with respect to the planar *trans* conformation, so as to enable an optimal fitting in the minor groove of $(dA\text{-}dT)_n$; an exploration of possible difference in the dehydration energies of the two BGHs, a factor which if present must be included in the overall energy balance of the interaction.

## 2. Computational details

The intermolecular interaction energies are computed by means of an additive procedure, elaborated in our laboratory [9], and applied to a number of problems related to binding specificities [10–14]. It was shown to reproduce satisfactorily the results of *ab initio* SCF supermolecule computations in representative cases [9, 10, 15] or experimental results when available [11, 16].

The interaction energy $\Delta E$ is computed as a sum of four components:

$$\Delta E = E_{MTP} + E_{pol} + E_{rep} + E_{dl}$$

where $E_{MTP}$ is the electrostatic interaction energy computed between the overlap multipole expansions of the charge distributions of the entities in interaction, $E_{pol}$ is the polarization energy, $E_{rep}$ is the sum of bond–bond repulsions, and $E_{dl}$ is a dispersionlike term, calibrated in [9].

The multicenter multipolar expansions (up to quadrupoles) of the charge distributions of the bisguanylhydrazones, required to compute the electrostatic and polarization contributions to the binding energy [9], are derived from *ab initio* SCF computations using the Melius–Topiol pseudopotentials [17, 18]; the minimal orbital basis set utilized is the one described in Ref. [19], with a *dzeta* exponent of 1.2 on the C—H hydrogens and 1.5 on the N—H hydrogens.

The input data for the BGHs use standard bond lengths and valence angles, close to the ones determined by an X-ray diffraction study of methylglyoxalbisguanylhydrazone [20] and its dimethyl derivative [21].

We have retained throughout this study the standard B DNA conformation for the oligomers, as given by the refined coordinates published by Arnott et al. in 1980 [22]. This choice was adopted on account of the results of recent theoretical computations of the proton shifts of the bases in a model dodecamer [23], which indicate the relevance of these coordinates to situations in solution.

The nucleic acid oligomers were constructed from their constituent fragments in the same fashion as that adopted for the computation of the molecular electrostatic potential of large macromolecules [24]. The constituent fragments are the two bases, deoxyribose and monomethylphosphate; their *ab initio* SCF wave functions were computed using our usual basis set [25].

The multicenter multipole expansions of the constituent subunits of DNA and of the BGHs were simplified according to a procedure recently developed in this laboratory [26] in which every dipole and quadrupole located on the center of a non-bonded pair of atoms is split between the two centers closest to it, either atom or bond barycenter (whether related to the corresponding pair or not).

The search for the optimal binding configurations of the BGHs was performed by means of an energy-minimization procedure [27]. We have proceeded in two steps. A prior determination of the optimal fitting of the BGH in the minor groove of the oligomer was performed first by computing the sole monopole-monopole contribution, together with $E_{rep}$ and $E_{dl}$ and the polarization energy due to the field exerted by the sole monopoles. In this step, conformational rearrangements are allowed during energy-minimization, along the C—C single bond of the aliphatic BGH and the two C—C single bonds of the aromatic BGH. For the most significant energy-wise configurations, the wave-function of the BGH is recomputed in its redetermined intramolecular conformation: this enables to derive the energy expenditure for conformational rearrangement along the C—C single bond(s), as well as the multipolar expansion in the new conformation. The energy minimization is then resumed in a second step, but the electrostatic and the polarization contributions are now computed by means of the complete multipolar expansion.

## 3. Results and discussion

### *3.1 The isolated* BGHs

The distributions of the Mulliken net charges in the two BGHs are represented in Figs. 2(a, b). These distributions are seen to be closely similar on all corresponding atoms in the two BGHs. Thus, replacement of an aliphatic carrier by an aromatic one does not alter significantly the charge distribution in the terminal cationic groups and affects only moderately that of the two neighboring nitrogens.

### *3.2 Interactions with the oligomers*

The notations adopted to denote the bases in the two strands at the undecamer level are indicated in Fig. 3.

