Explicit solvation of a single-stranded DNA, a binding protein, and their complex: a suitable protocol for fragment molecular orbital calculation

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Abstract

Fragment molecular orbital (FMO) calculations were performed for explicitly solvated single-stranded DNA (ssDNA), ssDNA binding protein, and their complex in order to assess the solvent effects on the solutes and thereby to find optimal solvation conditions for FMO calculation. A series of solvated structures were generated with different solvent thicknesses. The structures were subjected to FMO calculation at MP2/6-31G* to obtain the net charges and internal energies of the solutes and the solute–solvent interaction energies as functions of the solvent thickness. In all cases, the properties showed complete or marginal convergence at ca. 6 Å, regardless whether or not the system charge was neutralized. This suggested that the first and second solvent shells mainly determine the electronic structure of a solute while the outer solvent including ions has only minor effects, consistent with several preceding reports. In light of this, and considering safety as a factor, we conclude that a solvent shell thickness of ca. 8 Å suffices for FMO calculation of the solutes.

Key Words: FMO, explicit solvent, single-stranded DNA, protein

Area of Interest: Molecular recognition and molecular modeling
1. Introduction

The fragment molecular orbital (FMO) method [1] has been extensively applied to electronic structure calculations of large molecular systems, especially biological molecules (see [2-4] for recent reviews). As biological molecules usually function in the presence of solvent, namely water and ions, the solvent effect must be included in FMO calculations. This effect can be incorporated inexpensively by implicit solvent models [5-7]. However, it is more realistic to use an explicit solvent model. Hence, FMO calculations have been performed with explicit models for an oligopeptide [8], proteins [9-11], ligands [12], protein/ligand complexes [13-15], double-stranded (ds) DNAs [16, 17], and a protein/dsDNA complex [18]. To the list we would like to add a single-stranded DNA (ssDNA) and its complex with a protein.

In biology, ssDNA appears upon unzipping of dsDNA in replication, and some viruses exist as ssDNA. Hence, examination of the electronic structure of ssDNA can shed light upon some ubiquitous biological phenomena. In this paper, we investigate an ssDNA with six nucleotides, \((dT)_6\), as the target. We also examine an ssDNA complexed with the oligosaccharide/oligonucleotide binding domain of the *Sulfolobus solfataricus* ssDNA binding protein (SSB) [19] and the isolated SSB, using the NMR structure of the complex as the modeling template (Fig. 1). We have chosen this molecular system with a view toward extending the current study to detailed analyses of the ssDNA/SSB interaction. We will delve into the interaction between ssDNA and SSB elsewhere [20]; in the present short article we concentrate on solvent effects on solutes to find an optimal solvation condition for FMO calculation.

![Figure 1. NMR structure of the ssDNA:SSB complex](image)

The best conformation specified in PDB:2MNA [19]. SSB and ssDNA are shown in green and blue, respectively.
2. Methods

Hereafter, ssDNA, SSB, or their complex is referred to as the solute while the water and Na\(^+\)/Cl\(^-\) ions are the solvent.

The effects of solvent size on the solutes were systematically investigated by the following scheme: molecular-mechanics (MM)-based modeling and annealing of the solvated molecular structures of the solutes, preparation of the structures for FMO from the MM-modeled structures, and calculation of their electronic structures by FMO. This scheme was basically similar to that of our previous studies on a solvated protein [9] and dsDNA [17], except that in the present study we also examine the effect of neutralization. Note that the distance between a solvent or ion and the solute was measured as the distance between nonhydrogen atoms (Na, Cl, O, C, N, and P) to maintain consistency with the preceding studies. The molecular structures were visualized with CHIMERA ver. 1.11 [21].

2.1 Modeling of the solvated molecular structures via MM-based methods

We prepared the molecular structures for FMO calculations by MM-based molecular dynamics (MD) and energy minimization (EM), using the NMR structure of the ssDNA:SSB complex (PDB: 2MNA) [19] as the initial structure (Fig. 1). Each solute was immersed in a periodic box of TIP3P water molecules containing enough Na\(^+\) or Cl\(^-\) ions to neutralize the net charge of the solute. The minimum distance between the solute and the box wall was set to 12 Å for SSB and Complex and to 15 Å for ssDNA, because the ssDNA was considerably smaller than SSB and Complex.

The solvated molecular models were then optimized as follows. After EM for 1000 cycles, each model was heated from 5 to 300 K within 100 ps. The MD simulation was then continued at 300 K for 7 ns, 5 ns, and 10 ns for ssDNA, SSB, and Complex, respectively. From the generated MD trajectories, snapshots after 3 ns were chosen at 100 ps intervals and were subjected to annealing from 300 to 5 K within 100 ps and then to EM for 1000 cycles. During the MD and EM, the positions of the heavy (nonhydrogen) atoms of the solute molecules were restrained by a weak harmonic potential with a force constant of 1 kcal/mol/Å\(^2\), and the electrostatic interaction was calculated by the particle-mesh Ewald method with a cutoff of 12 Å for the real-space summation [22]. In the MD simulations, covalent bonds involving hydrogen atoms were constrained to their equilibrium values with the SHAKE algorithm [23] at a 1 fs time step. The temperature and pressure were controlled by Berendsen’s method [24] with a relaxation time of 5 ps. This fairly long relaxation time was chosen to minimize the artefact of the velocity and coordinate rescaling on the dynamics of the molecules. All calculations were performed by the AmberTools15 suite program [25] with the AMBER14SB force field, an updated version of AMBER99SB [26].

