Accuracy of the fragment molecular orbital (FMO) calculations for DNA: total energy, molecular orbital, and inter-fragment interaction energy.

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Abstract

The Fragment Molecular Orbital (FMO) method can calculate the electronic structure of macromolecules such as DNA by dividing them into several fragments and introducing suitable approximations. To establish guiding principles for FMO calculation of DNA, benchmark tests were performed for several small DNA models consisting of one or two bases or two base pairs. The effects of several factors on the accuracy of FMO calculations were investigated, including the methods used to fragment the nucleotide units, approximations for the electrostatic potential, charge neutralization, and electron correlation. It was found that charge neutralization is indispensable for the reliable calculation of energies and spatial distribution of molecular orbitals, but not necessarily so for inter-fragment interaction energy analyses, such as calculation of the base-base interaction. The electrostatic approximations were shown to have only an insignificant effect on the qualitative nature of the calculations. It was also confirmed that the base-base stacking energy can be reproduced semi-quantitatively by the Møller-Plesset second-order perturbation (MP2) method though with some overestimation, and that the overestimation can be alleviated by the spin-component-scaled MP2 method.

Keywords

Fragment Molecular Orbital method, DNA, polynucleotide, interaction energy, molecular orbital, MP2

1. Introduction

The importance of quantum chemistry for calculating the electronic structure of proteins, sugars, lipids, DNA, RNA and other biological macromolecules cannot be denied. Nevertheless, the large size of these macromolecules still hampers their all electron *ab initio* calculations. Fortunately, recent advances in fragment-based quantum chemical methods are rapidly improving this situation [1]. Among the fragment-based methods, the fragment molecular orbital (FMO) method proposed by Kitaura *et al.* [2] is a promising one that has already been applied to a vast array of macromolecules, particularly proteins. See Fedorov & Kitaura [3, 4] for extensive reviews.

FMO calculations of polynucleotides, *i.e.*, DNA and RNA, are more difficult than those of proteins, however. The difficulty originates mostly from the charged phosphate backbone of the polynucleotides. For this reason, molecular modeling methods such as neutralization and solvation should also be closely considered in addition to FMO calculation methods such as fragmentation and calculation levels. In calculations of polynucleotides, we thus need to investigate both the accuracy of the FMO calculation itself and the molecular modeling protocol.

Presumably reflecting this difficulty, only a limited number of papers have been published on the accuracy of FMO of DNA. The few papers include that by Watanabe *et al.* [5], in which the total energy was calculated at the Hartree Fock (HF)/STO-3G level under various conditions. Another paper by Sekino *et al.* [6] reported the total and orbital energies calculated at the HF/STO-3G and PW91/STO-3G levels. These earlier papers examined only small basis sets without dispersion; nonetheless, use of a larger basis-set and inclusion of dispersion via an appropriate electron correlation method would be necessary to reproduce the stacking interaction between DNA bases [7-10].

The first choice for inclusion of the electron correlation is the Møller-Plesset perturbation theory to the second order (MP2). Hence, various FMO-MP2 calculations have been published so far and have shown that upgrading the calculation level from Hartree-Fock (HF) to MP2 drastically improves the accuracy of the interaction between hydrophobic amino acid residues within a protein [11], that of the π - π base-base stacking interaction within a DNA [8], and so on. Nevertheless, MP2 is known to overestimate the stabilization of any interaction energy, and thus a higher-order correlation method such as a coupled-cluster method (CCSD(T)) would be desirable [7, 12]. Unfortunately, these higher-order methods are too costly to apply to macromolecules such as DNA and proteins, and therefore we have to seek a less expensive one. The spin-component-scaled MP2 approach (SCS-MP2) is one such method that can improve the overestimation by the original MP2 [13-15]. In this paper, we report FMO calculations of DNA at the HF, MP2, and SCS-MP2 levels with several basis sets and compare the accuracy of the calculations by these different FMO

methods.

In FMO, in addition to the electron correlation and basis set, one needs to find an optimal way to fragment the molecule(s) and to approximate the electrostatic potential (ESP) [16] from the environment. The most reasonable way to fragment DNA may be to regard the whole nucleotide unit consisting of a base, sugar, and phosphate as a fragment. However, division of the nucleotide unit into several fragments becomes necessary if the FMO calculation is performed for an inter-fragment interaction energy (IFIE) analysis [17], by which the base-base Watson-Crick hydrogen bonding and stacking interactions can be calculated. The approximation of ESP is another factor that can alter the accuracy of FMO of DNA, which is a highly charged molecular system. Thus we here investigate several options for the optimal division of a nucleotide unit for IFIE analysis, as well as the effect of the ESP approximation on the IFIE.

In structure modeling of DNA, charge neutralization of the molecular system is regarded as necessary for FMO, much as in the classical molecular dynamic simulations of DNA, though FMO calculations are sometimes performed for charged DNA molecules [5, 8, 18]. There are roughly two ways to neutralize the phosphate group of DNA: one is to place a counterion on the group and the other is to cap the group with hydrogen. Sengoku et al. [19] compared the two ways and found that the former is more effective. Thus, in this paper, we examined the effect of the counterions on FMO. For further comparison, we also examined neutralization by deletion of the phosphate. These test calculations were performed for small DNA models consisting of a base, two bases, and a double strand of two base pairs (bp).

