*Articles*

# Role of the Core Region of the PufX Protein in Inhibition of Reconstitution of the Core Light-Harvesting Complexes of *Rhodobacter sphaeroides* and *Rhodobacter capsulatus*[†]

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ABSTRACT: PufX, the protein encoded by the *pufX* gene of *Rhodobacter capsulatus* and *Rhodobacter sphaeroides*, has been further characterized. The mature forms of these proteins contain 9 and 12 fewer amino acids, respectively, at the C-terminal end of the protein than are encoded by their *pufX* genes. To identify the portion of PufX responsible for inhibition of LH1 formation in reconstitution experiments, different regions (N-terminus and several core regions containing different lengths of the C-terminus) of *Rb. sphaeroides* and *Rb. capsulatus* PufX were chemically synthesized. Neither the N- nor C-terminal polypeptides of *Rb. sphaeroides* were inhibitory to LH1 reconstitution. However, all core segments were active, causing 50% inhibition at a concentration ratio of between 3:1 and 6:1 relative to the LH1 α-polypeptides whose concentrations were 3−4 $\mu$M. CD measurements indicated that the core segment containing 39 amino acids of *Rb. sphaeroides* PufX exhibited 47% α-helix in trifluoroethanol while the core segment containing 43 amino acids of *Rb. capsulatus* PufX exhibited 59 and 55% α-helix in trifluoroethanol and in 0.80% octylglucoside in water, respectively. Approximately 50% α-helix was also indicated by a PHD (Burkhard−Rost) structure prediction. Binding of bacteriochlorophyll to these PufX core segments is implicated.

A supramolecular complex existing in the membranes of photosynthetic bacteria is responsible for absorbing light and converting this energy into an electrochemical gradient of protons. Structural elements of this complex include the reaction center (RC), a set of core light-harvesting pigments (LH1)[1] and possibly several other components (*1*−*4*). The structure of the RC as crystallized from isolated detergent complexes has been determined for several bacteria (*5*−*9*). Although a high-resolution structure of LH1 has not yet been obtained, the structure of a related peripheral light-harvesting complex (LH2) has been determined (*10*−*12*). In addition, a low resolution structure of LH1 of *Rhodospirillum rubrum* has been determined using electron cryomicroscopy (*13*, *14*). On the basis of these data, it has been suggested that LH1 has a ring-like structure that encircles the RC (*15*, *16*). This is problematic, however, in that if LH1 completely encircles the RC, it is unclear how the reducing equivalents collected in $Q_BH_2$ of the RC could reach the bc1 complex.

It is likely that the supramolecular photoreceptor complex (also called the core complex, containing LH1 and the RC) contains additional proteins, some of which may interface between LH1 and the RC. It has been proposed that in *Rb. capsulatus* and *Rb. sphaeroides* the *pufX* gene product is contained in the complex and enables reducing equivalents of $Q_BH_2$ to reach the bc1 complex (*17*−*19*). PufX was isolated from both *Rb. capsulatus* and *Rb. sphaeroides* and shown to inhibit reconstitution of LH1 and thus was proposed to interrupt LH1 encirclement of the RC (*20*). Recent experiments have indicated that PufX exists in a 1:1 mole ratio to the RC in *Rb. sphaeroides* and that it may be involved in dimerization of core complexes (*21*). Further suggestions that the core complex might be dimeric in vivo were based on electron microscopic measurements of an LH2(−) mutant of *Rb. sphaeroides* (*22*). In the latter report, it was suggested that the bc1 complex was also a component of this supramolecular complex. Working with this same LH2(−) mutant, linear dichroism measurements indicated that PufX was

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[1] Abbreviations: Bchl, bacteriochlorophyll *a*; LH1, core light-harvesting complex (also referred to as B875 after the near-infrared absorption maximum); LH2, peripheral light harvesting complex (also referred to as B800-850 after the near-infrared absorption maxima); subunit complex (also referred to as B820), subunit form of LH1 or LH2 either isolated from membranes or prepared by reconstitution using native α- and β-polypeptides and Bchl; subunit-type complex, in vitro reconstituted complex exhibiting absorption and CD spectra very similar to those of the native subunit complex but containing a non-native α- and β-polypeptide combination or only a native β polypeptide and Bchl; PufX, protein coded for by the *pufX* gene of *Rb. sphaeroides* or *Rb. capsulatus*; core region of PufX, middle region of PufX exhibiting the most similarity in amino acid sequence between *Rb. sphaeroides* and *Rb. capsulatus* (approximately from amino acid 20 to 66); CD, circular dichroism; OG, *n*-octyl-β-D-glucopyranoside; HFA, hexafluoroacetone trihydrate.

