$\pi-\pi$ Stacking Interactions in the Peridinin−Chlorophyll−Protein of *Amphidinium carterae*

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Carotenoids play an important role of light harvesting, photoprotection and structural stabilization in the photosynthetic organisms. Despite their functional importance, the molecular basis for binding of carotenoids in the photosynthetic pigment−protein complexes is poorly understood. On the basis of a recent discovery that carotenoids are surrounded either by aromatic residues or by chlorophylls in all known crystal structures of the photosynthetic pigment−protein complexes (*J. Am. Chem. Soc.* **2002**, *124*, 8445), it is hypothesized that $\pi-\pi$ stacking interactions are the molecular forces that bind carotenoids in the photosynthetic pigment−protein complexes. In this article, the $\pi-\pi$ stacking interactions between the carotenoid molecule peridinins and their surrounding aromatic groups (aromatic residues and chlorophyll-a) in the peridine−chlorophyll−protein complex of *Amphidinium carterae* are characterized by means of the supermolecular approach with the second-order Møller−Plesset perturbation method (MP2). The modified 6-31G*(0.25) basis set with diffuse d-polarization by Hobza et al. is adopted here. A representative peridinin chlorophyll pair (PID624···Chl602) is chosen to study the structural stabilization role of peridinin, and three unique peridinin and aromatic residue pairs (PID623···TYR270, PID624···PHE301, and PID624···PHE304) are chosen to study the configurational (orientation and distance) dependence of $\pi-\pi$ stacking interactions between peridinins and their interacting partners. The MP2/6-31G*(0.25) calculations yielded a favorable $\pi-\pi$ stacking interaction energy of −11.52 kcal/mol between the large conjugated tetrapyrrol π-system of chlorophyll Chl602 and the long conjugated π-electrons of peridinin PID624. For the parallelly oriented PID623···TYR270 pair, the MP2/6-31G*(0.25) calculations give rise to a stabilization energy of −7.25 kcal/mol. For the perpendicularly oriented pairs, the calculated MP2/6-31G*(0.25) $\pi-\pi$ stacking energies are −3.77 and −1.71 kcal/mol for PID624···F301 and PID624···F304, respectively. It is thus concluded that $\pi-\pi$ stacking interactions between peridinins and the nearby aromatic groups play a substantial role in binding peridinins in the peridine−chlorophyll−protein complex of *A. carterae*. Consequently, the molecular basis of the structural stabilization function of carotenoids in forming the pigment−protein complexes is unraveled.

1. Introduction

Life as we know it today exists because of photosynthesis, the process through which light energy is converted into chemical energy by plants, algae, and photosynthetic bacteria.[1−8] Photosynthetic organisms have developed complex and efficient apparatus to harvest the light of the sun and to convert the light energy into chemical energy.[8−11] The photosynthetic membranes of these organisms contain thousands of pigment molecules, mainly chlorophylls and carotenoids. The latter are noncovalently bound to proteins to form well organized pigment−protein complexes,[8,12−14] such as the light-harvesting complexes and the photosynthetic reaction center.

Tremendous progress in our understanding of photosynthesis has been achieved in the last two decades with the structural determination of the photosynthetic pigment−protein complexes.[15−20] These crystal structures provide detailed knowledge of the organization of pigment molecules in the photosynthetic membrane and stimulated a new wave of theoretical and experimental investigations of mechanisms of photosynthesis.[2,8−10,21−28] However, the focus of these past investigations had been on the electronic excited states in close association with the primary energy transfer and electron-transfer processes, which are the functional aspects of the photosynthetic systems. Little is known about the structural aspects of complex formation. We are interested in understanding binding of pigment molecules in the photosynthetic pigment−protein complexes, in particular, binding of carotenoids.

Carotenoids play multiple roles in photosynthesis, including light harvesting, photoprotection and structural stabilization.[29−31] The light-harvesting and photoprotection functions of carotenoids are well understood.[8,29,30,32,33] As accessory light-harvesting pigments, carotenoids absorb energy in a spectral region complementary to that of chlorophylls and transfer energy to the major pigments (chlorophylls). Most importantly, as photoprotective agents, carotenoids quench the excited triplet state of chlorophylls. The latter state would otherwise be long-lived and could readily react with molecular oxygen to generate singlet oxygen, which causes the photooxidative destruction of membranes.[30,34] In contrast, the structural stabilization role of carotenoids is less well characterized. A considerable amount of data has been accumulated which indicated that carotenoids are necessary for the assembly and stabilization of certain pigment−protein complexes in the photosynthetic bacteria and plants.[31,35,36] But the detailed mechanism for such a structural stabilization role of carotenoids is not clear. On the basis of careful examination of all known crystal structures of photosynthetic pigment−protein complexes,[15,16,18−20,37] we discovered that all carotenoids are surrounded either by aromatic residues or by chlorophylls.[38] We hypothesize that the $\pi-\pi$ stacking interactions are the molecular forces that bind carotenoids in

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the pigment−protein complexes. In ref 38, we calculated the strengths of these $\pi-\pi$ stacking interactions between carotenoid and its surrounding aromatic residues in the LH−II complex of *Rhodospirillum molischianum* by high level ab initio electronic structure calculations. The second-order Møller−Plesset perturbation method (MP2) calculations yielded a total stabilization energy of −15.66 kcal/mol between the carotenoid molecule lycopene and the four surrounding aromatic residues (α-Trp-23, β-Phe-20, β-Phe-24, β-Phe-27).[38] Although the absolute magnitude of the $\pi-\pi$ stacking interaction is inevitably dependent on the level of theory used, the attractive nature of the $\pi-\pi$ stacking interaction between lycopene and the surrounding aromatic residues in the LH−II complex of *Rs. molischianum*, as well as its substantial magnitude, is firmly established. In this article, we extend the quantum chemical analysis of intermolecular interactions into the peridinin−chlorophyll−protein (PCP) from *Amphidinium carterae*.[37] As detailed below, in addition to $\pi-\pi$ stacking interactions with aromatic residues, carotenoids in PCP from *A. carterae* are in close contact with chlorophylls. This work represents a first attempt to calculate $\pi-\pi$ stacking interaction energy between carotenoids and chlorophylls.