In this study, we investigate the effects of the fragmentation options, the ESP approximations, neutralization methods, basis sets, and incorporation of the electron correlation on the total energy, molecular orbital (MO) energies and spatial distribution, and IFIEs of several DNA models so as to identify guiding principles for FMO calculations. Thus, this study is intended to serve as a benchmark for future FMO calculations of DNA and, possibly, RNA.

2. Methods

2.1 DNA Models

We constructed DNA models 1-5 in order to test the FMO calculations (Fig. 1). Model 1 consisted of a single deoxyribonucleoside – the base and sugar without the phosphate (A, T, G, and C; we omit the prefix *d* for deoxy throughout this paper for the sake of simplicity). Model 2 was a single deoxyribonucleotide – the base, sugar, and phosphate. Model 3 was a single-stranded two base DNA (AT, CG, GA, and AA). Model 4 was a double-stranded 2 bp DNA (AT:AT, CG:GC, GA:CT, and AA:TT). **Model 5 was an H-capped two-bp DNA (AT:AT, CG:GC, GA:CT, and AA:TT) without sugar and phosphate groups.**

The models were generated as follows. Model 1 was optimized at MP2/6-31G** and model 2 at HF/6-31G** by the Gaussian 03 suite program [20]. Models 3 and 4 were constructed from the crystal structure of two strands of 5'-CGCGAATTCGCG-3' in the B-type conformation (PDB 335D) [21] as follows. The crystal structure was solvated with explicit water solvent and neutralized by a sufficient number of Na⁺ ions. The whole system was annealed by the AMBER99 classical force field [22]. Then, the water and ions were stripped off, and from the remaining 12 bp DNA, models 3 and 4 were constructed by excising appropriate nucleotide(s) and by capping them with H atoms at the 5' and 3' ends. Model 1 itself is neutral. Models 2 – 4 shown in Fig. 1 are negatively charged, but a neutral structure was also provided for each model by addition of an Na⁺ ion about 3 Å from a phosphate group (Fig. 1 inset). The added H and Na⁺ were optimized by the AMBER99 force field.

2.2 Fragmentation of DNA

We compared three options for DNA fragmentation (Fig. 2). In option 1, the one used in our previous study [8], the formal charge on each base becomes $-1e$, which might deteriorate the accuracy in calculating a larger DNA. Hence, we tried options 2 and 3, which set the formal charge of the base to $0e$. Both options 2 and 3 include some sugar carbons that bring about some drawbacks, however. In option 2, a carbon atom at the 1' position of sugar ring serves both as the bond detached atom (BDA) and bond attached atom (BAA), and the base fragment containing the carbon therefore has a formal charge of $0e$. In option 3, the sugar ring should be divided and assigned to two fragments. Carbon atoms at the 1' and 2' positions play roles of BDA and BAA, respectively, and the base fragment's formal charge becomes $0e$ also in this option. Each of the three options thus had a possible disadvantage for achieving a good level of accuracy, and hence their effects on the FMO accuracy were investigated in detail. Na⁺ ion was included in the phosphate fragment.

2.3 Parameters of FMO calculations

We also investigated several combinations of optional FMO parameters as follows. Calculations without the electron correlation were performed at the HF level and those with the correlation at MP2 and SCS-MP2 (Grimme [13] and Hill & Platts [14]). The basis set functions used were 6-31G, 6-31G*, and 6-31G**. When the ESP approximations were applied, the Mulliken orbital charge approximation (esp-aoc), point charge approximation (esp-ptc), and dimer-electrostatic approximation (dimer-es) were set to 0, 2 and 2, respectively (see [16] for the

definitions of these parameters). Sometimes reference energies were calculated by the conventional MO method to evaluate the quality of the FMO energies. We used the ABINIT-MP program [23] to make the MO and FMO calculations.

3. Results and discussion

We tested several conditions that may influence the accuracy of the FMO calculation of DNA. The investigated conditions included the fragmentation options, ESP approximations, neutralization methods, basis functions, and electron correlation. The influence and accuracy of the conditions were evaluated by comparing the total and orbital energies by FMO with those by conventional MO. Also, IFIE was calculated under various conditions to obtain more detailed information.

3.1. Total energy: tests of fragmentation and ESP approximation

We compared the total energy calculated by FMO with that by conventional MO to evaluate the three fragmentation options defined in Fig. 2. Presence and absence of the ESP approximations were also investigated in conjunction with the fragmentation options.

