CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200500725

Binding of Cationic and Neutral Phenanthridine Intercalators to a DNA Oligomer Is Controlled by Dispersion Energy: Quantum Chemical Calculations and Molecular Mechanics Simulations

Tomáš Kubař, Michal Hanus, Filip Ryjáček, and Pavel Hobza*[a]

Abstract: Correlated ab initio as well as semiempirical quantum chemical calculations and molecular dynamics simulations were used to study the intercalation of cationic ethidium, cationic 5-ethyl-6-phenylphenanthridinium and uncharged 3,8-diamino-6-phenylphenanthridine to DNA. The stabilization energy of the cationic intercalators is considerably larger than that of the uncharged one. The dominant energy contribution with all intercalators is represented by dispersion energy. In the case of the cationic intercalators, the electrostatic and charge-transfer terms are also important. The ΔG of ethidium intercalation to DNA was estimated at $-4.5\,\mathrm{kcal\,mol^{-1}}$ and this value agrees well with the experimental result. Of six contributions to the final free energy, the interaction energy value is crucial. The intercalation process is governed by the non-covalent stacking (including charge-transfer) interaction while the hydrogen bonding between the ethidium amino groups and the DNA backbone is less important. This is confirmed by the evaluation of the interaction energy as well as by the calculation of the free energy change. The intercalation affects the macroscopic properties of DNA in terms of its flexibility. This explains the easier entry of another intercalator molecule in the vicinity of an existing intercalation site.

Keywords: ab initio calculations · DNA · intercalations · molecular dynamics · thermodynamics

Introduction

Small organic molecules can bind to DNA by means of a non-specific (mainly electrostatic) binding along the DNA exterior, a specific groove binding and intercalation. Intercalators are drugs that may be inserted between adjacent base-pair steps of a nucleic-acid double helix, forming stable sandwich-like structures. It is especially the intercalators which are the point of interest for their mutagenic, teratogenic and carcinogenic effects and, conversely, antitumor and antiviral pharmacologic activity.[1]

The knowledge of the intercalation energetics gives deeper insight into the intercalation process.[2] An intercalator binds to the DNA double helix via the non-covalent stacking interaction with the DNA base pairs, and with hydrogen bonding between its polar groups and the DNA's sugar-phosphate backbone. The relative importance of these contributions was not clear and it was merely presumed that hydrogen bonding was more important. Only recently has it been shown that stacking interaction plays a more important role than expected and the strength of the stacked complexes is comparable with that of hydrogen-bonded ones.[3–7] There are also intercalators that additionally form a covalent bond to DNA using their side chains providing their sequence specificity.

The intercalation is believed to be at least a two-step reaction:[8–13] a relatively weak "outer complex" is formed at the diffusion controlled rate in the first step and the ligand is inserted in the second step. As a result, an additional base-pair separation by ca. 3.4 Å and a considerable unwinding of the DNA double helix are observed. Intercalation reactions have been studied by many research groups[2,14–20] but their conclusions sometimes differ.

Being a simple polycyclic aromatic molecule with only short side chains, ethidium represents a typical intercalator without any sequence specificity;[21–25] it is generally considered to be an ideal model compound for this type of binding to DNA.[26–28] Ethidium possesses all structural features im-

[a] T. Kubař, M. Hanus, Dr. F. Ryjáček, Prof. P. Hobza
Institute of Organic Chemistry and Biochemistry
Academy of Sciences of the Czech Republic
and Center for Biomolecules and Complex Molecular Systems
Flemingovo nám. 2, 166 10 Praha 6 (Czech Republic)
Fax: (+420) 220-410-320
E-mail: hobza@uochb.cas.cz

Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author.

portant in its class—a positive charge (almost ideally delocalized throughout the aromatic system), high polarizability, high electron affinity (ethidium as well as other cationic intercalators are good electron acceptors) and highly polar amino groups. These features a priori predicate ethidium to be involved in both stacking (including charge transfer) and hydrogen-bonding interactions. As a result of these features ethidium has been widely used as a common fluorescence stain and a lot of experimental data on ethidium have been collected over the last three decades.[21–25,29–33]

It has been shown experimentally[12] that the intercalation rate of ethidium to DNA is controlled by the insertion of an aromatic ring into the DNA structure. (Earlier, it was suggested that the process was controlled by the diffusion of ethidium toward the DNA.) Recent experimental data[13] indicate the existence of a groove binding intermediate formed by a fast relaxation process, which probably contains about 40% of the total ethidium bound to the double helix, although such an intermediate has not been identified. UV spectroscopic experiments have identified two strong complexes (intercalation) and a weak complex (only at lower temperatures). The crystal structures published[21,22,34] indicate the insertion of ethidium's condensed aromatic rings in between two successive base pairs and the localization of the phenyl residue into the minor groove.

The understanding of the intercalation process requires a detailed knowledge of the energetics, thermodynamics and dynamics of this process and such evidence can be consistently generated by theoretical calculations. It has been proven by QSAR studies[2] that the strength of the intercalator binding correlates with its biological effect. Despite the importance of the interaction energy, the change of free energy (ΔG) upon intercalation is the decisive factor and must be taken into consideration. Experimentally, ΔG is accessible by careful analysis of the DNA binding isotherms[14] (equilibrium studies, titrations), while ΔH may be directly measured by using calorimetric techniques[15] (ICT or DSC). The total binding free energy can be divided into enthalpic and entropic parts. The enthalpic term[35] is mainly a combination of the dispersion, electrostatic, induction and charge-transfer contributions (which are attractive) and the exchange-repulsion; the entropic term consists mainly of the repulsive formation entropy (discussed also later).

To correctly theoretically describe the DNA···ethidium complex it is absolutely necessary to adopt the correlated ab initio quantum-chemical (QC) approach. This is mainly due to the stacking interaction, which requires high-level ab initio treatment. Let us recall that low-level QC methods such as Hartree–Fock (HF) and the density-functional theory (DFT) fail to describe stacking complexes and are thus insufficient for the study of the intercalation process. The size of the DNA···intercalator complexes usually eliminates the use of ab initio QC methods from consideration and leads to the application of empirical molecular mechanics (MM). These methods do not contain the induction and charge-transfer terms explicitly, therefore they tend to underestimate the stabilization energy. From this point of view, it is clear that the quality of the empirical potential is at least of the same importance as the quality of the statistical methods used in thermodynamic calculations and in fact, it determines the reliability of the theoretical prediction.

In our previous study[35] we carefully analyzed the interaction of various intercalators (including ethidium) with the adenine···thymine and the guanine···cytosine base pairs. We evaluated the stabilization energy of these complexes using a correlated ab initio QM method and compared it with values yielded by the empirical force field by Cornell et al., which is widely used for the study of the intercalation process. It was clearly shown that the MM approach underestimates the stabilization energy and we suggested that this is due to the neglect of the induction (charge-transfer) term. Since the Cornell et al. force-field (as well as other empirical potentials in common use) does not contain this energy term, we compensated for it by increasing the attractive van der Waals (vdW) term. The modified potential accurately reproduced the results of the correlated QC calculations and this potential was also used in the MM part of this study.