The photosynthetic dinoflagellate *A. carterae* contains a water-soluble accessory light-harvesting complex, peridinin−chlorophyll−protein (PCP). The predominant carotenoid of dinoflagellate is peridinin, which enables the organism to capture blue-green light (470−550 nm) that is inaccessible by chlorophylls alone. The crystal structure of PCP from *A. carterae*[37] was determined at a resolution of 2.0 Å by X-ray crystallography, revealing a trimeric organization of the complex. Each monomeric PCP contains two pigment clusters; the latter comprises eight peridinins (PID) and two chlorophyll-a (Chl) molecules each. The exceptionally high ratio of four carotenoid to chlorophyll in PCP suggests a dominant light harvesting role of peridinins. It has been reported that singlet energy transfer from peridinin to chlorophyll is close to unity.[39,40] The light-harvesting and photoprotection functions of peridinin in PCP have been the subject of numerous experimental and theoretical investigations.[40−43] Here, the structural stabilization role of peridinin in PCP is studied. It is assumed that the $\pi-\pi$ stacking interactions between peridinin and its surrounding aromatic residues and chlorophylls are the molecular forces that bind peridinins in the PCP complexes. The strength of these $\pi-\pi$ stacking interactions in the PCP complex of *A. carterae* is characterized by means of high level ab initio electronic structure calculations.

$\pi-\pi$ stacking interaction is dominated by the dispersion force that arises from the mutual correlation of electrons that belong to interacting monomers (intermolecular correlation effects). Consequently, inclusion of electron correlation is necessary to adequately treat $\pi-\pi$ stacking interaction. There are three principal methods that include the correlation correction: (a) configuration interaction (CI) methods; (b) coupled cluster (CC) methods; and (c) many-body perturbation theory(MBPT), also known as Møller−Plesset perturbation theory (MP). As we have extensively discussed in ref 38, a popular and feasible way to include the correlation effects is the MP2 method, which usually covers a significantly large part of the correlation energy. The MP2 method has been applied to a wide variety of weakly bonded complexes, including $\pi-\pi$ stacking and hydrogen bonding in DNA, van der Waals complexes of atoms and molecules, etc.[44−47] Serving as the prototype for aromatic $\pi-\pi$ stacking, benzene dimer has been studied at several levels of theory.[48−55] One of the largest aromatic dimer systems studied at the MP2 level of theory up to date is the MP2/6-31G* calculation of bacteriochlorophyll dimer in the photosynthetic reaction center of the purple bacterium *Rhodobacter sphaeroides*.[56]

TABLE 1: Aromatic Groups Surrounding Peridinins[a] in Peridinin−Chlorophyll−Protein of *A. carterae*

peridinins[b]	chlorophyll-a[b] within 5 Å	aromatic residues[b] within 5 Å
PID611	Chl: 601	Phe17, Phe28, Trp23, Trp 136
PID612	Chl: 601	Phe28, Tyr122
PID613	Chl: 601, 602	Phe139, Tyr108, Tyr136
PID614	Chl: 601	Phe139, Phe142, Trp23, Trp85
PID621	Chl: 602	Phe180, Phe286, Tyr191
PID622	Chl: 602	Tyr191
PID623	Chl: 601, 602	Tyr270, Tyr302
PID624	Chl: 602	Phe301, Phe304, Trp186, Tyr247

[a] There are eight peridinins in PCP from *A. carterae* which form two clusters that are related by a pseudo-2-fold symmetry. [b] The naming convention for peridinins and chlorophylls follows ref 20, and the residue identification numbers are in accordance with the PDB file for the PCP (accession number 1PPR).

It should also be pointed out that the density functional approach (DFT) is gaining popularity for treating large biomolecules due to its low computing cost for including the correlation effect. Unfortunately, the DFT method is found inadequate for treating weakly bonded intermolecular complexes since it does not include the dispersion effect.[46,57]

In this article, we implement the MP2 method to calculate the strengths of $\pi-\pi$ stacking interactions between peridinin and its surrounding aromatic residues and chlorophylls in the PCP of *A. carterae*. The remainder of the article is organized as follows. In section II, we review structural details of PCP from *A. carterae*, with special emphasis on the surroundings of carotenoids. Detailed implementation of the MP2 method, along with the choice of basis set, is described in section III. Section IV presents the results of intermolecular interaction strengths, as well as an analysis of the physical origin of intermolecular forces. A brief summary is given in section V.

2. Binding Environment of Peridinins

In the crystal structure of PCP from *A. carterae*, the amino and carboxyl halves of the peptide form nearly identical domains related by a pseudo 2-fold symmetry.[37] The primary sequences of the two domains are homologous but not identical. Each domain contains a chlorophyll−peridinin cluster consisting of two pairs of mutually orthogonal peridinins and a chlorophyll-a molecule. The four peridinins associated with the amino domain are denoted PID611, PID612, PID613, and PID614, and the other four peridinins, PID621, PID622, PID623, and PID624, are bound to the carboxyl domain. Hereafter, the residue identification numbers for amino acids and peridinins are in accordance with the PDB file for the PCP (accession number 1PPR). A systematic examination of the binding pockets of all eight peridinins showed that all of the carotenoids are in van der Waals contact (<5 Å) with chlorophylls or aromatic residues. Table 1 lists all eight peridinins and their surrounding aromatic groups within a distance of 5 Å.