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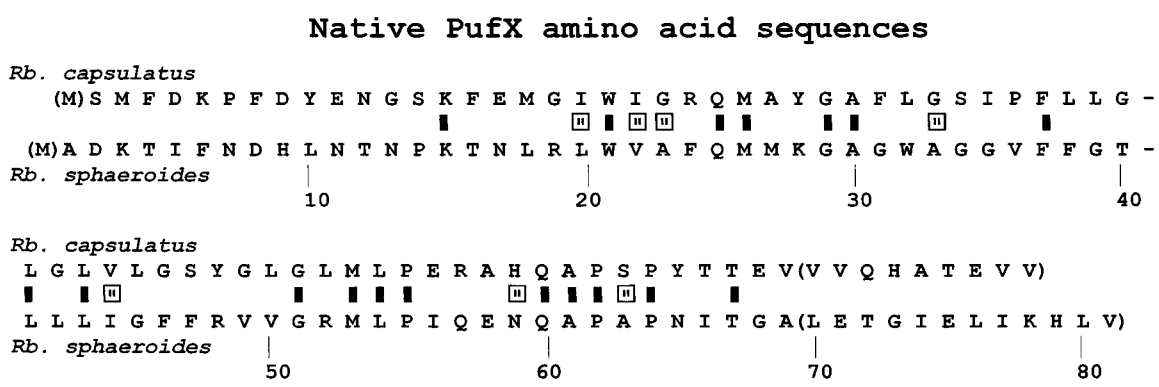


FIGURE 1: Amino acid sequence of PufX from *Rb. capsulatus* (*31*) and *Rb. sphaeroides* (*32*). The symbols (solid squares) and (open squares with two vertical black lines) indicate identical and very similar amino acids, respectively, between the two sequences. Parentheses indicate amino acids encoded by the *pufX* gene sequence, but not present in the isolated mature protein.

responsible for a specific orientation of LH1 to the RC and also enabled long-range order (*23*). Taken together, these results imply that PufX is intimately involved in the structure of the core complex in *Rb. capsulatus* and *Rb. sphaeroides*.

In this paper, PufX isolated from *Rb. sphaeroides* and *Rb. capsulatus* are further characterized. In addition, portions of PufX of *Rb. sphaeroides* and *Rb. capsulatus* were chemically synthesized and used to examine the ability of different regions of PufX to inhibit the formation of LH1. Only the core segments proved effective in this regard.

## MATERIALS AND METHODS

*n*-Octyl-$\beta$-D-glucopyranoside (OG) was obtained from the Sigma Chemical Co. Hexafluoroacetone trihydrate (HFA) and trifluoroethanol (TFE) were purchased from Aldrich Chemical Co. HPLC grade solvents were obtained from Burdick & Jackson. The PufX proteins (*20*) and the LH1 $\alpha$- and $\beta$-polypeptides (*4*, *24*−*26*) of *Rb. sphaeroides* and *Rb. capsulatus* were isolated as previously described.

Chemical syntheses of polypeptides were carried out in an atmosphere of ultrapure nitrogen on a Milligen 9600 Peptide Synthesizer using 9-fluorenylmethoxycarbonyl benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium-hexafluorophosphate (Fmoc-BOP) chemistry using BOP or PyBOP. Fmoc-L-amino acids were obtained from either Perseptive Biosystems or Novabiochem. The procedures followed were previously described (*27*). Mass spectral analyses were carried out by the Harvard Microchemistry Facility (Cambridge, MA) or the Macromolecular Structure Facility at Michigan State University. Determination of N- and C-terminal amino acid sequences were carried out by the Macromolecular Structure Facility at Michigan State University.