The binding of the BGH can occur *a priori* along one or the other of the two directions of the oligonucleotide. Let us consider the imino NH bond and the amino NH bond *cis* to it, of one terminal guanidinium group of the BGH. The two corresponding NH hydrogens will be bound to sites ($O_1'$, $O_2$ and $N_3$) belonging to one strand of the oligonucleotide in the minor groove. We define the binding of the BGH to occur along the direction 3′5′ of the oligonucleotide, if in order to reach the other extremity of the BGH, one runs along the direction 3′5′ of the considered strand, that is, in the sense of the arrow in Fig. 3. The direction 5′3′ denotes the reverse sense. Energy-minimization indicated the binding of both BGHs to occur more favorably along the 3′5′ direction than along the 5′3′ direction, the energy difference amounting to $\simeq$20 kcal/mole at the undecamer

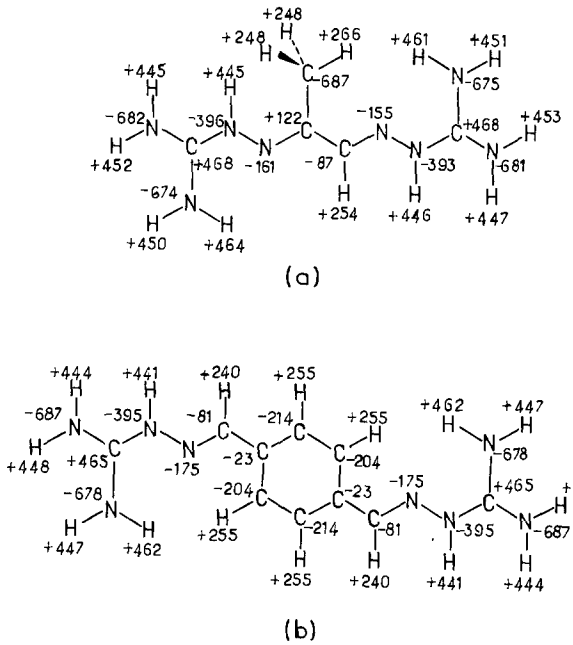



**Fig. 2a, b.** Distribution of the Mulliken net atomic charges in the investigated bisguanylhydrazones

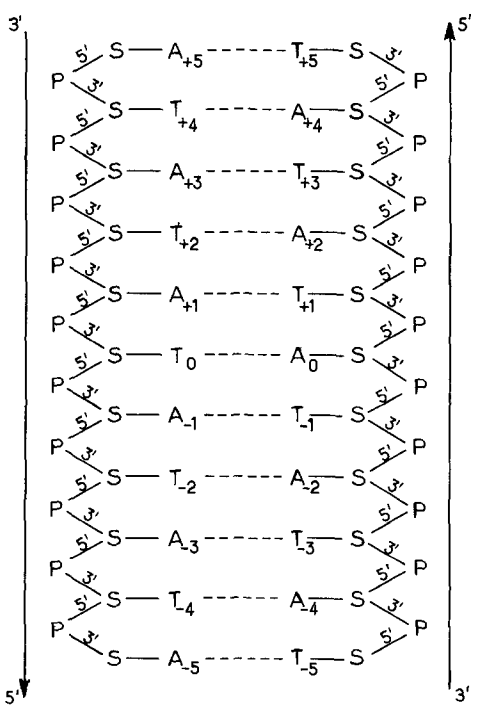



**Fig. 3.** $(dA\text{-}dT)_n$ at the undecanucleoside decaphosphate double helix level. Atom numbering

**Table 1.** Values of the optimized intermolecular interaction energies of MGGH and PBGH with double-stranded $(dA\text{-}dT)_n$ oligomers. Energies in kcal/mole

| | Heptanucleoside hexaphosphate | Undecanucleoside decaphosphate | Triple phosphate turn | Undecanucleoside decaphosphate screened by $M^+$ | Triple phosphate screened by $M^+$ |
|---|---|---|---|---|---|
| MGGH | | | | | |
| $E_{tot}$ | −893.1 | −1222.5 | −2033.1 | −295.6 | −311.5 |
| $E_{MTP}$ | −827.3 | −1152.0 | −1962.1 | −225.1 | −240.6 |
| $E_{pol}$ | −42.7 | −45.2 | −45.6 | −45.2 | −45.5 |
| $E_{rep}$ | +35.9 | +32.5 | +32.5 | +32.5 | +32.5 |
| $E_{dl}$ | −62.0 | −60.8 | −60.9 | −60.9 | −60.9 |
| $E_{conf.}$ | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| PBGH | | | | | |
| $E_{tot}$ | −877.0 | −1214.6 | −2028.5 | −287.3 | −307.0 |
| $E_{MTP}$ | −808.1 | −1143.0 | −1956.3 | −215.7 | −235.1 |
| $E_{pol}$ | −37.3 | −40.4 | −41.0 | −40.4 | −41.1 |
| $E_{rep}$ | +41.8 | +44.1 | +44.1 | +44.1 | +45.1 |
| $E_{dl}$ | −77.9 | −79.7 | −79.8 | −79.8 | −80.5 |
| $E_{conf.}$ | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |

level. The results presented below pertain thus to the energy values optimized along the 3′5′ direction.

