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Intermolecular hydrogen bonding between carotenoid and bacteriochlorophyll in LH2

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Abstract We have studied van der Waals contacts of the carotenoid rhodopin glucoside (RG) with the bacteriochlorophyll pigments absorbing at 800 nm (B800) in the crystal structure of *Rhodopseudomonas acidophila*, and the hydrogen positions were determined from quantum chemical calculations at the Hartree–Fock (6-31G) level. We have found strong evidence for hydrogen bonding between the B800 BChl and the RG from neighboring protomer units. The binding energy was estimated to be about 2 kcal/mol (700 cm^{-1}). CI-singles approach and time-dependent density functional theory calculations of the B800–RG dimer indicate a red-shift (ca 2 nm) of the B800 Q_y transition, along with a substantial increase of its oscillator strength, probably due to the hydrogen bonding. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Light-harvesting complex II; Bacteriochlorophyll; Carotenoid; Ab initio calculation; Hydrogen bond

1. Introduction

Pigment molecules in light-harvesting complexes are organized in a way that leads to a highly efficient excitation transfer and trapping in primary photosynthesis [1,2]. In most antenna systems, a protein scaffold forms the backbone for arranging the pigment molecules. Hydrogen bonding plays a decisive role in protein structure formation and stabilization [3]. Hydrogen bonding is also known to tune the energies of absorption bands of pigment–protein complexes [4]. For example, in the light-harvesting antenna II (LH2) of the photosynthetic purple bacterium *Rhodobacter sphaeroides* two distinct absorption bands related to the bacteriochlorophyll *a* (BChl *a*) Q_y transition occur at 800 and 850 nm and are called B800 and B850, respectively. At the same time BChl *a* in solution absorbs at around 780 nm. Most of the B800 shift can be assigned to a hydrogen bond between the βArg$_{-10}$ residue and the BChl *a* [5,6]. In case of B850 about 25 nm of the red-shift is due to the hydrogen bonding [4,7]. Only strongly electronegative atoms O and N are usually considered as possible proton-donors and -acceptors in hydrogen bonding. During recent years C–H···O interactions have attracted much attention [8]. A number of reports have indicated the significance of these non-classical hydrogen bonds in biological macromolecules [9].

In the present work we will study possible C–H···O hydrogen bonding between two pigment molecules, a carotenoid rhodopin glucoside (RG) and BChl *a* in LH2 of the purple bacterium *Rhodopseudomonas acidophila* with known structure [10], by means of the characteristic features of the C–H···O hydrogen bonds: (i) geometric parameters for an ideal C–H···O = C hydrogen bond; (ii) the H-bond strength; (iii) electron density transfer from the proton-acceptor to the proton-donor molecule. Finally, we will examine how the C–H···O = C hydrogen bonds affect excited states of BChl *a*.

2. Computational details

The present calculations are based on the crystal structure of *R. acidophila* (strain 10050) reported by McDermott et al. [10]. Our previous work shows that a B800 BChl and the carotenoid RG from the neighboring protomer units (labeled as RG′ in Figs. 1 and 2) make short van der Waals contacts (< 3.4 Å), leading to a significant blue-shift of the RG′ S_2 transition energy upon photoexcitation of the B800 [11]. In this work we will study the possibility that hydrogen bonds could be formed between the RG′ and B800. Since the hydrogen positions are not yet available in the current crystal structure, they must be determined by using geometry optimizations.

In order to identify the possible hydrogen bonds between the B800 and RG′, we employ a two-step procedure to generate the hydrogen atom positions: (i) First, the individual geometries of the RG and B800 are separately optimized for the hydrogen positions using restricted Hartree–Fock (RHF) computations with 6-31G basis set, where all heavy atoms are fixed according to the LH2 crystal structure. Then (ii) the hydrogen positions of the possible hydrogen-bond region are reoptimized for the B800–RG′ dimer illustrated in Fig. 1 at the RHF level. In the course of these calculations the positions of seven hydrogen atoms marked with asterisks are recomputed, and the phytyl chain is replaced by a hydrogen atom for simplification of the optimizations. In the second step, the two basis sets 6-31G and 6-31G* are employed. All calculations in the present work are performed with the Gaussian 98 program package [12]. In Section 3 we will see that the resulting structure suggests the presence of C–H···O = C hydrogen bonds between the B800 and RG′.