Many recent experimental studies[2,17,19,36–38] suggest that entropy also plays an important role. The release of water molecules and a monovalent cation from the DNA hydration shell upon the ethidium intercalation is a stabilizing effect and the hydrophobic effect associated with the ethidium transfer from bulk water to the hydrophobic core of DNA adds another significant contribution. On the other hand, the final complex is more rigid and both the DNA and the intercalator lose their translational and rotational degrees of freedom resulting in entropic destabilization.

Both theoretical and experimental attempts were made to evaluate all of these contributions for ethidium.[16,18,19,35] Here, we summarize the main (sometimes very controversial) results:

- the stacking interaction between the intercalator and the neighboring base pairs is considerably stronger than the stacking between two base pairs, and the respective stabilization energy is large
- ethidium is slightly TA-specific
- the unwinding of the helix helps to bind another ethidium molecule cooperatively, up to one ethidium per two base pairs
- hydration plays a major role in determining ethidium's binding affinity and specificity
- there is no net release or uptake of water molecules
- the free energy change is dominated by the entropic term
- the hydrophobic term plays a major role
- the individual components are only estimates or perhaps upper limits rather than absolute values.

From the findings mentioned above, it is evident that the nature of the intercalation process is still not fully understood and further studies (both theoretical and experimental) are needed for its elucidation. One of the most severe problems, typical not only for intercalation, concerns the rel-

ative importance of the enthalpy and entropy contributions. At this point, let us mention our recent studies which emphasized the role of enthalpy in the formation of the hydrophobic core of a protein[39] or in the stabilization of the DNA pseudo-base pairs.[40] A similar conclusion was made by Barratt et al.[41] on the basis of studies of 2-methoxy-3-isobutylpyrazine binding to the mouse major urinary protein.

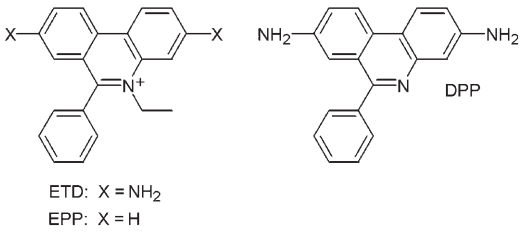


Figure 1. Ethidium and its derivatives.

The aims of this work are the following: First, to elucidate the role of enthalpy and entropy in the ethidium intercalation process. For the first time, the interaction energy of ethidium with the whole DNA structure (i.e., not only the base pairs) will be determined on the basis of highly reliable correlated ab initio calculations. Then, we will examine whether a difference between poly(AT) and poly(GC) DNA sequences could result in a preferred intercalation of ethidium into one of these sequences. We will consider not only the interaction between the ethidium molecule and the DNA but the hydration and dehydration processes will also be taken into account. Furthermore, we will make an attempt to evaluate the binding free energy change upon the modification of the ethidium molecule concerning its amino groups. Finally, we will explore the macroscopic properties of the DNA double strand and especially their changes upon intercalation. Specifically, we will show whether these changes could possibly facilitate the intercalation of another ethidium molecule.

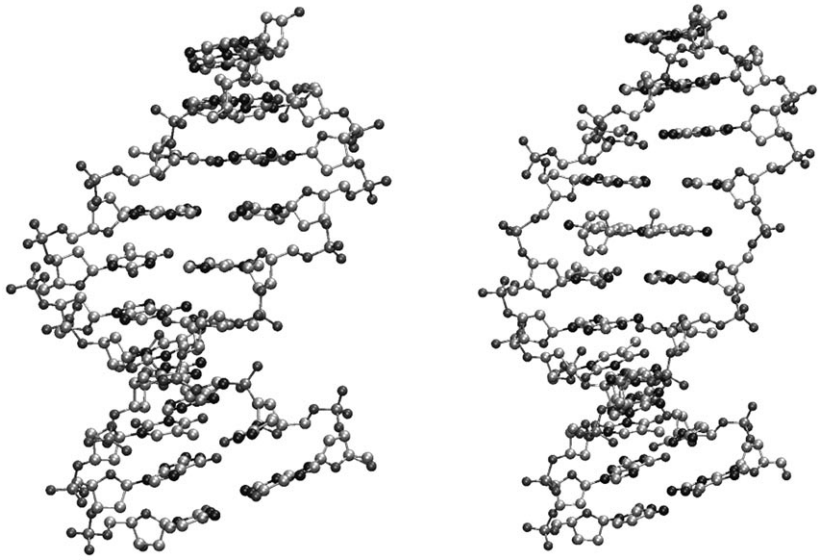


Figure 2. B-DNA decamer $(GCATATATGC)_2$: bare (left) and with an ethidium molecule intercalated (right).

Our study is based on both ab initio and semiempirical QC calculations combined with MM simulations performed using the modified Cornell et al. empirical force field.[42] The role of the solvent will be estimated on the basis of the continuum as well as explicit models.

Methods

Systems studied: We investigated several intercalating agents derived from phenanthridine. The following intercalators were considered: ethidium (3,8-diamino-5-ethyl-6-phenylphenanthridinium, ETD), 5-ethyl-6-phenylphenanthridinium (EPP), and 3,8-diamino-6-phenylphenanthridine (DPP; Figure 1). Both ETD and EPP carry a positive charge of +1 while DPP is uncharged.

In the MM simulations, we used B-DNA decamers $(GCATATATGC)_2$ and $(GCGCGCGCGC)_2$ as model DNA species. The intercalation site is located between the 4th and 5th base pair, that is, in a pyrimidine–purine base-pair step. Figure 2 shows the structure of the former decamer, both bare and with an ethidium molecule intercalated.

For higher-level determination of the interaction energy and the free energy evaluation, we used a "minimal model" consisting of an intercalator molecule and a B-DNA double-helical dimer (2TA or 2CG), that is, four nucleosides and two phosphate residues (Figure 3). In selected calculations, both phosphate residues in the minimal model were protonated in order to mimic the proximity of a sodium cation and reduced electrostatic charge of the phosphate.

The starting structure of the minimal model was adapted from RNA system NDB ID DRBB12[34] and the structure of the bare B-DNA dinucleotide was created by the AMBER NUCGEN module (see below).