Reconstitutions were performed as previously described (*28*), using *n*-octyl-$\beta$-D-glucopyranoside (OG). Bchl*a* with a geranyl-geraniol esterifying alcohol was isolated from the G9 carotenoidless mutant of *Rs. rubrum* following earlier procedures (*28*). This form of Bchl was chosen rather than Bchl*a* with a phytol esterifying alcohol, the form of Bchl found in *Rb. sphaeroides*, because the former is more easily isolated, and previous studies with *Rb. sphaeroides* and *Rs. rubrum* (*29*) have shown that the nature of the esterifying alcohol has no effect on formation of subunit and LH1 complexes.

Absorption spectra were recorded on a Shimadzu UV160 spectrophotometer. CD spectra were measured on a Jasco J500C spectropolarimeter using 0.1, 1, or 2 cm quartz cuvettes. The percent $\alpha$-helical content was calculated from the CD data on the basis of the molar ellipticity at 208 nm (*30*).

## RESULTS

*Mature PufX*. PufX was isolated from an LH1(−)LH2(−) mutant (RCO1(+)) of *Rb. sphaeroides* as previously described (*20*). A portion of one sample was submitted for N-terminal amino acid sequencing and a second portion was submitted for mass spectral analysis. Another sample from a separate preparation was also submitted for mass spectral analysis. The N-terminal amino acid sequence found was ADKTIF, which is in agreement with that previously found and that expected from the gene sequence except for the absence of the N-terminal formylmethionine (Figure 1). The two separate mass spectral analyses gave very similar values of 7576 and 7582, which are substantially smaller than the mass predicted by the gene sequence (*32*). A sample of isolated PufX was also submitted for determination of the C-terminal amino acid. The amino acid obtained was Ala, which is consistent with the mass expected if 12 C-terminal amino acids predicted by the gene sequence are not present in the mature protein; its mass is accordingly calculated to be 7575.

Similarly, PufX was isolated from an LH2(−) mutant (PTB999) of *Rb. capsulatus* (*20*), and samples were submitted for N-terminal amino acid sequencing and mass spectral analysis. The N-terminal amino acid sequence was SMFDK, which is in agreement with that previously obtained (*20*) and that predicted by the gene sequence minus the N-terminal formylmethionine (*31*) (Figure 1). Mass spectral analysis gave 7490, once again indicating that there were fewer amino acids present (in this case there were nine less) than predicted by the gene sequence. This is the predicted mass for a protein ending at Val66 (Figure 1) assuming an oxidized Met. Because of the quantity of protein required, it has so far not been possible to obtain the C-terminal amino acid sequence.

*Chemically Synthesized Polypeptides Reproducing Portions of Rb. sphaeroides PufX*. Isolation of PufX is labor intensive, and a great deal of effort results in only very small quantities of pure material. To obtain larger quantities for more complete characterization of this protein, and to evaluate which part of the protein might be responsible for inhibition of LH1 formation, three separate regions of *Rb. sphaeroides* PufX were chemically synthesized (Figure 2).

