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Characteristics of Light-Harvesting Complex II Mutant of *Rhodobacter sphaeroides* with Alterations at the Transmembrane Helices of β-Subunit

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Abstract—The peripheral light-harvesting complex II (LHII) is an important component of the photosynthetic apparatus of *Rhodobacter sphaeroides*. In this study, genetic, biochemical, and spectroscopic approaches were applied to investigate the spectral properties and functions of LHII in which two amino acid residues Phe32 and Leu42 in the transmembrane helix domain of *pucB*-encoded β-apoprotein were replaced by Leu and Pro. The mutated LHII complex showed blue shift of absorbance peaks in the near infrared region at ~801-845 nm in *R. sphaeroides*. It should be noted that the B800 peak was much lower than that of the native LHII, and transfer energy was efficient from the B800 to the B850 pigments in the LHII complex. The results suggest that the mutated *pucB* could be expressed in *R. sphaeroides*, and the functional LHII was assembled into the membrane of *R. sphaeroides* notwithstanding with the different spectral properties. These mutated residues were indeed critical for the modulation of characteristics and function of LHII complex.

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Key words: photosynthetic bacteria, light-harvesting complex II, *Rhodobacter sphaeroides*, mutagenesis, expression analysis

Photosynthesis is the single most important process developed by nature, providing all of the biological energy needed for higher forms of life to exist. The photosynthetic apparatus of the purple bacterium *Rhodobacter sphaeroides* consists of a photochemical reaction center (RC) surrounded and interconnected by light-harvesting (LH) complexes. The LHI complex is the immediate donor of excitation energy to the RC, and it receives energy in turn from the peripheral LHII complex [1, 2].

The bacteriochlorophyll (BChl)-containing LHII complex is an integral membrane protein that catalyzes the photosynthetic process in purple photosynthetic bacteria. The LHII complexes from *R. sphaeroides* show characteristic strong absorbance at 800 and 850 nm [3] due to the bacteriochlorophyll molecules confined in two separate areas of the protein and are composed of two pigmented integral membrane-spanning polypeptides, α and β, that anchor BChl and carotenoids in the membrane [4, 5].

It has been reported previously that the *puc* operon of *R. sphaeroides* consists of the *pucBA* structural genes encoding the LHII α- and β-polypeptides and an additional *pucC* gene; pucC affects the posttranscriptional expression and assembly of the LHII α- and β-polypeptides [6, 7].

LHII is a complicated complex. It is well established that assembly of the peripheral antenna complex, LHII, is required for proper photosynthetic membrane biogenesis in the purple bacterium *R. sphaeroides*. The underlying interactions of lipid–protein and BChl–protein are, as yet, not well understood [8-11].

It was reported by Kwa et al. that the LHII complex, specifically β-Glu20 and the polar head group of carotenoids, contributes to the shaping of the photosynthetic membrane by specific interactions with surrounding lipid molecules [12]. Previous work had shown that

Abbreviations: BChl, bacteriochlorophyll; ICM, intracytoplasmic membranes; LB, Luria–Bertani medium; LHI(II), light-harvesting complex I(II); PCR, polymerase chain reaction; PMSF, phenylmethylsulfonyl fluoride; RC, reaction center.

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changing β-His20 to Ser resulted in an LHII protein that lacked 800-nm-absorbing BChl in *R. capsulatus* [13].

Alterations at positions α-Tyr44 and α-Tyr45 in the LHII complex of the photosynthetic bacterium *R. sphaeroides* are known to produce blue shifts in bacteriochlorophyll absorbance [14].

