JCTC Journal of Chemical Theory and Computation

Influence of Stacking on the Hydrogen Bond Donating Potential of Nucleic Bases

K. Vanommeslaeghe,*[,†,‡] P. Mignon,[†,§] S. Loverix,[†,||] D. Tourwé,[‡] and P. Geerlings[†]

General Chemistry Group, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussel, Belgium, Organic Chemistry Group, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussel, Belgium, Centre for Surface Chemistry and Catalysis, Katholieke Universiteit Leuven, Kasteelpark Arenberg 23, B-3001 Heverlee, Belgium, and Department of Molecular and Cellular Interactions, Vlaams Interuniversitair Instituut voor Biotechnologie, Pleinlaan 2, B-1050 Brussel, Belgium

Received April 25, 2006

Abstract: Hydrogen bonding is the dominant interaction in the pairing of nucleic bases and largely determines the stability of the double-helical structure of DNA. In a previous study, we used the molecular electrostatic potential (MEP) near a hydrogen-bond (HB) *acceptor* to demonstrate that the *intra*strand $\pi-\pi$ stacking interaction influences the *inter*strand HB *accepting* capacity of DNA/RNA bases. In the present work, we first examined at the MP2/6-31G(d) level whether the MEP near a HB *donating* site of an aromatic or nucleic base can be used as a computationally inexpensive measure for its HB *donating* potential, quantified as the interaction energy with an HB acceptor probe, and whether this also holds in the presence of a stacking partner. A good correlation was found for substituted anilines in a vacuum, and this seemed to hold for cytosine, stacked with substituted benzenes. However, when stacked pairs of nucleic bases were studied, no correlation between the MEP and the HB strength was found. This turned out to be caused by the direct interaction of the HB donor's stacking partner with the probe molecule as well as its influence on the MEP. After this perturbation was eliminated, a significant correlation was found. The influence of stacking on the HB donating potential was shown to be dominated by the stacking geometry and not by the nature of the stacking partner. The present findings suggest that the $\pi-\pi$ interaction on itself does not have an overall strengthening on H bonding in DNA.

Introduction

Hydrogen bonds (HBs) are highly important in biological systems. In particular, hydrogen bonding is the dominant interaction in the pairing of nucleic bases and largely determines the stability of the double-helical structure of DNA.[1] Likewise, $\pi-\pi$ stacking interactions play an important role in the structure,[2] catalysis,[3−5] and inhibition[6−8] of proteins, and the DNA double helix exhibits prominent stacking interactions between the nucleic bases.[1] Thus, it can be said that, apart from the covalent bonds in the backbone, the double-helical structure of DNA is essentially held together by polar *inter*strand HBs[9] and apolar (London) *intra*strand $\pi-\pi$ interactions.[1,10−12] These different interactions are thought to be cooperative; however, to the best of our knowledge, no theoretical study has been able to confirm this. In the study of stair motifs at protein−DNA interfaces, cooperativity was investigated for H-bonded and stacked trimers of nucleic acid bases and a charged amino acid.[13] The three-body term contribution was found to vary from −0.4 to +7.4 kcal/mol, where significantly negative values

* Corresponding author fax: +32-2-26293317; e-mail: Kenno.Vanommeslaeghe@vub.ac.be.
† General Chemistry Group, Vrije Universiteit Brussel.
‡ Organic Chemistry Group, Vrije Universiteit Brussel.
§ Katholieke Universiteit Leuven.
|| Vlaams Interuniversitair Instituut voor Biotechnologie.

10.1021/ct600150n CCC: $33.50 © 2006 American Chemical Society
Published on Web 07/27/2006

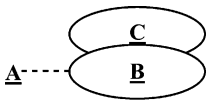

Figure 1. Naming conventions. **A**: ammonia. **B**: hydrogen-bonded base. **C**: stacking partner.

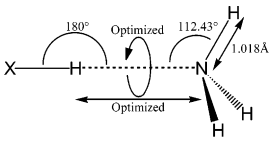

Figure 2. Partial optimization of an ammonia molecule as an HB acceptor.

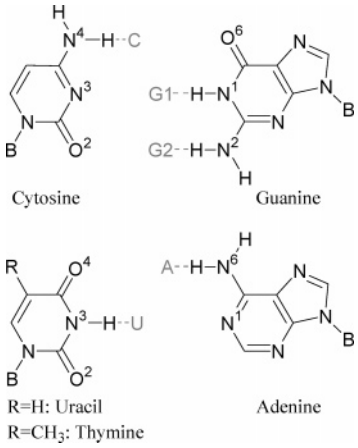

Figure 3. Four nucleic bases and their five biologically relevant HB donating sites, henceforward dubbed C, U, A, G1, and G2 (colored gray). B: sugar−phosphate backbone. R: variable group. DNA contains thymine (R = CH_3), while RNA contains uracil (R = H). Throughout this study, uracil was used for the sake of computational simplicity.

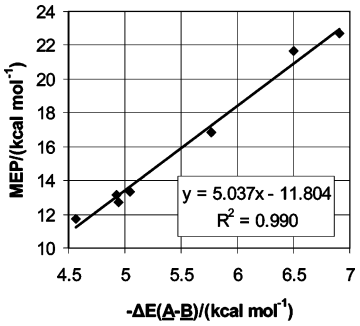

Figure 4. Correlation between the MEP and the strength of the HB between ammonia and a series of substituted anilines.

would have corresponded to cooperative binding. Also, electrostatics-based studies by Sivanesan et al. on hydrated nucleic bases show that stacked base pairs hydrate better than H-bonded ones.[14,15] They observed the most negative values of the molecular electrostatic potential (MEP) for the stacked pairs. Likewise, by calculating the MEP, we have demonstrated in a previous study that these *intra*strand stacking interactions influence the *inter*strand HB *accepting* capacity of DNA/RNA bases.[16]

As this influence is thought to be mainly the result of charge transfer, the impact of stacking on HB *donating* capacity may differ significantly. Also, the MEP has been

Table 1. Strength of the HB between Ammonia and a Series of Substituted Anilines, $-\Delta E(\mathbf{A}-\mathbf{B})$, and the MEP at 4 a_0 from the HB Donating Hydrogen Atom[a]

	$-\Delta E(\mathbf{A}-\mathbf{B})$	MEP
p-aminoaniline	4.57	11.71
aniline	5.05	13.31
p-hydroxyaniline	4.93	13.16
m-methylaniline	4.95	12.73
m-chloroaniline	5.76	16.87
m-nitroaniline	6.50	21.63
p-nitroaniline	6.90	22.68

[a]All energies are in kcal mol^{-1}.

