Dynamic Exchange Properties of the Antiparallel Bacteriochlorophyll *c* Dimers

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The dynamic exchange behavior of the (3^1R)-type bacteriochlorophyll (BChl) *c* dimer with an antiparallel piggy-back conformation has been investigated by two-dimensional nuclear magnetic resonance exchange spectroscopy (EXSY). The exchange rate between two BChl *c* molecules in the antiparallel dimer was evaluated from the integrated intensities of cross-peaks due to chemical exchange in the EXSY spectra, and its values were found to dramatically increase with the rise of solvent polarity. The temperature dependence of the exchange rates can be well expressed by the Arrhenius equation, the parameters of which show the correlation of the fast exchange rate with the stability of a transition state in the exchange process. Thermodynamic analysis was also applied to investigate the substituent effect on the exchange rate of the antiparallel dimer. The increase of the exchange rate upon addition of substituents at the peripheral 8-position was mainly attributed to the rise in frequency factor rather than activation energy. A high exchange of the (3^1R)-type BChl *c* dimer by a small amount of (3^1S)-type BChl *c* also resulted from the significant increase of the frequency factor. The substituent effects demonstrate that the antiparallel dimer formed by the most abundant BChl *c* homologue in the native light-harvesting entity, called chlorosome, can be highly exchanged by minor BChl *c* components. The results suggest that minor BChl *c* homologues in the chlorosome could bring about a phase transition of the most abundant BChl *c* from the stable dimer to a large aggregate.

Introduction

Bacteriochlorophyll (BChl) *c* is a major pigment in a special light-harvesting complex, the chlorosome, of green photosynthetic bacteria.[1,2] The chlorosome contains pigments of more than 50 wt %, and self-aggregation of BChl *c* plays an important structural role in the chlorosome. The $Q_y(0-0)$ transition of isolated BChl *c* monomer appears at 660−70 nm in hydrophilic organic solvents,[3] while chlorosome has an absorption maximum around 740 nm.[4] The large red-shift of the $Q_y(0-0)$ transition in vivo has been one of the major topics in the study of chlorosome antennae. A large number of recent studies have shown that, in hydrophobic solvents, isolated BChl *c* forms aggregates with various spectroscopic properties closely resembling those of native chlorosome (absorption,[4−7] circular dichroism (CD),[8] Fourier transform infrared (FT-IR),[9] and solid nuclear magnetic resonance (NMR) spectra[10,11]), and further, it is reported that the self-aggregates of isolated BChl *c* can be formed with glycolipid in water.[12] Therefore, self-aggregation of BChl *c* plays a predominant role in the pigment orientation in the chlorosome, and elucidating the mechanism of self-aggregation is generally considered essential for understanding the chlorosome structure.

The pathway to the 740-nm aggregates with absorption spectra similar to the spectrum of the chlorosome is dependent upon BChl *c* homologues. The major diastereomer in the chlorosome, (3^1R)-BChl *c*, is well-known to form two different small aggregates with absorption maxima at 680 and 710 nm.[13,14] For the small aggregates, a dimer structure was first revealed by the ring current effect observed from the ^{1}H NMR spectra for bacteriochlorophyllide (BChlide) *d*.[15] Recent small-angle neutron scattering (SANS) and ^{1}H, ^{13}C, and ^{15}N nuclear magnetic resonance (NMR) experiments for BChl *c* demonstrated that the 710-nm-rich species in neat CCl_4 predominantly consists of dimers with an antiparallel piggy-back conformation.[16−18] Further, recent CD and magnetic circular dichroism (MCD) studies proposed two different small aggregates:[19] one is an antiparallel dimer with two $Q_y(0-0)$ exciton components at 680 and 710 nm (Figure 1a), and the other is a transient (T-shaped) dimer with a single $Q_y(0-0)$ transition at 680 nm whose structure has been proposed for Chl *a* (Figure 1b).[20−22] Several models have been proposed for the correlation between the two small aggregates and the 740-nm aggregate.[23−26] However, critical verification is very difficult, because the formation of the 740-nm aggregate involves a phase transition from a soluble form (monomer and dimers) to the solid state (740-nm aggregate), and the solubilizing solvent might influence the pathway to the 740-nm aggregate. The 740-nm aggregate is formed through the dimers on drying BChl *c* in CH_2Cl_2 and CCl_4, while the drying of BChl *c* in diethyl ether suggests high aggregation directly from a monomer state.[9] The same situation is found when diluting BChl *c* solutions (BChl *c*−CH_2Cl_2 or BChl *c*−THF solution) into cyclohexane or hexane.[4,27]

In contrast to (3^1R)-BChl *c*, (3^1S)-BChl *c* forms large aggregates with a Q_y absorption maximum at 740−750 nm directly from a monomer in CH_2Cl_2 or CCl_4 instead of the small