We have reported in Table 1 the results of energy-minimization for both BGHs. The optimal configuration derived for binding MGGH involves a torsion of 40° along the $C_8—C_9$ single bond. The optimal configuration derived for binding PBGH involves conformational changes of 12° and 67° along its single bonds $C_8–C_9$ and $C_{14}—C_{15}$, respectively. In Table 1, $E_{tot}$ denotes the sum of the intermolecular interaction energy, $\Delta E$, of the BGH with the oligomers considered and of the conformational energy, $E_{conf.}$, required to generate the appropriate conformational change of the BGH with respect to the planar *trans* conformation.

Because previous studies from this laboratory have pointed out the importance of length effects on the electrostatic properties of oligonucleotides [28, 29], and because of the possible significance of end effects on the specificity of binding, the affinities of the BGHs were investigated for binding to:

1. A double helix built out of a heptanucleoside hexaphosphate; we have started this investigation at the heptamer level in order to ensure that the number of base pairs is slightly larger than the minimum number of bases spanned by the BGHs, which is estimated to be close to 5 [1, 2];

2. A complete helical turn of B DNA, i.e. a double helix built out of an undecanucleoside decaphosphate;

3. A complete double helical turn, as above, in which the two strands are moreover flanked with a supplementary complete helical turn of phosphodiester

**Table 2.** Values of the optimized interatomic distances between two BGHs and (dA)-dT)$n$ at the undecanucleoside decaphosphate levels. Distances in Å

| MGGH | | | | PBGH | | | |
|---|---|---|---|---|---|---|---|
| 5′3′ strand | | 3′5′ strand | | 5′3′ strand | | 3′5′ strand | |
| $H_1$—$O_1'S_3$ | 2.46 | $H_{25}$—$O_1'S_{-1}$ | 3.29 | $H_{21}$—$O_1'S_0$ | 2.75 | $H_{23}$—$O_1'S_{-2}$ | 2.75 |
| $H_1$—$O_2T_2$ | 2.65 | $H_{26}$—$O'S_{-1}$ | 2.14 | $H_{22}$—$O_1'S_0$ | 2.61 | $H_{24}$—$O_2T_{-1}$ | 2.93 |
| $H_{18}$—$O_2T_2$ | 2.17 | $H_{27}$—$O_1'S_{-1}$ | 2.43 | $H_{22}$—$O_2T_0$ | 3.10 | $H_{26}$—$O_1'S_{-1}$ | 2.60 |
| $H_{18}$—$O_1'S_2$ | 3.28 | $H_{17}$—$O_1'S_0$ | 2.40 | $H_{27}$—$O_1'S_1$ | 2.37 | $H_{31}$—$O_1'S_0$ | 2.86 |
| $H_{19}$—$O_1'S_2$ | 2.56 | $H_{20}$—$O_2T_{-1}$ | 2.76 | $H_{30}$—$O_2T_2$ | 2.72 | $H_{32}$—$O_1'S_0$ | 2.60 |
| $H_{21}$—$O_1'S_1$ | 2.38 | $H_{24}$—$O_1'S_{-2}$ | 3.06 | $H_{34}$—$O_2T_2$ | 2.44 | $H_{23}$—$N_3A_{-2}$ | 2.62 |
| $H_{21}$—$O_2T_0$ | 3.07 | | | $H_{30}$—$O_1'S_2$ | 2.29 | $H_1$—$O_1'S_{-3}$ | 2.42 |
| $H_{22}$—$O_2T_0$ | 2.74 | | | | | | |

groups, on both 3′ and 5′ termini (denoted as the "triple phosphate turn" below). This last computation was performed in order to investigate the effect of a higher buildup of the anionic charges of the phosphate backbone on the compared affinities of the two BGHs: we expect to be able to assess in this way whether a "polymeric" effect will exert itself on these affinities as contrasted to the "oligomeric" situation encountered at the undecanucleoside decaphosphate level.

