Electron Transfer in the c-Type Cytochrome Subunit of the Photosynthetic Reaction Center of *Rhodopseudomonas viridis*: *Ab Initio* Theoretical Study

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ABSTRACT: The pathways of the electron transfers (ETs) within the c-type cytochrome subunit and from the cytochrome subunit to the oxidized special pair (P^+) in the reaction center of *Rhodopseudomonas viridis* were studied by calculating the transfer integrals among the chromophores and the bridging amino acid residues by *ab initio* molecular orbital method. In the cytochrome subunit, the candidate of the bridge molecules was selected by the criterion of the distance from the two neighboring hemes, and the calculated results indicate that the ET occurs directly from a heme to the next heme. From c559, the proximate heme to the special pair, to P^+, the ET occurs mainly through TYR L162, which lies halfway between c559 and P^+, because of its proper location. Furthermore, the mutation experiments in which TYR L162 was replaced by phenylalanine and threonine were examined by the same theoretical method, and it was shown that the result of the mutation experiment was understood by the difference in the spatial distribution of the MOs between the wild type and mutants, and not by the energy difference of the MOs between donor (or acceptor) and bridge, though the latter factor had often been considered as the main factor controlling the rate of the ET. J Comput Chem 22: 521–527, 2001

Keywords: *Rhodopseudomonas viridis*; cytochrome subunit; electron transfer; pathway of electron transfer; *ab initio* molecular orbital method

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Introduction

Rhodopseudomonas viridis (*Rps. viridis*) is one of the purple bacteria that carry out photosynthesis.[1] The cycle of photosynthesis of the purple bacteria is simpler than that of green plants, and so it is basic to study purple bacteria for understanding the mechanism of photosynthesis. The reaction center of *Rps. viridis* is a membrane protein complex, and its X-ray structure was reported by Deisenhofer et al.:[2] it consists of the four subunits, L, M, H, and C, and the configurations of the chromophores in the reaction center are shown in Figure 1. The first step of the photosynthesis is an electronic excitation of special pair (P: bacteriochlorophyll b dimmer) in the reaction center. Charge separation occurs subsequently through L-subunit up to ubiquinone, and the oxidized P is reduced by the firmly bound c-type cytochrome (C) subunit. This subunit consist of the four c-type cytochromes, which are labeled as c554, c556, c552, and c559, the number being the wave number of the α-peak in the absorption spectra. The roles of these four hemes have been studied by various methods,[3–7] since the structure of the reaction center was determined by X-ray chrystallography.[2] Despite the large distance (8.7 Å) between P and c559, which is the proximate chromophore to P in the cytochrome subunit, rather fast electron transfer (120–220 ns) has been observed.[8–11] Several amino acid residues exist between c559 and P, and especially TYR L162 was considered to participate in this ET. Further, the role of TYR L162 in this ET has been examined in detail by mutation experiment[10, 11] in which the tyrosine was replaced by phenylalanine and threonine.

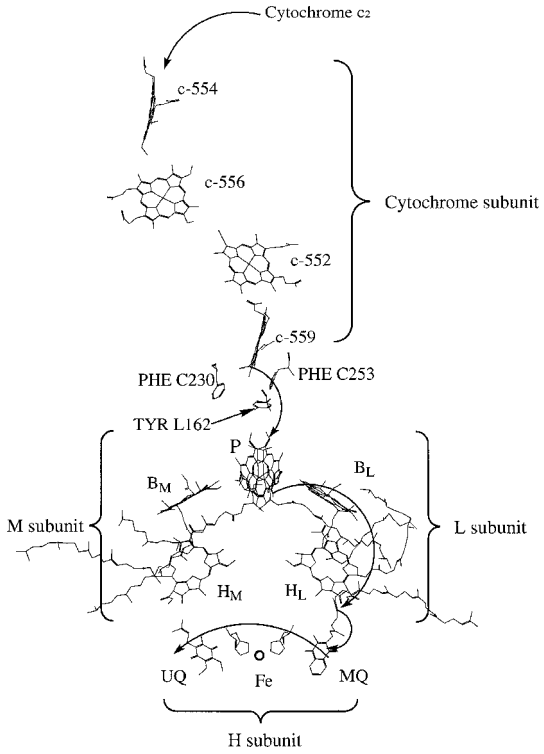

FIGURE 1. Overview of the reaction center.