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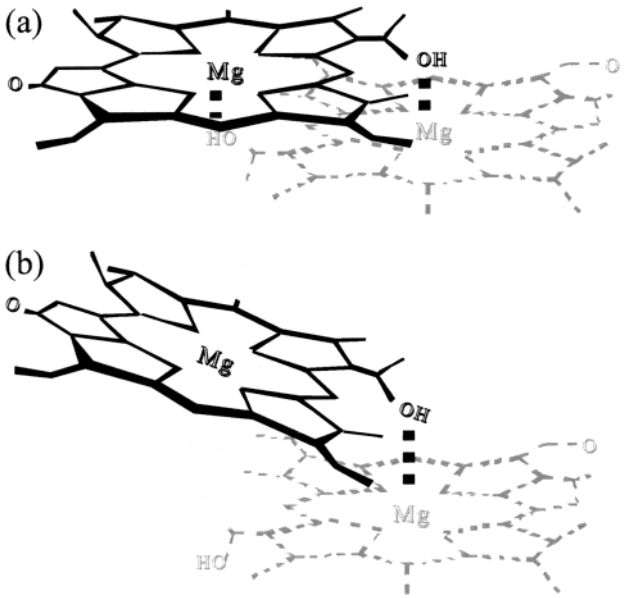

Figure 1. Proposed models for an antiparallel dimer model with a piggy-back comformation (a) and a T-shaped dimer (b). The antiparallel dimer has been determined by NMR results,[15] and the T-shaped dimer is proposed from CD spectra.[17]

intermediates.[13,24] Interestingly, it is reported that large aggregates of (3^1S)-BChl *c* bear more resemblance to the native chlorosome than the (3^1R)-BChl *c* aggregate, despite the fact that (3^1R)-BChl *c* is more abundant than (3^1S)-BChl *c* in chlorosomes.[11,24,28] Hence, the influence of (3^1S)-BChl *c* on the aggregation behavior of (3^1R)-BChl *c* is of importance for understanding the self-aggregation in chlorosome.

In this study, we have measured two-dimensional exchange spectroscopy (EXSY) spectra for the antiparallel dimer formed from (3^1R)-BChl *c*, to elucidate the influence of the solvent used and minor BChl *c* homologues on the antiparallel dimer prepared from the most abundant homologue of BChl *c* in the chlorosome of *Chlorobium tepidum*. The BChl *c* homologues used in this study were the (3^1R)-BChl *c* with ethyl groups at the 8- and 12-positions ((3^1R)-[E,E]BChl *c*, where the characters in the brackets represent the first letters (in capitals) of the substituents at the 8- and 12-positions, respectively) as the most abundant homologue, the (3^1R)-[P,E] BChl *c* and (3^1S)-[P,E] BChl *c*, with a propyl group at the 8-position as minor homologue and diastereomer, respectively (Figure 2). The thermodynamics of the antiparallel dimer was discussed in terms of the exchange rate between two BChl *c* molecules in the dimer, and the temperature dependence of the exchange rates was well expressed by Arrhenius equation. The Arrhenius parameters obtained indicate that the polarity of solvent stabilizes a transition state in the exchange process, and they also demonstrate that the substitution of a propyl group at C-8 and *S*-configuration at C-3^1 increases the exchange rate of the antiparallel dimer. Especially, the high exchange rate of the (3^1R)-[E,E] BChl *c* antiparallel dimer by a small amount of (3^1S)-[P,E] BChl *c* shows the possibility that minor BChl *c* homologues in chlorosome bring about a phase transition of (3^1R)-[E,E] BChl *c* from the stable dimer to a large aggregate.

Experimental Section

Materials and Sample Preparation. The thermophilic green photosynthetic bacterium *Chlorobium tepidum* was grown as previously reported.[29] BChl *c* was extracted from the dry cells with methanol and purified by reversed-phase HPLC as

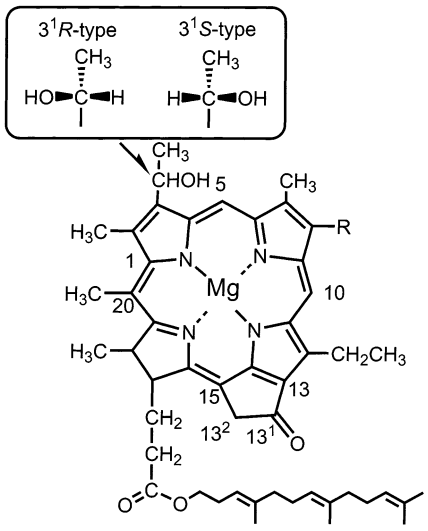

Figure 2. Molecular structure of (3^1R)-[E,E] BChl *c* (R = ethyl group) and (3^1R)-/(3^1S)-[P,E] BChl *c* (R = propyl group).

described previously.[3] The elution solvent was composed of methanol and water with a volume ratio of 98: 2 for the separation of the (3^1R)-[E,E] BChl *c* and (3^1R)/ (3^1S)-[P,E] BChl *c* fraction from crude BChl *c*. Each of the (3^1R)- and (3^1S)-[P,E] BChl *c* diastereomers was fractionated from the mixture using a methanol−water elution solvent with a volume ratio of 88:12. The separation and purification of (3^1R)- and (3^1S)-BChl *c* were verified by the position of the 5-CH signal in the ^{1}H NMR spectra.[30,31]