In LHII, large portions of the transmembrane helices make up the dimeric BChl or lipid-binding site [15, 16]. In this study, we replaced two residues near the BChl-binding site of the transmembrane helices of pucB of *R. sphaeroides*. Replacement of both residues results in a blue shift of antenna LHII complexes in the membrane, which have energy transfer function.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *Rhodobacter sphaeroides* W1 (wild type, Chinese Collection of Microorganisms, Beijing, China) and *R. sphaeroides* DD13 [17] (genomic deletion of *pucBAC* and *pufBALMX*, insertion of Sm^R and Km^R genes) were grown aerobically or semi-aerobically under dark conditions in medium M22+. When required, antibiotics were added to M22+ at the following final concentrations: streptomycin (10 μg/ml), tetracycline (2 μg/ml) [18]. The semi-aerobic incubation was achieved by growing the bacteria in 500 ml conical flasks filled with 70% volume medium at 34°C with 150 rpm agitation [19].

Escherichia coli JM109 and S17-1 were cultivated in Luria–Bertani (LB) medium at 37°C. The final concentrations of antibiotics were as follows: ampicillin (100 μg/ml), tetracycline (50 μg/ml).

Bacterial strains, plasmids, and oligonucleotides used for PCR amplification are listed in the table.

DNA preparation and PCR amplification. Cultures of *R. sphaeroides* W1 from the logarithmic growth phase were collected, and chromosomal DNA was extracted as

Bacterial strains, plasmids, and primers

Description	Reference
Plasmids	
pMD18-T Amp^r; 2.8 kb, PCR product cloning vector	Takara
pMD18-T-*pucBA* Amp^r; pMD18-T with 346 pb PCR product containing *pucBA*	this work
pRK*puc*C; pRK415 containing *puc* promoter and *puc*C of *R. sphaeroides*, Tc^r	Fig. 1
pRK*pucBA*C; pRK*puc*C containing the *Sac*I/*BamH*I fragment form pMD18-T-*pucBA*, Tc^r	this work
pRK*pucBmAC*; mutagenesis *pucB* in pRK*pucBAC*, Tc^r	–"–
Bacterial strains	
R. sphaeroides W1	wild type
R. sphaeroides DD13; genomic deletion of *pucBA*, *pufBALMX*, insertion of Sm^R and Km^R	[17]
Escherichia coli	
JM109 recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 Δ(lac-proAB)	Takara
S17-1 Tp^r Sm^r hsdR pro recA RP4-2-Tc::Mu-Km::Tn7 in chromosome	[19]
DD13(pRK415); DD13 containing the plasmid pRK415	this work
DD13(pRK*pucBA*C); DD13 containing the plasmid pRK*pucBA*C	–"–
DD13(pRK*pucBmAC*); DD13 containing the plasmid pRK*pucBmAC*	–"–
Primers	
pucBA (F): 5'-AAGCTGAGCTCCATATGACTGACGATCTGAACAAAG-3'	
pucBA (R): 5'-AAGGATCCTTACTCGGCCGCGACCGCAGCCGAGCCTTG-3'	(cloned *pucBA*)
pucBm (F): 5'-CGGCACCCGCGTCTTGGGTGGCATGGCGCTCATCGCGCACTTCCCC-GCCGCCGCTGCG-3'	
pucBm (R): 5'-CGCAGCGGCGGCGGGGAAGTGCGCGATGAGCGCCATGCCACCCAA-GACGCGGGTGCCG-3'	(mutagenesis for *pucB*)

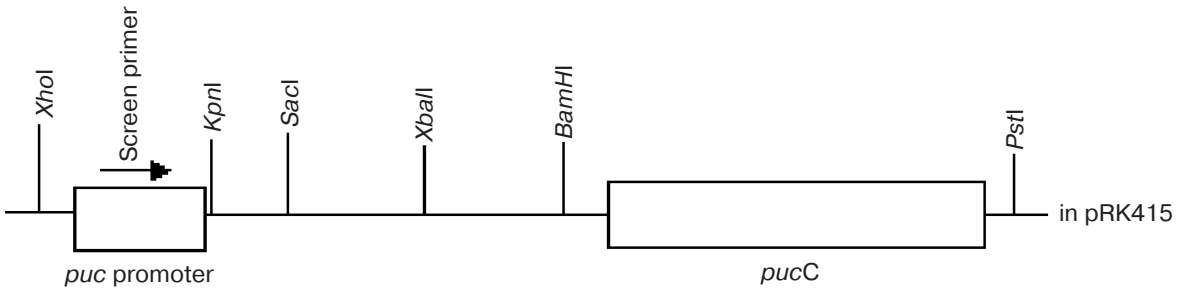

Fig. 1. Structure of LH expression vector pRK*pucC* (derived from pRK415).