To clarify the influence of the possible hydrogen bonds on the excitation energies for the B800 pigment, we consider the dimer B800–RG′ surrounded by one carotenoid RG and the five BChls (α, β B850, α, β B850′, and B800′) shown in Fig. 2. The surrounding molecules are modeled via atomic point charges to represent the electrostatic field of LH2 near this dimer. The point charges are obtained from the RHF wavefunction by using the Mulliken population analysis [13] with 6-31G, as described in our previous paper [11]. We have calculated the excited states of the dimer in the point charge field

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Abbreviations: LH2, light-harvesting antenna II; BChl, bacteriochlorophyll; B800, bacteriochlorophyll absorbing at 800 nm; HF, Hartree–Fock calculation; CIS, CI-singles approach; TDDFT, time-dependent density functional theory

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using the CI-singles approach (CIS) [14] and time-dependent density functional theory (TDDFT) [15] based on the three-parameter Lee–Yang–Parr (B3LYP) functional [16]. Additionally, excitation energies of a monomer B800 can also be computed in the charge field produced by one more carotenoid RG′. The comparison of the dimer and monomer calculations indicates that the two hydrogen bonds have a significant effect on the B800 Q_y transition energy.

3. Results and discussion

We now present computational evidence suggesting that two C–H···O=C arrangements between the RG′ and the B800 have stereochemical features, typical of hydrogen bonds. In an ideal C–H···O=C hydrogen bond, the H···O and C···O distances should be less than 2.7 and 3.8, which are, respectively, the sums of the van der Waals radii of the two atoms; the C–H-bond should point toward the oxygen giving a C–H···O angle of 180°, where the hydrogen would align with the lone pair of the oxygen giving an H···O=C angle of 120°; and finally the H···O hydrogen bond would lie in the plane of the carbonyl carbon with an elevation angle of 0°. Studies of actual C–H···O=C hydrogen bonds in proteins indicate that the H···O=C angles are mostly within about 20° of the ideal value of 120°, while the C–H···O angles are greater than 90°, and the absolute values of the elevation angles are less than 45° [9]. Table 1 summarizes the calculated structural parameters for three hydrogen atoms in the RG′ that may be H-bonded with the oxygen atom of the B800 (see Fig. 1). We can clearly identify two C–H···O=C hydrogen bonds in the dimer B800–RG′. Moreover, we found that the calculated structural parameters for these H atoms only change slightly in the individual optimization and reoptimization with 6-31G. It implies that the structure of a portion of the polyene chain involving the van der Waals contacts with B800 originally favors the formation of the hydrogen bonds. Obviously, no significant changes appear in the reoptimized hydrogen positions (see Table 1) as 6-31G is extended to the 6-31G* basis set.

Next, we estimate the strength of the two C–H···O=C H-bonds in the B800–RG′ dimer. In general, the difference between the ground-state energy for the optimized hydrogen-bond geometry of the dimer and the sum of the ground-state energies of the two individual molecules provides the binding energy ($-\Delta E$) for the hydrogen bonding of RG′ with B800. RHF/6-31G and RHF/6-31G* computations yield $-\Delta E = 1.53$, and 2.38 kcal/mol, respectively, as the intermolecular binding energy, which we assign to the hydrogen-bond energy. This corresponds to relatively weak H-bonds.

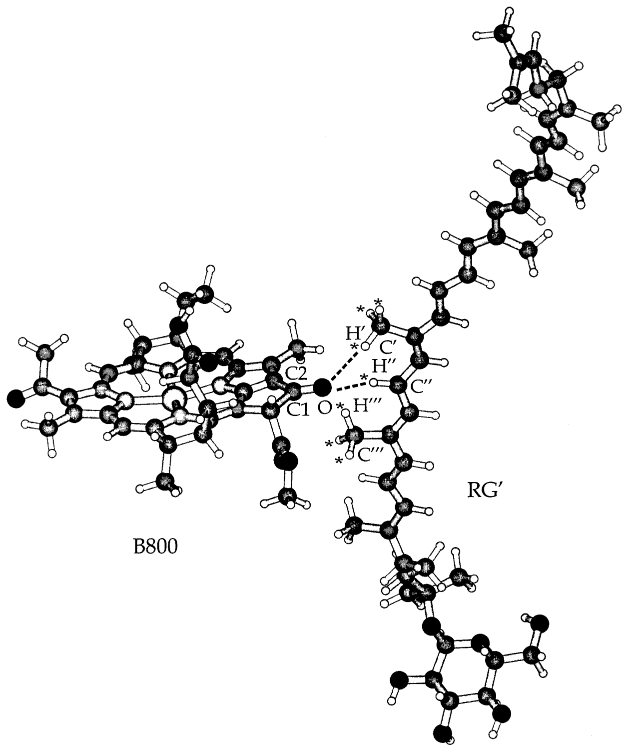

Fig. 1. A B800–RG′ dimer used for optimization of the hydrogen bond geometries. The dashed lines indicate the two intermolecular hydrogen bonds.