First, the neutralized single-stranded 2 base DNA (model 3) and double-stranded 2 bp DNA (model 4) were subjected to conventional MO and FMO calculations under the three fragmentation options (Fig. 2), and their energy difference was investigated (Table 1). Among the three options, option 3 gave the largest error in each set of the calculations. Either option 1 or option 2 gave the smallest error. Sometimes option 2 gave an unacceptable level of error as large as 9 kcal/mol. In contrast, the largest error by option 1 was only 4 kcal/mol; the average error per base was ca. 0.9 kcal/mol independent of the basis-set with and without MP2. Thus, option 1 was considered the most appropriate. Relatively larger errors for some molecules, GA, GA:CT, and CG:GC, were observed. Numerical data indicate that the precision became the worst when the 5'-terminal is G, the base having the smallest ionization potential (the highest HOMO energy) (Table 1). Nonetheless, the precision should be subject to change according to the structure of the model molecule. Hence it is difficult to draw a concrete conclusion here.

--- Table 1 ---

Secondly, we examined the effects of the ESP approximations and counterions on the total energy. Similarly to Table 1, we calculated the difference in the total energy between conventional MO and FMO with/without the ESP approximations, with the three fragmentation options, and

with/without counterions (Table 2). The overall trend was invariant: fragmentation option 1 was consistently the best. In addition, the accuracy of FMO was uninfluenced by the counterions: the effect ranged from only 0.1-1 kcal/mol with/without the ESP approximations. On the other hand, the ESP approximations affected the accuracy by a few kcal/mol. In particular, fragmentation option 1 without the ESP approximations reduced the error to 1/2 – 1/10. Note also that in option 1 the original error itself was small enough that summation with the error due to the ESP approximations still remained within an acceptable range.

We thereby observed that fragmentation option 1 was always acceptable, that option 2 was sometimes acceptable but sometimes unacceptable, and that option 3 was always unacceptable. The robustness of option 1 indicates that the formal charge of $-1e$ assigned to the base portion does not degrade the accuracy. The instability of option 2 may originate from the two fragmented bonds assigned on an atom [3, 4, 24]. The pathological behavior of option 3 may be attributable to division of the sugar ring and, possibly, to the proximity of the charged phosphate to the fragmentation site.

In this subsection, we have used the difference in total energy between conventional MO and FMO calculations. We have found that option 1 is the most appropriate way to fragment a nucleotide unit. We have also observed that the energy difference becomes somewhat large by use of the ESP approximations but is fairly uninfluenced by the presence and absence of counterions.

--- Table 2 –

3.2 Energy and spatial distribution of molecular orbitals: test of neutralization

We then examined the charge neutralization methods by evaluating the energy and spatial distribution of HOMO and nearby MOs. As is explained below, we found that these MO characters are highly sensitive to the charge neutralization of DNA. We first performed conventional MO calculations to obtain the general MO characteristics and then compared them with the FMO calculations.

Conventional MO calculations

To assess the neutralization methods, we performed conventional MO calculations on the single-base models, namely model 1 (neutral), model 2 without Na^+ (charged), and model 2 with Na^+ (neutral). Orbital energies from HOMO to HOMO-4 were calculated for the models (Table 3). In all the models, the obtained MO energies were in the reverse order of ionization potential (IP), $G < A < C < T$, consistent with Koopmans's theorem and with a preceding study [25]. Differences in the HOMO to HOMO-4 energies between model 1 and model 2 with Na^+ , both neutral, were fairly small,

the former being ca. 0.5 eV lower than the latter. In contrast, the difference between model 2 without Na^+ (charged) and that with Na^+ (neutral) was as large as ca. 2.0-2.5 eV. These conventional MO calculations on the single base DNA models thus indicated that neutralization is more important in stabilization of the molecules than the existence of the phosphate group.

--- Table 3 ---

We further analyzed the effect of charge neutralization on the orbital energies and spatial distribution more closely for models 3 and 4, single- and double-stranded two-base DNAs. The orbital energies and **localizations** of HOMO to HOMO-3 of each model were obtained with/without Na^+ ions (Table 4). The MOs of AT and AT:TA are shown as representative examples (Fig. 3).

In model 3, neutralization by Na^+ was shown to stabilize these four MOs and to localize them on the base portion (Table 4A and Fig. 3). Na^+ lowered the HOMO energy by ca. 1.7 eV, and this trend was also seen in the HOMO-1, HOMO-2, and HOMO-3 energies, indicating that neutralization stabilized these occupied MOs. Also, the **localizations** of the MOs differed between the neutralized and charged models. In the presence of Na^+ , the four MOs resided at the bases; in particular, HOMO was localized at either one of the two bases and HOMO-1 at the other. In the absence of Na^+ , HOMO still remained at the base, but two of HOMO-1, HOMO-2, and HOMO-3 were localized at the phosphate, indicating the destabilization of the phosphate group.

The above trend found on model 3, the single-stranded DNAs, mostly holds in model 4, the double-stranded DNAs. That is, the orbital energies were on average 2.6 eV more stabilized by neutralization with Na^+ . Also, with Na^+ ions, all of the four MOs were localized on the bases, but without them, HOMO-2 and HOMO-3 were **localized** on the phosphate groups. These findings were consistent with previous reports that HOMO and the nearby occupied orbitals exclusively resided on the base portion of double-stranded DNAs in the presence of Na^+ and water [26, 27].