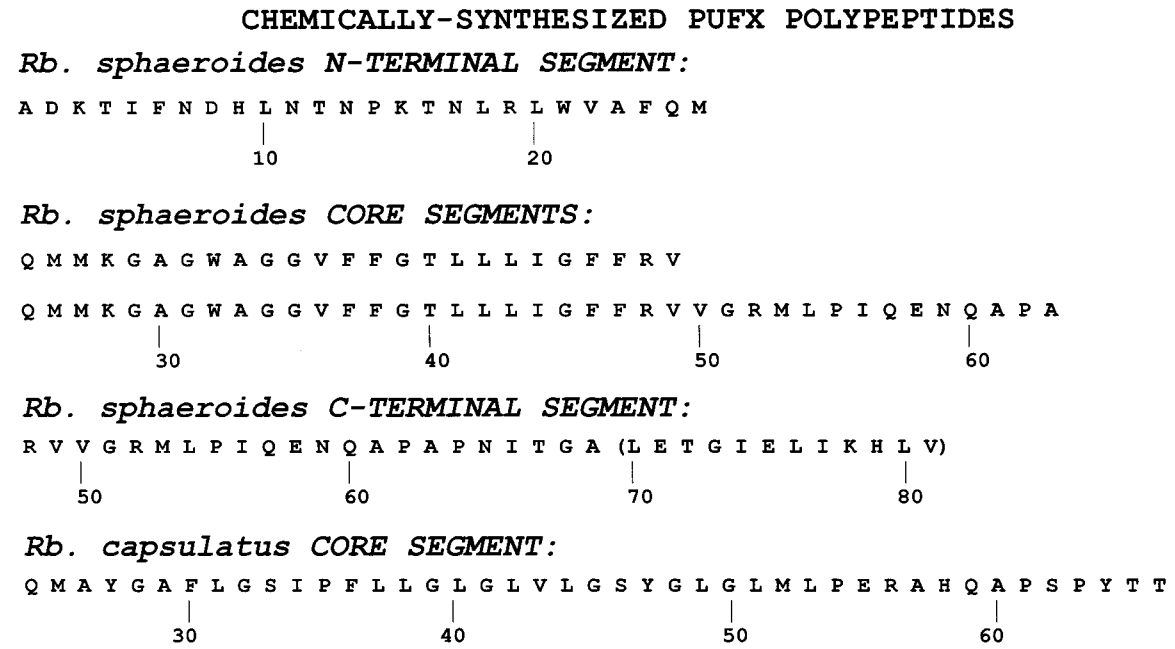


FIGURE 2: Chemically synthesized polypeptides reproducing part of the *Rb. sphaeroides* or *Rb. capsulatus* PufX sequence. Amino acids in parentheses were predicted by the *pufx* gene sequence but not present in the mature protein.

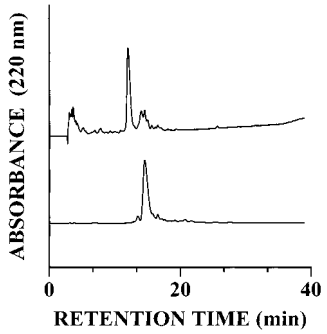


FIGURE 3: HPLC chromatograms of the synthetic C- and N-terminal polypeptides reproducing the amino acid sequences of the last 34 (top trace) and the first 31 (except for fMet) amino acids (bottom trace), respectively, of PufX from *Rb. spheroides* according to the gene sequence. A reverse-phase C18 column was used following procedures described in ref *28*. For the C-terminal polypeptide, the gradient used was previously described in ref *28*. For the N-terminal polypeptide, the column was first equilibrated with an 80:20 mixture (v/v) of A and B solutions. Solution A contained water and acetonitrile in a 50:50 (v/v) ratio with 0.1% trifluoroacetic acid. Solution B contained acetonitrile and 2-propanol in 50:50 (v/v) ratio with 0.1% trifluoroacetic acid. After equilibration, the sample was injected and the column was exposed to a linear gradient varying from 20 to 100% B.

The N- and C-terminal segments were easily synthesized and purified as they were water-soluble polypeptides. The HPLC profiles of these segments are shown in Figure 3. Exposing the isolated polypeptides to a second HPLC resulted in a single peak at the same elution time as the original. Each of these polypeptides was found to be without effect on the reconstitution of LH1 even at high concentration ratios (e.g., 10:1) (data not shown). The C-terminal segment represents the entire C-terminal end as predicted by the gene sequence (Figures 1 and 2) as it was synthesized before the smaller size of the mature protein was known. It is of value to consider because it is water soluble and also contains 21 amino acids of the core region.

Two different portions of the core region of *Rb. sphaeroides* PufX were synthesized. The first of these reproduced the amino acid sequence from residue 25 to 49 which contains six Gly and, with the exception of a Lys near the N-terminus and an Arg near the C-terminus, is very hydrophobic. Mass spectral analysis indicated a mass of 2704 in excellent agreement with that expected (2704). This polypeptide proved to be very difficult to dissolve and manipulate. A tripartite solvent consisting of formic acid/acetonitrile/HFA in a 1:1:1 ratio was somewhat effective and allowed partial characterization of this segment. As shown in Figure 4A, this polypeptide inhibited LH1 formation causing 50% inhibition (based on the size of the 874 nm peak expected in the absence of PufX material) at an approximately 3:1 ratio to the *Rb. sphaeroides* α-polypeptide which was 3 $\mu$M.