Ab initio QC calculations: The structures of ETD, EPP and DPP were optimized at the HF/6-31G* level. The atomic charges were determined using two methods: the restrained electrostatic potential (RESP) fitting procedure[43] and natural-bond-orbital (NBO) analysis; both types of calculations were based on the DFT wavefunction obtained at the B3LYP/cc-pVTZ level. The same wavefunction was used for the molecular isotropic polarizability calculations. The fron-

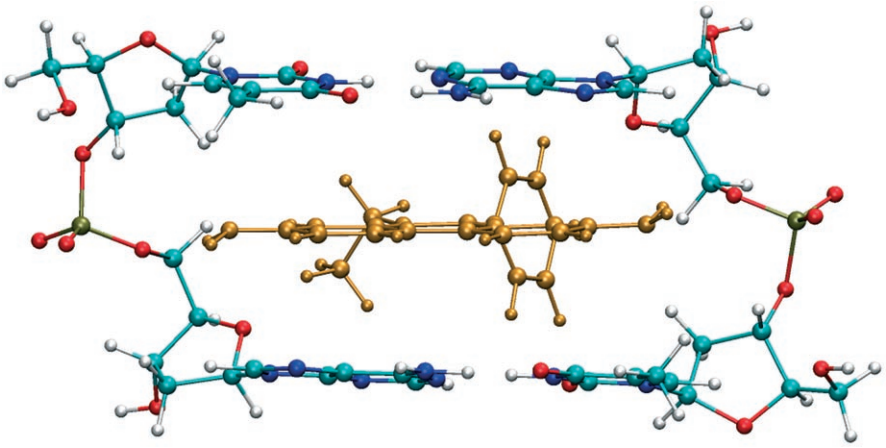


Figure 3. Minimal model of an intercalation site.

tier orbital energy was obtained at the HF/6-31G** level. The amount of charge transfer in the complex of an intercalator molecule and two base pairs was estimated by using the RESP and the NBO charges determined at the DFT/B3LYP/6-31G** level. The hydration free energy was obtained using a polarizable continuum model (C-PCM)[44] at the HF/6-31G* level. The program package GAUSSIAN 03[45] was used for these ab initio calculations.

The minimal model interaction energy was determined as the energy difference of the molecular cluster and its components (DNA and the intercalator). The geometry of the complex and the subsystems was determined by the semiempirical SCC-DFTB-D method (see below). To cover all the interaction energy contributions and to obtain the benchmark data, the energy was also determined at the correlated MP2 level using the resolution-of-identity (RI)[46] approximation and the SVP(0.25, 0.15) basis set.[47] This basis set differs from the standard SVP in the values of the exponents of the d and p polarization functions: instead of the values 1.2 (oxygen), 1.0 (nitrogen) and 0.8 (carbon and hydrogen), more diffuse exponents of 0.25 and 0.15 were used on heavy atoms and hydrogen atoms, respectively. Consequently, a better description of the dispersion energy was achieved. The RI-MP2/SVP(0.25, 0.15) stabilization energy of hydrogen-bonded and stacked DNA base pairs was found to agree well with the stabilization energy yielded by the much larger aug-cc-pVDZ basis set.[4] The basis set superposition error (BSSE) was eliminated using the function counterpoise procedure by Boys and Bernardi.[48] Furthermore, several popular density functionals were used to compute the interaction energy, namely B3LYP, BLYP, PBE and TPSS. The TZVP basis set was used in all DFT calculations. The program package TURBOMOLE (version 5.7)[49] was used for these calculations.

Semiempirical QC calculations: These calculations were performed by using the approximative self-consistent-charge density-functional tight-binding method augmented by an empirical term accounting for the correct description of dispersion energy (SCC-DFTB-D).[50] The structure of the model complex was optimized by using the SCC-DFTB-D method; the DNA base non-hydrogen atoms were excluded form the optimization process for the complex to maintain the geometry as in a larger DNA fragment. The energy minimization was performed with the algorithm implemented in the TURBOMOLE package (script JOBEX).

The SCC-DFTB-D calculations were used to determine the structure and the stabilization energy of the minimal model of the intercalator···DNA complex. The respective stabilization energy value was not corrected for the BSSE since the introduction of a tight-binding scheme leads to negligible BSSE values.

Molecular dynamics simulations: The molecular dynamics (MD) simulations were performed by using the AMBER package (version 7, modules NUCGEN, LEAP, SANDER and CARNAL)[51] with the Cornell et al. force field.[42] While the standard parameter set was used for all DNA atoms, a modified set was used to describe ethidium in order to account for the missing charge-transfer term.[35] The NUCGEN module was used to generate the geometry of the unperturbed B-DNA decamers $(GCATATATGC)_2$ and $(GCGCGCGCGC)_2$. To obtain the initial geometry of the ethidium molecule intercalated into the poly(AT) decamer, we modified the structure of the ethidium···RNA complex NDB ID DRB018.[22] The initial geometry of the ethidium··· poly(GC)-decamer complex was generated by substituting guanine and cytosine for adenine and thymine, respectively. In this way, a 1:1 complex with the intercalator molecule between 4th and 5th base pair for both decamers was prepared.

We ran the simulations for data collection for 9 ns (for the details see Supporting Information). The coordinates and energy information were recorded every 1 ps.

For the analysis, the time-averaged structure of every species was calculated using CARNAL. To calculate the quantities concerning DNA flexibility, the method developed by Lankaš et al.[56] was applied on the set of helical parameters provided by the 3DNA package[53] for every frame recorded.

Binding free energy: The use of a variety of computational methods was required to evaluate the free (Gibbs) energy of intercalation of ethidium into the DNA. Our model of this reaction consisted of an ethidium molecule being bound to the DNA dimer (2TA, the minimal model introduced earlier) with two sodium cations near the phosphate residues.

The formation of the complex was followed by the release of a sodium cation. The entire process took place in an aqueous environment:

$$2TA \cdot Na_2\ (aq)\ +\ ETD^+\ (aq)\ \rightarrow\ 2TA \cdot Na \cdot ETD\ (aq)\ +\ Na^+\ (aq) \qquad (1)$$

The overall free energy corresponding to the ethidium binding consisted of the following contributions:

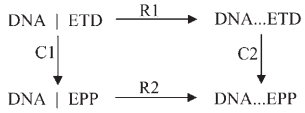
1) *The free energy of the dehydration of reactants and the hydration of products.* Both contributions were determined by using the C-PCM method. The geometry of both complexes containing sodium cations had already been obtained by the partial energy minimization of the minimal model described above. These minimizations were performed at the HF/STO-3G level and only the sodium cations and the closest oxygen atoms were relaxed.
2) *The interaction energy of the ethidium···DNA complex.* This term was calculated using the SCC-DFTB-D method. These data correspond to the formation of an intermediate complex $2TA{\cdot}Na_2{\cdot}ETD^+$. The energy required to separate a sodium cation was calculated by using the AMBER package (version 6, module SANDER_CLASSIC) equipped with the Cornell et al. force field.
3) *The difference of both entropy and zero point vibrational energy between the reactants and the products.* Both contributions were determined by vibrational analysis performed using the AMBER package (version 8, module NMODE). Prior to every calculation of vibrational frequency values, the respective system was energy-minimized using the Newton-Raphson algorithm implemented in NMODE.

Free energy difference calculations on the ethidium···DNA decamer complex: The free energy changes accompanying the binding of two different intercalators to DNA were determined by molecular dynamics–thermodynamic integration (MD-TI) calculations.