A closer inspection of the MOs revealed differences in their distribution between the single- and double-stranded DNAs, however. HOMO, which resided within the base portion with/without Na^+ both in models 3 and 4, was localized within a base in model 3 but was extended within a base pair in model 4 (Table 3 HOMO). Note that this base pair refers to a diagonal or stack pair, not to the Watson-Crick pair. For example, compare AT (Figs. 3 ab) and AT:TA (Figs. 3 cd); the HOMO was localized in the A base in AT, but was extended within A:A in AT:TA. HOMO-1 showed different behavior upon charge neutralization between models 3 and 4; in model 3, HOMO-1 stayed in the base portion with/without Na^+ , but in model 4 it moved to the phosphate upon deletion of Na^+ . We also observed penetration of some MOs into stack pairs made of the same base, such as AA in AA:TT, but the penetration was too small to list in Table 4.

We have so far found the followings through the conventional MO calculations of the

single- and double-stranded DNAs. Charge neutralization is of critical importance to stabilization of the **orbital energies of** DNA molecules. Each of HOMO to HOMO-3 reside in the base portion if neutralized by Na^+ counterions, but if not neutralized HOMO-2 and HOMO-3 (and also HOMO-1 in the single-stranded DNAs) shift their position to the phosphate groups. In the double-stranded DNA, HOMO and HOMO-1 are delocalized within one of the diagonal base pairs. Based on these findings, we will now consider the FMO calculations on the same DNA models.

--- Table 4 ---

FMO calculations

We then calculated the orbital energies of models 3 and 4 by FMO and contrasted them with those by conventional MO presented so far. The HOMO energies of the fragments comprising the DNA were calculated (Table 5) with fragmentation option 1, the most appropriate one (subsection 3.1). See Fig. 4 for the definition of the fragments listed in Table 5.

The FMO calculations on model 3, the single-stranded DNA, mostly agreed with the conventional MO calculations. The HOMO energies of the two fragments by FMO corresponded to HOMO and HOMO-1 by conventional MO and the order of the orbital energies also agreed between FMO and conventional MO. In the AT strand, for example, the HOMO energies of fragments 1 and 3 by FMO corresponded to the HOMO and HOMO-1 energies by conventional MO, respectively. The orbital energies with Na^+ were on average 1.7 eV lower than those without them. This result was comparable to that by the conventional MO calculations.

The FMO calculations on model 4, the double-stranded 2 bp DNA, again agreed with the conventional MO calculations. In the presence of Na^+ , the HOMO energies of the four fragments corresponded to the HOMO, HOMO-1, HOMO-2, and HOMO-3 energies of the conventional MO calculation. Also, the FMO orbital energies of the two bases with a delocalized orbital in the conventional MO calculation were almost degenerate. For instance, in AT:TA the two delocalized Aoo:ooA orbitals (HOMO and HOMO-1) by the conventional MO calculation (Fig. 3) corresponded to the HOMOs of fragment 1 (A) and fragment 5 (A) by the FMO calculation (Fig. 5). On closer inspection, each of the two A bases had an amino group, and the π orbitals of the two amino groups overlapped and made an anti-bonding orbital and a bonding orbital, the former corresponding to HOMO and the latter to HOMO-1 obtained by the conventional MO calculation. The HOMO and HOMO-1 were quasi-degenerate, and corresponded to the two HOMOs of Fragments 1 and 5 via FMO. Similarly to the conventional MO, the quasi-degeneracy of the two HOMOs via FMO was as small as 0.0 – 0.2 eV. The stabilization of HOMO energies by the counterions ranged from 2.67-3.03 eV, again being comparable to that by the conventional MO. In summary, the FMO calculations mostly agreed with the reference conventional MO calculations.

We also investigated the effect of the ESP approximations for model 4. Note that models 1-3 are too small to apply the approximations. The effect of the counterions was similar with and without the ESP approximations. In contrast, with the ESP approximations, the FMO orbital energies increased by ca. 1.2 eV and became more distant from the conventional orbital energies. The quasi-degeneracy discussed above became less intense with the ESP approximations, implying an influence of the approximations on the environmental potentials. Thus the ESP approximations had some negative influence on the accuracy of FMO. Nevertheless, the computation time was ca. 3.6 times faster with the approximations than without it (data not shown); hence, the approximations may be used unless minute analyses of MOs are the purpose of the FMO calculation.

--- Table 5 ---

Let us summarize this subsection. We have investigated the neutralization effect on several DNA models. We have found that the orbital energies are destabilized without neutralization even in the small DNA models containing only a base or two. HOMO and nearby occupied MOs reside in the base portion in the neutral state, but some of them transfer to and destabilize the phosphate group upon deletion of the neutralizing counterions. Hence, neutralization is inevitable in both conventional MO and FMO calculations of DNA to obtain reliable energies and spatial distribution of MOs. We have also investigated the effects of ESP approximations on the MO characters and found that they have some quantitative, though not qualitative, influence on the accuracy and should be generally avoided in minute characterization of MOs. We should note that direct comparison between conventional MO and FMO became possible because MOs obtained by the conventional MO calculations were localized in the two-base and two-bp DNA models and did not extend along two fragments. Also, the energy level order of the MOs was invariable between the conventional MO and FMO calculations because the MOs by the conventional MO calculation were regarded as a collection of MOs by the FMO calculation. We will nonetheless need either the FMO-LCMO [28, 29] or FMO-MO [5, 30] methods if we have to consider the distribution of MOs extending along the whole molecule.