For absorption, CD, and MCD measurements, BChl *c* was dissolved in methanol, CH_2Cl_2, and CCl_4. All the solvents used were spectral grade, and CH_2Cl_2 and CCl_4 were treated with Na_2CO_3 before use. Deuterated methanol and dichloromethane (CD_3OD and CD_2Cl_2, D > 99.9%), purchased from Cambridge Isotope Laboratories Inc., were used for solution NMR experiments. Extinction coefficients used for BChl *c* were calculated as described previously.[3]

Instruments. Absorption spectra were measured on a Beckman DU-640 spectrophotometer. CD and MCD spectra were measured on a Jasco J-720w spectropolarimeter. The conditions of CD and MCD measurements were the same as described previously.[3]

NMR spectra were obtained with a Bruker Avance DRX-400 spectrometer at various temperatures. For the measurements in CCl_4 solution, deuterated water (D_2O) in a 1-mm-inner-diameter tube was inserted in the 5-mm NMR tube. 2-D EXSY spectra were measured with a pulse sequence of NOESY and acquired using TPPI for phase-sensitive detection. The 2-D spectra were collected with 256 t_1 points, 2048 data point in t_2, and 32 scans for each t_1 point using a repetition time of 1 s.

Results

Exchange between Two BChl *c* Molecules in the Antiparallel Dimer in CH_2Cl_2 (CD_2Cl_2) and CCl_4. Figure 3 shows the absorption spectra of (3^1R)-[E,E] BChl *c* in methanol, CH_2Cl_2, and CCl_4 at concentrations of 0.05 mM (in methanol) and 2 mM (in CH_2Cl_2 and CCl_4). BChl *c* existed as a monomer in methanol with a $Q_y(0-0)$ transition at 661 nm (solid line), while aggregation of BChl *c* caused band-splitting and a red-shift of $Q_y(0-0)$ to 680 and ~710 nm in CH_2Cl_2 (dotted line) and CCl_4 (dashed line). The ratio of the 680-nm absorption intensity to the 710-nm peak was larger in CH_2Cl_2 than in CCl_4. In organic solvents, BChl *c* forms two types of dimers with different

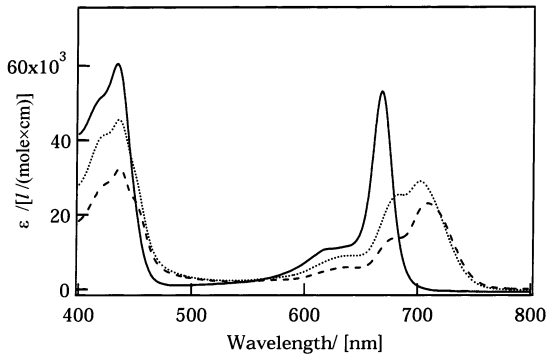

Figure 3. Absorption spectra of (3^1R)-[E,E] BChl *c* in methanol (solid line), in CH_2Cl_2 (dotted line), and in CCl_4 (dashed line). The concentration of BChl *c* was 0.05 mM (in methanol) and 2 mM (in CH_2Cl_2 and CCl_4).

conformations, called T-shaped and antiparallel dimers (Figure 1).[19] The T-shaped dimer shows only a $Q_y(0-0)$ absorption band at 680 nm, while the antiparallel dimer has two $Q_y(0-0)$ exciton components at 680 and 710 nm and is predominately formed in the nonpolar solvents CCl_4 and benzene. Therefore, the difference of the absorption intensity at 680 nm is assigned to the coexistence of the T-shaped and antiparallel dimers in CH_2Cl_2.[19]

In the ^{1}H NMR spectra of the CD_2Cl_2 and CCl_4 solutions, BChl *c* had two resonances of 5-H with a large upfield shift, both of which are derived from the antiparallel dimer (Figure 4a).[17] The 5-H doubling signal gives rise to cross-peaks due to chemical exchange in the EXSY spectra of both the CD_2Cl_2 and CCl_4 solutions (I_{AB} and I_{BA} in Figure 4b),[17,19] showing that two BChl *c* molecules in the antiparallel dimer exchange their sites. In CD_2Cl_2, no resonance from the T-shaped dimer was detected, despite the recognition of the T-shaped dimer from the ratio of the 680-nm absorption intensity to the 710-nm peak in the absorption spectrum (Figures 3 and 4a). This indicates that the T-shaped dimer is a short-lived transient intermediate in the exchange process of the antiparallel dimer. In a previous study,[19] we estimated the exchange rate between pigments in the antiparallel dimer from the 2-D EXSY spectra and revealed that the polarity of solvent strongly influences the exchange rate. Here, we measured the exchange rate at various temperatures; the temperature was decreased in CD_2Cl_2 and increased in CCl_4, and then the stability of the antiparallel dimer was compared using the thermodynamic parameters of the Arrhenius equation. The exchange rate *k* is expressed as the slope of the correlation line between the value of $\ln\{(r+1)/(r-1)\}$, calculated from the cross-peak intensities (I_{AA}, I_{BB}, I_{AB}, and I_{BA}) and the mixing time (τ_m) (Figure 5), with the assumption that the exchange system of BChl *c* in CD_2Cl_2 and CCl_4 can be approximated as a two-site exchange between two uncoupled systems of spin A and B.[32] At each temperature in the CD_2Cl_2 and CCl_4 solutions, the values of $\ln\{(r+1)/(r-1)\}$ calculated from the 5-H intensities were proportional to the τ_m time in each appropriate range (Figure 5). This indicates that the assumption of the two-site uncoupled systems is appropriate in both solutions.