2.2 Preparation of the molecular structures for FMO

The solvated structures for FMO were prepared by modifying the annealed structures. A structure was chosen for each solvated solute based on the criterion that all the ions were farther than 6 Å from the surface of the solute. The 5.0 ns, 5.0 ns, and 8.5 ns snapshots were chosen for ssDNA, SSB, and Complex, respectively. From each chosen structure, we made two series of structures with varying thicknesses of shell water with and without neutralization. See Fig. 2 for a schematic presentation of the procedure, in which “with neutralization” (“w/ neut.”) means all Na\(^+\)
and Cl\(^-\) ions were included even if they were not within the solvent shell, while “without neutralization” (“w/o neut.”) means only those within the shell were included. See also Fig. 3 for the constructed series of ssDNA structures with and without neutralization. Similar series were also generated for SSB and Complex.

We address three important points about the molecular structures thus prepared. One point is that the constructed solute structures were almost invariant from the original NMR structure (the root mean square deviation was ca. 0.5 Å for heavy atoms; data not shown) because the heavy atoms of the solutes were restrained by the harmonic potential to their original positions during the MM-MD/EM calculations. We used this restraint because the objective of these MM-MD simulations was not to sample the solute structures but to optimize the solvent configurations. The second point is that we chose configurations in which Na\(^+\)/Cl\(^-\) ions were not directly coordinated to the solutes. This criterion was necessary to avoid large errors observed for FMO calculation of Na\(^+\) ions [27]. The third point is that the molecular structures were minutely annealed and energy minimized. Hence they were not snapshots at finite temperature.

Thus, we took this rather conservative approach to prepare molecular structures. Another approach would be use of multiple snapshots generated by MM-MD to include thermal fluctuations of the structures and physical properties [10, 14, 15, 28, 29, and so on]. We do not deny the advantage of the latter approach, but this time we took the former approach because the main purpose was to determine the solvation effect on the solutes whose structures were not too deviated from the experimental ones.

**Figure 2.** Schematic of the strategy for constructing solvated structures of the solutes for FMO calculation
Figure 3. Examples of ssDNA structures constructed by excising the solvent.

Two series were constructed: one with neutralization (A) and the other without neutralization (B). The water molecules are shown by red teardrop shapes while the Na\(^+\) ions are shown by blue spheres. \(R\) is the solvent shell thickness.
2.3 FMO calculations

The solute–solvent structures thus annealed, chosen, and modified were subjected to FMO calculation as follows. All FMO calculations were performed with the ABINIT-MP program [3, 30] to the two-body expansion (FMO2) at the MP2/6-31G* level [31-33]. An amino acid residue or a nucleotide was regarded as a fragment, while each water molecule and counter ion (Na⁺ or Cl⁻) was regarded as a separate fragment. The thresholds for electrostatic approximations were \( L_{aoe} = 0 \), \( L_{ptc} = 2 \), and \( L_{dimer} = 2 \) (see [34] for definitions of the thresholds). See Table 1 for numbers related to the system size and for formal charges of the solute molecules.

Table 1. System size and formal charges of the solute molecules

| Solute          | ssDNA | SSB  | Complex |
|-----------------|-------|------|---------|
| Atoms           | 191   | 1,807| 1,998   |
| Residues/Nucleotides | 6     | 117  | 123     |
| Fragments       | 6     | 117  | 123     |
| Formal charges (e) | -5e   | +2 e | -3 e    |

2.4 Post-processing of the FMO results

The output of the FMO calculations was post-processed similarly to our previous reports [9, 17]. The net charges of the solutes were calculated as summations of the net charges of the constituent atoms obtained by either Mulliken population analysis (MPA) or natural population analysis (NPA) [35]. Interaction energies within the solvated systems were calculated based on inter-fragment interaction energy analysis (IFIE) [34, 36, 37]. In FMO2, the total energy of the system \( E \) is obtained as the sum of internal-fragment energies \( E'_I \) and \( \Delta E_{IJ} \) as follows:

\[
E = \sum_i E'_i + \sum_{i<j} \Delta E_{ij}.
\]

Let \( A \) be a partial group of the fragments in the molecular complex. The internal energy within group \( A \) \( (E_A) \) is then obtained by summation of \( E'_i \) and \( \Delta E_{ij} \):

\[
E_A = \sum_{i \in A} E'_i + \sum_{i<j; j \neq A} \Delta E_{ij}.
\]

The interaction energy of \( A \) with another group \( B \) \( (\Delta E_{AB}) \) is calculated as:

\[
\Delta E_{AB} = \sum_{i \in A} \sum_{j \in B} \Delta E_{ij}.
\]

Groups \( A \) and \( B \) can be any pair of clusters of fragments. In this study, the solutes and solvent were regarded as groups \( A \) and \( B \).