In our series of *ab initio* theoretical studies on the reaction center of *Rps. viridis*,[12–16] we have studied the absorption spectra of the whole of the reaction center by applying the SAC (symmetry adopted cluster)/SAC-CI method,[17–20] which is an *ab initio* method including electron correlation for ground, excited, ionized, and electron attached states of molecular systems, and given reasonable assignment of the observed peaks.[12, 13] We have also studied in some detail the excited states of the four hemes in the cytochrome subunit.[15] These studies mean that we have good wave functions of the ground, excited, ionized, and anion states of the chromophores that participate in the ETs as the initial and final states. We then studied the pathways of the ET from excited P (P*) to bacteriopheophytins in L and M regions by calculating the transfer integrals involved, and showed the mechanism and the origin of the unidirectionality and selectivity of the ET.[14] More recently, we studied the pathway of the ET from bacteriopheophytin to ubiquinone and clarified the important roles of proteins in the ET mechanism.[16] This study for the ET in the cytochrome subunit constitutes a part of this series of studies.

The rate constant of ET reaction studied in this article may be expressed by the formula given by Marcus[21] as

$$k_{\mathrm{ET}} = \left(\frac{2\pi}{\hbar}\right) H_{IF}^2 \frac{1}{\sqrt{4\pi\lambda k_B T}} \exp\left\{-\frac{(\Delta G^\circ + \lambda)^2}{4\lambda k_B T}\right\}. \quad (1)$$

The electronic factor is represented by the square of the transfer integral H_{IF}, which represents the electronic coupling between initial and final states. The rest of eq. (1) represents the nuclear factor in which ΔG° is the free energy difference between these states, and λ is the reorganization energy in the ET reaction. Two mechanisms have been proposed to understand the long-distance and efficient ET. One is the sequential mechanism, and the other is the superexchange mechanism. In the former mechanism, an electron transfers from molecule to molecule sequentially, staying in a certain lifetime on each molecule. While, in the latter mechanism, the ET from initial and final state occurs utilizing the

(virtual) states of the bridge molecules. Namely, in the latter mechanism, the wave functions of the donor and acceptor spread into the region of the bridge molecules and interact more strongly than in the case they are restricted to the regions of the donor and acceptor alone, so that the long-range electron transfer is accomplished. In this article we study the ET mechanism by the *ab initio* molecular orbital method for the ETs within the cytochrome subunit and from the cytochrome subunit to P^+.

Calculation of Transfer Integral

The transfer integral can be expressed by the perturbation theory as

$$H_{IF} = \langle I|H|F\rangle + \sum_s \frac{\langle I|H|V_s\rangle\langle V_s|H|F\rangle}{E_I - E_{V_s}} + \sum_{s,t} \frac{\langle I|H|V_s\rangle\langle V_s|H|V_t\rangle\langle V_t|H|F\rangle}{(E_I - E_{V_s})(E_I - E_{V_t})} + \cdots \quad (2)$$

where I and F denote the initial and the final states in ET, H is the total hamiltonian of this system, and V_s and V_t are sth and tth virtual states, respectively, and E_I and E_{V_s} are the energies of the initial and sth virtual states, respectively.

The first term of eq. (2) is called direct term that represents the direct ET from the donor to the acceptor. The next term is called one bridge term: the ET or hole transfer (HT) involving only one bridge molecule, and subsequent terms correspond to two bridge, three bridge, etc. terms. Generally speaking, the molecules near to the donor, another bridge molecules and acceptor are important as bridge molecules. When the bridge molecule is reduced by electron donor in the virtual state, it is called electron transfer state. When the bridge molecule is oxidized by the acceptor molecule, it is called the hole transfer state. When we calculate the transfer integrals of the system, we can say which term is large, what pathway is important, and whether ET or HT is dominant. For the total understanding of the ET rate, the nuclear factor is important, but from *ab initio* theoretical point of view, the nuclear factor requires a much larger amount of calculations than the electronic factor. In this article, we study the mechanism of the ET through the calculation of the transfer integral alone.

In the previous article of this series,[16] we have summarized the method of calculations of the transfer integral within the Hartree–Fock level of approximation. We use the same level of approximation throughout the present studies. The effect of electron correlation on the transfer integral was studied in some detail in the former article of this series:[14] although it was important, it was not so much as to change the pathway.