4. Double helices in which the anionic charges of the phosphate are screened by the presence of a bound cation in the plane of the anionic oxygens [25, 30].

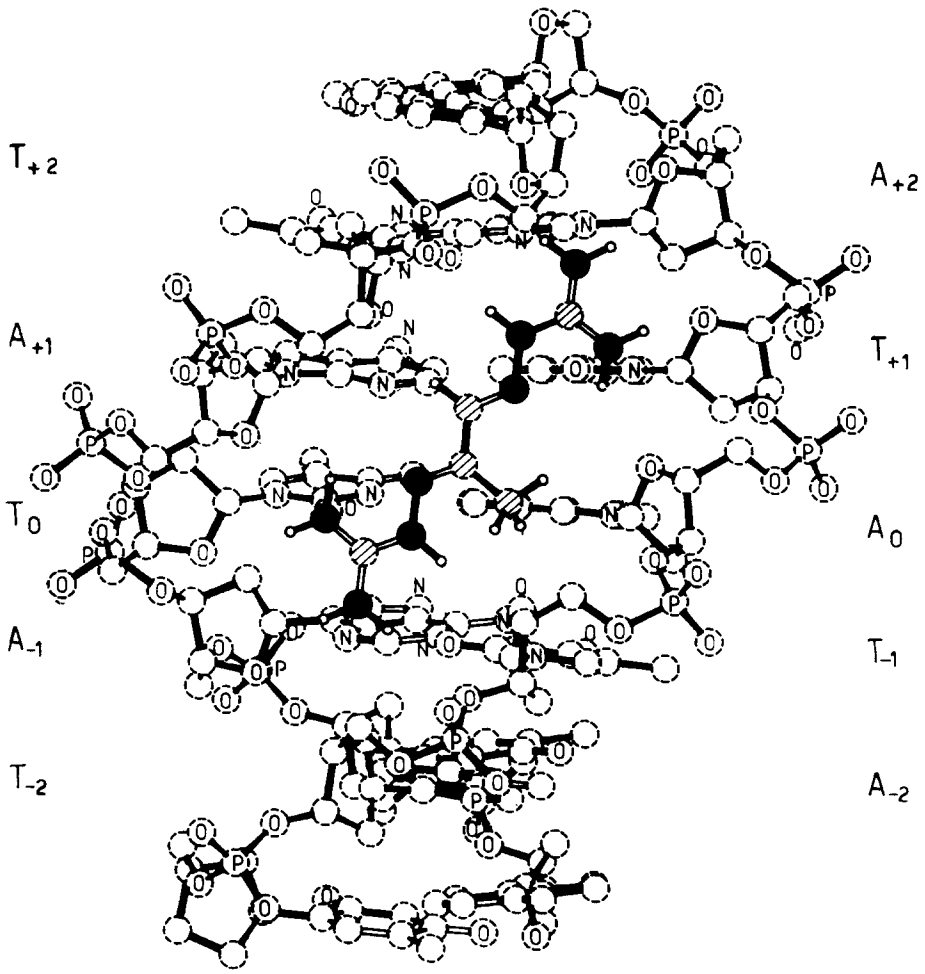
Table 2 reports the most significant intermolecular distances between the hydrogen atoms of the BGHs and the binding sites of the oligomer, as derived at the undecanucleoside decaphosphate level.

The optimal configurations derived for MGGH and PBGH binding are shown in Fig. 4(a, b). For the sake of clarity, only the shortest heptanucleoside double helix is represented. These figures were drawn with the help of FIGATOM program [31] for drawing stereoscopic views by a graphic plotter.

The results of Table 1 lead to the following conclusions:
a) the values of $E_{tot}$ are larger with the aliphatic BGH than with the aromatic BGH.
b) the values of $E_{tot}$ increase when the oligomer chain is lengthened.
c) *The energy difference between corresponding values of* $E_{tot}$ *for the two* BGHs, $\delta$, *decreases markedly when the oligomer chain is lengthened.* Thus, whereas $\delta$ amounts to 16.1 kcal/mole for the heptamer, it drops to 7.9 kcal/mole for the undecamer. It is further reduced to 4.6 kcal/mole when the two undecanucleoside decaphosphate strands are flanked with a complete turn of phosphates on their 3′ and 5′ termini.