As seen in Table 1, there exist a multitude of intermolecular interactions between peridinins and their surrounding aromatic groups. Our long-term goal is to understand how these intermolecular interactions stabilize the pigment protein complex. Here, we focus on studying the configurational (orientation and distance) dependence of $\pi-\pi$ stacking interactions between peridinins and their interacting partners. As shown in Figure 1 are two representative peridinin molecules (PID623, PID624)

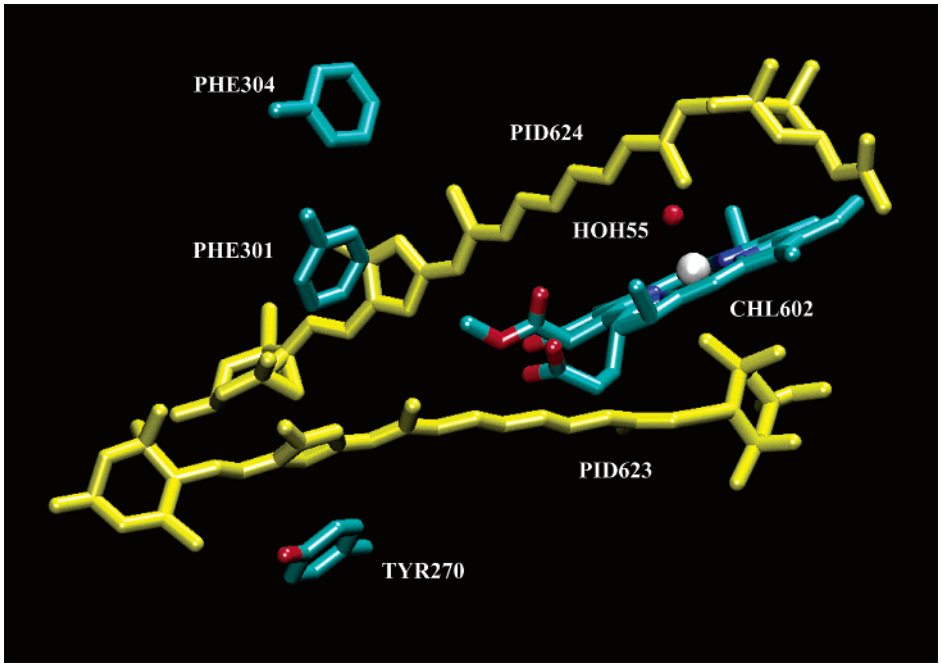

Figure 1. Binding pocket of PID623 and PID624 in PCP from *A. carterae*.[37] For clarity, only aromatic groups involved in the representative configurations of intermolecular interactions chosen for the quantum analysis are shown (see Table 1 for a complete list of surrounding aromatic residues of PID623 and PID624). Two peridinin molecules PID623, PID624 (yellow), one chlorophyll-a (Chl602), and three aromatic residues (Tyr270, Phe301, Phe304) are in a licorice representation. The phytyl tail of Chl602 is truncated for clarity, and the central Mg atom of Chl602 is shown as a silver sphere. Also shown is a structured water molecule HOH55. (This figure is produced with the program VMD[59].)

surrounded by one chlorophyll-a (Chl602) and three aromatic residues (Phe301, Phe304, Try270). We have selected three representative configurations to study the $\pi-\pi$ stacking interactions between peridinin and aromatic residues (PID623···TYR270, PID624···PHE301, and PID624···PHE304), and one configuration to study the $\pi-\pi$ stacking interaction between peridinin and chlorophyll (PID624···Chl602). The peridinin chlorophyll pair PID624···Chl602 is representative of all peridinin chlorophyll interactions, and the three peridinin aromatic residue pairs are chosen due to their unique orientations. Specifically, the aromatic π-plane of residue Tyr270 and the conjugated π-plane of PID623 are nearly parallel to each other with a crossing angle of 18°. The nearest atom to atom distance between Tyr270 and PID623 is 3.4 Å. Residues Phe301 and Phe304 are almost perpendicular to the PID624; Phe301 approaches the conjugated π-plane of PID624 from top while Phe304 is positioned alongside of the π-plane (see Figure 1). The nearest atom to atom distance between the aromatic residues and PID624 is 3.66 and 3.85 Å for Phe301 and Phe304, respectively. The tetrapyrrol plane of Chl602 is nearly perpendicular to the conjugated π-plane of PID624 with a crossing angle of 76 degree and a nearest atom to atom distance of 3.33 Å. It is worth noting that due to its overall T-shaped arrangement of the two π-electron systems, the T-shaped $\pi-\pi$ stacking interaction between PID624 and Chl602 can also be referred to as a CH$-\pi$ interaction.[58] However, for consistence, we will used the term "$\pi-\pi$ stacking interaction" to denote the T-shaped stacking throughout this article. As shown in Figure 1, the X-ray crystal structure also revealed a structural water molecule HOH55 in close geometric proximity of the central Mg atom of Chl602.[37]

3. Methods

The $\pi-\pi$ stacking interactions between peridinins and their surrounding aromatic groups (aromatic residues and chlorophyll-a) in the PCP complex of *A. carterae* are characterized by ab initio electronic structure calculations at the MP2 level. As in all other quantum mechanical calculations, the quality of calculated results depends on the choice of the basis set. It has been shown that for a proper treatment of $\pi-\pi$ stacking interactions, inclusion of diffuse basis sets is required.[46,51] These diffuse basis sets are localized sufficiently far from the atomic nuclei and thus fill the empty space between two interacting monomers. The latter is where a substantial portion of correlation energy originates. At the MP2 level, Dunning's correlation consistent basis sets (cc-pVXZ, X = D, T, Q, and 5) and the augmented aug-cc-PVXZ basis sets are desirable, and they have been applied to both $\pi-\pi$ stacking and hydrogen-bonding complexes of small molecules.[60,61] However, such huge basis sets are not computationally feasible for the large system of our interest here. A more feasible choice for our system is a medium sized basis set, such as the polarization augmented double-ζ 6-31G* basis set. In a series of studies of DNA base stacking, Hobza and co-workers employed a modified 6-31G* basis set with diffuse (momentum optimized, dispersion energy-optimized) d-polarization at the MP2 level of theory.[44,46,62,63] In the conventional 6-31G* basis set, the d-polarization functions for non-hydrogen atoms (C, N, and O atoms) are energy-optimized with an exponent of 0.8. In the modified basis set, an exponent of 0.25 is used for the d-polarization functions of C, N, and O atoms, instead. Following the author's convention,[44,64] the modified basis set is designated 6-31G*(0.25). Inclusion of more diffused d-polarization functions in the 6-31G*(0.25) basis set improves the electron correlation stabilization energy of stacked DNA base dimers substantially.[44,65] A recent comparison of MP2/6-31G*(0.25) treatment of DNA base stacking with that of the theoretically more rigorous CCSD(T) has shown that MP2/6-31G*(0.25) recovered 75−90% of the intermolecular correlation stabilization energy.[55] The 6-31G*(0.25) basis set was adopted in all of our calculations.