We calculate the energies and the wave functions of all the chromophores and amino acid residues by putting them within the reaction center represented by the point charge model. When we calculate one molecule, all the other molecules are also represented by the point charge model. The difference of the energies between two states are approximated by the difference of the energies of the orbitals having different occupation numbers between the two states. The initial, final, and bridge states are approximated by the product of the Hartree–Fock wave functions of each system. The HF SCF calculations were performed by using HONDO program.[22] The method of calculation of transfer integral is the same as that in the previous article[16] except for the electric state of the initial and final state. Here, one electron is missing from the closed-shell state, while there was one additional electron in the previous study.[16]

Computational Detail

X-ray crystallographic structure[2] (1PRC in Brookhaven Data Bank) is used for the geometries of the reaction center. The geometry of the heavy atoms in PSRC are taken from X-ray structure. The positions of hydrogens are estimated by using the molecular-modeling software SYBYL. For calculational efficiency, some substituents of the chromophores were replaced with hydrogens, except for the substituents, which can π-conjugate with the rings.

We use common quality of basis sets as the previous series of articles.[12–16] For the C, N, and O atoms of both the chromophores and amino acids, we use Huzinaga's (63/5)/[63/41] sets,[23] and for the H atoms, the (4)/[4] set[24] are used. For Mg, Huzinaga's (533/53)/[5321/521] set[23] plus two p-type polarization functions (ζ = 0.045 and 0.143) and d-type polarization functions (ζ = 1.01) are used. For S, Huzinaga's (533/53)/[5321/521] set[23] plus p-type anion basis (ζ = 0.041) and d-type polarization functions (ζ = 0.421) are used. For Fe, Huzinaga's (5333/53/5)/[53321/53/41] set plus p-type basis (ζ = 0.082) were used. The effect of proteins and waters are introduced by a point charge model. The published charges[25, 26] are put on all atoms of protein and waters. The ionized form is

adopted for the ionizable residues, ASP, LYS, ARG, and GLU. For the other chromophores surrounding the chromophore under consideration, the Mulliken charges at the Hartree–Fock level are put on the constituent atomic positions.

Results and Discussion

ET WITHIN THE CYTOCHROME SUBUNIT

The cytochrome subunit reduces the special pair oxidized by light absorption and subsequent ET, and then, the oxidized cytochrome subunit is reduced by soluble cytochrome c_2.[1] Therefore, the ET pathway in the cytochrome subunit depends on the docking site of cytochrome c_2. It was observed that the ET rate decreased when ionic strength of the solvent increased.[27] This means the binding between the cytochrome subunit and cytochrome c_2 is controlled by electrostatic interaction. Further, cytochrome c_2 has LYS residue on its surface, and the cytochrome subunit has four glutamic acids on its surface near c554. The mutation of these glutamic acids of the bacteria very resemblant to *Rps. viridis* made the rate of ET slower.[28] These results indicate that c554 is the chromophore first reduced by cytochrome c_2. Based on this reason, we investigated ET pathway starting from c554.

In the cytochrome subunit, the molecules as candidates of bridges are selected based on the distances from the two neighboring hemes: the donor and acceptor. Generally speaking, the amino acids having π-conjugated rings, for instance, tyrosine, phenylalanine, and tryptophan, are good bridge molecules because of small energy differences of orbitals from both the donor and acceptor, which are usually π-conjugated molecules. After searching the bridge molecules among all amino acids in proteins, we found only two candidates. One is TYR C89, which is near both c554 and c556, and the other is PHE C246, which lies near both c552 and c559. Their locations are shown in Figure 2. We summarize in Table I the transfer integrals calculated between hemes in C subunit. The direct term between c554 and c556 is 1.92×10^{-6} a.u. and the one-bridge term, c554–TYR C89–c556 is -2.25×10^{-9} a.u.: the direct path is much more preferable to the one-bridge path. The direct term between c556 and c552 is -2.99×10^{-7} a.u., but no preferable bridge was found between them. The direct term between c552 and c559 is -1.90×10^{-5} a.u. and the one-bridge term, c552–PHE C246–c559 is -2.97×10^{-8} a.u.: again, the direct path is preferable. Thus, the calculated results show that the ET occurs through the direct path from the heme to the next heme within the cytochrome subunit. This is because the distances between the hemes are rather close, and there are no molecules that come in between the two hemes and work well as bridges.