3.3 IFIE: tests of dimer-es approximation, counterions, and electron correlation

In this subsection, we turn to another criterion, IFIE, to test the effect of the ESP approximations, counterions, and electron correlation on the FMO calculation of DNA. The IFIE diagram with respect to base-base interactions was drawn for model 4, the double-stranded 2 bp DNAs, under several conditions (Fig. 6). In the diagram, the horizontal lines correspond to Watson-Crick hydrogen bonds, the vertical lines to intra-strand stackings, and the diagonal lines to

inter-strand stackings (note that kcal/mol is used instead of eV in presenting IFIE, 1 kcal/mol = 0.04337 eV). We also compared IFIE with the conventional supermolecule calculation to discuss the advantages and disadvantages of the two methods.

We first investigated the effects of the counterions and the ESP approximations at the HF/6-31G** level (Figs. 6 d-g). The ESP approximations influenced the IFIE values by ca. 10%. In contrast, the effect of the counterions on IFIE was negligible. This suggests that an FMO calculation of DNA without neutralization may be acceptable if it is only intended for an IFIE analysis.

We then investigated the effect of the electron correlation on IFIE by comparing the HF (Fig. 6 d), MP2 (Fig. 6e), and SCS-MP2 (Fig. 6f, Grimme [13], and Fig. 6g, Hill & Platte, [14]) methods as applied to the Watson-Crick hydrogen bonding and the inter- and intra-base stacking interactions.

The Watson-Crick hydrogen bond interactions in CT and AT pairs were -12.0 ~ -12.5 kcal/mol at HF, -18.4 ~ -19.5 kcal/mol at MP2, -16.6 ~ -17.8 kcal/mol at SCS-MP2 by Grimme, and -17.6 ~ -18.8 kcal/mol at SCS-MP2 by Hill. The estimations of Watson-Crick hydrogen bonds by MP2 were thereby 1.2 – 1.5 times larger than those by HF. The MP2 values should contain some overestimation, which nonetheless was reduced to 90% by the SCS-MP2 corrections. Generally, the Watson-Crick hydrogen bond interactions obtained by HF, MP2, and SCS-MP2 semi-quantitatively agreed with each other.

In contrast to the Watson-Crick hydrogen bond, the stacking interactions showed qualitative disagreement between HF and MP2. The intra- and inter-strand base-base stacking interactions showed considerable variation, but their summation amounted to positive values (repulsive) at all the HF calculations and to negative values (attractive) at all the MP2 calculations. Each of the MP2 stacking energies was ca. 10 kcal/mol lower, *i.e.*, more stabilized, than the HF stacking energy. This result reiterates previous findings that stacking stabilization is correctly reproduced only if the dispersion energy is incorporated with the electron correlation [7, 8]. Again, an important problem should be the overestimation of the stacking energy by MP2. The MP2 stacking interactions were corrected to 70% by SCS-MP2, the effect of the correction being more evident than in the Watson-Crick hydrogen bonding interaction. Thus, the SCS-MP2 methods enable semi-quantitative evaluation of the base-base interactions at about the same computation cost of MP2.

Finally, we compared the FMO-IFIE and the conventional supermolecule calculation. Note that the essential difference between the two methods is that the former neglects but the latter considers the reorganization of the electrons upon complex formation. Namely, in the supermolecule calculation the electronic energy of the complex and those of the individual isolated molecules are solved independently to obtain the binding energy (BE) as their difference, while in

the FMO-IFIE the interaction energy between molecules is obtained from a single electronic structure of the molecular complex. To evaluate the difference between BE and IFIE, we calculated the hydrogen bonding and stacking interactions in H-capped two base pairs without the main chain (Fig. 1 Model 5) by both methods (Fig. 7). We thus obtained the interaction energy for two Watson-Crick hydrogen bonds (Table 6A) and the base-base stacking energy between two base pairs (Table 6B). BE of a hydrogen bond for single base pair was -17.3 kcal/mol for A:T and -32.6 kcal/mol for G:C [8]. However, BE of double hydrogen bonds, AT:TA and CG:GC, were not equal to twice of A:T and C:G, respectively, suggesting that diagonal interactions disturb their additivity. The absolute value of the hydrogen bonding energy by IFIE was larger by 20-30 % than that by BE, thus IFIE overestimated the stabilization by the hydrogen bond. We also observed an overestimation by IFIE in the stacking interaction though only by ca. 10%. The overestimation of both hydrogen-bonding and stacking interactions of IFIE should originate from the destabilization of each fragment monomer in the molecular complex from that in an isolated environment. FMO-IFIE thus showed some overestimation compared with BE by the supermolecule calculation. This overestimation is nonetheless only semi-quantitative and does not impede the utility of FMO-IFIE in calculation of interactions within a large molecular complex such as longer DNA molecules, the size of which often obstructs the supermolecule calculation [22].