A second, somewhat longer region of *Rb. sphaeroides* PufX core was synthesized to include more of the C-terminal portion. In addition to the 25 amino acids of the first core segment (residues 25 to 49 of *Rb. sphaeroides* PufX), an additional 14 amino acids were included at the C-terminus, seven of which are identical between PufX of *Rb. sphaeroides* and that of *Rb. capsulatus* (compare Figures 1 and 2). This polypeptide, although larger, was much better behaved than the first core segment synthesized, could be dissolved in many solvents we have commonly used, and was easily isolated by HPLC (Figure 5 top; 15 min peak). Exposing the isolated polypeptides to a second HPLC run resulted in a single peak at the same elution time as the original. Mass spectral analysis gave a value of 4213, which is in excellent agreement with the expected value of 4210. When this longer core polypeptide segment was tested for its effect on LH1 reconstitution, an inhibition similar to that observed with the first core segment was found (Figure 4B). The concentration ratio of PufX to the α-polypeptide at which a 50% inhibition was observed (based on the size of the 874-nm peak expected in the absence of PufX material) was again about 3:1.

*Chemically Synthesized Polypeptides Reproducing Part of Rb. capsulatus PufX*. After working with the synthetic polypeptides that reproduced part of the *Rb. sphaeroides*

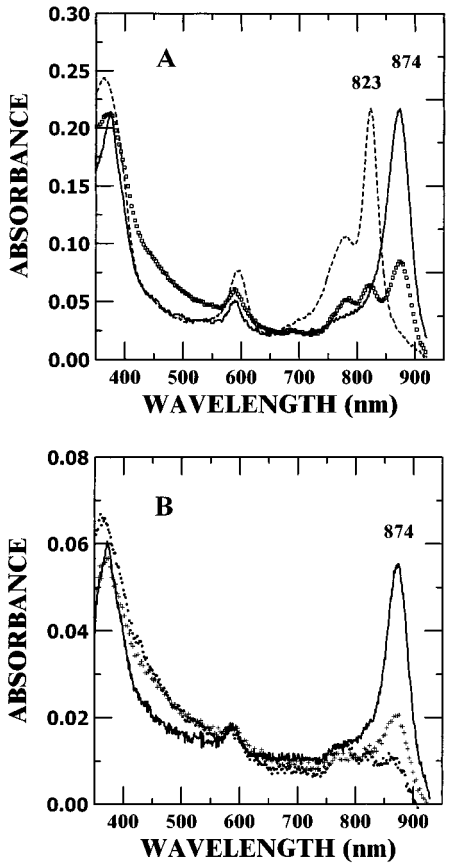


FIGURE 4: Inhibition of *Rb. sphaeroides* LH1 formation by the 26mer (A) and 39mer (B) core segments of *Rb. sphaeroides* PufX. (A) Dashed line, subunit complex at 0.90% OG; solid line, LH1 formed upon standing overnight at 4 °C in 0.66% OG; squares, LH1 formed upon standing overnight at 4 °C in 0.66% OG in the presence of a 6:1 ratio of PufX 26mer/LH1 α. Concentrations at 0.90% OG were $\alpha = 2.9$ $\mu$M, $\beta = 2.9$ $\mu$M, PufX 26mer = 19.2 $\mu$M, and Bchl = 3.7 $\mu$M. (B) Solid line, LH1 formed in the absence of PufX 39mer upon standing overnight at 4 °C in 0.66% OG; plus signs, LH1 formed in the in the presence of PufX 39mer at a 6:1 ratio of PufX 39mer/LH1α; dotted curve, LH1 formed in the presence of PufX 39mer at a 10:1 ratio of PufX 39mer/LH1α. Concentrations at 0.66% OG were $\alpha = 3.0$ $\mu$M, $\beta = 2.1$ $\mu$M, 39mer (solid circles) = 18 $\mu$M, 39mer (open circles) = 30 $\mu$M and Bchl = 0.64 $\mu$M. To enable a more quantitative comparison of the three spectra, those at 0.75% OG and 0.66% OG were adjusted for dilution relative to the spectra at 0.90% OG.