It is interesting to compare the individual contributions to the binding energy between the two BGHs, in order to delineate the energetical factors involved in their relative affinities for the oligonucleotides.

The values of the electrostatic contribution to the binding of the BGHs, $E_{MTP}$, increase considerably from the heptamer to the undecamer, to the triple




**Figs. 4a, b.** Representation of the complexes of MGGH (**a**) and PBGH (**b**) with $(dA\text{-}dT)_n$ at the heptanucleoside hexaphosphate double helix level

phosphate turn, owing to the increase of the total number of phosphates (12, 20 and 60 respectively). By contrast, the difference $\delta_{MTP}$ of the $E_{MTP}$ values between the two BGHs, favoring the aliphatic BGH decreases in a manner parallel to that of the corresponding total energy difference $\delta : \delta_{MTP}$ drops from 19.2 kcal/mole at the heptamer level, to 9.0 and 5.8 kcal/mole at the undecamer and triple phosphate levels, respectively.

The polarization energy, $E_{pol}$, is also larger with the aliphatic BGH than with the aromatic BGH. The energy difference amounts to 5 kcal/mole and is not markedly sensitive to the oligomer chain lengthening. The predominant component of $E_{pol}$ is the polarization of the oligonucleotide by the positive charges of the BGH. MGGH being smaller, can approach closer to its binding sites, $O_2(T)$, $N_3(A)$ and $O'_1$, than PBGH, as can be seen from the comparison between

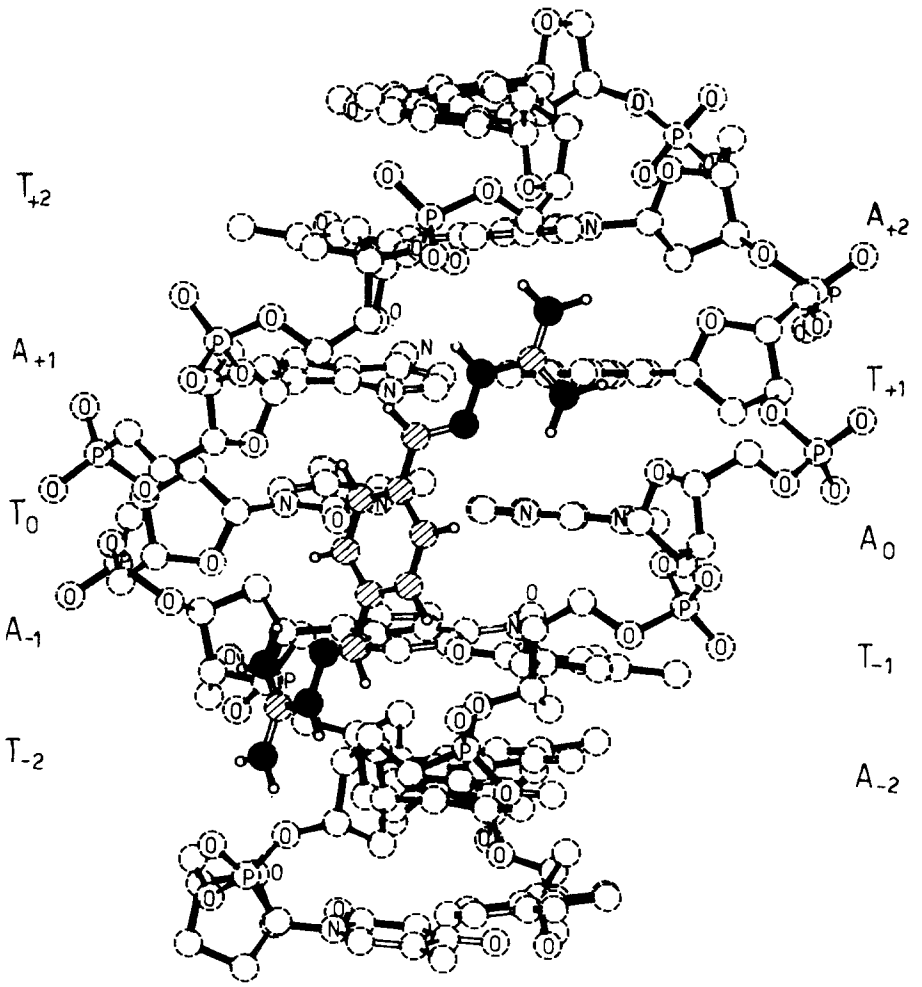



**Fig. 4b**

the interatomic distances of the involved sites of the oligonucleotide to the hydrogen atoms of either BGH (table 2).