The exchange rates *k* calculated from the EXSY spectra at various temperatures were plotted in Arrhenius representation ($k = A_0 \exp(-\Delta E/RT)$) (Figure 6). The series of exchange rates showed a good correlation to the Arrhenius equation in both CD_2Cl_2 and CCl_4 solutions. Both the frequency factor (A_0) and the activation energy (ΔE) for the CD_2Cl_2 solution were lower than those for the CCl_4 solution, clearly revealing that the high exchange rate in CD_2Cl_2 results from the low activation energy.

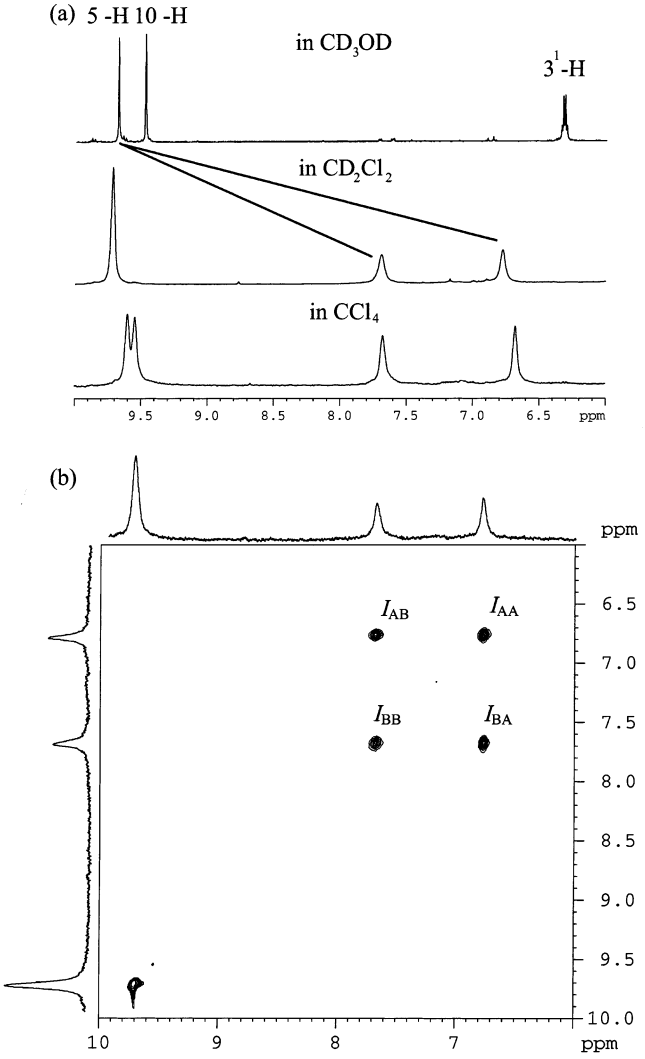

Figure 4. ^{1}H NMR spectra of (3^1R)-[E,E] BChl *c* (2 mM) in methanol-d_4, CD_2Cl_2, and CCl_4 at 298 K (a), and negative-phase contour plot of the 2-D EXSY spectrum for (3^1R)-[E,E] BChl *c* (2 mM) in CD_2Cl_2 with a mixing time of 60 ms at 298 K (b).

This suggests that the polarity of CD_2Cl_2 stabilized a transition state in the exchange process of the antiparallel dimer. Considering the observation of the T-shaped dimer in CD_2Cl_2 (Figure 3), the transient dimer can be a transition state in the exchange process.

Difference in the Stability of the Antiparallel Dimer between (3^1R)-[E,E] BChl *c* and (3^1R)-[P,E] BChl *c*. Figure 7 shows the absorption, CD, and MCD spectra of (3^1R)-[E,E] BChl *c* (solid line) and (3^1R)-[P,E] BChl *c* (dotted line) in CCl_4. All the spectra of (3^1R)-[P,E] BChl *c* were identical with those of (3^1R)-[E,E] BChl *c* (Figure 7). Our previous study demonstrates that (3^1R)-[E,E] BChl *c* forms predominantly the antiparallel dimer and a minute amount of monomer in CCl_4 and that the T-shaped dimer is not formed in CCl_4 at high concentrations.[19] Therefore, the similarity of the spectra suggests that (3^1R)-[P,E] BChl *c* forms the antiparallel dimer in CCl_4, too, without any T-shaped dimer.