previously described [20]. The *pucBA* genes from the *R. sphaeroides* were isolated using PCR, cloned into the pMD18-T plasmids, and sequenced to check their authenticity.

Construction of plasmids. The mobilizable plasmids used in this study were based on pRK*pucC* (see Fig. 1), which was used to express the *pucB* and *pucA* of *R. sphaeroides*. The plasmid pRK*pucC* (Tc^r) was derived from pRK415 encompassing *puc* promoter and *pucC* gene of *R. sphaeroides*. Standard procedures were used for plasmid isolation, restriction endonuclease digestion, ligation, and other molecular biological techniques [21].

The *pucB* fragments (containing engineered *Sac*I-*Xba*I ends) and *pucA* fragments (containing engineered *Xba*I-*BamH*I ends) of *R. sphaeroides* were cloned into the *Sac*I-*Xba*I and *Xba*I-*BamH*I sites of pRK*pucC* to produce pRK*pucBAC*, and the resulting constructions were confirmed by DNA sequencing.

Construction of *pucB* mutant containing LHII. LHII with Leu32 and Pro42 instead of Phe32 and Leu42 was constructed using a Site-Directed Mutagenesis Kit by directly mutating *pucB* in plasmid pRK*pucBAC* according to the manufacturer's instructions. The oligonucleotides of PCR used for mutagenesis *pucB* are listed in the table, so pRK*pucBmAC* was constructed [22, 23].

Conjugation techniques. The constructs were introduced into *R. sphaeroides* DD13 by conjugative transfer. The mobilizable plasmids to be introduced into *R. sphaeroides* were first transformed into *E. coli* strain S17-1, and then matings were performed as described in [24]. Transconjugants were grown aerobically in the dark on plates of medium M22+ supplemented with appropriate antibiotics: 2 μg/ml tetracycline and 10 μg/ml streptomycin.

Preparation of intracytoplasmic membranes (ICM). Following conjugative transfer, antibiotic-resistant strains were further screened for the presence or absence of light-harvesting complexes by absorption spectroscopy. *Rhodobacter sphaeroides* strains were grown under semi-aerobic or aerobic conditions in the dark at 34°C. Cultures were centrifuged, washed, and resuspended in 50 mM Tris-HCl (pH 8.0) containing 2.5 mM magnesium acetate and 1 mM phenylmethylsulfonyl fluoride (PMSF). Cells were disrupted in a French pressure cell, followed by centrifugation at 2000*g* for 20 min. The ICM were purified by harvesting from the interface of sucrose step gradients (15%/40%, w/w) after centrifugation in a Beckman Ti45 rotor at 27,000 rpm for 8 h.

Absorption spectroscopy. The absorption spectra of transconjugant strains were measured from 700 to 900 nm at room temperature on a Lambda-2 spectrometer (Perkin-Elmer, USA); DD13(pRK415) was used as the reference sample.

ICM were suspended in 10 mM Mops, 50 mM KCl (pH 7.2). Absorbance spectra in the VIS/NIR spectral region at room temperature were recorded on a Lambda-2 spectrometer; the reference was the ICM of DD13(pRK415) [25, 26].

Excitation transfer measurement. Fluorescence emission spectra were measured at room temperature using an LS50B spectrofluorimeter (Perkin-Elmer). ICM from semi-aerobically grown cells were diluted to absorbance $A_{680}^{1cm} = 0.05$ in 50 mM Tris-HCl, pH 8.0. The sample was excited at different wavelengths, and the fluorescence was measured with the spectrofluorimeter as described in [18, 27].