PufX, a somewhat larger segment of the *Rb. capsulatus* PufX core region was synthesized which reproduced the amino acid sequence from Gln24 through Val66 (Figures 1 and 2). Thus, it contains all of the C-terminus of mature PufX except for the last two amino acids that are not conserved between *Rb. capsulatus* and *Rb. sphaeroides* (Figure 1). This 43mer readily dissolved in the solvent systems usually employed in purification and reconstitution studies, and it gave a major peak on HPLC (Figure 5, bottom, 18 min peak). Exposing the isolated polypeptides to a second HPLC resulted in a single peak at the same elution time as the original. Mass spectral analysis of this material gave a value of 4511, which is in excellent agreement with the expected value of 4509. When assayed in a reconstitution system containing *Rb. capsulatus* LH1 α- and $\beta$-polypeptides and Bchl, inhibition of both subunit and LH1 formation was observed (Figure 6). This result is parallel to that obtained with native PufX of *Rb. capsulatus* (*20*) except that a higher concentration of

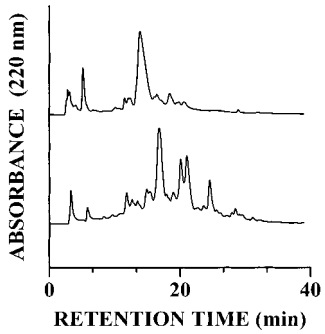


FIGURE 5: HPLC chromatograms of the 39mer core segment of PufX from *Rb. sphaeroides* (top) and the 43mer core segment of PufX from *Rb. capsulatus* (bottom) having the amino acid sequences indicated in Figure 2. The peak at 15 min in the top trace was collected and that at 18 min in the bottom trace was collected. The solvent gradients used have been previously described (*20*, *28*).

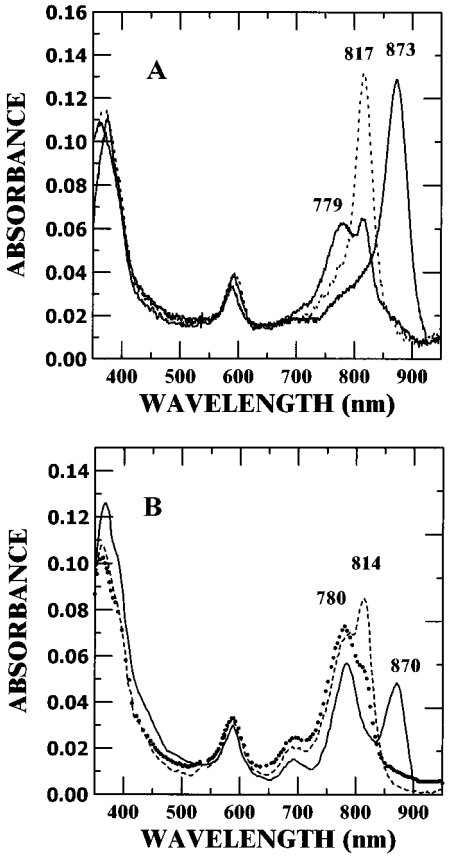


FIGURE 6: Inhibition of *Rb. capsulatus* LH1 formation by the 43mer core segment of *Rb. capsulatus* PufX. (A) Control reconstitution assay utilizing the LH1 α- and $\beta$-polypeptide of *Rb. capsulatus* and Bchl. Spectra were recorded at 0.90% OG (solid curve with maxima at 779 and 817 nm), 0.75% OG (dashed curve), and 0.66% OG after standing overnight at 4 °C (solid curve with maximum at 873 nm). Concentrations at 0.90% OG were $\alpha = 4.1$ $\mu$M, $\beta = 2.9$ $\mu$M, and Bchl = 1.3 $\mu$M. (B) Same reconstitution as panel A but in the presence of *Rb. capsulatus* 43mer. Spectra were recorded at 0.90% OG (dotted line), 0.75% OG (dashed line), and 0.66% OG after standing overnight at 4 °C (solid curve with absorption maximum at 870 nm). Concentrations at 0.90% OG were $\alpha = 4.1$ $\mu$M, $\beta = 2.9$ $\mu$M, Bchl 1.2 $\mu$M, and *Rb. capsulatus* 43mer = 24 $\mu$M. To enable a more quantitative comparison of the three spectra, those at 0.75% OG and 0.66% OG were adjusted for dilution relative to the spectra at 0.90% OG.

the synthetic polypeptide was required (50% inhibition was observed at an approximately 5:1 ratio of 43mer to *Rb.*