The 1-D ^{1}H NMR spectrum in CCl_4 supports that (3^1R)-[P,E] BChl *c* forms the same antiparallel dimer as (3^1R)-[E,E] BChl *c* (Figure 8). In CCl_4, all ^{1}H signals from (3^1R)-[P,E] BChl *c* were identical with those of (3^1R)-[E,E] BChl *c*, except for the signal of 8^3-CH_3 which is absent in (3^1R)-[E,E] BChl *c*. However, 2-D EXSY spectra revealed the differences in the exchange rates between the two pigments in the antiparallel

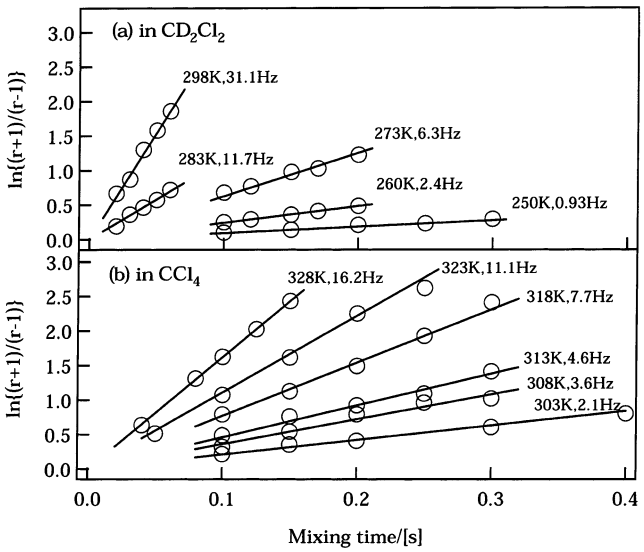

Figure 5. Correlation of $\ln\{(r+1)/(r-1)\}$ with mixing time τ_m in the (3^1R)-[E,E] BChl *c*-CD_2Cl_2 (a) and (3^1R)-[E,E] BChl *c*-CCl_4 (b) solutions at various temperatures. The temperatue was decreased from 298 to 250 K in CD_2Cl_2, and increased from 303 to 328 K in CCl_4. *r* is $(I_{AA} + I_{BB})/(I_{AB} + I_{BA})$, where I_{AA} and I_{BB} are the intensities of diagonal peaks, and I_{AB} and I_{BA} are the intensities of cross-peaks. The 5-H proton resonances at 6.8 and 7.7 ppm in CD_2Cl_2 (6.7 and 7.7 ppm in CCl_4) were applied for the calculation. The numbers in this figure are the applied temperature (K) and the slope values (Hz) of the least-squares lines.

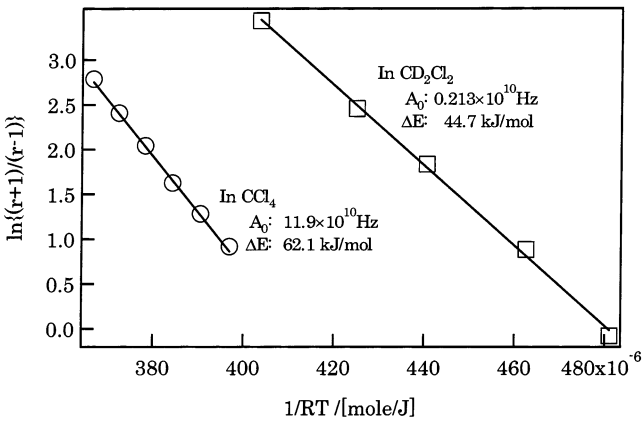

Figure 6. Arrhenius plot for the exchange rate of the (3^1R)-[E,E] BChl *c* antiparallel dimer in CD_2Cl_2 and CCl_4.

dimer. Table 1 lists the exchange rates calculated from the correlation between the 5-H signal intensities (I_{AA}, I_{BB}, I_{AB}, and I_{BA}) and the mixing time τ_m in 2-D EXSY. Just as was the case with (3^1R)-[E,E] BChl *c*, the values of $\ln\{(r+1)/(r-1)\}$ calculated from the 5-H intensities were proportional to the τ_m time in an appropriate range at each temperature (data not shown). However, the exchange rates were higher than those of (3^1R)-[E,E] BChl *c* at all the temperatures studied (Table 1), revealing that the antiparallel dimer formed from (3^1R)-[P,E] BChl *c* is more dynamic than that from (3^1R)-[E,E] BChl *c*. The Arrhenius parameters calculated from the exchange rates can account for the dynamics of the (3^1R)-[P,E] BChl *c* antiparallel dimer (Table 2). The activation energy of the (3^1R)-[P,E] BChl *c* dimer was larger than that of the (3^1R)-[E,E] BChl *c* dimer, while the frequency factor was 50-fold increased as compared to that of the (3^1R)-[E,E] BChl *c* dimer. Therefore, the substitution of a propyl group at the 8-position increases the activation energy in the exchange process of the antiparallel dimer, but instead increases the frequency factor so that the