We have investigated only the electronic structure of DNA *in vacuo* throughout this paper, but here we would like to comment on the solvation effect. The electronic structure of DNA is usually regarded as largely influenced by the presence of the solvent consisting of water molecules and ions. This view will be reconfirmed in our next paper [22], which reports the electronic structure change of 12 bp DNA upon solvation. The destabilization of the HOMO energy by the negative charge on the phosphate shown in this paper (ca. +1.7 eV/base, see subsection 3.2) accumulates even to +16 eV in the charged 12 bp DNA (-22e) *in vacuo*. This significant destabilization is completely relieved by neutralization and solvation by the solvent. In contrast to the HOMO energy, IFIE is rather unaffected by the solvent and hence the discussion based on IFIE in this paper essentially holds for the solvated DNA. Thus the influence of the solvent on the electronic structure of DNA varies according to the investigated physical quantities. Further details will be presented elsewhere [22].

In this subsection, we have shown intra- and inter-molecular interactions of DNA via the IFIE analyses. IFIE is fairly uninfluenced by neutralization with counterions, in contrast to the orbital energies (subsection 3.2). Inclusion of the electron correlation by MP2 and MP2-SCS brings about only semi-quantitative change to the Watson-Crick hydrogen bonding, but qualitative change to the base-base stacking; the stacking energies become repulsive without the correlation but attractive with it. SCS-MP2 alleviates overestimation by MP2. IFIE overestimates the interactions within a molecular complex compared with the conventional supermolecule calculation by 10-30 %.

4. Conclusion

We have investigated the influence of several factors on the accuracy of FMO calculation of small DNA molecules so as to lay the foundation for future FMO studies of large DNA and RNA molecules. Specifically, we have studied the effects of the fragmentation options, the ESP approximations, the neutralization methods, the basis sets, and the electron correlation via MP2 or MP2-SCS on the total energy, MO properties, and IFIEs of one- or two-base DNAs. Whenever possible, we have used conventional MO calculations as the reference against which the FMO calculations were tested.

We summarize the results here by giving a list of guiding principles for FMO calculation of DNA based on the findings of the current and previous studies.

- (1) Neutralize the molecular charge either by adding counterions near the phosphate groups or by deleting them. Both neutralization methods are acceptable, but the former is preferable in the simulation of realistic DNA molecules. Neutralization is inevitable in the calculation of energies and spatial distribution of MOs but not necessarily so in the IFIE analysis.
- (2) Use fragmentation option 1 (Fig. 2) if fragmentation of a nucleotide unit is necessary. In addition to its good energy accuracy, option 1 is also the simplest fragmentation, dividing the base and the sugar-phosphate backbone into two separate fragments. In any case, avoid fragmentation of the nucleotide unit if good energy accuracy is the supreme objective of the FMO calculation.
- (3) The ESP approximations may be applied without significant degradation of the results if used with appropriate neutralization and fragmentation. Nevertheless, it is preferable to avoid the approximations in minute analysis of the characteristics of MOs such as quasi-degeneracy.
- (4) Include the electron correlation effect to reproduce the base stacking interactions. HF is adequate to reproduce the Watson-Crick hydrogen bonding, but not to reproduce the stacking, which originates from the dispersion energy. Inclusion of the electron correlation via MP2 suffices for semi-quantitative representation of the stacking, though MP2 overestimates the energy value. This overestimation can be overcome by MP2-SCS with negligible computational cost. Also, MP2.5 will be available soon, which gives good accuracy comparable to that by CCSD [31].

These principles will serve for FMO and related *ab initio* calculations of DNA and RNA molecules and their complexes with proteins. Based on these principles, we are now studying a longer DNA double helix with explicit solvent and ions [22] with a view to understanding the electronic structure of DNA within a more realistic environment.

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Figure Captions

Fig. 1. Chemical definitions of models 1-4. BASE refers to A, T, G, or C. Inset: neutralization of models 2-4.

Fig. 2 Three options for fragmentation of a nucleotide unit. Different fragments are classified by color.

Fig. 3 Visualization of HOMO, HOMO-1, HOMO-2, and HOMO-3 of AT and AT:AT with/without Na^+ calculated by the conventional MO at HF/6-31G**.

Fig. 4 Definition of fragments for model 3 of GA and model 4 of GA:CT. 3' and 5' indicates 3'- and 5'-terminals of DNA, respectively.

Fig. 5 Visualization of the HOMOs of fragment 1 (A) and fragment 5 (A) calculated by FMO at HF/6-31G** without ESP approximations for AT:TA with Na^+ .

Fig. 6 IFIE diagram of base-base interactions within model 4, the double-stranded 2 bp DNAs.

Fig. 7 Definitions of the interactions obtained by the supermolecules calculation for DNA model 5 in Fig. 1.