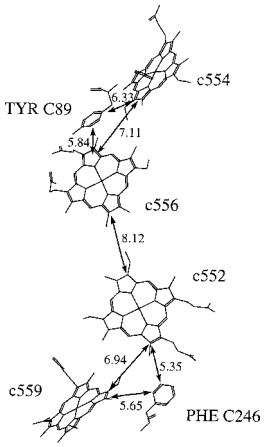

FIGURE 2. Schematic view of the chromophores and the bridge molecules around the C subunit of *Rps. viridis*. The distances between terminal conjugated atoms are shown in Å.

ET BETWEEN c559 AND OXIDIZED SPECIAL PAIR

It has been suggested that the protein between c559 and special pair works as a bridge to explain the efficient ET in this system, and three amino acids, TYR L162 included in the L subunit, and PHE C230 and PHE C253 included in the cytochrome subunit, may work as bridge molecules. Their loca-

TABLE I.
Transfer Integral ($\times 10^{-6}$ a.u.) between the Hemes of the Cytochrome Subunit of *Rps. viridis*.

Pathway	Direct Term	Pathway	One-Bridge Term
c554–c556	1.92	c554–TYR C89–c556	−0.00225
c556–c552	−0.299	—	—
c552–c559	−19.0	c552–PHE C246–c559	−0.0297

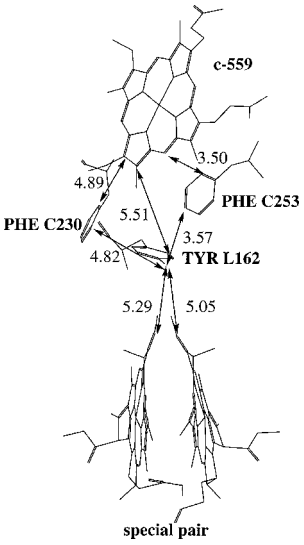

FIGURE 3. Schematic view of the region between c559 and special pair in *Rps. viridis*. Distances between terminal π-conjugated atoms of chromophores and bridges are shown in Å.

tions are shown in Figure 3. No other amino acid and water were found between c559 and P. The calculated results of the transfer integral are given in Table II. The direct term between c559 and P^+ is 4.06×10^{-11} a.u. The one-bridge term through either of TYR L162, PHE C230, or PHE C253 is 5.90×10^{-6}, -3.50×10^{-10}, or -4.94×10^{-9} a.u., respectively. Only two two-bridge pathways are considered from the locations of the three amino acid residues and two chromophores. The two-bridge term through PHE C253 and TYR L162 is -2.60×10^{-6} a.u., and the one through PHE C230 and TYR L162 is -5.06×10^{-7} a.u.

Thus, the direct term between c559 and P^+ is very small compared with the other bridge terms because of the long distance between them. Among the three one-bridge terms, the term through TYR L162 is the largest. This is because PHE C253 and PHE C230, which belong to the C subunit, are nearer to c559, but are more distant from P than TYR L162. TYR L162, which belongs to the L-subunit, is about halfway between c559 and P. The orbital energies of these three amino acids, which determine the energy difference denominator, are similar. The transfer integrals in this level of theory are determined mainly by the electronic coupling term, which is the numerator of eq. (2). This is the result similar to the previous case.[16] The electronic coupling between c559 with PHE C230 or PHE C253 is larger than that between c559 and TYR L162, but the electronic coupling between P^+ with PHE C230 or PHE C253 is much smaller than that between P^+ and TYR L162 because of their large distances. Therefore, the one-bridge term through TYR L162 becomes the largest. TYR L162 works not only as a bridge by itself in the one-bridge term, but also works in the two-bridge term, cooperating with PHE C253 and PHE C230, as seen from Table II. Note, however, that this two-bridge term works to cancel the one-bridge term for its opposite phase.

ET RATE IN MUTANTS

Dohse et al.[10] performed interesting mutation experiment for the rate of ET between c559 and P^+. They replaced experimentally TYR at L162 with PHE (phenylalanine) and THR (threonine), and observed the lifetime for the ET from c559 to P^+ as 174 and 1000 ns, respectively, compared with that of the wild type at 185 ns. The rate of ET does not change much by the replacement of TYR with PHE, but decreases by more than five times with the replacement by THR (see Table III), although the structures of these two mutants are not quite different from that of the wild type. The nuclear factors may not change much in these mutants because the

TABLE II.
Transfer Integral ($\times 10^{-8}$ a.u.) between c559 and Oxidized Special Pair of *Rps. viridis*.