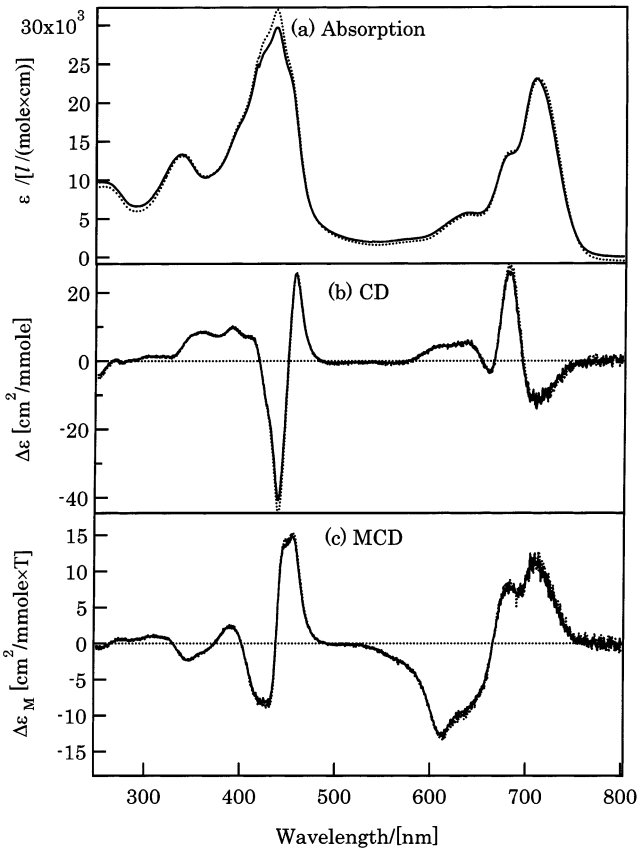

Figure 7. Absorption, CD, and MCD spectra of (3^1R)-[E,E] BChl *c* (solid line) and (3^1R)-[P,E] BChl *c* (dotted line) in CCl_4 at 2 mM concentration.

TABLE 1: Exchange Rates (Hz) between Two BChl *c* Molecules in the Antiparallel Dimer at Each Temperature

	temp (K)					
	303	308	313	318	323	328
(3^1R)-[E,E]	2.1	3.6	4.6	7.7	11.1	16.2
(3^1R)-[P,E]	5.0	7.4	12.2	18.2	27.6	40.3
(3^1R)-[E,E]/(3^1R)-[P,E][a]	2.3	3.6	5.9	9.9	13.6	18.3
(3^1R)-[E,E]/(3^1S)-[P,E][a]	4.8	8.2	13.3	20.1	31.3	50.0
(3^1R)-[P,E]/(3^1S)-[P,E][a]	7.0	11.2	16.2	25.9	37.1	54.4

[a] The (3^1R)- or (3^1S)-[P,E]BChl *c* was added to the (3^1R)-[E,E] or [E,E]BChl *c*-CCl_4 solution in a ratio of 1:9.

TABLE 2: Arrhenius Parameters Calculated from the Exchange Rates between Two BChl *c* Molecules in the Antiparallel Dimer[a]

	A_0 (10^{10} Hz)	ΔE (kJ/mol)	coefficient of determination
(3^1R)-[E,E]	11.9	62.1	0.996
(3^1R)-[P,E]	586	70.2	0.999
(3^1R)-[E,E]/(3^1R)-[P,E]	291	70.2	0.993
(3^1R)-[E,E]/(3^1S)-[P,E]	4789	75.3	0.998
(3^1R)-[P,E]/(3^1S)-[P,E]	448	68.5	0.998

[a] The Arrhenius parameter was calculated from the exchange rate *k* in Table 1 according to the equation $\ln k = \ln A_0 - \Delta E/RT$.

exchange between (3^1R)-[P,E] BChl *c* molecules become faster than that between (3^1R)-[E,E] BChl *c* molecules.

Influence of Minor BChl *c* Homologues upon the Stability of the (3^1R)-[E,E] BChl *c* Antiparallel Dimer. To investigate the effect of the BChl *c* homologue and diastereomer on the exchange dynamics of the (3^1R)-[E,E] BChl *c* antiparallel dimer, we added a small amount (1:9) of (3^1R)- or (3^1S)-[P,E] BChl *c* to the (3^1R)-[E,E] BChl *c* in CCl_4. The addition of (3^1R)- or

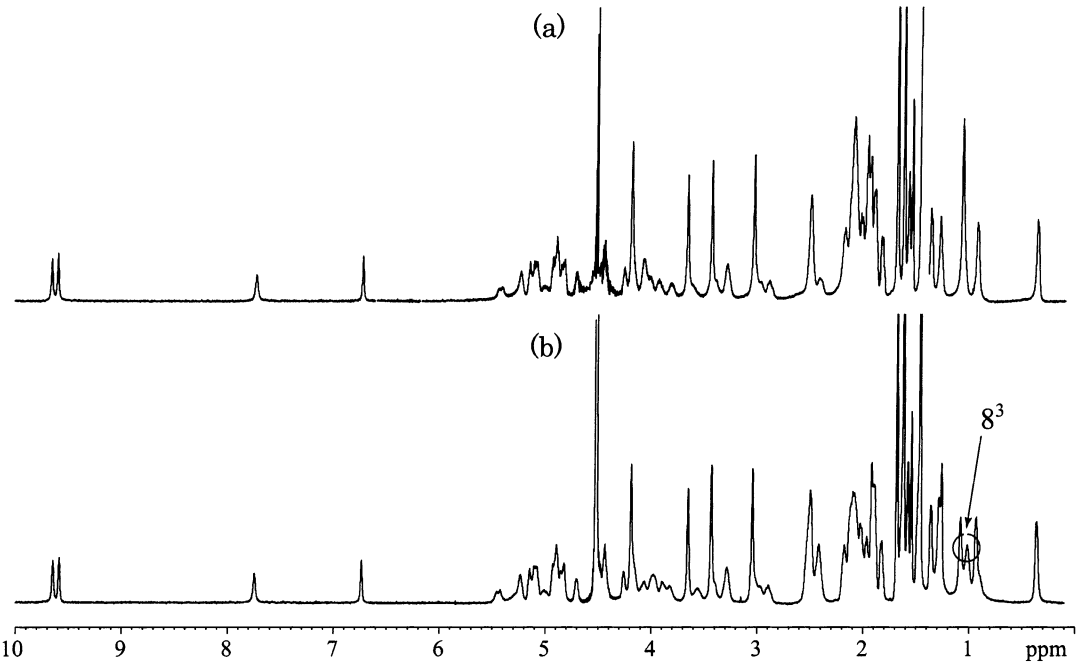

Figure 8. ^{1}H NMR spectra of (3^1R)-[E,E] BChl *c* (a) and (3^1R)-[P,E] BChl *c* (b) in CCl_4 at the BChl *c* concentration of 2 mM.