The intermolecular interaction energy was calculated at the MP2/6-31G*(0.25) level using the supermolecular approach. In

the supermolecular approach, the molecular Schrödinger equations for the dimer AB, and the two monomers A and B

$$\hat{H}_i\psi(i) = E_i\psi(i) \quad i = AB, A, B \tag{1}$$

are solved. Here, $\hat{H}_i$,$\psi(i)$, and E_i are the Hamiltonian, wave function, and energy for the molecular species i, respectively. The energy of interaction between molecules A and B is defined as the difference between the energy of the interacting dimer E_{AB} and the energies of the monomers E_A and E_B

$$E_{int} = E_{AB} - E_A - E_B \tag{2}$$

In our calculations, the coordinates of non-hydrogen atoms in peridinins and their interacting partners (i.e., aromatic residues and chlorophylls) (see Figure 1) were extracted from the 2.0 Å X-ray crystal structure of PCP from *A. carterae*[37] (PDB accession number 1PPR). Therefore, the internal coordinates of the monomers used in computing E_A and E_B are the same as within the dimer AB.

All the calculations were carried out using the GAUSSIAN98 program[66] on a LINUX workstation cluster and a SGI ONYX2 server in our laboratory. The basis set superposition error (BSSE) is corrected by the Boys and Bernardi counter poise method.[67]

In addition to the intermolecular interaction energy, we are also interested in determining the physical origin of intermolecular interactions. In particular, we want to find out contributions of electrostatic interaction and dispersion force to the overall intermolecular interaction for a particular $\pi-\pi$ stacking. As described in ref 38, intermolecular $\pi-\pi$ stacking interactions are essentially a juxtaposition of several elements, including electrostatic interactions, exchange repulsion interactions, induction, and dispersion forces.[68] Dispersion forces arise from the mutual correlation of electrons that belong to interacting monomers (intermolecular correlation effects). In the variational supermolecular approach adopted here, the correlation component of the MP2 interaction energy corresponds primarily to the dispersion interaction energy, as well as correlation corrections to the electrostatic interaction and induction force. The upper bound of dispersion interaction energy can be estimated as the correlation energy; the latter is simply the difference between the MP2 energy and the Hartree−Fock (HF) energy. The electrostatic interaction energies were analyzed by means of the distributed multipole method of Stone et al. as implemented in the program ORIENT 3.2.[69] Distributed multipoles[70] themselves are evaluated from the GAUSSIAN98 output wave functions by means of the GDMA 1.0 program.[69]

4. Results and Discussion

Intermolecular $\pi-\pi$ stacking interaction energies between the peridinin molecules and each of the surrounding aromatic groups (aromatic residues or chlorophyll-a) are calculated in a pairwise manner. The coordinates of all the non-hydrogen atoms of the interacting molecules, including peridinin, chlorophyll-a and the three amino acids, are taken directly from the X-ray crystal structure of PCP from *A. carterae*.[37] The positions of all the hydrogen atoms are placed by ab initio geometry optimization at the HF/6-31G* level with all the heavy (non-hydrogen) atom positions fixed. Figure 2 depicts the structural formula for the peridinin molecule, chlorophyll-a, and the two aromatic amino acids phenylalanine and tyrosine.

For $\pi-\pi$ stacking interactions between peridinin and aromatic residues, the entire peridinin molecule and the side chain atoms of aromatic amino acids are included in the MP2/6-31G*(0.25) calculation; the α-carbon atom and its associated main chain groups are excluded (see Figure 2). The α-carbon itself is replaced by a hydrogen atom. The PID623···Tyr270 and PID624···Phe301 (same for PID624···Phe304) pairs contain a total of 112 and 111 atoms, respectively. With the 6-31G*(0.25) basis set, the PID623···Tyr270 complex consists of 398 electrons with a total of 926 basis functions (1744 primitive Gaussians); the PID624···Phe301 complex contains 390 electrons with a total of 911 basis functions (1716 primitive Gaussians).

For the $\pi-\pi$ stacking interaction between peridinin and the chlorophyll-a molecule, the combined total of 233 atoms (137 atoms in chlorophyll-a and 96 atoms in peridinin) far exceeds the memory and disk capacity of currently available computers. To proceed, both the chlorophyll-a and the peridinin molecules are truncated. For the chlorophyll-a molecule Chl602, the phytol tail is omitted, and other nonpolar side groups of Chl602 are replaced by hydrogen atoms as depicted in Figure 2. The replaced nonpolar side groups are circled in shaded squares in Figure 2, including the methyl groups associated with C-2, C-7, C-12, C-18, the ethyl group on C-8, and the entire 17-propionic acid side chain. For the peridinin molecule PID624, all the methyl groups except one are replaced with H atoms as shown in Figure 2. The methyl group attached to C21 is kept since it is located between PID624 and Chl602. The replacement retains the essential tetrapyrrol plane of Chl-a and the conjugated π-system of peridinins. Shown in Figure 3 is the three-dimensional structure of the PID624···Chl602 complex after truncation of nonessential bulk groups and addition of hydrogen atoms. Also shown in Figure 3 are a water molecule HOH55 and the side chain of the amino acid GLY240. The water molecule HOH55 is involved in ligation of the central Mg atom of Chl602. The main chain carboxyl oxygen atom of GLY240 forms a hydrogen bond with the hydroxyl group attached to C29 of PID624.