Alternatively, analysis of the charge distributions of the B800–RG′ dimer and a single B800 provides information about the hydrogen bonding. Comparison of the two partial atomic charge distributions derived from Mulliken population analysis at RHF/6-31G level reveals that the largest difference of the charges appears on the B800 oxygen atom indicated by O in Fig. 1, i.e. the acceptor of the hydrogen bonds. Its atomic charge in the dimer (−0.5903) is more negative than that of the single molecule (−0.5498), strongly supporting the formation of the hydrogen bonds between B800 and RG′. Furthermore, the partial charges obtained for the B800 and RG′ molecules from the RHF/6-31G calculation indicate that a partial intermolecular charge transfer accompanies the hydrogen bonding: 0.028 electron charge is transferred from the B800 to RG′. Charge transfer from the proton-acceptor to the proton-donor is one of the generally accepted common features of H-bonds.

In order to investigate the effect of the hydrogen bonds on the B800 excitation energies, we compare the calculated exci-

Table 1
The calculated hydrogen-bond geometry parameters between the B800 and RG′ from the neighboring protomer unit[a]

	$d_{\mathrm{H\cdots O}}$ (Å)	$d_{\mathrm{C\cdots O}}$ (Å)	∠CH···O (°)	∠H···O=C1 (°)	Θ^b (°)
Ideal	≤2.7	≤3.8	180	120	0
6-31G					
C′H′(RG′)···O(B800)	2.498	3.536	160.65	144.57	30.24
C″H″(RG′)···O(B800)	2.323	3.380	170.85	143.91	−19.72
C‴H‴(RG′)···O(B800)	2.342	3.255	142.21	98.95	−59.22
6-31G*					
C′H′(RG′)···O(B800)	2.507	3.536	158.55	144.75	30.42
C″H″(RG′)···O(B800)	2.322	3.380	170.55	143.83	−19.70
C‴H‴(RG′)···O(B800)	2.342	3.255	142.11	98.88	−59.20

[a] The labels for the atoms are taken from Fig. 1.

[b] Θ is the elevation angle defined as the angle between the H′ (or H″)···O bond and the OC1C2 plane (also see Fig. 1).

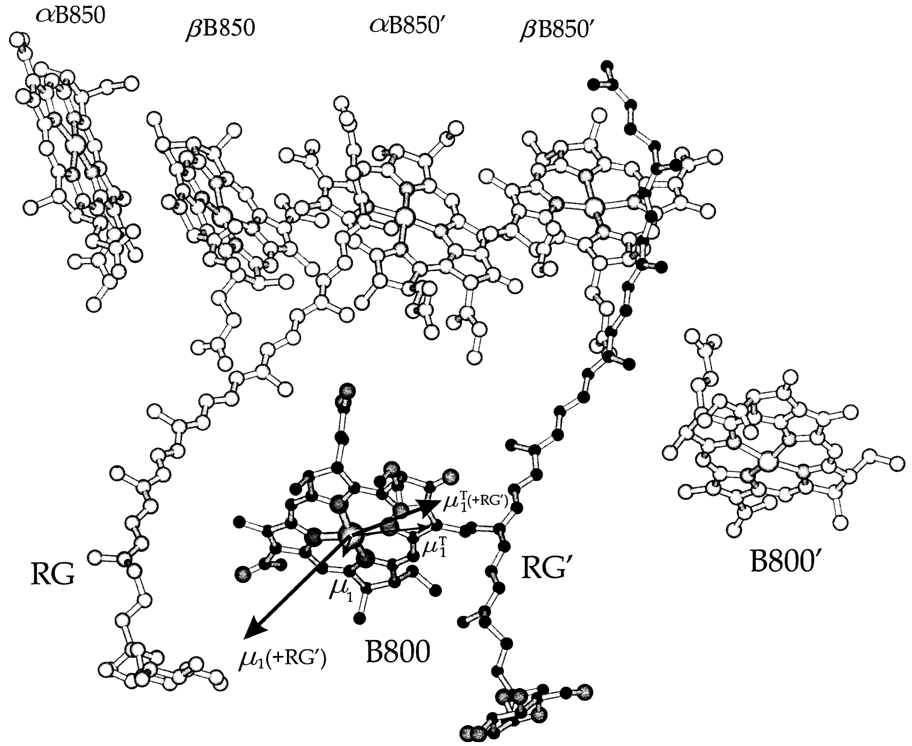

Fig. 2. Schematic representation of electric (μ_1) and transition (μ_1^T) dipole moments of the B800 for the S_1 state in the CIS/6-31G calculations, where the dimer B800–RG′ (the monomer B800) was considered in a charge field consisting of four B850, one B800, and one (two) RG: α, β B850, α, β B850′, B800, RG (RG′). Note that the μ_1(+RG′) (μ_1) and μ_1^T(+RG′) (μ_1^T) denote the dipole moments for the dimer (the monomer). For the simplicity the phytyl chains of the BChl have been removed from the figure, but included in the calculations.