(3^1S)-[P,E] BChl *c* resulted in no change of the absorption, CD, and MCD spectra (data not shown). The exchange rate in the presence of (3^1R)-[P,E] BChl *c* was only little faster than that of pure (3^1R)- [E,E] BChl *c* at each temperature (Table 1). From the Arrhenius parameters, this is attributed to the canceling of the change in the frequency factor and activation energy (Table 2). However, the addition of (3^1S)-[P,E] BChl *c* to the (3^1R)-[E,E] BChl *c* antiparallel dimer made the exchange rate much faster (Table 1), indicating that the (3^1S)-[P,E] BChl *c* makes the (3^1R)-[E,E] BChl *c* antiparallel dimer more dynamic than (3^1R)-[P,E] BChl *c*. The Arrhenius parameters showed the increase of both the frequency factor and the activation energy (Table 2). The significant increase in the frequency factor far outweighs the impeding effect of the activation energy change. The (3^1S)-epimer has a greater effect on the exchange dynamics of the (3^1R)-[E,E] BChl *c* dimer than the (3^1R)-epimer with a propyl group at the 8-position. We also added (3^1S)-[P,E] BChl *c* to the (3^1R)-[P,E] BChl *c* antiparallel dimer. The exchange rate became a little faster (Table 1), and the Arrhenius parameters were comparable to those of pure (3^1R)-[P,E] BChl *c* (Table 2). The exchange dynamics of the (3^1R)-[P,E] BChl *c* dimer was less affected by the (3^1S)-epimer than the (3^1R)-[E,E] BChl *c* dimer.

Discussion

In this study, we have quantitatively investigated the dynamics of an antiparallel dimer formed from the most abundant homologue, (3^1R)-[E,E] BChl *c*, in the chlorosome of *Chlorobium tepidum*. The EXSY measurement clearly demonstrates the exchange between two BChl *c* molecules in the antiparallel dimer. The exchange rate was strongly dependent upon the polarity of solvent, and the increase in the exchange rate by solvent resulted from the lowering of the activation energy. The (3^1R)-[E,E] BChl *c* has two $Q_y(0-0)$ transitions (680 and ~710 nm) in CH_2Cl_2 and CCl_4, but the behavior of the two absorption components varies according to solvent (Figure 3).[9,19,24] In CH_2Cl_2 (dipole moment (μ) = 1.55), the $Q_y(0-0)$ transition predominantly appears around 680 nm at a BChl *c* concentration of ~1 mM, but the absorption maximum shifts from 680 to ~710 nm with the increase of the BChl *c* concentration.[9] In contrast, the 710-nm peak is the major component in CCl_4 ($\mu = 0$) even at low concentration, and the ratio of the 680-nm intensity to the 710-nm intensity changed only a little with the increase of the BChl *c* concentration to 3 mM.[9] Our recent CD and MCD studies revealed two kinds of dimers in CH_2Cl_2: an antiparallel dimer with two $Q_y(0-0)$ exciton components at 680 and 710 nm (Figure 1a), and a T-shaped dimer with a single $Q_y(0-0)$ transition at 680 nm (Figure 1b).[19] In CD_2Cl_2, the exchange system of the antiparallel dimer could be approximated by a two-site exchange mechanism, and no 5-H proton resonance was observed for the T-shaped dimer. These facts suggest that the T-shaped dimer is formed as a short-lived transition state in the exchange process of the antiparallel dimer. The proposed structure for the transient dimer is T-shaped with only a ligation of the 3^1-hydroxyl group to Mg (Figure 1b), which is supported by CD spectra.[19] Considering that the T-shaped dimer has an uncoordinating hydroxyl group different from the antiparallel dimer, the stabilization of the free hydroxyl group in the T-shaped dimer by CD_2Cl_2 decreases the activation energy. Additionally, we compared the exchange rate difference in CH_2Cl_2 and CCl_4 in terms of viscosity (CH_2Cl_2, 0.449 mM·s/m^2 at 288 K; CCl_4, 0.965 mM·s/m^2 at 293 K). The fact that the increase in the viscosity of the solvent led to the slowing of the exchange rate suggests that the viscosity is one of the factors slowing the exchange rate.