RESULTS

Analysis of mutagenesis of *pucB*-encoded polypeptides. Through site-directed mutagenesis, C^{96} and T^{125} of *pucB* were replaced by G and C, so Phe32 and Leu42 of pucB were replaced by Leu32 and Pro42 in pucBm; they are near putative H-bonded residues (see Fig. 2). Leu, Pro, and Phe are nonpolar hydrophobic amino acids, so the mutations did not change the characteristics of the amino acids, only their category.

The DAS Transmembrane Prediction server predictions suggested that the pucBm polypeptide contains only one transmembrane helix domain between Val21 and Ala46, and 3D structure predicted by SwissModel Automatic Modelling Mode revealed that the templates of pucB and pucBm polypeptides were automatically based

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RA-10050-b          ----------ATLTAEQSEELHKYVIDGTRVFLGLALVAHFLAFSATPWLH
R. sphaeroides-b    MTDDLNKVWPSGLTVAEAEEVHKQLILGTRVFGGMALIAHFLAAAATPWLG
R. sphaeroides-bm   MTDDLNKVWPSGLTVAEAEEVHKQLILGTRVLGGMALIAHFPAAAATPWLG
                      **      ** **  *  ***  *  * *** *  *****
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Fig. 2. Comparison of the apoprotein between pucB and pucBm of *Rhodobacter sphaeroides* and pucB of *Rhodopseudomonas acidophila* 10050. The underlined and residues in the β-subunits have been shown to have a role in the regulation of the spectral properties of the LHII. The polypeptide contains the transmembrane helix domain between Val21 to Ala46; the transmembrane helices are indicated by boldface type.

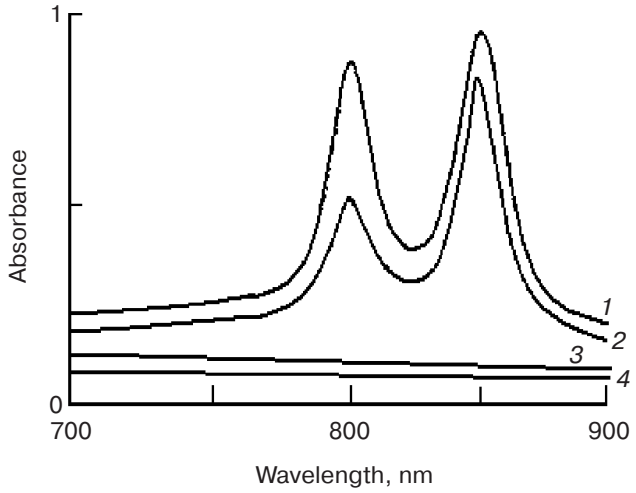

Fig. 3. Absorption spectra of membrane of transconjugant strains: *1*) DD13(pRK*pucBAC*); *2*) DD13(pRK*pucBmAC*); *3*) DD13(pRK415); *4*) DD13.

on β-apoprotein of LHII from *Rhodopseudomonas acidophila* 10050 (RA 10050), whose three-dimensional crystal structure has been determined with a high degree of resolution [28]; their sequence identities were 66 and 61%, respectively. Sequence analyses were performed with DNAMAN or web tools (http://www.sbc.su.se/~miklos/DAS/ and http://swissmodel.expasy.org/workspace).

Absorption spectroscopy of transconjugant strains and membranes. The absorption spectrum of the transconjugant strain DD13(*pRKpucBmAC*) was similar to that of strain DD13(*pRKpucBAC*), which showed the typical LHII absorption peaks at 800 and 850 nm. This means that the pucB mutant containing LHII has the same characteristics as *R. sphaeroides* DD13 (data not shown).