Table 2. Strength of the HB between Ammonia and Cytosine, Stacked with Substituted Benzenes, and the MEP at 4 a_0 from the HB Donating Hydrogen Atom[a]

	$-\Delta E(\mathbf{A}-\mathbf{BC})$	MEP
aniline	6.36	5.68
toluene	6.51	6.91
benzene	6.53	7.38
phenol	6.60	7.96
benzaldehyde	6.65	9.26
fluorobenzene	6.72	8.98
nitrobenzene	6.88	13.30

[a]All energies are in kcal mol^{-1}.

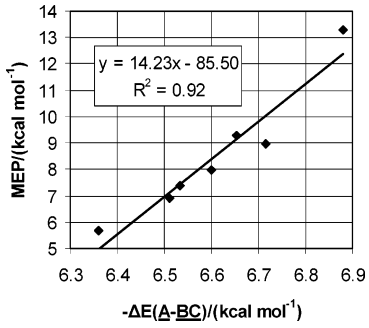

Figure 5. Correlation between the MEP and the strength of the HB between ammonia and cytosine stacked with a series of substituted benzenes.

known for a long time[17] as a reliable descriptor of the hydrogen-bond *accepting* strength: the deeper the electrostatic potential, the stronger the electrostatic interaction with water molecules and with hydrogen-bond donors in general.[18−21] However, we presently wish to use the MEP as a descriptor of the HB *donating* strength. An indication that this approach might be rewarding is the work by Politzer et al, showing successful correlations in the case of relatively simple systems.[22−24] Nevertheless, we cannot be fully confident that it will be accurate for the more complicated stacked systems presently studied. Consequently, our first goal is to find out whether the MEP near a hydrogen-bond donating site of a nucleic base in the presence of a stacking partner can be used as a computationally inexpensive way to estimate its HB donating potential, quantified as the binding affinity for a probe HB acceptor. Then, the influence of stacking on this HB donating potential will be investigated. To meet these goals, we first considered substituted anilines in order to test whether the correlation between the MEP and the HB strength is linear over a relevant range. Then,

Table 3. Strength of the HB between Ammonia and the Five Biologically Relevant HB Donating Sites on Four Nucleic Bases and the MEP at 4 a_0 from the HB Donating Hydrogen Atom[a]

B	$-\Delta E^{AB}(A-B)$	MEP	μ/D	AVG(ΔHB)	σ(ΔHB)	AVG(ΔHB/HB)
C	7.33	6.57	7.41	−0.52	0.52	−6.83%
U	8.66	7.22	5.03	−0.65	0.19	−7.86%
A	6.82	8.93	2.53	−0.69	0.22	−8.70%
G1	9.11	19.55	7.34	−0.71	0.35	−8.19%
G2	9.66	27.94	7.34	−0.71	0.35	−8.19%

[a] Also included are dipole moments (μ) of the isolated bases, as well as averages (AVG) and standard deviations (σ) of the influence of stacking on the HB donating potential; see the section "Corrected Results for Stacked Nucleic Bases" and Figure 12. All energies are in kcal mol^{-1}.

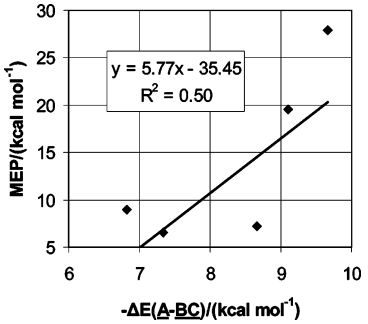

Figure 6. Poor correlation between the MEP and the strength of the HB between ammonia and the five biologically relevant HB donating sites on four nucleic bases.

this approach is extended to systems comprising cytosine, stacked with substituted benzenes. Finally, stacked pairs of nucleic bases are studied.

Methodology

Because stacking interactions are mainly determined by dispersion forces, they can only be properly described at a level of theory that includes electron correlation.[25−27] As density functional theory methods do not correctly reproduce the dispersion component of stacking interactions, at least second-order Møller−Plesset (MP2) theory must be used.[25,28] Consequently, all calculations were carried out at the MP2/6-31G(d) level of theory, using the Gaussian 03 software.[29] Ammonia was used as a HB acceptor and placed adjacent to the HB donating sites under investigation. After optimization of the hydrogen-bond geometry, the interaction energy was calculated as a measure for the HB strength, rigorously applying the counterpoise method to correct the basis set superposition error.[30] Wherever a two-body subset of the complete three-body system is considered (e.g., for the calculation of the $\Delta E(\underline{A}-\underline{C})$ term, see below), the basis set of the whole system was used, as recommended in the literature.[5,31,32] (The alternative approach, using the two-body basis set for the two-body interactions, was also attempted. The results deviated only 0.06 kcal mol^{-1} on average from those presently presented, with a maximum deviation of 0.15 kcal mol^{-1}. Nevertheless, the correlations with the MEP were slightly worse.)

Additionally, the MEP of the complex at a distance of 4 a_0 from the hydrogen atom of the same HB donating sites was determined, as earlier studies demonstrate that this descriptor correlates well with the acidity.[6,33,34] (Although it is likely that the Hartree−Fock level would be sufficiently accurate to calculate the MEP, MP2 results were used for the sake of consistency.)