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Figure 1

Model 1

Model 2

Model 3

Model 4

Model 5

Figure 2

Option1

Option2

Option3

Figure 3

Model 3

Model 4

Figure 6

Two base pairs
GA:CT

Hydrogen bond
interaction

Stacking interaction

Table 1 Effect of fragmentation options on the difference in total energy between FMO and MO ($E_{FMO}-E_{MO}$, kcal/mol). Both models were neutralized by Na^+ ions.

		HF			MP2		
fragmentation option		6-31G	6-31G*	6-31G**	6-31G	6-31G*	6-31G**
Model 3 with Na^+							
AT	1	-1.026	-0.693	-0.925	-1.753	-1.602	-1.898
	2	0.394	0.564	0.034	-0.500	-0.762	-1.429
	3	17.176	16.586	14.968	16.151	14.959	13.165
CG	1	-0.510	-0.361	-0.769	-1.104	-1.315	-1.842
	2	0.351	0.330	-0.248	-0.184	-0.691	-1.411
	3	17.336	16.682	15.133	17.437	16.075	14.386
GA	1	1.169	1.791	1.435	1.002	1.362	0.986
	2	4.635	4.551	3.855	4.510	3.979	3.212
	3	21.302	20.160	18.351	21.208	19.418	17.494
AA	1	0.828	0.801	0.408	0.307	-0.094	-0.585
	2	0.326	0.446	0.003	-0.354	-0.812	-1.414
	3	17.874	17.882	16.142	17.159	16.379	14.476
Model 4 with Na^+							
AT:TA	1	-2.407	-2.125	-2.618	-4.006		
	2	-0.052	0.089	-1.024	-2.058		
	3	34.315	32.797	29.549	32.249		
CG:GC	1	-1.760	-1.999	-2.711	-3.030		
	2	-5.391	-6.005	-8.528	-6.564		
	3	32.667	31.193	28.366	32.782		
GA:CT	1	1.201	2.198	1.550	0.412		
	2	9.118	8.739	7.464	8.858		
	3	43.090	40.690	37.060	42.970		
AA:TT	1	-1.528	-1.160	-1.739	-3.086		
	2	2.090	2.400	1.313	0.575		

3	34.495	33.720	30.336	33.363
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Table 2 Effect of the ESP approximations and counterions on the difference in total energy between FMO and MO ($E_{\text{FMO}}-E_{\text{MO}}$, kcal/mol) calculated for model 4 (double-stranded 2 bp DNAs).

ESP approximations		yes	yes	no	no
Na ⁺ ions		yes	no	yes	no
		fragmentation			
AT:TA	1	-2.618	-2.695	-1.306	-1.465
	2	-1.024	-1.249	1.875	1.715
	3	29.549	29.334	26.578	26.268
CG:GC	1	-2.711	-2.621	-0.483	-0.655
	2	-8.528	-7.486	-1.464	-1.329
	3	28.366	28.378	24.842	24.694
GA:CT	1	1.550	1.306	1.424	1.228
	2	7.464	7.101	8.295	8.175
	3	37.060	36.213	28.090	27.763
AA:TT	1	-1.739	-1.857	0.292	0.104
	2	1.313	1.130	3.536	3.392
	3	30.336	29.939	26.401	26.161

Table 3 Orbital energies (eV) of the four highest occupied MOs of DNA models 1 and 2 calculated by the conventional MO at HF/6-31G**.

Base	HOMO	HOMO-1	HOMO-2	HOMO-3	HOMO-4
Model 1 (neutral)					
A	-7.572	-8.976	-10.285	-10.343	-11.246
T	-8.572	-11.079	-11.314	-12.292	-12.707
G	-7.437	-9.653	-10.806	-11.166	-11.259
C	-8.344	-9.639	-10.585	-11.391	-12.405
Model 2 without Na ⁺ (charged)					
A	-5.121	-6.399	-7.848	-7.942	-8.827
T	-5.475	-8.403	-8.533	-9.521	-9.528
G	-4.814	-6.737	-8.015	-8.708	-8.859
C	-5.323	-7.069	-7.821	-8.627	-9.256
Model 2 with Na ⁺ (neutral)					
A	-7.262	-8.626	-9.967	-10.037	-10.931
T	-7.873	-10.591	-10.765	-11.789	-11.969
G	-6.954	-9.077	-10.297	-10.746	-10.843
C	-7.722	-9.173	-10.045	-10.899	-11.715

Table 4 Orbital energies (eV) of the four highest occupied MOs of DNA models 3 and 4 calculated by the conventional MO at HF/6-31G**. The label “energy” refers to the orbital energy and “MO” to the localization of the orbital. The MO localizations are represented by the following rule. The two digit-label corresponds to the two bases comprising the DNA strand, *i.e.*, two of “A,” “T,” “G,” and “C,” but label “P” indicates the phosphate backbone and label “o” is null, showing that no MO is localized in this position. In the case of AT, for example, if an MO is labeled with “Ao” it is localized in the A base, if it is labeled with “P” it is localized in the phosphate, and so on. The same rule applies to the two-stranded DNAs, such as AT:TA, where “Ao:oA” implies that the MO is extended along the AA base pair.