We used the GROMACS 3.1.4 molecular simulation package[54] with the Cornell et al. force field. To avoid unstable simulations and incorrect free energy accumulation arising from singularities in the van der Waals and Coulomb potential energy terms, soft core potential energy scaling[55] was used systematically.

The configuration space was sampled according to various simulation protocols. The simulations were divided into so-called windows with fixed values of the coupling parameter λ. First, there was a group of simulations in which the amount of sampling in every window was fixed and divided into an equilibrium phase and a data-collection stage (for the details see Supporting Information). Then the reverse cumulative averaging (RCA) procedure[56] was adopted and implemented in GROMACS. When using RCA, the data-collection stage commenced when the system was equilibrated at the 85% confidence level and it was terminated when the uncertainty of the free energy derivative went under 1.5 kcal mol^{-1}. This procedure ensured control of the uncertainty of the free energy estimate and represents the most reliable simulation protocol used. Its disadvantage lies in the a priori unknown length of the simulation.

The free energy difference was determined for complexes of ETD and EPP with a common DNA decamer $(GCATATATGC)_2$. In order to obtain these characteristics, the following thermodynamic cycle was adopted (Scheme 1).



Scheme 1. Thermodynamic cycle. "DNA | X": DNA and X separated in bulk water; "DNA···X": complex of DNA and X in bulk water.

The direct calculation of the formation free energy of a complex (processes R1 and R2 in Scheme 1) is quite difficult and large uncertainty is introduced. On the other hand, the calculation of the free energy difference for the "alchemical" change of one intercalator into another (no matter whether intercalated or not; processes C1 and C2 in Scheme 1) should be both easier and accurate enough provided there is little chemical difference between both intercalators. Since free energy is a state function, the following equation holds:

$$\Delta\Delta G\ =\ \Delta G(R2) - \Delta G(R1)\ =\ \Delta G(C2) - \Delta G(C1) \qquad (2)$$

Thus, we performed the MD-TI calculations for the "alchemical" change of the ETD molecule to EPP twice: first, for the intercalator bound to the DNA decamer and second, for the free intercalator dissolved in water. Then, the free energy difference of the intercalation of EPP and ETD was obtained as the difference of the two ΔG values calculated.

Results and Discussion

Ethidium and its derivatives

The ethidium molecule is a planar aromatic system and only the phenyl and ethyl groups deviate from the molecular plane. The positive charge of the cationic molecule is significantly delocalized; it is worth emphasizing that the charge delocalization was confirmed by two entirely different methods (RESP fitting and NBO methodology) providing this important finding with firm support. This result sharply contrasts with the recent assumption of charge localization on the amino groups made by Luedtke et al.[57] (For the optimum structure and the atomic charge values of ethidium molecule see Supporting Information.)

The isotropic molecular polarizability values of ETD, EPP and DPP determined at the HF/6-31G** level amount

to 35.8, 32.5 and 33.1 Å^3, respectively. These values differ only slightly and thus the dispersion energy contribution to the interaction energy in complexes with a common partner can be expected similar.

The electron donor–acceptor capabilities of ethidium and its derivatives can be estimated from the energy of their frontier orbitals. The DNA bases are good electron donors while the cationic intercalator molecule is expected to be an electron acceptor. The energy of the lowest unoccupied molecular orbital (LUMO) was determined for ETD, EPP and DPP from the HF/6-31G* wavefunction and amounts to −2.10, −2.40 and +2.09 eV, respectively. Consequently, both cationic intercalators ETD and EPP exhibit good electron-acceptor properties (due to their low LUMO energy values) while the neutral DPP can hardly act as an electron acceptor.

The DNA decamer

The averaged structure of both double-helical B-DNA decamers $(GCATATATGC)_2$ and $(GCGCGCGCGC)_2$ generated by MD simulations with the Cornel et al. force field are presented in Figure 2.

The interaction energy of the DNA···ethidium complex—The minimal model

Figure 3 shows the optimal structure of the minimal model consisting of the thymine···adenine base-pair step and an ethidium molecule. In other words, a non-covalently bound intercalator molecule interacts with four base residues, four sugar molecules and two phosphate units, which have been protonated to neutralize the negative charge of the phosphate and thus to mimic the proximity of a positively charged counterion.

The interaction energy values of the complex intercalator (ETD, EPP and DPP)···DNA step (both TA- and CG-) determined by the SCC-DFTB-D method are presented in Table 1. The overall stabilization energy is expressed as a sum of the net stabilization energy and the deformation energy, which represents the energy required for DNA to adapt from its optimal structure (without an intercalator).

The first and most important result is the finding that the net stabilization and total stabilization energy values are large, much larger than might have been expected. Comparing the different base-pair steps, we find rather surprisingly that the total interaction energy as well as its component differs only slightly. The systematically smaller values of the total stabilization energy for the GC-step go to the account of the larger value of the deformation energy. Following this expectation, the positively charged intercalator molecules bind to DNA much more strongly than the neutral intercalator. From the data presented in Table 1 it further follows that the dispersion energy contribution to the interaction energy is decisive and of comparable magnitude in all cases while the difference in the interaction energy comes from the non-dispersive term. This finding is not surprising since the values of polarizability are similar for all intercalator molecules (see above). On the other hand, the charge and the electron affinity of these systems differ considerably (cf. previous section) leading to a large difference in the electrostatic and donor–acceptor contributions to the interaction energy.

Reliable decomposition of the interaction energy can only be carried out using the symmetry-adapted perturbation theory approach.[58] However, the size of our minimal model prevents us from using such a computationally extensive procedure. So, our aim must be simpler—just to identify the critical component of interaction energy and to connect it with the properties of interacting subsystems. This "decomposition" applies only to the interaction energy and does not concern free energies (see next chapter) at all.

The electrostatic and charge-transfer energy contributions can be determined only indirectly. The electrostatic term will be discussed first. On the basis of the atomic charge analysis (RESP and NBO), we can estimate the charge–charge electrostatic contribution to the interaction energy of the intercalator···dinucleotide complex. The electrostatic energy values based on the RESP and NBO charges are very similar and amount to −49.7 and −51.0 kcal mol^{-1} for ETD···DNA and to −6.3 and −7.6 kcal mol^{-1} for DPP···DNA. Evidently, the large difference in the binding of ETD and DPP to DNA stems from the different electrostatic contribution, which comes from the different total charge and charge distribution of ETD and DPP.