To test whether the correlation between the MEP and the HB strength is linear over a relevant range, a series of substituted anilines was constructed and optimized. Then, the MEP was calculated at a distance of 4 a_0 from one of the hydrogen atoms attached to the aniline nitrogen along the N−H bond axis. The same hydrogen atom was subsequently used as a HB donor for a newly added NH_3 molecule. The resulting complex was fully optimized, whereupon the interaction energy between ammonia and the substituted aniline was calculated. As this yielded an excellent correlation (see "Results and Discussion"), a similar procedure was applied to cytosine, stacked with a series of substituted benzenes. Cytosine-substituted benzene complexes were already constructed and optimized in a previous study.[16] On each of these complexes, the MEP at a distance of 4 a_0 from the hydrogen atom of the biochemically relevant HB donating site of cytosine, was calculated. In parallel, an ammonia molecule was added to all of these HB donating sites (Figure 1), using the geometry from the fully minimized complex with *p*-nitroaniline. Then, the hydrogen-bond length and torsion angle were optimized (see Figure 2). Finally, the interaction energy between the ammonia molecule and the pair of stacked bases was calculated.

Using the resulting linear fit as a calibration curve should make it possible to infer the HB donating potential from the MEP, which is desirable because adding and optimizing an ammonia molecule and calculating the interaction energy becomes computationally expensive for larger systems. However, although a fair correlation was obtained, we cannot yet be sure that the HB donating potential can always be inferred from the MEP. Therefore, both descriptors were calculated for the biologically relevant HB donating sites of stacked pairs of nucleic bases (Figure 3). All possible combinations of bases were studied this way, each time considering both an optimized and an experimentally observed stacking orientation. The optimized geometries were taken from a study by Šponer et al., wherein pairs of separately optimized nucleic bases were positioned at a fixed distance, after which the twist angle and the parallel displacement between the rigid bases were optimized.[35] The experimental orientations were based on X-ray structures from the Research Collaboratory for Structural Bioinformatics Protein Data Bank,[36] on which the above-mentioned preoptimized nucleic bases were superimposed, as described in an earlier publication.[16] The MEP and the ammonia affinity were calculated for all biologically relevant HB

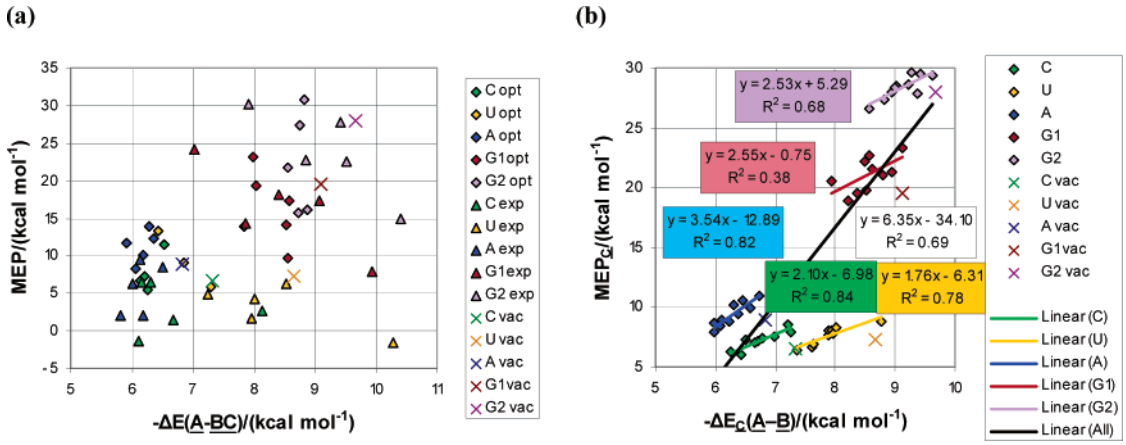

Figure 7. Correlation between the MEP and the interaction energy between ammonia and stacked pairs of nucleic bases before (a) and after (b) correction of the direct influence of base **C** on the MEP and the interaction energy. See the section "Corrected Results for Stacked Nucleic Bases" for an explanation.

donating sites (Figure 3), applying exactly the same procedure as that for cytosine stacked with substituted benzenes.

Throughout this article, the ammonia molecule will be named **A**, the base from which it receives a hydrogen bond **B**, and its stacking partner **C**, as schematized in Figure 1.

Results and Discussion

Substituted Anilines. The preliminary study of substituted anilines, aimed at establishing the range in HB strengths and MEPs and the linear correlation between both, yielded encouraging results. Indeed, Figure 4 shows an excellent correlation ($R^2 = 0.99$) for this series. Furthermore, the fact that a 12 kcal mol^{-1} range in MEP is translated into a 2.5 kcal mol^{-1} range in binding affinity (Table 1) indicates that possible inaccuracies in the MEP (see below) are not likely to have a significant influence on the predicted HB strengths.

Cytosine Stacked with Substituted Benzenes. As mentioned in the Introduction, a calibration curve was established for cytosine by calculating ammonia affinities and MEPs for this base, stacked with substituted benzenes with a wide range of electron-donating properties (Table 2 and Figure 5).[16] Nevertheless, the range in HB strengths is a factor 5 smaller than that for the substituted anilines. Although this results in a lower correlation coefficient ($R^2 = 0.92$), it should still be useful for extrapolation. Remarkably, the ratio between the MEP and the binding affinity is about a factor of 3 higher than that for the substituted anilines (Figure 5).

Isolated Nucleic Bases. Prior to starting the calculations on the stacked pairs of nucleic bases, HB strengths and MEPs were calculated for isolated bases, as a reference for calculating the influence of stacking on the HB donating potential. Table 3 shows that the exocyclic amine of adenine is the weakest HB donor and the exocyclic amine of guanine the strongest. As the bases differ significantly in chemical nature, there is a poor correlation with the MEP (Figure 6).