Term	Pathway	Transfer Integral
Direct term	c559–P^+	0.00406
One-bridge term	c559–TYR L162–P^+	590
	c559–PHE C253–P^+	−0.494
	c559–PHE C230–P^+	−0.0350
Two-bridge term	c559–PHE C253–TYR L162–P^+	−260
	c559–PHE C230–TYR L162–P^+	−50.6
H_{IF} sum		279

TABLE III.
Transfer Integrals ($\times 10^{-8}$ a.u.) between c559 and Oxidized Special Pair of the Mutants of *Rps. viridis* Compared with the Experimental Time for ET.

Term	Pathway	L162 TYR	L162 PHE	L162 THR
One-bridge term	c559–L162–P^+	590	406	35.0
Two-bridge term	c559–PHE C230–L162–P^+	−50.6	−44.9	1.41
	c559–PHE C253–L162–P^+	−260	14.2	−112
H_{IF} total		279	375	−76.1
$\lvert H_{IF}\rvert^2$ ($\times 10^{-12}$ a.u.)		7.78	14.1	0.58
Estimated relative ET rate		1	1.81	0.074
Experimental time for ET (ns)[a]		185	174	1000
Experimental relative ET rate[a]		1	1.06	0.185

[a] Ref. 10.

donor and the acceptor molecules are the same and the geometry do not change much. So, we may attribute the change of the ET rate as mainly being due to the change in the electronic factors. We calculated the electronic factors of the mutants and compared them with that of the wild type. In this calculation of transfer integrals, only the geometries of the amino acid residues that are replaced in mutants are optimized using the molecular mechanics method with freezing the geometries of their α carbons, other amino acids of proteins, and chromophores in the reaction center. The method and the basis sets are the same as those used in the calculations of the transfer integral of the wild type.

The result of the calculation is shown in Table III, in which the transfer integrals for the three systems are summarized. Although tyrosine and phenylalanine have a π-ring, threonine does not. The square of the transfer integral for the wild type (TYR), PHE mutant, and THR mutant are 7.78×10^{-12}, 14.1×10^{-12}, and 0.058×10^{-12} a.u., respectively. The ratio of the ET rate estimated from the electronic factor alone is 1:1.8:0.07 for the TYR, PHE, and THR systems, while the ratio of the experimental ET rate is 1:1.06:0.185 in the same order. Thus, the calculated electronic factors of these three systems roughly reproduce the experimental trend of the ET rate, supporting the argument given in the above paragraph. Referring to Table III, we see that the order of the electronic factor is due not only to the one-bridge term but also to the two-bridge term. By the one-bridge term alone, the order between TYR and PHE systems is not reproduced, although it is reproduced if we consider both one- and two-bridge terms.

Conclusion

This article is one of our series of theoretical studies on the electronic structure of the photosynthetic reaction center of *Rhodopseudomonas viridis*.[12–16] In this report, we studied the mechanism, the rate, and the pathway of the ET within the cytochrome subunit and from c559 to the oxidized special pair, P^+. We have calculated the transfer integrals for various possible routes of the ET, and based on such calculations, we could show the best possible pathway of the ET and the estimate of the transfer rate.

For the ET within the cytochrome subunit, the direct sequential pathway connecting four different hemes c554–c556–c552–c559 is most probable. Along this pathway, there seems to be no important bridging molecules and residues. The electronic spectra of these four different hemes were studied in a separate article.[15]

For the ET from c559 to P^+, the bridging protein residues play an important role. Among others, the π-ring of the tyrosine of TYR L162 is very important in the one-bridge term and also in the two-bridge term, together with the π rings of phenylalanines of PHE C253 and PHE C230. Experimentally, the time for ET from c559 to P^+ is observed to be 1.2–2.2×10^{-7} s.[8–11] The time for ET from excited P (P^*) to bacteriopheophytin b in the L-region (H_L) is estimated to be 3×10^{-12} s. Therefore, the first process is about 10^{-5} times slower than the second process. In the previous study on the ET from P^* to H_L, the electronic factor, $\lvert H_{IF}\rvert^2$ was calculated to be 2.5×10^{-8} a.u. in fair agreement with the experimental value.[14] If we neglect the effect of electron correla-

tion, the calculated value was 3.7×10^{-6} a.u. On the other hand, the present results of the electronic factor for the ET from c559 to P^+ is 7.78×10^{-12} a.u. Then, our theoretical results estimate the ET rate of c559 to P^+ to be 3×10^{-4} times (at the same HF level, 2×10^{-4} times) slower than the ET from P^* to H_L, if we can assume the nuclear factors are roughly equal for both processes.