To quantify the charge-transfer energy, we evaluated the amount of charge transfer between the electron donor (presumably the base-pair step) and the acceptor (presumably the intercalator). ESP analysis was performed and provided us with atomic charge values for the complex. Based on these values, we obtained the following charge transfer amount: DNA→ETD 0.22 e, DNA←DPP 0.05 e. The differ-

Table 1. Interaction energy and its components (ΔE, kcal mol^{-1}) of the complexes of the intercalators ETD, DPP and EPP with DNA steps TA and CG.[a]

Complex	TA···ETD		TA···DPP		TA···EPP	
	total	dispers.	total	dispers.	total	dispers.
ΔE^{INT}	−71.9	−47.6	−49.9	−45.3	−66.1	−42.7
ΔE^{DEF}	21.8	15.7	20.6	15.8	20.7	15.9
ΔE^{TOT}	−50.0	−31.8	−29.4	−29.5	−45.4	−26.8
Complex	CG···ETD		CG···DPP		CG···EPP	
	total	dispers.	total	dispers.	total	dispers.
ΔE^{INT}	−72.6	−48.5	−51.0	−45.9	−66.5	−43.6
ΔE^{DEF}	24.2	16.8	22.1	16.9	22.8	17.0
ΔE^{TOT}	−48.4	−31.7	−29.0	−29.0	−43.7	−26.6

[a] ΔE^{INT}: net interaction energy, ΔE^{DEF}: deformation energy, ΔE^{TOT}: total interaction.

ence between the intercalators ETD and DPP seems substantial. However, a question arises concerning the quality and suitability of the ESP fitting procedure for the intercalator···DNA complex where the intercalator molecule is located ("buried") in the cavity formed by the DNA and so the charge on its atoms may easily become incorrectly defined. As opposed to this, the NBO methodology does not suffer from this problem. The charge-transfer values based on the NBO charges differ considerably from the ESP values and now the intercalator is always the electron acceptor. In the case of ETD···DNA and DPP···DNA, 0.09 and 0.02 electron was transferred, respectively. These data show that the charge transfer is larger in the ETD···DNA complex but the difference is not as significant as indicated by the results of the ESP analysis. The charge-transfer contribution to the interaction energy can be estimated by calculating the E2 perturbation energy from the NBO analysis. The total E2 energy obtained at the B3LYP/6-31G** level for ETD→DNA bases is 43.5 kcal mol^{-1} while that for DPP→DNA bases is 40.3 kcal mol^{-1}. Putting all these data together, we may conclude that the charge-transfer is similar for the complexes of charged and uncharged intercalators with DNA. The significant difference in the respective stabilization energy values should thus be assigned to the different magnitudes of the electrostatic contribution.

We can conclude that the strong non-covalent binding of cationic intercalators derived from phenanthridine to DNA is due to dispersion and electrostatic contributions; the charge-transfer term contributes to the overall stabilization as well but its role is incidental. The binding of a neutral intercalator to DNA occurs exclusively due to the dispersion contribution to stabilization energy. So, the dispersion energy thus represents the dominant stabilization energy contribution in the intercalation process. Consequently, any theoretical procedure unable to cover the dispersion energy correctly is not suitable for the intercalation description.

The total interaction energy of complexes containing cationic intercalators is, in all cases, very large and represents about half the value typical for covalent bonds. However, the net stabilization energy is even larger and reaches 70 kcal mol^{-1} if the intercalator carries a positive charge. To demonstrate the reliability of the values calculated by the SCC-DFTB-D procedure, we determined the net interaction energy of the ETD···DNA and DPP···DNA complexes using the correlated ab initio method, RI-MP2/SVP(0.25, 0.15) as well. The resulting values of the interaction energy (Table 2) fully confirm the excellent performance of the SCC-DFTB-D method. Table 2 also contains the stabilization energy values yielded by several popular density functionals for complexes of ETD and DPP with the TA dinucleotide.

Table 2. Stabilization energy [kcal mol^{-1}] of the intercalator···DNA complex obtained by using the following methods: SCC-DFTB-D, correlated MP2, HF and DFT with various functionals.

Method	SCC-DFTB-D	MP2	HF	B3LYP	BLYP	PBE	TPSS
Basis set	N/A	SVP(0.25, 0.15)		TZVP			
ETD···DNA	−71.9	−69.3	+5.7	+31.4	+7.0	−15.1	−4.0
DPP···DNA	−49.9	−47.1	+25.2	+15.5	+24.8	+3.6	+14.4

It is evident that all functionals fail completely similar to the HF method; the best performance was shown by the PBE functional. This conclusion is by no means surprising and merely confirms that DFT cannot be used to describe processes governed by dispersion energy.

It is worth mentioning the value of deformation energy (Table 1), which represents the energy required to separate two base pairs from their relaxed distance of 3.4 Å to the geometry suitable for the intercalation, where the distance is increased to about 6.5 Å. This energy is similar for both the TA- and GC-steps and unexpectedly modest in magnitude, therefore confirming the flexibility of the DNA double helix.

Until now, the role of hydrogen bonding in the stabilization of an intercalator in DNA was undetermined and no common perspective had been established thus far. The data presented in Table 1 provide clear evidence that the hydrogen bonds existing between the amino groups of ethidium and the DNA backbone contribute to the interaction energy only marginally. The absolute value of the interaction energy of the complex containing the deamino derivative of ethidium (EPP) is only 5.8 kcal mol^{-1} less than that of the complex containing ethidium itself, that is, two hydrogen bonds contribute less than 10% to the overall stabilization.

Free energy of the ethidium intercalation into DNA

DNA dinucleotide: The intercalation process is very complex and may consist of six distinct partial steps depicted below (2TA: dinucleotide, ETD^+: ethidium, Na^+: sodium cation, (aq): hydrated species).

1) $2TA \cdot Na_2(aq) \rightarrow 2TA \cdot Na_2$ (DNA hydration)

2) $ETD^+(aq) \rightarrow ETD^-$ (ETD^+ hydration)

3) $2TA \cdot Na_2 \cdot ETD^+ \rightarrow 2TA \cdot Na_2 \cdot ETD^+$ (ETD^+ binding)

4) $2TA \cdot Na_2 \cdot ETD^+ \rightarrow 2TA \cdot Na \cdot ETD + Na^+$ (Na^+ release)

5) $2TA \cdot Na \cdot ETD \rightarrow 2TA \cdot Na \cdot ETD(aq)$ (complex hydration)

6) $Na^- \rightarrow Na^+(aq)$ (Na^+ hydration)

The resulting values for the six steps mentioned are summarized in Table 3. (Principally, free energy cannot be split into different contributions like dispersive and electrostatic energy. Thus, only the total interaction energy is taken into account for the process No. 3.)

Having investigated various individual processes we found that the hydration of Na^+ (no. 6) and the dehydration of

Table 3. Free energy change [kcal mol^{-1}] of the ethidium intercalation to DNA.