A. Model 3

	HOMO		HOMO-1		HOMO-2		HOMO-3	
	energy	MO	energy	MO	energy	MO	energy	MO
with Na ⁺ (neutral)								
AT	-7.80	Ao	-8.69	oT	-9.38	Ao	-10.48	Ao
CG	-7.06	oG	-7.98	Co	-8.86	Co	-9.94	CG
GA	-7.22	Go	-7.92	oA	-9.51	oA	-9.98	Go
AA	-7.51	Ao	-7.82	oA	-9.05	Ao	-9.33	oA
without Na ⁺ (charged)								
AT	-6.21	Ao	-6.50	P	-6.95	P	-6.95	oT
CG	-5.54	oG	-6.21	P	-6.29	Co	-6.73	P
GA	-5.55	Go	-6.20	oA	-6.68	P	-7.10	P
AA	-5.55	Ao	-6.33	oA	-6.38	P	-6.87	P

B. Model 4

	HOMO		HOMO-1		HOMO-2		HOMO-3	
	energy	MO	energy	MO	energy	MO	energy	MO
with Na ⁺ (neutral)								
AT:TA	-7.29	Ao:oA	-7.44	Ao:oA	-8.34	oT:To	-8.37	oT:To
CG:GC	-6.42	oG:Go	-6.60	oG:Go	-8.31	Co:oo	-8.40	Co:oo
GA:CT	-6.36	Go:oo	-6.98	oA:oo	-8.54	oo:oT	-8.71	oA:oo
AA:TT	-6.94	AA:oo	-7.20	AA:oo	-8.39	oo:TT	-8.56	oo:TT
without Na ⁺ (charged)								
AT:TA	-4.70	Ao:oA	-4.84	Ao:oA	-5.51	oo:P	-5.56	P:oo

CG:GC	-3.85	oG:Go	-4.02	oG:Go	-5.42	oo:P	-5.44	P:oo
GA:CT	-3.63	Go:oo	-4.16	oA:oo	-5.44	P:oo	-5.78	oo:CT
AA:TT	-4.37	Ao:oo	-4.64	oA:oo	-5.39	P:oo	-5.70	oo:oPT

Table 5 HOMO energies calculated by FMO-HF/6-31G**. See Figure 4 for the definition of the fragments.

A. Model 3

	fragment 1	fragment 3
with Na ⁺ (neutral)		
AT	-6.88	-7.70
CG	-7.07	-6.28
GA	-6.34	-7.25
AA	-6.62	-6.90
without Na ⁺ (charged)		
AT	-5.22	-5.86
CG	-5.30	-4.67
GA	-4.60	-5.42
AA	-4.97	-5.32

B Model 4

	fragment 1	fragment 3	fragment 5	fragment 7
with Na ⁺ (neutral), without ESP approximations				
AT:TA	-7.45	-8.35	-7.46	-8.34
CG:GC	-8.38	-6.63	-8.34	-6.53
GA:CT	-6.43	-7.05	-8.54	-8.86
AA:TT	-7.08	-7.23	-8.48	-8.44
without Na ⁺ (charged), without ESP approximations				
AT:TA	-4.87	-5.65	-4.88	-5.63
CG:GC	-5.79	-4.06	-5.76	-3.97
GA:CT	-3.71	-4.24	-5.85	-5.98
AA:TT	-4.52	-4.69	-5.93	-5.74
with Na ⁺ (neutral), with ESP approximations				
AT:TA	-6.18	-7.23	-6.31	-7.12
CG:GC	-7.11	-5.57	-7.17	-5.40
GA:CT	-5.30	-6.04	-7.36	-7.62

AA:TT	-5.76	-5.90	-7.27	-7.32
without Na ⁺ (charged), with ESP approximations				
AT:TA	-3.48	-4.38	-3.60	-4.27
CG:GC	-4.39	-2.86	-4.46	-2.70
GA:CT	-2.45	-3.08	-4.54	-4.59
AA:TT	-3.07	-3.22	-4.60	-4.48

Table 6 Comparison between binding energy and IFIE for capped two base pairs calculated by FMO-MP2/6-31G* (in kcal/mol).

A. Hydrogen bond interaction

	Energy associated hydrogen bonds		
	BE	IFIE	ΔE
AT:TA	-32.29	-39.84	7.55
CG:GC	-68.89	-91.44	22.55
GA:CT	-46.51	-62.59	16.08
AA:TT	-34.51	-43.23	8.72
<i>A:T^a</i>	-17.3	-	-
<i>G:C^a</i>	-32.6	-	-

^aref. [8]

B. Stacking interaction

	Energy associated with stacking		
	BE	IFIE	ΔE
AT:TA	-15.62	-16.88	1.26
CG:GC	-18.74	-21.03	2.29
GA:CT	-13.78	-13.93	0.15
AA:TT	-16.08	-16.58	0.50