Stacked Nucleic Bases. As described under the Methodology section, ammonia affinities and MEPs were calculated for the 48 biologically relevant HB donating sites in 20 geometrically different stacked pairs of bases. The results are summarized in Figure 7a. Surprisingly, correlation between the MEP and the HB strength is completely absent

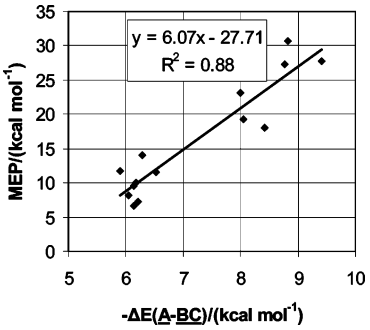

Figure 8. Correlation between the MEP and ΔE(**A**−**BC**) for **A**−**C** distances larger than 5 Å.

for the whole data set ($R^2 = 0.18$), as well as for each nucleic base on its own. Also remarkable is the presence of two negative MEPs in the data set, which can be explained by observing that a lone electron pair of base **C** points roughly in the direction of the point at which the MEP is calculated. Further inspection of the NH_3 binding geometries reveals a few cases where the ammonia molecule *donates* a HB *to base* ***C***. Although these undesired HBs feature such a strongly distorted geometry that they may more accurately be called "HB-like polar interactions", we deem them capable of ruining the correlation on their own right, given the narrow range of interaction energies observed for each base **B**.

To test whether the bad correlation is indeed caused by direct interference of the base **C** with both the ammonia affinities and the MEP, correlation was sought in two subsets of the original data set, where only the points were retained in which the distance between base **C** and ammonia is larger than 4 and 5 Å, respectively. Although the former yields a definite improvement, the correlation is still rather poor ($R^2 = 0.57$). Indeed, to get a reasonable correlation, the cutoff distance must be set as high as 5 Å ($R^2 = 0.88$; Figure 8). The ratio is comparable to the value for substituted anilines but not to the value for cytosine stacked with substituted benzenes.

Corrected Results for Stacked Nucleic Bases. Although Figure 8 exhibits a reasonable correlation, it contains only 14 of our 48 original data points. Moreover, only three of the experimental—and thus biologically relevant—geometries

Table 4. Corrected Interaction Energies, ΔHBs, and MEPs for the Optimized (a) and Experimental (b) Geometries[a]

Part a

B	**C**	$-\Delta E$(**A**−**BC**)	$-\Delta E$(**A**−**C**)	$-\Delta E_C$(**A**−**B**)	ΔHB	ΔHB/HB	MEP(**BC**)	MEP(**C**)	MEP_C(**B**)	ΔMEP_C(**B**)
C	C	6.52	0.09	6.43	−0.90	−12.34%	11.50	5.45	6.05	−0.52
C	U	6.25	−0.54	6.79	−0.54	−7.41%	5.49	−1.94	7.43	0.87
C	A	6.14	−0.56	6.70	−0.63	−8.57%	6.56	−0.58	7.14	0.58
C	G	6.22	−0.44	6.66	−0.67	−9.16%	7.20	0.21	6.99	0.42
U	C	6.44	−0.92	7.37	−1.29	−14.92%	13.29	6.86	6.43	−0.79
U	U	7.83	0.22	7.61	−1.04	−12.06%	13.92	7.23	6.69	−0.53
U	A	7.31	−0.58	7.89	−0.77	−8.92%	5.86	−1.83	7.69	0.48
U	G	6.86	−0.79	7.65	−1.01	−11.68%	9.06	2.13	6.93	−0.29
A	C	6.29	0.31	5.98	−0.85	−12.39%	13.96	6.04	7.93	−1.01
A	U	6.36	0.12	6.23	−0.59	−8.69%	12.33	3.52	8.80	−0.13
A	A′	6.18	0.07	6.11	−0.71	−10.47%	9.99	1.13	8.87	−0.07
A′	A	6.05	−0.33	6.39	−0.44	−6.38%	8.21	−1.24	9.45	0.51
A	G	5.90	−0.17	6.07	−0.75	−10.98%	11.75	3.37	8.39	−0.55
G1	C	8.52	−0.26	8.79	−0.32	−3.55%	14.00	−7.06	21.07	1.52
G1	U	8.59	0.06	8.53	−0.58	−6.36%	17.27	−2.47	19.74	0.19
G1	A	8.05	−0.33	8.37	−0.73	−8.06%	19.24	−0.30	19.54	−0.01
G1	G′	7.99	−0.23	8.22	−0.88	−9.71%	23.05	4.11	18.95	−0.60
G1′	G	8.55	−0.39	8.94	−0.17	−1.82%	9.67	−11.66	21.33	1.79
G2	C	8.87	−0.55	9.42	−0.25	−2.54%	16.08	−13.47	29.54	1.60
G2	U	8.56	−0.47	9.03	−0.64	−6.60%	21.69	−6.77	28.47	0.52
G2	A	8.76	−0.18	8.94	−0.72	−7.48%	27.29	−0.63	27.92	−0.03
G2	G′	8.83	0.00	8.83	−0.84	−8.66%	30.71	3.35	27.36	−0.58
G2′	G	8.73	−0.55	9.28	−0.39	−3.99%	15.76	−13.82	29.58	1.63