Our primary objective here is to study the $\pi-\pi$ stacking interaction between PID624 and Chl602. It is evident from Figure 3 that the methyl group attached to C21 of PID624 is likely involved in the so-called CH$-\pi$ interaction[58] with the conjugated chlorophyll molecule. In addition, the water molecule HOH55, as one of the ligands for the central Mg atom, can be considered part of the Chl602 system. Given its geometric proximity to PID624, the water molecule HOH55 can be involved in OH$-\pi$ interactions with PID624. To sort out contributions of these various components to the overall intermolecular interaction between PID624 and Chl602, we have set up three sets of interacting pairs denoted "PID624··· Chl602", "PID624···Chl602 with HOH55", and "PID624 without CH_3···Chl602", respectively. The first pair PID624···Chl602 consists of the truncated PID624 (all the atoms belonging to PID624 in Figure 3) as monomer A and the truncated Chl602 (all the atoms belonging to Chl602 in Figure 3) as monomer B. This PID624···Chl602 pair contains a total of 121 atoms. Using the 6-31G*(0.25) basis set, the complex consists of 506 electrons with a total of 1169 basis functions (2212 primitive Gaussians). The second pair "PID624···Chl602 with HOH55" is set up almost the same as the first pair except that the water molecule HOH55 is added to Chl602 as monomer B. The third pair "PID624 without CH_3···Chl602" is essentially the same as the first pair except that the methyl group attached to C21 of PID624 is replaced by a hydrogen atom in monomer A.

Table 2 presents results of MP2/6-31G*(0.25) energy calculations for all molecular species of pairwise intermolecular interactions PID623···TYR270, PID624···PHE301, PID624···

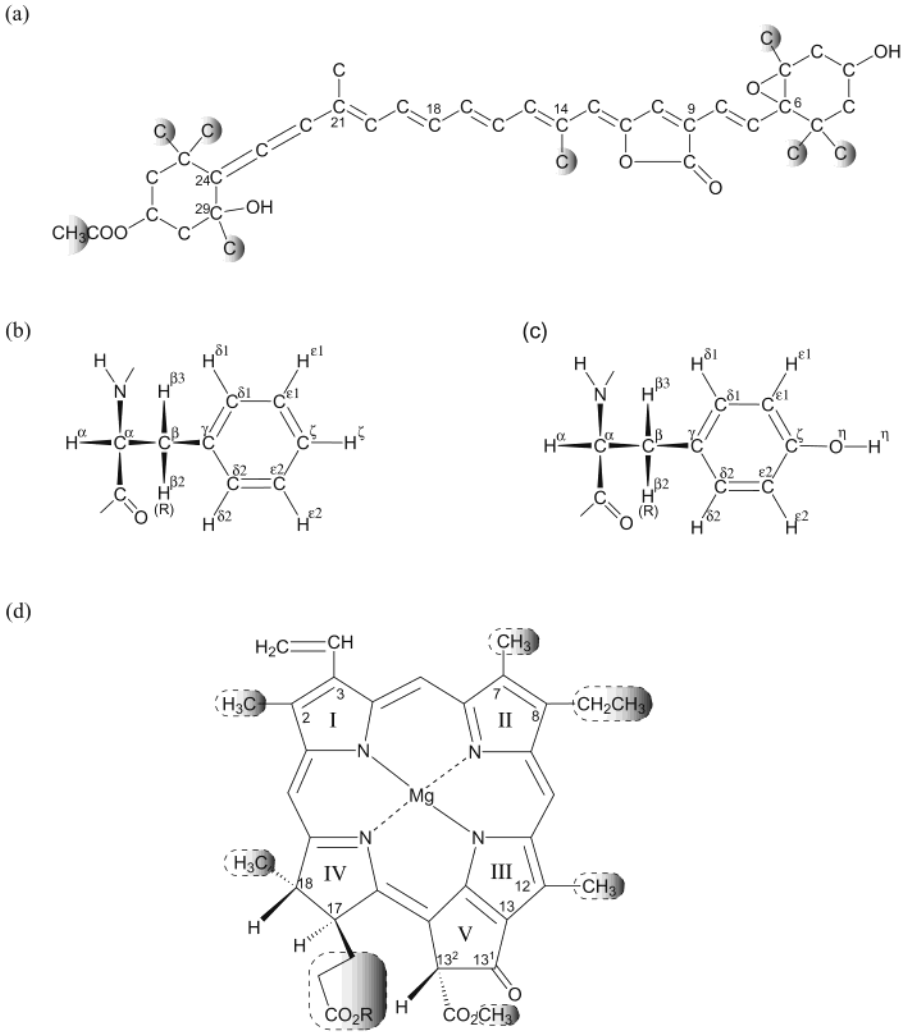

Figure 2. Molecular structures of intermolecular interaction partners: (a) peridinin with hydrogen atoms omitted, and labeled in accord with atom names in the PDB file 1PPR; (b) phenylalanine; (c) tyrosine; and (d) chlorophyll-a, labeled according to the IUPAC−IUB carbon numbering system.[71] The nonpolar side groups circled by the shaded squares in chlorophyll-a and peridinin indicate groups that are replaced by H atoms in the MP2/6-31G*(0.25) calculations of the PID624···Chl602 pair (see text for details).