	Process	Quantity	Value	Method
1	2TA·Na_2 dehydration	ΔG	+75.0	C-PCM
2	ETD^+ dehydration	ΔG	+49.9	C-PCM
3	ETD^+ binding to 2TA·Na_2	ΔE	−50.0	SCC-DFTB-D
4	Na^+ release from 2TA·Na_2·ETD^+	ΔE	+19.9	force-field
3+4	whole reaction in vacuo	$-T\Delta S$	+15.6	force-field
3+4	whole reaction in vacuo	ΔE^{ZPV}	+7.2	force-field
5	2TA·Na·ETD hydration	ΔG	−24.5	C-PCM
6	Na^+ hydration	ΔG	−97.6	C-PCM
	total	ΔG	−4.5	

2TA·Na_2 (no. 1) exhibit the largest ΔG values. However, by summing up the ΔG values of all four hydration and dehydration processes (5, 6, 1, 2) we obtain a rather small value of +2.8 kcal mol^{-1}. How accurate is this value? Since we used the same procedure to calculate the ΔG values for hydration and dehydration, we believe that errors occurring on both sides of the equilibrium compensate for each other and the resulting ΔG difference is rather robust (i.e., independent of the computational procedure). We believe that the same statement is true regarding the change of entropy and ZPVE ascribed to processes 3 and 4 in vacuo. Both values are repulsive and do not depend much on the nature of the complexation process. Studying the formation of various DNA base pairs in vacuo by ab initio QC calculations,[59] we found that ΔE differed considerably while $-T\Delta S$ and ΔE^{ZPV} exhibited almost constant values for various base pairs. The critical values determining the final ΔG are thus the stabilization energy of the ethidium···DNA complex and the energy needed to release a sodium cation from the intercalation site.

In our previous study,[35] we showed that the SCC-DFTB-D value of the interaction energy of the complex of ethidium and a TA base pair agrees well with the MP2 value. To verify the performance of SCC-DFTB-D procedure, we determined the stabilization energy of process 3 using the correlated RI-MP2 method with the SVP(0.25,0.15) basis set. The SCC-DFTB-D stabilization energy value of −71.9 kcal mol^{-1} is in excellent agreement with the RI-MP2 value of −69.3 kcal mol^{-1}. Also, Table 2 shows that the SCC-DFTB-D value of the interaction energy of the DPP···DNA complex agrees fairly well with the MP2 value and for both complexes, the stabilization energy is only slightly overestimated (by 2.6 and 2.8 kcal mol^{-1} for ETD···DNA and DPP···DNA, respectively). Thus, the SCC-DFTB-D procedure is considered reliable for the study of the intercalation process in general.

The release of Na^+ from the 2TA·Na_2·ETD^+ complex was described by using the Cornell et al. force field. This process is dominated by the electrostatic interaction and the use of the empirical potential is fully justified.

Summing all values together, we obtain the total ΔG value of −4.5 kcal mol^{-1}, that is, the ethidium intercalation to DNA is an exergonic process. This result compares well with the experimental values around −7 kcal mol^{-1} (e.g. −6.9 kcal mol^{-1} by Garbett et al.[36]) and this agreement verifies the ΔE and ΔG values of distinct partial processes shown in Table 3. It is possible to conclude that among various individual terms the interaction energy of the ethidium binding to DNA represents the crucial contribution to the total ΔG.

We are aware that to a certain extent, the final agreement with experimental value might be due to a fortuitous cancellation of errors. In previous paragraphs, we discussed the accuracy of our calculations and we rely on the fact that the same method is used for the calculation of every quantity of both reactants and products. Generally, the accuracy of interaction energy is much larger than that of solvation free energy.

DNA Decamer: The binding free energy difference of ETD and EPP was determined by using the thermodynamic cycle (Scheme 1) and MD-TI simulations of the mutation of ETD to EPP both inside DNA and in pure water. Various simulation protocols differing in the number of λ windows and the amount of sampling in every window (see Supporting Information) were applied.

Originally, we intended to determine the ΔG of the intercalation process directly, that is, to "mutate" the ethidium molecule to a "ghost" system. After many fruitless attempts we realized that such a procedure is impractical and only a much smaller mutation can be performed. Finally, we decided to investigate the role of ethidium's amino groups in the binding process since it was known that these are responsible for the hydrogen bonding of the intercalator to the sugar–phosphate backbone of DNA which may affect the overall strength of the interaction of the intercalator and DNA. To achieve that, the amino groups were substituted by hydrogen atoms to form an EPP molecule (Figure 1).

The results for various simulation protocols are presented in Table 4.

From the first five rows in Table 4 we can see that the simulations with a fixed window width (i.e., a fixed amount of both equilibration and data sampling for every value of the coupling parameter λ) did not lead to converged results. Moreover, the error of these results cannot be estimated reliably. On the other hand, the simulations with the RCA setup (the last row in Table 4) both yield data of controlled

Table 4. Calculated values of the free energy [kcal mol^{-1}] associated with the mutation ETD to EPP for various simulation protocols used.

Simulation protocol[a]	ΔG in DNA	ΔG free	$\Delta\Delta G$
4	93.7	89.8	3.9
7	89.0	92.7	−3.7
14	91.0	88.0	3.0
21	90.6	92.0	−1.4
16	87.2	80.2	7.0
RCA[b]	70.4(0.8)	68.0(1.1)	2.4(1.9)

[a] For the definition of protocols see Supporting Information. [b] The uncertainty is given in parentheses for RCA calculations.

uncertainty and utilize the computational time efficiently; the total sampling time in this case was 4.9 ns in the simulation of the DNA···intercalator complex and 3.7 ns in the simulation of the free intercalator. The uncertainty carried by the value of the binding free energy difference is considerable. However, it can be reduced by setting the target error of the free energy lower than 1.5 kcal mol^{-1} at the expense of increased sampling time. Regardless, the resulting value of $+2.4\pm1.9$ kcal mol^{-1} agrees qualitatively with the experimental value of $+1.6$ kcal mol^{-1} reported by Garbett et al.[36] This fact encourages us to use the MD-TI methodology with RCA analysis in further studies of the interactions of DNA···intercalator, protein···ligand and others.

Rather than giving evidence of the absolute value of the intercalation ΔG, the results obtained provide us with a relatively accurate image of a ΔG change (i.e., $\Delta\Delta G$) upon the amino groups' disappearance. The resulting value of $+2.4\pm1.9$ kcal mol^{-1} should be compared with the $\Delta\Delta E$ value of $+5.8$ kcal mol^{-1} determined earlier. It must be concluded that the interaction energy difference is reduced significantly if entropy is considered.

Conformational and flexibility changes of DNA upon the intercalation of ethidium

Intercalation affects the macroscopic properties of DNA and these changes might provide feedback on further action of the intercalator. In this section we compare the conformation and deformability of the bare DNA decamer and the 1:1 complex of this decamer and ethidium. We focused our attention on the properties of base-pair steps, namely various helical parameters[53] for the description of conformation, and also the "rise" harmonic force constants[52] as the characteristics of stretching deformability.

Neither in the case of the poly(AT) nor the poly(GC) decamer was any significant change of base-pair step helical parameters upon ethidium intercalation revealed (Table 5). Note that both decamers are symmetric and so in non-intercalated decamers, the 4th and the 6th base-pair steps are identical.

Ethidium intercalation strongly distorts the base-pair step forming the intercalation site. As expected, this step exhibits an increased rise (i.e., vertical distance between base-pair planes) and lowered twist (expressing the DNA molecule being unwound). But, it is clearly seen (Table 5, columns headed "step 4") that the intercalation of an ethidium molecule does not affect the conformation of the second-next base-pair step.