Part b

B	**C**	$-\Delta E$(**A**−**BC**)	$-\Delta E$(**A**−**C**)	$-\Delta E_C$(**A**−**B**)	ΔHB	ΔHB/HB	MEP(**BC**)	MEP(**C**)	MEP_C(**B**)	ΔMEP_C(**B**)
C	C′	6.15	−0.10	6.25	−1.08	−14.70%	6.45	0.25	6.20	−0.36
C′	C	8.13	0.88	7.25	−0.08	−1.11%	2.66	−5.26	7.92	1.36
C	U	6.67	−0.30	6.97	−0.36	−4.87%	1.45	−6.09	7.54	0.98
C	A	6.31	−0.21	6.52	−0.81	−11.08%	6.38	−0.84	7.22	0.65
C	G	6.12	−1.09	7.21	−0.12	−1.63%	−1.41	−9.96	8.55	1.99
U	C	10.28	1.50	8.77	0.11	1.31%	−1.49	−10.22	8.73	1.51
U	U′	8.02	0.14	7.88	−0.78	−8.97%	4.21	−3.83	8.04	0.83
U′	U	8.53	0.55	7.98	−0.68	−7.87%	6.31	−1.41	7.72	0.50
U	A	7.24	−0.69	7.94	−0.72	−8.35%	4.91	−3.16	8.07	0.85
U	G	7.97	−0.05	8.03	−0.63	−7.29%	1.59	−6.68	8.28	1.06
A	C	6.49	0.20	6.30	−0.53	−7.72%	8.48	−1.71	10.19	1.26
A	U	6.01	−0.45	6.46	−0.37	−5.38%	6.17	−4.36	10.53	1.59
A	A′	6.14	0.17	5.97	−0.85	−12.47%	9.48	0.84	8.65	−0.29
A′	A	6.19	−0.39	6.58	−0.25	−3.62%	1.98	−7.98	9.96	1.03
A	G	5.81	−0.92	6.73	−0.09	−1.35%	2.01	−8.97	10.99	2.05
G1	C	9.93	0.81	9.12	0.01	0.10%	7.87	−15.38	23.25	3.70
G1	U	9.08	0.58	8.50	−0.61	−6.66%	17.28	−4.90	22.18	2.64
G1	A	7.85	−0.77	8.63	−0.48	−5.31%	14.37	−7.15	21.52	1.98
G1	G′	8.41	−0.15	8.56	−0.55	−6.05%	18.03	−4.63	22.66	3.12
G1′	G	7.02	−0.92	7.94	−1.17	−12.86%	24.11	3.63	20.48	0.93
G2	C	10.41	0.78	9.63	−0.03	−0.35%	14.83	−14.53	29.36	1.42
G2	U	9.52	0.29	9.23	−0.44	−4.53%	22.61	−5.96	28.57	0.62
G2	A	8.86	−0.53	9.38	−0.28	−2.91%	22.81	−5.03	27.84	−0.10
G2	G′	9.41	0.40	9.01	−0.66	−6.80%	27.79	−0.57	28.35	0.41
G2′	G	7.91	−0.66	8.57	−1.09	−11.30%	30.20	3.58	26.62	−1.32

[a] If the nature of the two bases is the same, a choice has to be made as to which base is **B** and which is **C**. In these cases, both possibilities were considered, where the base **B** that has the longest distance between its HB donating site(s) and base **C** is notated as X and the other, "most hindered" base is notated as X′. Note that, for the optimized structures of C−C and U−U, this is irrelevant because their geometries are perfectly symmetrical. All energies are in kcal mol^{-1}.

satisfied the criterion for this data set. Clearly, if any relevant insights into the influence of stacking on the HB donating potential are to be obtained, a method should be devised to eliminate the *direct* influence of the stacking partner, so that the influence *via the stacking interaction* becomes apparent. The interaction energy between **A** and **B** *in the presence of*

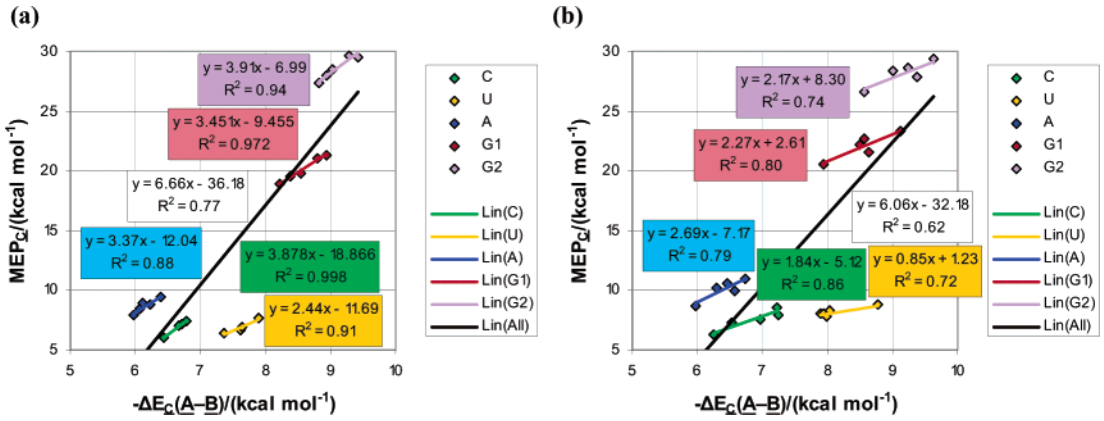

Figure 9. Correlation between the corrected MEP and the final corrected interaction energy for the optimized (a) and the experimental (b) structures.

C, $\Delta E_{\underline{C}}(\underline{A}-\underline{B})$, was calculated as $\Delta E(\underline{A}-\underline{BC}) - \Delta E(\underline{A}-\underline{C})$. Similarly, the corrected MEP was calculated by subtracting the MEP of base **C** from the MEP of the **BC** system. The results of the final corrected calculations of the ammonia affinity and the MEP are summarized in Table 4 and Figure 7b. In contrast with Figure 7a, reasonable correlations are obtained for all of the biologically relevant HB donating sites except the endocyclic N^1—H of guanine (G1), with ratios between 1.8 and 3.5. Also, there is a low but significant correlation across the whole data set, with a ratio between the MEP and the HB strength that is on the same order of magnitude as the ratio for the substituted anilines.

Remarkably, separating the optimized and the experimental data significantly improves correlation in nearly all cases, as can be seen by comparing Figures 9a and b to Figure 7b. This improvement is especially pronounced for site 1 of guanine (G1); here, the extremely bad correlation in Figure 7b results from the fact that the optimized and the experimental geometries are clearly located on two distinct lines, each with a rather good correlation (Figure 9a and b). It is also apparent that, for each base, the range in HB strengths is significantly smaller for the optimized structures than for the experimental data. Nevertheless, the correlations are much better for the optimized structures. This can be attributed to the fact that the experimental geometries are imposed by the overall structure of the RNA/DNA double helix. From the point of view of an isolated duo of stacked bases in a vacuum, these experimental geometries are rather arbitrary. In particular, the tilt angle between the two bases cannot be justified without considering the double-helical structure.[16] This can be regarded as a perturbation of the optimized geometry, which results in a perturbation of the correlations. When comparing parts a and b of Figure 9, it is tempting to speculate that a tilt angle differing slightly from zero has a smaller effect on the MEP than on the "internal electronical structure" of base **B** and thus on the HB strength. In any case, one should be careful when trying to correlate the HB strength with the MEP of nonoptimized structures.