After spectroscopic analysis of the strains, their membranes were extracted for further spectroscopic analysis. The room temperature absorption spectra of membranes prepared from the transconjugant strains are shown in Fig. 3. The absorption spectra of the membranes from DD13(*pRKpucBmAC*) and DD13(*pRKpucBAC*) showed the typical LHII absorption peaks at 800 and 850 nm. Furthermore, the absorption properties of the synthesized LHII complexes containing pucB mutant differed from those of *R. sphaeroides* native LHII (λ_{max} = 800 and 850 nm). The LHII complex from the *pucBmA* gene pair showed blue shifted absorption peaks in the near infrared at 801 and 845 nm, and the 800 nm peak of DD13(*pRKpucBmAC*) was much lower than that of DD13(*pRKpucBAC*).

The spectra are scaled to reflect the level of LHII complex per cellular membrane amount as quantified by total membrane protein. It is clear that LHII arising from the gene *pucBmA* is present at comparatively low level; the expressed mutated LHII is less than that of native LHII of *R. sphaeroides* in DD13.

Energy transfer within LHII complexes. The expression of mutated LHII complexes depends on demonstration that the complexes are still functional, i.e. that they can harvest and transfer light energy.

To measure the ability of the 800 nm absorbing pigments to transfer energy to the 850 nm pigments within

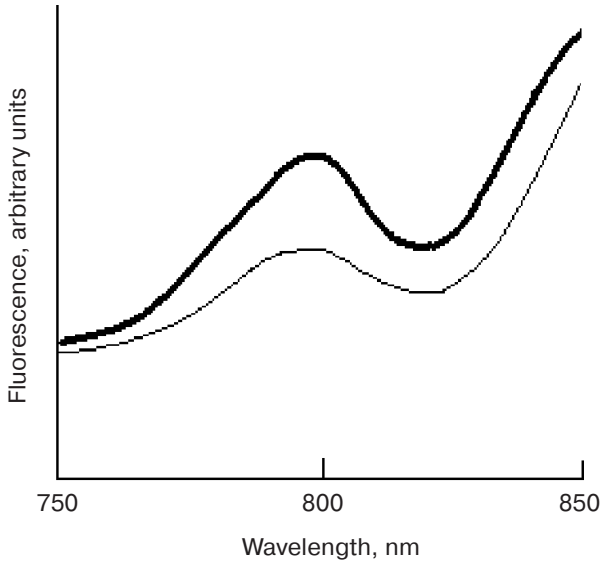

Fig. 4. Near infrared region of the fluorescence excitation spectra for membranes from the following strains: DD13(pRK*pucBmAC*) (solid curve), DD13(pRK*pucBAC*) (bold curve). As the wavelength of excitation was varied from 750 to 850 nm, the emission of fluorescence was registered at 900 nm.

the synthesized mutated LHII complexes, excitation spectra in the near infrared region of the spectrum were measured. This technique measures the ability of the 800 nm absorbing pigments to transfer energy to the 850 nm pigments, from which fluorescence is detected at 900 nm.

The absorption and emission spectra suggest that the mutated LHII complexes are able to transfer energy from their B800 to their B850 pigments. It can be seen that the 800 nm absorbing pigments within the mutated LHII are able to produce emission at 900 nm from the B850 band. The *R. sphaeroides* native LHII is the same.

Figure 4 shows the near infrared region of the fluorescence excitation spectra for membrane-bound complexes from the LHII-only strains DD13(*pRKpucBmAC*) and DD13(*pRKpucBAC*). It shows that energy is efficiently transferred from the B800 to the B850 pigments in the synthesized mutated LHII complexes.

DISCUSSION

In recent years, understanding of membrane protein structure and function has been greatly advanced. The interplay of lipids, pigments, and membrane proteins has been recognized to be vital for maintaining and optimizing their functions [29]. The ways through which membranes are formed into particular functional structures has gained recent attention. The number of factors known to be involved in membrane maturing and maintenance of particular membrane structures is continually increasing and has given some insight into the complexity of these processes.