The entry of an intercalator molecule into a DNA duplex may be made easier or harder not only by a conformational change, but also by a change in deformability. Therefore, we also evaluated the characteristics of the DNA base-pair step related to the force necessary to remove its base pairs one from the other. The respective force constants for both decamers (with and without the intercalator) are listed in Table 6.

Table 6. Harmonic (Hooke's law) force constants [kcal mol^{-1} Å^{-2}] for the vertical displacement (rise) of the nonterminal base-pair steps in two DNA decamers, both bare and with an ethidium molecule intercalated.

Base-pair step	Poly(AT)		Poly(GC)	
	Bare	Intercalated	Bare	Intercalated
2–3	4.8	5.9	5.4	6.1
3–4	10.2	10.7	9.1	9.3
4–5	5.2	2.8[a]	5.6	6.1[a]
5–6	11.1	11.0	9.7	8.9
6–7	5.0	2.9	6.1	6.0
7–8	10.1	10.3	9.4	9.5
8–9	4.9	5.0	4.2	4.9

[a] With an ethidium molecule intercalated here.

The force constants of the purine–pyrimidine steps (3–4, 5–6, 7–8) are roughly twice as large as those of the pyrimidine-purine steps (2–3, 4–5, 6–7, 8–9). Consequently, the purine–pyrimidine steps are much stiffer than the pyrimidine–purine ones. This conclusion is in perfect agreement with previous findings by Lankaš et al.[52]

Furthermore, poly(AT) and poly(GC) decamers exhibit a difference in rigidity of the base-pair steps near the intercalated one. While the deformability characteristics of poly(GC) remain unchanged upon intercalation, in the case of poly(AT), the rise force constant of the second-next step (6–7) to the intercalated one (4–5) is nearly halved. Consequently, the entry of another ethidium molecule into the poly(AT) DNA duplex that already contains will be facilitated due to an easier loosening of the second-neighboring thymine···adenine step. This feature of poly(AT) duplexes may lead to a stronger cooperativity of the intercalation of ethidium into these DNA structures.

Table 5. Helical parameters of selected base-pair steps in two DNA decamers, both bare and with an intercalated ethidium molecule.

Helical parameter		Poly(AT)				Poly(GC)			
		Bare		Intercalated		Bare		Intercalated	
		Step 4	Step 6	Step 4[a]	Step 6	Step 4	Step 6	Step 4[a]	Step 6
shift	[Å]	0.0	−0.1	−0.5	0.1	−0.1	0.0	0.7	−0.1
slide	[Å]	−1.1	−1.1	−0.5	−1.1	−0.4	−0.4	0.1	−0.4
rise	[Å]	3.4	3.4	6.6	3.5	3.1	3.2	6.5	3.2
tilt	[°]	0	0	−1	0	0	1	4	0
roll	[°]	12	12	6	13	8	9	8	8
twist	[°]	29	30	2	31	22	29	2	28

[a] With an ethidium molecule intercalated here.

Conclusion

i) The stabilization energy of cationic intercalators with DNA is considerably larger than that of uncharged intercalators and in both

cases, the dispersion energy represents the dominant energy contribution. While it is similar for all intercalators, the charge-transfer and mainly electrostatic contributions are considerably larger for the charged intercalators. The stabilization energy of ethidium with the TA and GC DNA dinucleotide is comparable and amounts to 70 kcal mol^{-1}.

ii) The free energy change upon intercalation determined as the sum of the free energy changes of six distinct individual processes amounts to -4.5 kcal mol^{-1} and the process is thus exergonic. The theoretical value agrees well with the experimental value of -7 kcal mol^{-1}. The largest free energy changes accompanied the hydration/dehydration processes and these contributions practically cancel each other. The key contribution represents the stabilization energy of the ethidium···DNA complex.

iii) Hydrogen bonding of ethidium to DNA contributes less than 10% to the overall stabilization energy and the main contribution originates from the stacking interaction of the ethidium molecule with the base pairs.

iv) Upon mutation of ETD to DPP (the amino groups are converted to hydrogen atoms), the calculated energy difference agrees well with the experimental value. The contribution of hydrogen bonding to the overall free energy is more significant than its contribution to the stabilization energy.

v) Intercalation affects the macroscopic properties of a DNA double strand and the largest change is its stretching flexibility. The base-pair step second-next to the intercalation site exhibits significantly higher deformability. Such a change may facilitate the introduction of another intercalator molecule into this base-pair step.

vi) The stabilization energy values of the intercalator··· DNA complex yielded by the SCC-DFTB-D method agree very well with the results of the correlated ab initio method. On the other hand, DFT calculations performed with various functionals failed completely and standard DFT calculations are not suitable for the description of the intercalation process.

Acknowledgements

We thank Dr. Filip Lankaš for his kind help. This work was supported by grants from the Grant Agency of the Czech Republic (grant No. 203/05/0009) and the Ministry of Education of the Czech Republic (grant No. LC512). It was a part of research project Z4 055 905.