As the data sets for the different bases **B** occupy discrete regions in Figure 7b, we tried to correlate the influence of stacking on the HB strength ΔHB, defined as $\Delta E^{AB}(\underline{A}-\underline{B}) - \Delta E_{\underline{C}}(\underline{A}-\underline{B})$, with the influence of stacking on the MEP: $\Delta\text{MEP} = \text{MEP}_{\underline{C}}(\underline{B}) - \text{MEP}(\underline{B})$. However, as can be seen in

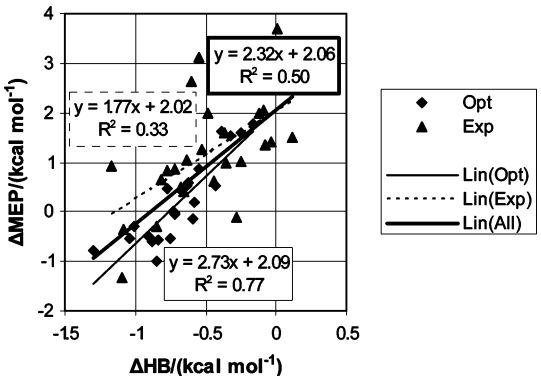

Figure 10. Correlation between ΔMEP and ΔHB, for the optimized, the experimental, and all geometries of the stacked pairs of nucleic bases.

Figure 10, there is no clear advantage to this approach. Nevertheless, ΔHB is an interesting quantity in its own right, as it is the most direct measure for the influence of stacking on the HB donating potential. First, we tried to correlate ΔHB with the nature of the stacking partner **C** (Figure 11). Overall, the range of ΔHBs, quantified as its standard deviation [σ(ΔHB) in Table 3], is largest when base **C** is cytosine and smallest in the case of uracil. This range might be related to the diversity of relative orientations, on one hand, and on the dipole moment of the stacking partner **C**, on the other hand. The former effect is difficult to quantify; for the latter effect, Figure 12 suggests a weak correlation, although this result is inconclusive. Likewise, it is difficult to draw general conclusions about the effect of the nature of stacking partner **C** on the HB strength itself. Although it is clear that stacking generally weakens the hydrogen-bond donating capacity—on average, by 0.59 kcal mol^{-1} [see AVG(ΔHB) in Table 3]—the influence of the nature of molecule **C** seems to be subordinate to the influence of its orientation, to the extent that it cannot be inferred from our data which of the nucleic bases **C** has the strongest intrinsic electron-donating effect on its stacking partner **B**.

In our previous work,[16,37] a comparable approach yielded evidence that the HB accepting capacity increases upon stacking. In the present study, we observe a decrease of the HB donating capacity. Both observations are consistent with the notion that the stacking partner "pushes electron density into the ring", as proposed in our previous work.[16] As a result,

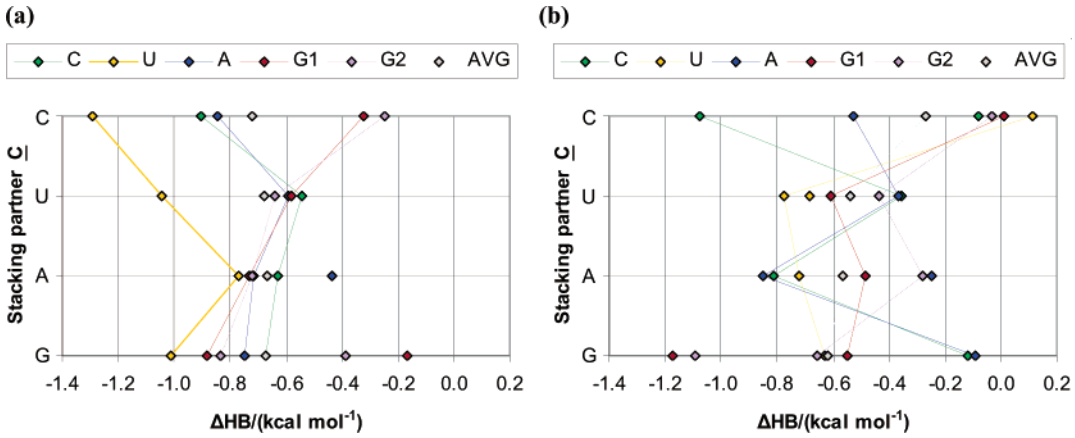

Figure 11. Influence of stacking on the HB donating potential for the optimized (a) and the experimental (b) structures. The difference in HB strength with respect to the unstacked base **B** is plotted in the horizontal axis, so that negative values represent a decrease in hydrogen-bond strength upon stacking. The color coding represents the nature of the base **B** as defined in Figure 1, while the nature of the base **C** is plotted on the vertical axis.

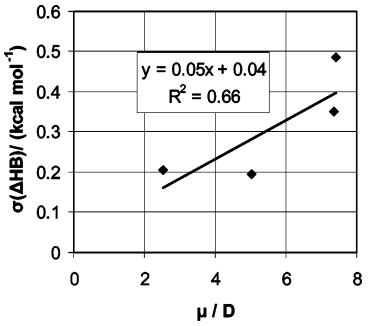

Figure 12. Correlation between the standard deviation of the influence of stacking on the HB strength and the dipole moment μ.

the influence of the stacking interaction on the HB donating ability counteracts its effect on the HB accepting capacity. To test the relative strength of these opposing influences, NH_3 was used as a HB donor for both of the HB accepting sites of cytosine and for the single HB accepting site of adenine. To determine the influence of stacking on the resulting HB strengths, these calculations were repeated with adenine and guanine as stacking partners for cytosine and with uracil as the stacking partner for adenine, using the optimized stacking geometries. The results for this set are summarized in Table 5 and show only small increases in HB strength (0.21 kcal mol^{-1} at most).