PHE304, and PID624···Chl602. On the basis of these data, intermolecular interaction energies for each interacting pair are calculated according to eq 2 and are listed in Table 3. The magnitude of BSSE correction for each interaction is listed in parentheses.

As seen in Table 3, for all pairwise intermolecular interactions analyzed here, the MP2/6-31G*(0.25) calculations yielded negative $\pi-\pi$ stacking energies. This suggests that $\pi-\pi$ stacking interactions between peridinins and their surrounding aromatic residues and chlorophylls play a stabilization role in binding peridinin in PCP from *A. carterae*.

In the case of peridinin−chlorophyll interaction, three sets of interacting pairs (see above) are studied. The MP2/6-31G*(0.25) calculation gives rise to a stabilization energy of −11.52 kcal/mol after BSSE correction for the first pair "PID624···Chl602". This arises from favorable stacking between the large conjugated tetrapyrrol π-system of chlorophyll Chl602 and the long conjugated π-electrons of peridinin PID624. Potentially, the methyl group attached to C21 of PID624 can be involved in the so-called CH$-\pi$ interactions with the conjugated chlorophyll molecule.[58] The MP2/6-31G*(0.25) calculation for the third pair, "PID624 without CH_3···Chl602" without the methyl group, results in an intermolecular interaction energy of −9.92 kcal/mol after BSSE correction. The magnitude of the CH$-\pi$ interactions between the methyl group and the chlorophyll molecule Chl602 can be estimated as the difference of interaction energies between the first pair and the third pair. This yields −1.6 kcal/mol for the CH$-\pi$ interactions. We have also considered the OH$-\pi$ interactions between the water molecule HOH55 and the conjugated π system of PID624. The MP2/6-31G*(0.25) calculation for the second pair, "PID624··· Chl602 with HOH55" with the water molecule HOH55 included as part of the Chl602 system, produces an intermolecular interaction energy of −18.81 kcal/mol after BSSE correction. The difference in interaction energies between the first pair and the second pair is substantial, i.e., 7.29 kcal/mol. Part of this difference can be accounted for by the OH$-\pi$ interactions between the water molecule HOH55 and the conjugated π system of PID624. The rest may arise from the coordination effect of water on electronic structure of the chlorophyll molecule Chl602.

The above quantum chemical analysis indicates that intermolecular $\pi-\pi$ stacking interactions between the carotenoid molecule PID624 and the chlorophyll molecule Chl602 resulted

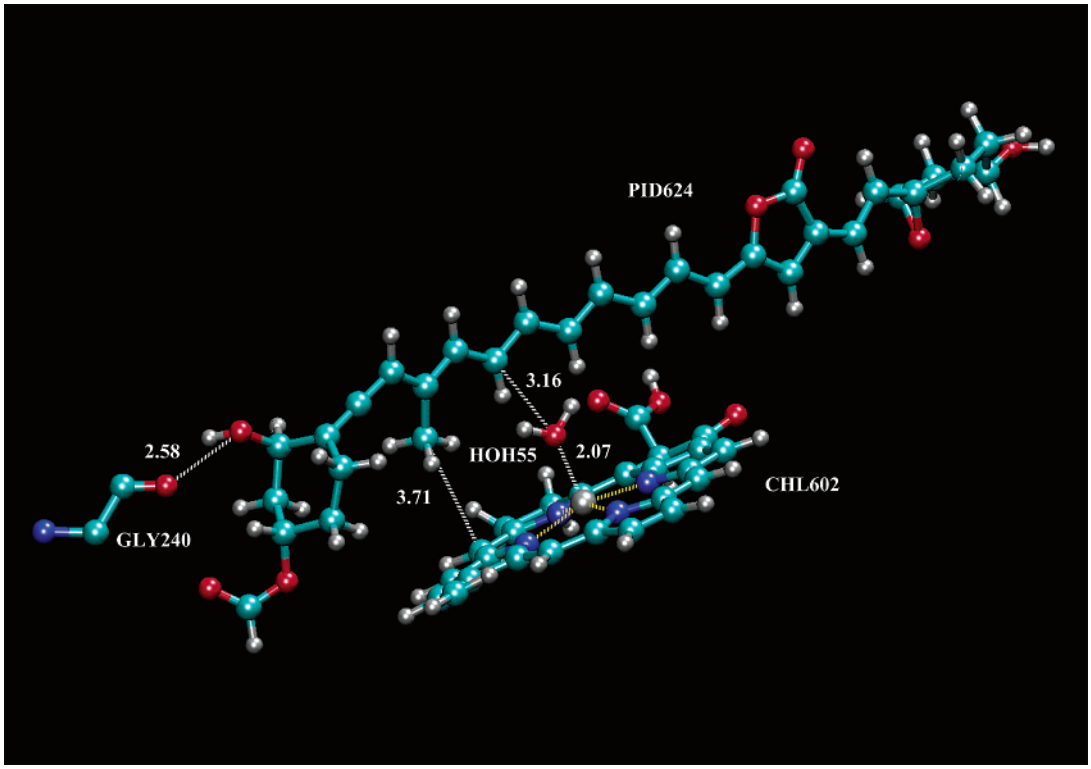

Figure 3. Structure of the interacting partner PID624 and CLA602 after truncation of nonessential bulk groups and addition of hydrogen atoms. Also shown are a water molecule HOH55 and a surrounding amino acid GLY240. The dashed lines indicate atom-to-atom distance in Å. (This figure is produced with the program VMD[59]).