The value of the "dispersionlike" contribution, $E_{dl}$, is much more in favour of the aromatic BGH. This result may be considered as a reflection of the larger "hydrophobicity" of the compound. The energy difference, close to 19 kcal/mole, is markedly greater than the corresponding value of $\delta_{MTP}$ when the two longer oligomers are considered. This greater $E_{dl}$ value is, however, opposed by the value of the repulsive contribution, $E_{rep}$, which is also greater for the aromatic BGH, owing to its larger molecular size. The sum $E_{rep} + E_{dl}$ remains, nevertheless, distinctly in favor of the aromatic BGH: the corresponding energy difference amounts to 7 kcal/mole and is not markedly sensitive to the oligomer chain length.

The conformational rearrangements of the BGHs, required for an optimal fitting in the minor groove, entail a more unfavorable conformational energy expenditure $E_{conf.}$, in the aromatic BGH (4.5 kcal/mole) than in the aliphatic BGH (3.0 kcal/mole).

It thus appears that $E_{dl}$ is the sole contribution to the binding energy that favors the aromatic BGH over the aliphatic BGH. The corresponding energy difference, although sizeable ($\simeq$19 kcal/mole) is opposed by the summed differences between the other contributions to the binding of the two BGHs, which all favor the aliphatic BGH: this results in an overall value of $\delta$ in favor of the aliphatic BGH. However, the numerical value of $\delta$ decreases when the chain is lengthened and its evolution is dictated by the corresponding evolution of $\delta_{MTP}$. This result of decreasing $\delta$ and $\delta_{MTP}$ values upon oligomer chain lengthening can be interpreted in terms of the molecular electrostatic potential (the MEP) on the surface envelopes of oligonucleotides. These potentials were shown to have their maximal values, in oligonucleotides of a finite chain length, in the middle of the oligomer [28, 29] and these maximal values have been shown to increase with increasing $n$, a situation which accounts for observation *b*) above. In the present case, the middle designates sites $O_2$ and $N_3$ of $T_0$ and $A_0$ in the minor groove. The attractive values of the MEP decrease steadily upon going from the central base pairs towards both ends of the oligomer. Configurations locating the "center" of the BGH in the region of the center of the oligomer will thus be favored by the electrostatic contribution to the binding. In the shorter aliphatic BGH, the two guanidinium moieties are closer to the center of the molecule and closer to each other, than in the aromatic BGH, and are thus closer to the zone of maximal MEP values.

Such an oligomeric effect is most conspicuous at the level of the heptanucleoside double helix and shifts the result in favor of the aliphatic BGH by a considerable energy difference $\delta$, amounting to 16.1 kcal/mole. It is still appreciable at the level of a complete helical turn ($\delta = 7.9$ kcal/mole). When a triple phosphate turn is considered, it is reduced to 4.6 kcal/mole.

*Effect of countercation binding to the phosphates*

As a result of countercation binding to the phosphates (Table 1), the values of $E_{MTP}$ are reduced to five to six-fold with respect to their values with the unscreened phosphates, the other contributions being rather unsensitive to its effect. The value of the energy difference between the two BGHs on the other hand, is not markedly affected with respect to its value with the unscreened phosphates.