tation energies of B800 in three different configurations: a B800 monomer and the two dimers, B800–RG and B800–RG′ (see Fig. 2 and Tables 2 and 3). In some of the calculations we have included the influence of the surrounding pigment molecules via their charge fields [11] (see Table 3). Comparison of the results shows that the RG′ exerts a stronger influence on the B800 excitation energy than the RG. The Q_y transition energies are red-shifted by 2.3 nm (RG′) and blue-shifted 1.2 nm (RG). Remarkably, the oscillator strength in the B800–RG′ dimer increases by 54% in the CIS calculation, and even more in the TDDFT treatment, when compared with the B800 monomer (see Table 3). Herek et al. [17] pointed out that the calculated B800 to B850 transfer rate is significantly below the experimentally measured values. The enhanced B800 oscillator strength would speed up this transfer rate which may explain the above controversy. A similar conclusion was recently drawn by Krueger et al. [18]. We suggest that this behavior arises at least partially from the hydrogen bonds between the B800 and RG′. In contrast, the hydrogen bonds have a minor influence on the S_2 state of the B800 (Q_x band) at the CIS/6-31G level.

We have also studied the effects of the hydrogen bonds on orientation of the electric (μ) and transition (μ^T) dipole moments of B800. Fig. 2 shows the dipole moments calculated by the CIS method for S_1 state of the B800 in the monomer (μ_1 and μ_1^T) and the dimer B800–RG′ [(μ_1(+RG′) and μ_1^T(+RG′)]. One can easily notice the changes in both dipole directions of the B800. Interestingly, the transition dipole moment of B800 tilts about 13° towards the oxygen atom involved in the hydrogen bonding with the RG′. This is consistent with recent calculations of carotenoid-mediated B800–B850 electronic coupling in LH2 [18,19], where a change in dipole moment direction of B800 is 15.13° owing to mixing with the carotenoid transition. We can also see that the value of the electric dipole moment change, $\Delta\mu = \mu_1(\text{CIS}) - \mu_0(\text{RHF})$, for the dimer (8.29 D) is much larger than that for the monomer (4.09 D), reflecting strikingly different influence of the RG′ on the S_1 and the ground-state S_0 of the B800.

Table 2
Comparison of CIS excitation energies for the B800 in an isolated monomer, and two dimers B800–RG, B800–RG′ without a charge field; all calculations use 6-31G basis set

States	B800		B800–RG		B800–RG′	
	E^a	Osc.^{b}	E	Osc.	E	Osc.
S_1	1.8752(661.18)	0.6903	1.8787(659.96) (−1.22)	0.6151	1.8687(663.48) (2.30)	1.0600
S_2	2.7579(449.56)	0.0242	2.7588(449.41)	0.0249	2.7552(450.00)	0.0235

[a] Energies are in eV (nm), or the wavelength shifts of the S_1

[b] Oscillator strengths.

Table 3
Comparison of calculated B800 excitation energies in two systems: the monomer B800 and the dimer B800–RG′ in the charge fields using 6-31G basis set[a]

States	B800		B800–RG′	
	E[b]	Osc.[c]	*E*	Osc.
CIS				
S_1	1.8915(655.48)	0.6916	1.8866(657.17) (1.69)	1.0651
S_2	2.7587(449.43)	0.0240	2.7581(449.52)	0.0229
B3LYP				
S_1	1.8955(654.08)	0.0187	1.8888(656.40) (2.32)	0.4180

[a] The charge fields cover four B850, B800 and RG molecules: α, β B850, α, β B850′, B800′, and RG depicted in Fig. 2, while one more carotenoid RG′ is included in the field for the monomer B800 calculations.
[b] Energies are in eV (nm), or the wavelength shifts of the S_1.
[c] Oscillator strengths.

4. Conclusions

We have studied interactions between the B800 BChl and the carotenoid RG′ from the neighboring protomer units by using ab initio quantum chemical methods. We have performed a series of CIS and TDDTF/B3LYP calculations with 6-31G for the dimer and the monomer B800 in the charge field of surrounding pigment molecules. From the calculations, two conclusions can be drawn: (1) Two C–H···O=C hydrogen bonds are formed between the B800 and RG′. The binding energy is 2.4 kcal/mol at the RHF/6-31G* level; it is predicted that a partial intermolecular charge transfer from the B800 to the RG′ accompanies the hydrogen bonding. (2) The hydrogen bonding leads to a red-shift (ca 2 nm) of the B800 Q_y band. The Q_y transition dipole moment is increased and tilted towards the oxygen atom involved in the hydrogen bonding.

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