However, one could argue that this result is biased because ammonia is a better HB acceptor than a HB donor, as the absolute HB strengths are about half as high when ammonia is used as a HB donor instead of an acceptor. If, for the sake of argument, we suppose that the influence of stacking on the HB strength is proportional to the HB strength itself (thus ignoring the cases where the influence is opposite in sign), we should consider percentages rather than absolute differences in HB strength. The ΔHB/HB values in Tables 4 and 5 and the averages [AVG(ΔHB/HB)] in Table 3 indicate that, even under this bold supposition, the average negative influence of stacking on the HB donating capacity of nucleic bases is higher than its positive influence on their HB accepting capacity. Although more data are needed to obtain a definitive conclusion in this respect, the present findings suggest that the electron-transfer accompanying $\pi-\pi$ stacking on itself does not strengthen interstrand hydrogen bonding in DNA.

Corrected Results for Cytosine Stacked with Substituted Benzenes. The same corrections as discussed above were applied on cytosine, stacked with substituted benzenes (Table 6). Here, a slight worsening of the correlation is observed ($R^2 = 0.88$; see Figure 13), which can be explained by considering that the range of HB strengths decreased from 0.52 to 0.16 kcal mol^{-1}. More importantly, the (previously anomalous) ratio is now in line with the results for the substituted anilines and the stacked nucleic bases. Thus, it can be concluded that, in the uncorrected calculations, we were mainly seeing the *direct effects* of the stacking partner on the MEP and the HB strength, while the *effects via the stacking interaction* are much more subtle.

Conclusions

In line with previous results,[24,33,34] it was shown that the MEP is an excellent measure for the HB donating potential of substituted anilines. Likewise, a good correlation between this MEP and the HB donating potential was found for a fixed nucleic base (cytosine) stacked with substituted benzenes in similar orientations. However, the observed differences were found to be mostly the result of the direct influence of the stacking partner **C** on the HB acceptor's binding site, while the electronic influence of **C** on the hydrogen-bonded base **B** was shown to be weaker by factors on the order of 3 (HB strength) and 15 (MEP). Moreover, the correlation completely breaks down when substantially differing stacking partners such as different nucleic bases are used, or if geometric variations of the stacking partner are allowed.

Correlation was partially restored by eliminating the direct influence of **C** out of the MEP as well as the binding energy of the HB accepting probe. Yet, for the pairs of stacked nucleic bases that were studied, no definite conclusions could be drawn regarding the influence of the nature of base **C** on the hydrogen-bond donating capacity of a parallel stacked base **B**. Nevertheless, the present results suggest that this

Table 5. Interaction Energies for Stacked Pairs of Nucleic Bases and Isolated Nucleic Bases *Receiving* a HB from NH_3

B	**C**	$-\Delta E$(**A**−**BC**)	$-\Delta E$(**A**−**C**)	$-\Delta E_{\underline{C}}$(**A**−**B**)	$-\Delta E^{AB}$(**A**−**B**)	ΔHB	ΔHB/HB
C2	A	4.08	0.85	3.23	3.11	0.12	3.88%
C2	G	4.30	0.99	3.31	3.11	0.21	6.61%
C3	A	4.40	−0.43	4.83	4.78	0.05	1.06%
C3	G	4.33	−0.51	4.84	4.78	0.06	1.25%
A	U	3.27	−0.19	3.46	3.50	−0.04	−1.08%

Table 6. Corrected Interaction Energies and MEPs for Cytosine, Stacked with Substituted Benzenes[a]

	$-\Delta E$(**A**−**BC**)	$-\Delta E$(**A**−**C**)	$-\Delta E_{\underline{C}}$(**A**−**B**)	MEP(**BC**)	MEP(**C**)	$MEP_{\underline{C}}$(**B**)
aniline	6.36	−0.22	6.58	5.68	−1.34	7.02
toluene	6.51	−0.05	6.56	6.91	0.02	6.89
benzene	6.53	−0.08	6.61	7.38	0.30	7.08
phenol	6.60	0.01	6.59	7.96	1.03	6.92
benzaldehyde	6.65	0.06	6.59	9.26	2.26	7.00
fluorobenzene	6.72	0.06	6.65	8.98	1.93	7.05
nitrobenzene	6.88	0.15	6.73	13.30	5.85	7.45

[a] All energies are in kcal mol^{-1}.

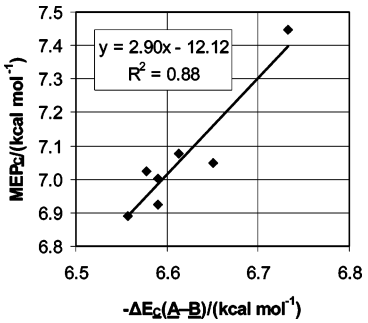

Figure 13. Correlation between the corrected MEP and the final corrected interaction energy between ammonia and cytosine stacked with a series of substituted benzenes.

influence varies more with the stacking geometry if the stacking partner **C** has a larger dipole moment. Also, the set of optimized geometries behaved qualitatively differently from the set of experimental geometries. Stacking was shown to weaken the HB *donating* capacity in 46 out of 48 cases, with a mean weakening of 0.59 kcal mol^{-1}. Conversely, the previously observed strengthening effect on the HB *accepting* capacity[16,37] was found to be 0.21 kcal mol^{-1} at most, albeit on a very limited data set and using a poor HB donor probe. Although a more thorough study is required to obtain a definitive conclusion in this respect, the present findings suggest that the $\pi-\pi$ interaction on itself has no net potentiating effect on the interstrand hydrogen bonds in DNA. It should also be noted that both the weakening effect on the donating capacity and the strengthening effect on the accepting capacity are consistent with the notion that the stacking partner "pushes electron density into the ring", as proposed in our previous work.[16]

On a practical note, the present study demonstrates that optimized or at least similar stacking geometries are required in order to have any meaningful correlation between the MEP and the HB donating potential. Even under these premises, final correlations are clearly not good enough for predicting hydrogen-bond strengths based on the MEP. Nevertheless, for a series of optimized geometries with the same HB donor **B** and similar stacking partners **C**, a ranking of the (corrected) MEPs should be useful as a rough measure for the ranking of the HB donating potentials, where a difference of two to four energy units in the MEP very approximately corresponds to a difference of one energy unit in the HB donating potential.