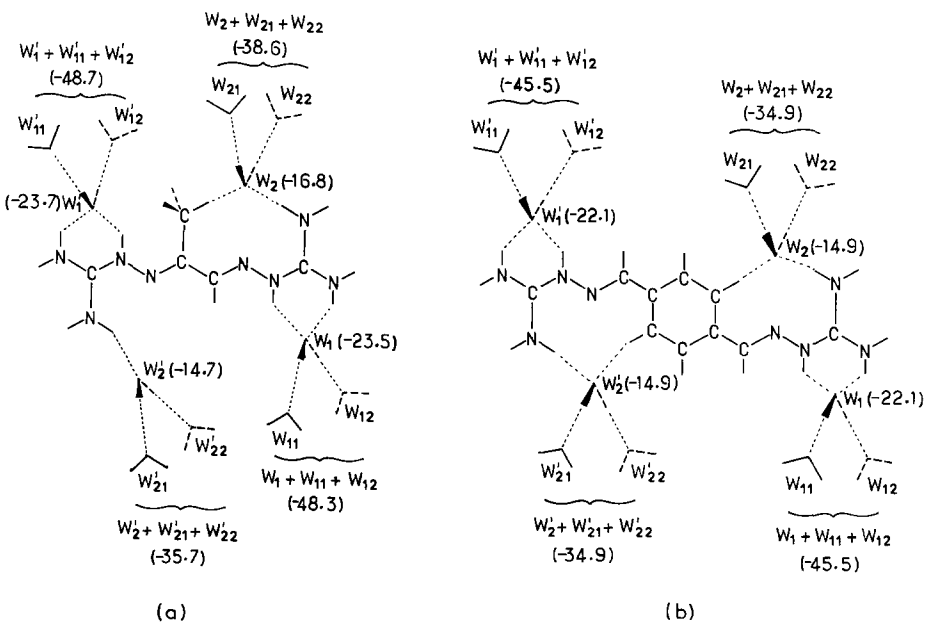
### *3.3. Interaction of the* BGHs *with water*

Our previous studies devoted to binding specificities have put into evidence the role played by dehydration in the comparative energy balances for competitive binding of molecules [11, 12] or cations [10, 13, 14] to a given ligand or receptor site. In the present case, the aliphatic BGH, owing to its smaller molecular size, can be expected to interact more strongly with water than the aromatic BGH:

the binding energy of a water molecule bound to one guanidinium moiety will be reinforced by the other guanidinium, and this reinforcement will be more pronounced with the aliphatic BGH, owing to the greater proximity of the two terminal fragments.

In an attempt to quantify the difference in dehydration energy between the two BGHs, we have computed the energies required to remove water from the sites of the BGHs involved in their interaction with the oligonucleotides. This was done by optimizing the interaction energy of water with the BGH, in the sites indicated in Figs. 5(a, b). The intrinsically preferred configuration of approach locates the plane of water molecule approximately perpendicular to the solvated NH/CH bond(s). Each water molecule was hydrogen-bonded, through its H atoms, to two water molecules in a second shell, on account of the finding [32, 33] that doubly charged cations are able to strongly bind and structure a second solvation shell.

The interaction energy values are reported in Figs. 5(a, b). For each investigated sites, we report the value of the interaction energy of the individual water molecule bound in the first shell, and the value of the total interaction energy at the site when the two second shell water molecules are further added: this last quantity also includes the water–water interaction energy, namely −5.3 kcal/mole in the framework of the present procedure. It appears from Figs. 5(a, b) that the interaction energies of water molecules in the first shell are large and the overall interaction energies are appreciably enhanced when the second shell is incorporated.




**Figs. 5a, b.** Representation of the hydration mode of MGGH (**a**) and PBGH (**b**)

The summed interaction energies, $E_W$, at sites $W_1, W_2, W_1'$ and $W_2'$ have the values $-171.3$ and $-160.8$ kcal/mole for the aliphatic and the aromatic BGH respectively, hence a difference in the hydration energies of the two BGHs in these sites amounting to 10.5 kcal/mole. We may note that this value is significantly larger than the value of the difference between the interaction energies of the BGHs derived at the triple phosphate level, namely 4.6 kcal/mole. This result implies that when the energies required to dehydrate the BGHs are taken into account by substracting the respective values of $E_W$ from the ones computed for $E_{tot}$ at the triple phosphate level, the resulting energy balance for complexation of the BGHs by the oligonucleotide is distinctly in favor of the aromatic BGH rather than of the aliphatic BGH.

The absolute values of $E_{tot}-E_W$ depend on the state of neutralization of the phosphates. Considering the "triple phosphate" level, these values drop 13-fold when passing from the unscreened to the totally screened phosphate states, owing to the decrease of $E_{MTP}$. The latter state may possibly be considered as more representative of the state of the nucleotide in solution. It must be realized however, that even under these conditions the values of the corresponding differences $E_{tot}-E_W$ ($-140.5$ and $-146.2$ kcal/mole for MGGH and PBGH respectively) should not be correlated quantitatively with experimental values of the enthalpies of complexation of the BGHs by the oligonucleotides (unavailable at present). Such a correlation would require a more exhaustive inclusion of the dielectric effects of the interaction in solution. (For a discussion of a similar case and suggestion for a formal treatment see e.g. [34]).