The β-Arg30 and β-His40 residues of pucB from *R. sphaeroides* LHII are putative ligands of B800-850 pigments [2, 3]. Visschers reported that pigment was not incorporated in the complex for the site-specific mutant (βHis22→Ser) of the peripheral (B800-850) light-harvesting complex of *R. sphaeroides* [30]. Alteration of the residue β-Glu20 and the carotenoids of LHII results in altered ICM morphology [12].

In this study, only two amino acid residues near β-Arg30 and β-His40 in the transmembrane helix domain of *pucB*-encoded β-apoprotein, Phe32 and Leu42, were replaced by Leu32 and Pro42 in the *pucBm*-encoded polypeptide. The mutated LHII complex showed blue shifted absorption peaks in the near infrared at 801 and 845 nm, and the 800 nm peak of DD13(pRK*pucBmAC*) was much lower than that of DD13(pRK*pucBAC*). These characteristics were possibly induced by the 3D structure change of LHII for the pucB mutant. The mutagenesis near β-Arg30 and β-His40 of pucB affected the interactions lipid–protein and BChl–protein, the "integral protein lipids" and "integral protein BChl" were usually within a membrane protein or a membrane protein complex [10, 11], and the assembly of the LHII complexes is highly dependent on the particular side chain of the residues at this position in the helix.

Substitution of the residues at position β-32 and β-42 with different residues but still nonpolar hydrophobic amino acids residues, LHII shows a lowered 800 nm absorption peak (see Fig. 3). Figure 4 (solid line) shows that for the mutated LHII complex the excitation peak at 800 nm demonstrates that some B800→B850 transfer occurs; there are indications of some energy transfer from the B800 to the B850 pigments, but the efficiency of this process needs to be quantified.

The mutagenesis studies suggest that these residues are indeed critical for the binding and functional modulation of the LHII complex. We demonstrate that mutagenesis does induce significant alterations to the tertiary structure of the LHII complex.

The crystal structures of LHII proteins isolated from *Rps. acidophila* strain 10050 [28] and *Rhodospirillum molischianum* have been reported, and this work is helpful in understanding the crystal structure of LHII from *R. sphaeroides* [31], which is still not determined.

This work opens up the possibility of creating site-directed LHII mutants from bacteria for which no genetic system is available. This is particularly significant for understanding the structures and functions of LHII from purple photosynthesis bacteria.

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REFERENCES

1. Bahatyrova, S., Frese, R. N., Siebert, C. A., Olsen, J. D., van der Werf, K. O., van Grondelle, R., Niederman, R. A., Bullough, P. A., Otto, C., and Hunter, C. N. (2004) *Nature*, **430**, 1058-1062.
2. McDermott, G., Prines, S. M., Freer, A. A., Hawthornthwaite-Lawless, A. M., Paplz, M. Z., Cogdell, R. J., and Isaacs, N. W. (1995) *Nature*, **374**, 517-521.
3. He, Z., Sundstrom, V., and Pullerits, T. (2001) *FEBS Lett.*, **496**, 36-39.
4. Gall, A., Cogdell, R. J., and Robert, B. (2003) *Biochemistry*, **42**, 7252-7258.
5. Kiley, P. J., and Kaplan, S. (1987) *J. Bacteriol.*, **169**, 3268-3275.
6. Lee, J. K., Kiley, P. J., and Kaplan, S. (1989) *J. Bacteriol.*, **171**, 3391-3405.
7. Choudhary, M., and Kaplan, S. (2000) *Nucleic Acids Res.*, **28**, 862-867.