TABLE 2: MP2/6-31G*(0.25) and HF/6-31G*(0.25) Energies for All Molecular Species of Intermolecular Pairs

intermolecular pair	molecular species	E_{HF} (hartree)[a]	E_{MP2} (hartree)[a]
PID623···Y270	PID623···Y270	−2373.743209	−2380.141550
	PID623···[GhostY270]	−2029.240385	−2034.707504
	[GhostPID623]···Y270	−344.509680	−345.422493
	PID623	−2029.237243	−2034.701709
	Y270	−344.506541	−345.416280
PID624···F301	PID624···F301	−2298.926063	−2305.179668
	PID624···[GhostF301]	−2029.259071	−2034.724966
	[GhostPID624]···F301	−269.670394	−270.448691
	PID624	−2029.257681	−2034.721718
	F301	−269.669131	−270.446822
PID624···F304	PID624···F304	−2298.927660	−2305.175301
	PID624···[GhostF304]	−2029.259377	−2034.724185
	[GhostPID624]···F304	−269.669125	−270.448388
	PID624	−2029.257681	−2034.721718
	F304	−269.668319	−270.447000
PID624···Chl602[b]	PID624···Chl602	−3314.785491	−3323.088453
	PID624···[GhostChl602]	−1717.056323	−1721.533341
	[GhostPID624]···Chl602	−1597.730386	−1601.536757
	PID624	−1717.053695	−1721.527930
	Chl602	−1597.723145	−1601.525177
PID624···Chl602 with HOH55[b]	PID624···Chl602	−3390.776806	−3399.246990
	PID624···[GhostChl602]	−1717.057060	−1721.527930
	[GhostPID624]···Chl602	−1673.719320	−1677.689079
	PID624	−1717.053695	−1721.527930
	Chl602	−1673.711161	−1677.675698
PID624···Chl602 without CH_3[b]	PID624···Chl602	−3275.782763	−3283.936780
	PID624···[GhostChl602]	−1678.053553	−1682.385946
	[GhostPID624]···Chl602	−1597.729335	−1601.535029
	PID624	−1678.051099	−1682.381074
	Chl602	−1597.723145	−1601.525177

[a] Energies at both the HF (E_{HF}) and the MP2 (E_{MP2}) are calculated using the modified 6-31G*(0.25) basis set with diffuse d-polarization. [b] See text for a detailed definition of the three PID624···Chl602 pairs.

in a stabilization energy of substantial magnitude. It has long been believed that the geometric proximity of carotenoid and chlorophyll is necessary for carotenoid to transfer the excitation energy to chlorophyll (light-harvesting role) and to quench the triplet excited state of chlorophyll (photoprotection role).[19,72−74] Our quantum chemical analysis suggested a third role for carotenoid and chlorophyll stacking, i.e., the structure stabilization role.

TABLE 3: MP2/6-31G*(0.25) and HF/6-31G*(0.25) Pairwise Intermolecular Interaction Energies

intermolecular pair	E_{HF} (kcal/mol)[a]	E_{MP2} (kcal/mol)[a]
PID623···Y270	4.30 (3.94)	−7.25 (7.54)
PID624···F301	2.31 (1.66)	−3.77 (3.21)
PID624···F304	0.53 (1.57)	−1.71 (2.42)
PID624···Chl602[b]	0.76 (6.19)	−11.52 (10.66)
PID624···Chl602 with HOH55[b]	−0.27 (7.23)	−18.81 (8.40)
PID624···Chl602 without CH_3[b]	0.08 (5.43)	−9.92 (9.24)

[a] Intermolecular interaction energies at both the HF (E_{HF}) and the MP2 (E_{MP2}) levels after BSSE correction. The BSSE value for each complex is shown in parentheses. [b] See text for a detailed definition of the three PID624···Chl602 pairs.

In the case of peridinin aromatic residue interactions, a strong configurational (orientation and distance) dependence of the $\pi-\pi$ stacking energy is observed. The stabilization energy between PID623 and the Tyr270 residue is −7.25 kcal/mol after BSSE correction, which is much larger than those of *peridinin···phenylalanine* pairs. As described in section II, the π-planes of PID623 and Tyr270 are nearly parallel to each other with a nearest atom to atom distance of 3.4 Å whereas residues Phe301 and Phe304 are almost perpendicular to the PID624. This large difference in stabilization energies is a clear indication that the parallelly oriented π-planes is energetically much more stable than the perpendicularly oriented π-planes for the interacting peridinin and aromatic side chains.

The BSSE-corrected MP2/6-31G*(0.25) intermolecular $\pi-\pi$ stacking energies are −3.77 and −1.71 kcal/mol for PID624···F301 and PID624···F304, respectively. This difference in interaction energies between PID624 and the two phenylalanine residues may be accounted for by geometrical difference. The nearest atom to atom distance between PID624 and Phe301 (3.66 Å) is slightly closer than that between PID624 and Phe304 (3.85 Å). Also, Phe301 approaches the conjugated π-plane of PID624 from a different orientation than Phe304 (see Figure 1). It is worth mentioning that this kind of T-shaped $\pi-\pi$ stacking interaction between PID624 and Phe304 is also commonly referred to as CH$-\pi$ interaction.[58]

The basis set superposition error (BSSE), as determined by the Boys and Bernardi counter poise method, is shown in Table 3. The BSSE is found to be substantial for the current system. At the MP2/6-31G*(0.25) level of theory, BSSE correction ranges from 2.42 kcal/mol for the PID624···F304 pair to 10.66 kcal/mol for the PID624···Chl602 pair. Since the fundamental cause of BSSE is basis set incompleteness, this calls for larger and more balanced basis sets [i.e., Dunning's correlation consistent basis sets (cc-pVXZ, X = D, T, Q, and 5) and the augmented aug-cc-PVXZ basis sets] to be used in future as the computational power evolves for treating large dimers as reported here.