[1] M. F. Brana, M. Cacho, A. Gradillas, B. de Pascual-Teresa, A. Ramos, *Curr. Pharm. Des.* **2001**, *7*, 1745.
[2] I. Haq, J. Ladbury, *J. Mol. Recognit.* **2000**, *13*, 188.
[3] Dabkowska, H. V. Gonzalez, P. Jurečka, P. Hobza, *J. Phys. Chem. A* **2005**, *109*, 1131.
[4] P. Hobza, J. Šponer, *Chem. Rev.* **1999**, *99*, 3247.
[5] P. Hobza, J. Šponer, *J. Am. Chem. Soc.* **2002**, *124*, 11802.
[6] J. Pittner, P. Hobza, *Chem. Phys. Lett.* **2004**, *390*, 496.
[7] J. Šponer, P. Hobza, *Collect. Czech. Chem. Commun.* **2003**, *68*, 2231.
[8] J. L. Bresloff, D. M. Crothers, *Biochemistry* **1981**, *20*, 3547.
[9] M. L. D′Amico, V. Paiotta, F. Secco, M. Venturini, *J. Phys. Chem. B* **2002**, *106*, 12635.
[10] R. B. Macgregor, R. M. Clegg, T. M. Jovin, *Biochemistry* **1985**, *24*, 5503.
[11] R. B. Macgregor, R. M. Clegg, T. M. Jovin, *Biochemistry* **1987**, *26*, 4008.
[12] F. J. Meyeralmes, D. Porschke, *Biochemistry* **1993**, *32*, 4246.
[13] D. Porschke, *Biophys. J.* **1998**, *75*, 528.
[14] J. B. Chaires in *Drug-Nucleic Acid Interactions* (Eds.: J. B. Chaires, M. J. Waring), Academic Press, San Diego, **2001**, pp. 3–22.
[15] I. Haq, B. Z. Chowdhry, T. C. Jenkins in *Drug-Nucleic Acid Interactions* (Eds.: J. B. Chaires, M. J. Waring), Academic Press, San Diego, **2001**, pp. 109–149.
[16] I. Haq, *Arch. Biochem. Biophys.* **2002**, *403*, 1.
[17] X. G. Qu, J. B. Chaires, *J. Am. Chem. Soc.* **2001**, *123*, 1.
[18] J. S. Ren, J. B. Chaires, *Biochemistry* **1999**, *38*, 16067.
[19] J. S. Ren, T. C. Jenkins, J. B. Chaires, *Biochemistry* **2000**, *39*, 8439.
[20] D. Suh, J. B. Chaires, *Bioorg. Med. Chem.* **1995**, *3*, 723.
[21] S. C. Jain, H. M. Sobell, *J. Biomol. Struct. Dyn.* **1984**, *1*, 1179.
[22] S. C. Jain, H. M. Sobell, *J. Biomol. Struct. Dyn.* **1984**, *1*, 1161.
[23] M. L. Lamos, G. T. Walker, T. R. Krugh, D. H. Turner, *Biochemistry* **1986**, *25*, 687.
[24] G. T. Walker, M. P. Stone, T. R. Krugh, *Biochemistry* **1985**, *24*, 7462.
[25] S. A. Winkle, L. S. Rosenberg, T. R. Krugh, *Nucleic Acids Res.* **1982**, *10*, 8211.
[26] N. V. Hud, S. P. Jain, F. Anet, *Abstr. Pap. Am. Chem. Soc.* **2004**, *228*, U694.
[27] S. S. Jain, F. A. L. Anet, C. J. Stahle, N. V. Hud, *Angew. Chem.* **2004**, *116*, 2038; *Angew. Chem. Int. Ed.* **2004**, *43*, 2004.
[28] S. S. Jain, N. V. Hud, *Abstr. Pap. Am. Chem. Soc.* **2004**, *228*, U177.
[29] D. B. Davies, A. N. Veselkov, *J. Chem. Soc. Faraday Trans.* **1996**, *92*, 3545.
[30] J. Duhamel, J. Kanyo, G. DinterGottlieb, P. Lu, *Biochemistry* **1996**, *35*, 16687.
[31] R. Krautbauer, L. H. Pope, T. E. Schrader, S. Allen, H. E. Gaub, *FEBS Lett.* **2002**, *510*, 154.
[32] D. C. Suh, *Exp. Mol. Med.* **2000**, *32*, 204.
[33] A. N. Veselkov, L. N. Dymant, P. A. Bolotin, S. F. Baranovskii, O. S. Zavyalova, D. A. Veselkov, K. Parkes, D. Davis, *Biofizika* **1995**, *40*, 1189.
[34] S. C. Jain, C.-C. Tsai, H. M. Sobell, *J. Mol. Biol.* **1977**, *114*, 317.
[35] D. Řeha, M. Kabeláč, F. Ryjáček, J. Šponer, J. E. Šponer, M. Elstner, S. Suhai, P. Hobza, *J. Am. Chem. Soc.* **2002**, *124*, 3366.
[36] N. C. Garbett, N. B. Hammond, D. E. Graves, *Biophys. J.* **2004**, *87*, 3974.
[37] F. X. Han, T. V. Chalikian, *J. Am. Chem. Soc.* **2003**, *125*, 7219.
[38] I. Haq, P. Lincoln, D. C. Suh, B. Norden, B. Z. Chowdhry, J. B. Chaires, *J. Am. Chem. Soc.* **1995**, *117*, 4788.
[39] J. Vondrášek, L. Bendová, V. Klusák, P. Hobza, *J. Am. Chem. Soc.* **2005**, *127*, 2615.
[40] D. Řeha, M. Hocek, P. Hobza, **2005**, unpublished results.
[41] E. Barratt, R. J. Bingham, D. J. Warner, C. A. Laughton, S. E. V. Phillips, S. W. Homans, *J. Am. Chem. Soc.* **2005**, *127*, 11827.
[42] W. D. Cornell, P. Cieplak, C. I. Bayly, I. R. Gould, K. M. Merz, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell, P. A. Kollman, *J. Am. Chem. Soc.* **1995**, *117*, 5179.
[43] C. I. Bayly, P. Cieplak, W. D. Cornell, P. A. Kollman, *J. Phys. Chem.* **1993**, *97*, 10269.
[44] S. Miertus, E. Scrocco, J. Tomasi, *Chem. Phys.* **1981**, *55*, 117.
[45] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Menucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Moro-

kuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, M. W. Wong, C. Gonzalez, J. A. Pople, Gaussian 03, Gaussian, Inc., Wallingford, CT (USA), **2004**.
[46] M. Feyereisen, G. Fitzgerald, A. Komornicki, *Chem. Phys. Lett.* **1993**, *208*, 359.
[47] L. M. J. Kroon-Batenburg, F. B. van Duijneveldt, *THEOCHEM* **1985**, *121*, 185.
[48] S. F. Boys, F. Bernardi, *Mol. Phys.* **1970**, *19*, 553.
[49] R. Ahlrichs, M. Bär, M. Häser, H. Horn, C. Kölmel, *Chem. Phys. Lett.* **1989**, *162*, 165.
[50] M. Elstner, P. Hobza, T. Frauenheim, S. Suhai, E. Kaxiras, *J. Chem. Phys.* **2001**, *114*, 5149.
[51] D. A. Pearlman, D. A. Case, J. W. Caldwell, W. S. Ross, T. E. Cheatham, S. Debolt, D. Ferguson, G. Seibel, P. Kollman, *Comput. Phys. Commun.* **1995**, *91*, 1.
[52] F. Lankaš, J. Šponer, P. Hobza, J. Langowski, *J. Mol. Biol.* **2000**, *299*, 695.
[53] W. K. Olson, M. Bansal, S. K. Burley, R. E. Dickerson, M. Gerstein, S. C. Harvey, U. Heinemann, X. J. Lu, S. Neidle, Z. Shakked, H. Sklenar, M. Suzuki, C. S. Tung, E. Westhof, C. Wolberger, H. M. Berman, *J. Mol. Biol.* **2001**, *313*, 229.
[54] E. Lindahl, B. Hess, D. van der Spoel, *J. Mol. Model.* **2001**, *7*, 306.
[55] T. C. Beutler, A. E. Mark, R. C. van Schaik, P. R. Gerber, W. F. van Gunsteren, *Chem. Phys. Lett.* **1994**, *222*, 529.
[56] W. Yang, R. Bitetti-Putzer, M. Karplus, *J. Chem. Phys.* **2004**, *120*, 2618.
[57] N. W. Luedtke, Q. Liu, Y. Tor, *Chem. Eur. J.* **2005**, *11*, 495.
[58] B. Jeziorski, R. Moszynski, K. Szalewicz, *Chem. Rev.* **1994**, *94*, 1887.
[59] P. Hobza, J. Šponer, *Chem. Phys. Lett.* **1996**, *261*, 379.

Received: June 24, 2005
Published online: November 18, 2005