Nevertheless, the present results distinctly show the trend followed by the compared affinities of the BGHs for the oligonucleotide as a function of chain length and strongly suggest a preference for the interaction with the aromatic BGH at the polynucleotide level. This last conclusion is in agreement with experimental results.

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## References

1. Braithwaite, A., Baguley, B.: Biochemistry **19**, 1101 (1980)
2. Baguley, B.: Mol. Cell. Biochem. **43**, 167 (1982)
3. Dave, C., Ehrke, J., Mihich, E.: Chem. Biol. Interactions **12**, 183 (1976)
4. Dave, C., Ehrke, J., Mihich, E.: Chem. Biol. Interactions **16**, 57 (1977)
5. Denny, W., Cain, B.: J. Med. Chem. **22**, 1234 (1979)
6. Zimmer, C.: Progr. Nucl. Acid. Res. Mol. Biol. **15**, 285 (1975)
7. Denny, W., Atwell, G., Baguley, B., Cain, B.: J. Med. Chem. **22**, 134 (1979)
8. Sartorelli, A., Iannotti, A., Booth, B., Schneider, F., Bertino, J., Johns, D.: Biochim, Biophys. Acta **103**, 174 (1965)
9. Gresh, N., Claverie, P., Pullman, A.: Int. J. Quantum Chem., Symp. **13**, 243 (1979)
10. Gresh, N.: Biochim. Biophys. Acta **597**, 345 (1980)

11. Gresh, N., Pullman, B.: Biochim. Biophys. Acta **608**, 47 (1980)
12. Gresh, N., Pullman, B.: Biochim. Biophys. Acta **625**, 356 (1980)
13. Gresh, N., Etchebest, C., de la Luz Rojas, O., Pullman, A.: Int. J. Quantum Chem., Quantum Biol. Symp. **8**, 109 (1981)
14. Gresh, N., Pullman, A.: Int. J. Quantum Chem. **22**, 709 (1982)
15. Gresh, N., Pullman, B.: Theoret. Chim. Acta (Berl.) **52**, 67 (1979)
16. Langlet, J., Claverie, P., Caron, F., Boeuve, J. C.: Int. J. Quantum Chem. **20**, 299 (1981)
17. Melius, C., Goddard III, W.: Phys. Rev. **A10**, 1528 (1974)
18. Topiol, S., Moskowitz, J., Newton, M., Jafri, J., Courant Institut of Mathematical Science, ERDA Research and Development Report (C00-3077-105)
19. Gresh, N., Pullman, A.: Theoret. Chim. (Berl.) **49**, 283 (1978)
20. Hamilton, W., La Placa, S.: Acta Crystallographica **B24**, 1147 (1968)
21. Edmonds, J., La Placa, W.: Acta Crystallographica **B28**, 1362 (1972)
22. Arnott, S., Chandrasekaran, R., Birdsall, D., Leslie, A., Ratliff, R.: Nature **283**, 743 (1980)
23. Giessner-Prettre, C., Pullman, B.: Biochem. Biophys. Res. Comm. **107**, 1539 (1982)
24. Pullman, A., Zakrzewska, K., Perahia, D.: Int. J. Quantum Chem. **16**, 395 (1979)
25. Pullman, B., Gresh, N., Berthod, H., Pullman, A.: Theoret. Chim. Acta (Berl.) **44**, 151 (1977)
26. Maeder-Vigne, F., Claverie, P.: to be published.
27. Fletcher, R.: FORTRAN Subroutines for Minimization by Quasi Newton Methods A.I.R.E. Report R 7125 (1972)
28. Lavery, R., Pullman, B.: Nucl. Acids Res. **9**, 3765 (1981)
29. Lavery, R., Zakrzewska, K., Pullman, B.: Biophys. Chem. **15**, 343 (1982)
30. Cauchy, D., Lavery, R., Pullman, B.: Theoret. Chim. Acta (Berl.) **57**, 323 (1980)
31. Langlet, G.: J. Appl. Crystallogr. **5**, 66 (1972)
32. Veillard, H.: J. Am. Chem. Soc. **99**, 7194 (1977)
33. Berthod, H., Pullman, A., Pullman, B.: Int. J. Quantum Chem., Quantum Biol. Symp. **5**, 79 (1978)
34. Nuss, M., Marsh, F., Kollman, P.: J. Am. Chem. Soc. **101**, 825 (1979).

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