Also listed in Table 3 are intermolecular interaction energies at the HF/6-31G*(0.25) level. HF treatment results in positive intermolecular interaction energies (i.e., unstable complexes) for all the pairwise intermolecular interactions except the "PID624···Chl602 with HOH55" pair. This further underscores the point made earlier about the necessity of including correlation correction when dealing with weakly bonded complexes, which is consistent with observations on many other $\pi-\pi$ stacking and hydrogen-bonded complexes.[38,44−46,56,75]

In addition to the total intermolecular interaction energies, its two major components, the dispersion interaction energy and the electrostatic interaction energy, are estimated for each of the pairwise $\pi-\pi$ stacking interactions. Results are listed in Table 4. The upper bound for the dispersion interaction energy

TABLE 4: Elements of the Pairwise Intermolecular Interaction Energies (in kcal/mol)

intermolecular pair	E_{MP2}[a]	E_{MP2}-E_{HF}[b]	$E_{electrostatic}$[c]
PID624···Chl602	−11.52	−12.28	−6.01
PID624···F301	−3.77	−5.91	−0.08
PID624···F304	−1.71	−2.24	−1.03
PID623···Y270	−7.25	−11.55	−0.87

[a] E_{MP2}: Total intermolecular interaction energies calculated at the MP2/6-31G*(0.25) level with BSSE correction. [b] $E_{MP2} - E_{HF}$: Difference between MP2 and HF energies calculated with the 6-31G*(0.25) basis set with BSSE correction, which corresponds to the correlation component of the intermolecular interaction energy. [c] $E_{electrostatic}$: Electrostatic interaction energies calculated based on a multipole analysis of the MP2/6-31G*(0.25) wave functions using the ORIENT 3.2 program.[69]

of the stacking complex was estimated by the correlation component of the MP2 interaction energy (i.e., the difference between the MP2 energy and the HF energy). The correlation component of the MP2 interaction energy ranges from −2.24 kcal/mol for the PID624···Phe304 complex to as high as −11.55 kcal/mol for the PID623···Tyr270 pair. The electrostatic interaction energies are determined by the ORIENT 3.2 program using multipoles extracted from the MP2/6-31G*(0.25) wave functions of the interacting complex as mentioned in the Method section. For all the pairwise intermolecular interactions treated here, the electrostatic interactions are attractive. In particular, the electrostatic interaction (−6.01 kcal/mol) contributed significantly to the stabilization energy between PID624 and Chl602.

Finally, it is interesting to compare intermolecular interactions responsible for binding of peridinin in the peridinin−chlorophyll−protein and those for binding lycopene in the light-harvesting complex II of *Rs. molischianum*.[38] While lycopene is simply a straight carbon chain with alternating single-double bonds, peridinin possesses cyclic six member rings linked with carbonyl and hydroxyl groups. The latter facilitates possibility of hydrogen bonding between peridinin and surrounding peptides. Indeed, the crystal structure of PCP showed a close contact between the hydroxyl group attached to C29 of PID624 and the main chain carboxyl oxygen atom of GLY240 of PCP (see Figure 3). A MP2/6-31G*(0.25) calculation of the hydrogen bond based on atomic coordinates from the crystal structure resulted in an interaction energy of −1.0 kcal/mol. This suggests that in addition to $\pi-\pi$ stacking interaction and CH$-\pi$ interactions, hydrogen bonding may also play a role in binding of peridinin in peridinin−chlorophyll−protein. It should be cautioned that the relatively weak calculated hydrogen bonding strength for this particular hydrogen bond may be an artifact due to the limited resolution power of the X-ray crystallographic structure determination method. The crystal structure showed an O−O distance of 2.58 Å for the hydrogen bond, which is shorter than the normal O−O distance of about 2.7 Å in a conventional hydrogen bond.

5. Conclusion

The $\pi-\pi$ stacking interactions between peridinins and their surrounding aromatic groups (i.e., aromatic residues and chlorophyll-a) in the PCP complex of *A. carterae* are characterized by means of the supermolecular approach at the MP2 level using the modified 6-31G*(0.25) basis set with diffuse d-polarization by Hobza et al. The MP2/6-31G*(0.25) calculations are based on the 2.0 Å resolution crystal structure of PCP from *A. carterae* as reported by Hofmann et al.[37] A representative peridinin chlorophyll pair (PID624···Chl602) is chosen to study the structural stabilization role of peridinin in addition to its well-

known functions of light harvesting and photoprotection of chlorophylls. The MP2/6-31G*(0.25) calculations yielded a favorable $\pi-\pi$ stacking interaction energy of −11.52 kcal/mol between the large conjugated tetrapyrrol π-system of chlorophyll Chl602 and the long conjugated π-electrons of peridinin PID624. Thus, our quantum chemical analysis revealed a new role for the carotenoid and chlorophyll stacking, i.e., structure stabilization.

Three unique peridinin and aromatic residue pairs (PID623···TYR270, PID624···PHE301, and PID624···PHE304), are chosen to study the configurational (orientation and distance) dependence of $\pi-\pi$ stacking interactions between peridinins and their interacting partners. For the parallelly oriented PID623···TYR270 pair, the MP2/6-31G*(0.25) calculations result in a stabilization energy of −7.25 kcal/mol after BSSE correction. For the perpendicularly oriented pairs, the BSSE-corrected MP2/6-31G*(0.25) $\pi-\pi$ stacking energies are −3.77 and −1.71 kcal/mol for PID624···PHE301 and PID624···PHE304, respectively. These results suggest that the strengths of $\pi-\pi$ stacking interactions between carotenoids and their surrounding aromatic groups depend strongly on both the distance and orientation of adjacent aromatic residues. Overall, all pairwise $\pi-\pi$ stacking interaction energies are substantially negative, indicating that $\pi-\pi$ stacking interactions between peridinins and the nearby aromatic groups play a substantial role in binding peridinins in the PCP complex of *A. carterae*. Therefore, the molecular basis for the long suggested structural stabilization role of carotenoids in forming the pigment−protein complexes is established.

Furthermore, the physical nature of the intermolecular interactions between peridinins and their surrounding aromatic groups was analyzed. The dispersion interaction is found to be the dominant intermolecular attraction force. There is also a substantial contribution of electrostatic attraction to the overall intermolecular stabilization energy.

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