Self-aggregation of BChl with a hydroxyl group at the 3^1-position has been studied using various BChl molecules (*c*, *d*, and *e*).[13,15,33−35] For BChlide *d*, which differs from BChl *c* at the 20^1-position with a methyl group replaced by a proton and with the farnesyl chain replaced by a methyl group, the stability of the dimers had been estimated in CD_2Cl_2 from the line width of proton resonance[33] and the dynamic NMR experiments;[34] the activation energy of the dimers was estimated to be 48 and 51 kJ from the line broadening and the coalescence of the two 5-H resonances at 268 K, respectively. Considering the difference of the estimation methods, the activation energy is not strongly influenced by the methylation at the 20^1-position and the long ester chain at 17^3-position.

The BChl homologues also have variations in the substituents at the 8- and 12-positions.[27,36,37] Some previous studies have reported that the small aggregates (T-shaped and antiparallel dimers) formed from (3^1R)-[P,E] BChl *c* show spectroscopic properties similar to those of (3^1R)-[E,E] BChl *c*,[19,36,37] and the structural similarity of the (3^1R)-[P,E] BChl *c* antiparallel dimer was shown in this study (Figure 8). However, the EXSY measurements demonstrate that the propyl group at the 8-position destabilized the antiparallel dimer. The Arrhenius parameters suggest that the high exchange by the propyl group results from the increase of the frequency factor (Table 2). We also have measured the absorption and EXSY spectra in the BChl *c* concentration range of 0.5−3 mM for the purpose of observing the intermolecular effect from monomers and neighboring dimers (data not shown); in this concentration range, a monomer state of BChl *c* remained little[19] and the exchange rate at each temperature changed only little with concentration. This indicates that the exchange process is intramolecular, suggesting that the propyl group increases the steric effect. For large aggregates, some differences between (3^1R)-[E,E] and [P,E] BChl *c* have been reported. Ishii et al. reported that (3^1R)-[P,E] BChl *c* always forms aggregates absorbing at a longer wavelength than those of (3^1R)-[E,E] BChl *c* in acetone−water solution at various pH values,[38] and Mizoguchi et al. showed that (3^1R)-[P,E] BChl *c* is more likely to form a high aggregate with an absorbance at 740 nm in the mixture of CH_2Cl_2 and hexane than (3^1R)-[E,E] BChl *c*.[25] The high exchange dynamics of the antiparallel dimer by the 8-propyl group probably promotes the aggregation from dimer to high aggregates.

Recently, a relatively large number of studies have been made for investigation of the 3^1-epimer effect on aggregation behavior.[24,25,35] The abundant BChl *c* homologue in chlorosome is generally (3^1R)-type BChl *c*, and the ratio of (3^1S)-type BChl *c* to the whole BChl *c* amount in *Chlorobium tepidum* was only 6% according to our HPLC result ((3^1S)-[P,E] BChl *c*, 4%; (3^1S)-[I,E] BChl *c*, 2%). However, recent spectroscopic studies have suggested that (3^1S)-type BChl *c* is essential for the formation of chlorosome-type aggregates in vitro.[24,25,35] (3^1R)-type BChl *c* only forms the small aggregates with absorbance at 680 and 710 nm in CH_2Cl_2 and CCl_4, while (3^1S)-type BChl *c* easily forms large aggregates absorbing at 740 nm.[13,24] The 710-nm band for the (3^1R)-type BChl *c* in CH_2Cl_2 solution remains, even in the presence of (3^1S)-type BChl *c*,[24] but the (3^1S)-type BChl *c* promotes the formation of large 740-nm aggregates from (3^1R)-type BChl *c* in hexane.[25] The present EXSY studies clearly show that a small amount of (3^1S)-[P,E] BChl *c* enhances the exchange dynamics of (3^1R)-[E,E] BChl *c* dimer in CCl_4. Interestingly, the enhancement is not caused by the decrease of the activation energy which results from the stabilization of a transition state in the exchange process, but is caused by the significant increase of the frequency factor. This indicates that the interaction of (3^1S)-[P,E] BChl *c* with the (3^1R)-[E,E] BChl *c* dimer increases the chance for the dissociation of two BChl *c* molecules in the dimer, in agreement with the fact that (3^1S)-type BChl *c* promotes (3^1R)-type BChl *c* forming large aggregates. Our EXSY experiments show that the exchange dynamics of the (3^1R)-[P,E] BChl *c* dimer was less enhanced by (3^1S)-[P,E] BChl *c* than that of the (3^1R)-[E,E] BChl *c* dimer. This suggests that the simultaneous differences at the 3^1- and 8-positions generate a synergistic effect.

In conclusion, application of EXSY spectra to the BChl *c* dimer was effective for estimating the dynamics of the antiparallel dimer, which has not been quantitatively detected. The high polarity of solvent gives rise to a T-shaped dimer as a transient in the exchange process of an antiparallel dimer by solvation of the free hydroxyl group, resulting in the decrease of the activation energy, whereas the substituent variation at the 8-position changes the frequency factor, and the increase in the number of methylene makes the exchange rate fast. A small amount of (3^1S)-type BChl *c* also enhanced the dynamics of the (3^1R)-type BChl *c* dimer, and the effect was most obvious when the (3^1S)-[P,E] BChl *c* was added in the (3^1R)-[E,E] BChl *c* dimer. This indicates that the antiparallel dimer formed from the most abundant homologue of BChl *c* can be destabilized kinetically by one of the homologous minor component of BChl *c* in vivo.

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