3. Results and discussion

We investigated the effect of solvent shell size on the electronic structure of each solute. Specifically, the net charge and internal energy of the solute molecules and the interaction between solvent and solute were obtained as functions of the solvent shell thickness (Figs. 4-6). The
influence of system neutralization was also examined.

3.1 Net charges of the solutes

The net charges of the solutes are plotted as functions of the solvent thickness (Fig. 4).

The overall shapes of the graphs in Fig. 4 are similar: the amount of charge transfer to the solvent is mostly established at 3 Å, but the graphs show a small dip at 4 Å and become stable after 6 Å. This pattern was invariant regardless of the kind of solute or the calculation method used (MPA or NPA). Similar, if not identical, patterns were seen in the previous FMO studies of a solvated protein (ubiquitin) [9] and dsDNA [17], as well as in a semi-empirical quantum mechanical study of a solvated dsDNA [29]. Note also that the solute net charges were unchanged by neutralization.

These results indicated that a solvent thickness of 6 Å is enough for the net charges to converge, that there was a minus charge transfer from the solute to the solvent, and that the net charges were unchanged by neutralization.

We have concern about the minus charge transfer from the solutes to the solvent. The minus charge transfer is natural for ssDNA because its formal charge is \(-5e\), but not so for SSB whose formal charge is \(+2e\) (Table 1). Minus charge transfer from solutes to solvents were also seen in several FMO and semi-empirical quantum mechanical (QM) calculations of biological molecules [9, 17, 28, 29], in all of which MM-based methods were used for preparation of the molecular structures. Interestingly, plus charge transfer was observed in a QM calculation of bovine pancreatic trypsin inhibitor whose solvated structure was prepared by a QM/MM-MD simulation [38]. Hence, the inconsistency between the MM-based structure and QM energy might be a cause. However, it is only a speculation and there is no definite conclusion about the origin of the minus charge transfer from the positively charged SSB protein. As biological macromolecules have quite complicated surface structures, further studies are necessary to elucidate the charge transfer phenomenon around their surfaces.

3.2 Internal energies of the solutes

The internal energies within the solutes (Eq. 2) were then calculated as functions of solvent thickness (Fig. 5). The internal energy of ssDNA became almost stable at 4 Å and completely converged at 8 Å. In SSB and Complex, the convergence was rather incomplete within the range investigated this time, presumably because they are both larger than ssDNA. Roughly speaking, however, their internal energies showed marginal convergence after 6 Å.

Note that the graphs were identical regardless of neutralization.

3.3 The solute-solvent interaction energy

The solute–solvent interaction energy (Eq. 3) showed a convergence trend similar to the case with internal solute energy. Namely, though the graphs did not converge completely, they showed marginal convergence after 6 Å. This trend is again in accordance with preceding studies [9, 10, 17].

A small, but significant difference was seen between ssDNA with and ssDNA without neutralization (Fig. 6 A). This should be attributed to the long-range nature of the electrostatic interaction. As ssDNA carried a considerable charge (-5e, Table 1) concentrated on its small body
of six nucleotides, enlargement of the solvent shell corresponded to increases in monopole–monopole (ssDNA and Na⁺ ions) and monopole–dipole (ssDNA and water) interactions, both of which are only conditionally convergent.

**Figure 4.** NPA and MPA charges of the solutes as functions of solvent thickness
The solute charge was calculated as the summation of partial atom charges comprising the solute calculated at FMO2/MP2/6-31G*. 
Figure 5. Internal energies (Eq. 2) of the solutes as functions of the solvent thickness calculated at FMO2/MP2/6-31G*.

Note that the y-axis of A (ssDNA) is eight times smaller than that of B (SSB) or C (Complex).
Figure 6. Interaction energies (Eq. 3) between the solutes and the solvent as functions of the solvent thickness calculated at FMO2/MP2/6-31G*

Note that the y-axis of A (ssDNA) is four times smaller than that of B (SSB) or C (Complex).

4. Conclusion

We have found that the physical properties of the solutes (net charge, internal energy, and interaction energy with solvent) were determined primarily by the first solvation shell and secondarily by the second shell, and were nearly uninfluenced by the outer shells including counterions. The present results are essentially similar to those of previous FMO studies of solvated proteins [9, 10] and dsDNA [17], and they reiterate the general picture of a water–solute interface; namely, the charge transfer from the solvent is mostly governed by the first solvation shell [28, 29]. Neutralization of the system had only negligible effects on these physical properties except in the ssDNA-solvent interaction.

Though there was a minor convergence problem in the solute–solvent interactions, we conclude here that a solvent shell thickness of 8 Å should suffice to incorporate the solvent effect in the calculation of the internal properties of the solute molecules and their local interactions with nearby solvent molecules. On the basis of the present findings, we are now analyzing the interaction between ssDNA and SSB by decomposing the interactions into base-amino acid residue interactions, and will publish the results elsewhere [20].
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