The role of TYR L162 in the ET from c559 to P^+ was studied by mutation experiment by Dohse et al.[10] in which tyrosine at L162 was replaced by phenylalanine and threonine, the former having a π-ring but the latter not. They concluded that TYR L162 was not required for fast ET from c559 to P^+.[10] The present calculated results show that the molecule at L162 need not be a tyrosine, but the molecule at L162 plays a critically important role in the ET as a bridge molecule in the superexchange mechanism.

Thus, we were able to trace this mutation, experiment by the present theoretical mutation, although the implication is somewhat different from the experimental one. Theoretical mutation can be done more easily than the real mutation experiment. We propose, in conclusion, that theoretical mutation should be done more frequently in the future before doing a truly meaningful mutation experiment.

References

1. Voet, D.; Voet, J. G. Biochemistry; John Wiley & Sons, Inc.: New York, 1995, 2nd ed., chap. 22.
2. Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. J Mol Biol 1984, 180, 395.
3. Shinkarev, V. P.; Drachev, A. L.; Dracheva, S. M. FEBS Lett 1990, 261, 11.
4. Nitschke, W. N.; Rutherford, A. W. Biochemistry 1989, 28, 3161.
5. Hubbard, J. A. M.; Evans, M. C. W. FEBS Lett 1989, 244, 71.
6. Vermeglio, A.; Richaud, P.; Breton, J. FEBS Lett 1989, 243, 259.
7. Nabedryk, E.; Berthomieu, C.; Vermeglio, A.; Breton, J. FEBS Lett 1991, 293, 53.
8. Ortega, J. M.; Mathis, P. Biochemistry 1993, 32, 1141.
9. Ortega, J. M.; Mathis, P. FEBS Lett 1992, 301, 45.
10. Dohse, B.; Mathis, P.; Wachtveilt, J.; Laussermair, E.; Iwata, S.; Michel, H.; Oesterhelt, D. Biochemistry 1995, 34, 11335.
11. Ortega, J. M.; Dohse, B.; Oesterhelt, D.; Mathis, P. Biophys J 1998, 74, 1135.
12. Nakatsuji, H.; Hasegawa, J.; Ohkawa, K. Chem Phys Lett 1998, 296, 499.
13. Hasegawa, J.; Ohkawa, K.; Nakatsuji, H. J Phys Chem B 1998, 102, 10410.
14. Hasegawa, J.; Nakatsuji, H. J Phys Chem B 1998, 102, 10420.
15. Ohkawa, K.; Hada, M.; Nakatsuji, H. J Porphyrins and Phtalocyanines, to appear.
16. Ito, H.; Nakatsuji, H. J Comput Chem, to appear.
17. Nakatsuji, H.; Hirao, K. J Chem Phys 1978, 68, 2053.
18. Nakatsuji, H. Chem Phys Lett 1978, 59, 362.
19. Nakatsuji, H. Chem Phys Lett 1989, 67, 329.
20. Nakatsuji, H. Chem Phys Lett 1989, 67, 334.
21. Marcus, R. A.; Sutin, N. Biochim Biophys Acta 1985, 811, 265.
22. Dupuis, M.; Farazdel, A. MOTECC-91; Center for Scientific and Engineering Computations, IBM.
23. Huzinaga, S.; Andzelm, J.; Klobukowski, M.; Radzio-Andzelm, E.; Sakai, Y.; Tatewaki, H. Gaussian Basis Set for Molecular Calculations; Elsevier: New York, 1984.
24. Huzinaga, S. J Chem Phys 1965, 42, 1293.
25. Cornell, W. D.; Cieplack, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Freguson, D. R.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. J Am Chem Soc 1995, 117, 5197.
26. Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. J Chem Phys 1983, 79, 926.
27. Knaff, D. B.; Willie, A.; Long, J. E.; Kriauciunas, A.; Durham, B.; Millett, F. Biochemistry 1991, 30, 1303.
28. Osyczka, A.; Nagashima, K. V. P.; Sogabe, S.; Miki, K.; Yoshida, M.; Shimada, K.; Matsuura, K. Biochemistry 1998, 37, 11732.