Acknowledgment. P.G. thanks the "F.W.O.-Vlaanderen" and the "Vrije Universiteit Brussel" for continuous support of his research group.

References

(1) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: Berlin, Germany, 1984.

(2) McGaughey, G. B.; Gagné, M.; Rappé, A. K. *J. Biol. Chem.* **1998**, *273*, 15458−15463.

(3) Versées, W.; Loverix, S.; Vandemeulebroucke, A.; Geerlings, P.; Steyaert, J. *J. Mol. Biol.* **2004**, *338*, 1−6.

(4) Steyaert, J. *Eur. J. Biochem.* **1997**, *247*, 1−11.

(5) Raines, R. T. *Chem. Rev.* **1998**, *98*, 1045−1065.

(6) Finnin, M. S.; Donigian, J. R.; Cohen, A.; Richon, V. M.; Rifkind, R. A.; Marks, P. A.; Breslow, R.; Pavletich, N. P. *Nature* **1999**, *401*, 188−193.

(7) Wang, D.-F.; Wiest, O.; Helquist, P.; Lan-Hargest, H.-Y.; Wiech, N. L. *J. Med. Chem.* **2004**, *47*, 3409−3417.

(8) Nielsen, T. K.; Hildmann, C.; Dickmanns, A.; Schwienhorst, A.; Ficner, R. *J. Mol. Biol.* **2005**, *354*, 107−120.

(9) Guerra, C. F.; Bickelhaupt, F. M.; Snijders, J. G.; Baerends, E. J. *Chem.—Eur. J.* **1999**, *5*, 3581−3594.

(10) Suzuki, M.; Amano, N.; Kakinuma, J.; Tateno, M. *J. Mol. Biol.* **1997**, *274*, 421−435.

(11) Mathews, D. H.; Sabina, J.; Zuker, M.; Turner, D. H. *J. Mol. Biol.* **1999**, *288*, 911−940.

(12) Bommarito, S.; Peyret, N.; SantaLucia, J., Jr. *Nucleic Acids Res.* **2000**, *28*, 1929−1934.

(13) Wintjens, R.; Biot, C.; Rooman, M.; Lievin, J. *J. Phys. Chem. A* **2003**, *107*, 6249−6258.

(14) Sivanesan, D.; Babu, K.; Gadre, S. R.; Subramanian, V.; Ramasami, T. *J. Phys. Chem. A* **2000**, *104*, 10887−10894.

(15) Sivanesan, D.; Sumathi, I.; Welsh, W. J. *Chem. Phys. Lett.* **2003**, *367*, 351−360.

(16) Mignon, P.; Loverix, S.; Steyaert, J.; Geerlings, P. *Nucleic Acids Res.* **2005**, *33*, 1779−1789.

(17) Kollman, P.; McKelvey, J.; Johansson, A.; Rothenberg, S. *J. Am. Chem. Soc.* **1975**, *97*, 955−965.

(18) Baeten, A.; De Proft, F.; Geerlings, P. *Chem. Phys. Lett.* **1995**, *235*, 17−21.

(19) Baeten, A.; De Proft, F.; Geerlings, P. *Int. J. Quantum Chem.* **1996**, *60*, 931−939.

(20) Mishra, P. C.; Kumar, A. In *Molecular Electrostatic Potentials: Concepts and Applications*; Murray, J. S., Sen, K., Eds.; Elsevier: Amsterdam, The Netherlands, 1996; Chapter 6, pp 257−296.

(21) Kushwaha, P. S.; Mishra, P. C. *Int. J. Quantum Chem.* **2000**, *76*, 700−713.

(22) Murray, J. S.; Politzer, P. *J. Org. Chem.* **1991**, *56*, 6715−6717.

(23) Murray, J. S.; Politzer, P. *J. Chem. Res. Synop.* **1992**, 110−111.

(24) Hagelin, H.; Murray, J. S.; Brinck, T.; Berthelot, M.; Politzer, P. *Can. J. Chem.* **1995**, *73*, 483−488.

(25) Hobza, P.; Šponer, J. *Chem. Rev.* **1999**, *99*, 3247−3276.

(26) Sinnokrot, M. O.; Valeev, E. F.; Sherrill, C. D. *J. Am. Chem. Soc.* **2002**, *124*, 10887−10893.

(27) Sinnokrot, M. O.; Sherrill, C. D. *J. Phys. Chem. A* **2004**, *108*, 10200−10207.

(28) Černý, J.; Hobza, P. *Phys. Chem. Chem. Phys.* **2005**, *7*, 1624−1626.

(29) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision B.03; Gaussian, Inc.: Wallingford, CT, 2003.

(30) Boys, S. F.; Bernardi, F. *Mol. Phys.* **1970**, *19*, 553−566.

(31) Van Duijneveldt, F. B.; Van Duijneveldt-Van De Rijdt, J. G. C. M.; Van Lenthe, J. H. *Chem. Rev.* **1994**, *94*, 1873−1885.

(32) Turi, L.; Dannenberg, J. J. *J. Phys. Chem.* **1993**, *97*, 2488−2490.

(33) De Proft, F.; Amira, S.; Choho, K.; Geerlings, P. *J. Phys. Chem.* **1994**, *98*, 5227−5233.

(34) Olasz, A.; Mignon, P.; De Proft, F.; Veszprémi, T.; Geerlings, P. *Chem. Phys. Lett.* **2005**, *407*, 504−509.

(35) Šponer, J.; Leszczyński, J.; Hobza, P. *J. Phys. Chem.* **1996**, *100*, 5590−5596.

(36) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res.* **2000**, *28*, 235−242.

(37) Guo, D. W.; Sijbesma, R. P.; Zuilhof, H. *Org. Lett.* **2004**, *6*, 3667−3670.

CT600150N