8. Fowler, G. J. S., Visschers, R. W., Grief, G. G., van Grondelle, R., and Hunter, C. N. (1992) *Nature*, **355**, 848-850.
9. Burgess, J. G., Ashby, M. K., and Hunter, C. N. (1988) *J. Gen. Microbiol.*, **135**, 1809-1816.
10. Palsdottir, H., and Hunte, C. (2004) *Biochim. Biophys. Acta*, **1666**, 2-18.
11. Hunte, C. (2005) *Biochem. Soc. Trans.*, **33**, 938-942.
12. Kwa, L. G., Wegmann, D., Brugger, B., Wieland, F. T., Wanner, G., and Braun, P. (2008) *Mol. Microbiol.*, **67**, 63-77.
13. Buche1, A., Ellis, G., and Ramirez, J. M. (2001) *Eur. J. Biochem.*, **268**, 2792-2800.
14. Fowler, G. J. S., Sockalingam, G. D., Robert, B., Grief, G. G., and Hunter, C. N. (1994) *Biochem. J.*, **299**, 695-700.
15. Olsen, J. D., Sturgis, J. N., Westerhuis, W. H., Fowler, G. J. S., Hunter, C. N., and Robert, B. (1997) *Biochemistry*, **36**, 12625-12632.
16. Garcia-Martin, A., Kwa, L. G., Strohmann, B., Robert, B., Holzwarth, A. R., and Braun, P. (2006) *J. Biol. Chem.*, **281**, 10626-10634.
17. Jones, M. R., Fowler, G. J. S., Gibson, L. C. D., Grief, G. G., Olsen, J. D., Crielaard, W., and Hunter, C. N. (1992) *Mol. Microbiol.*, **6**, 1173-1184.
18. Fowler, G. J. S., and Hunter, C. N. (1996) *J. Biol. Chem.*, **271**, 13356-13361.
19. Fowler, G. J. S., Gardiner, A. T., Mackenzie, R. C., Barratt, S. J., Simmons, A. E., Westerhuis, W. H., Cogdell, R. J., and Hunter, C. N. (1995) *J. Biol. Chem.*, **270**, 23875-23882.
20. Raiger Iustman, L. J., Kerber, N. L., Pucheu, N. L., Bornmann, M. J., Kohler, S., Labahn, A., Tadros, M., Drews, G., and Augusto, F. G. (2006) *Arch. Microbiol.*, **185**, 407-415.
21. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*, 2nd Edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
22. Rashtchian, A., Thornton, C. G., and Heidecker, G. (1992) *PCR Meth.*, **2**, 124-130.
23. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 488-492.
24. Hunter, C. N., and Turner, G. (1988) *J. Gen. Microbiol.*, **134**, 1471-1480.
25. Gall, A., Fowler, G. J. S., Hunter, C. N., and Robert, B. **(**1997) *Biochemistry*, **36**, 16282-16287.
26. Davis, C. M., Bustamante, P. L., Todd, J. B., Parkes-Loach, P. S., McGlynn, P., Olsen, J. D., McMaster, L., Hunter, C. N., and Loach, P. A. (1997) *Biochemistry*, **36**, 3671-3679.
27. Hess, S., Visscher, K. J., Pullerits, T., Sundstrom, V., Fowler, G. J. S., and Hunter, C. N. **(**1994) *Biochemistry*, **33**, 8300-8305.
28. Cogdell, R. J., Isaacs, N. W., Freer, A. A., Arrelano, J., Howard, T. D., Papiz, M. Z., Hawthornthwaite-Lawless, A. M., and Prince, S. (1997) *Prog. Biophys. Mol. Biol.*, **68**, 1-27.
29. Braun, P., Olsen, J. D., Strohmann, B., Hunter, C. N., and Scheer, H. (2002) *J. Mol. Biol.*, **318**, 1085-1095.
30. Visschers, R. W., Crielaard, W., Fowler, G. J. S., Hunter, C. N., and Vangrondelle, R. (1994) *Biochim. Biophys. Acta*, **1183**, 483-490.
31. Walz, T., Jamieson, S. J., Bowers, C. M., Bullough, P. A., and Hunter, C. N. (1998) *J. Mol. Biol.*, **282**, 833-845.