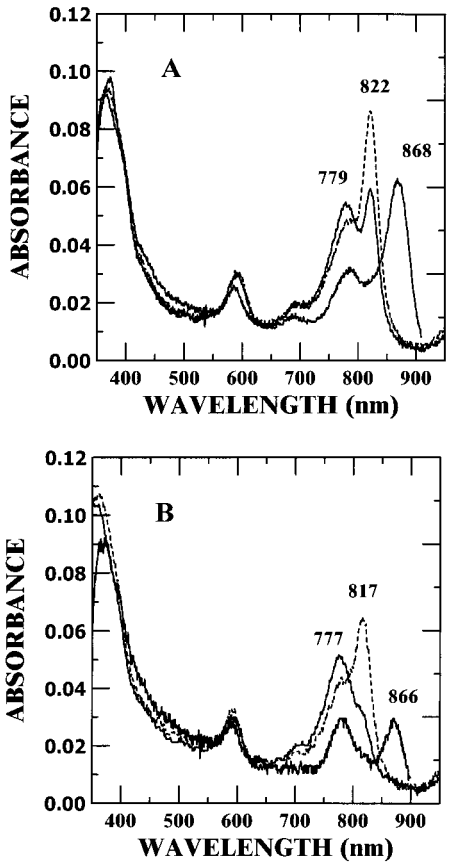


FIGURE 7: Inhibition of LH1 formation of *Rb. sphaeroides* by the 43mer of *Rb. capsulatus* PufX (A) and LH1 formation of *Rb. capsulatus* by the 39mer of *Rb. sphaeroides* PufX (B). At 0.90% OG, each α-polypeptide was 4.1 $\mu$M and each $\beta$-polypeptide was 2.9 $\mu$M. The *Rb. capsulatus* 43mer and the *Rb. sphaeroides* 39mer were six times the concentration of the α-polypeptide. Bchl concentrations were 1.2 $\mu$M (A) and 0.8 $\mu$M (B). Spectra were recorded at 0.90% OG [solid curves with maxima at 779 nm (A) and 777 nm (B)], 0,75% OG (dashed curves) and 0.66% OG after standing overnight at 4 °C [solid curves with maxima at 868 nm (A) and 866 nm (B)]. To enable a more quantitative comparison of the three spectra, those at 0.75% OG and 0.66% OG were adjusted for dilution relative to the spectra at 0.90% OG.

*capsulatus* LH1 α-polypeptide which was 4 $\mu$M as compared with a 0.5:1 ratio required for 50% inhibition by native PufX, based on the size of the 873-nm peak expected in the absence of PufX material (*20*)).

*Assay for Cross-Reactivity.* Native *Rb. sphaeroides* PufX had very little effect on reconstitution systems using *Rb. capsulatus* LH1 α- and $\beta$-polypeptides and Bchl (*20*). Similarly, native *Rb. capsulatus* PufX had very little effect on reconstitution systems using *Rb. sphaeroides* LH1 α- and $\beta$-polypeptides and Bchl (*20*). Since the synthetic core segments of PufX lack significant portions of the N-terminal region of native PufX, it was of interest to determine whether these polypeptides would exhibit inhibition in both reconstitution systems or would exhibit the species specificity of the corresponding native PufX. Also, because a plentiful supply of the synthetic polypeptides were available, much higher concentrations could be tested than was possible with the native polypeptides (*20*). As can be seen in Figure 7A, the 43mer of *Rb. capsulatus* PufX is inhibitory to subunit and LH1 formation in reconstitution experiments with *Rb. sphaeroides* LH1 α- and $\beta$-polypeptides and Bchl. More striking is the inhibitory effect of the 39mer of *Rb. sphaeroides* PufX on subunit and LH1 formation in reconstitution experiments with Bchl and the α- and $\beta$-polypeptides of *Rb. capsulatus* (Figure 7B).

Because it was previously reported that native PufX did not inhibit formation of a subunit-type complex when only the $\beta$-polypeptide and Bchl were used (*20*), the effect of the synthetic core segments was examined in reconstitution experiments with Bchl and the *Rb. sphaeroides* or *Rb. capsulatus* $\beta$-polypeptide. In the case of the *Rb. sphaeroides* $\beta$-polypeptide reconstitution system, only a modest inhibition was observed at 0.90% OG that largely disappeared at 0.75 and 0.66% OG. On the other hand, with the *Rb. capsulatus* $\beta$-polypeptide system, very strong inhibition (>80% at a 5:1 ratio of core segment/LH1 α-polypeptide) was observed with both *Rb. sphaeroides* and *Rb. capsulatus* PufX core segments (data not shown). As will be discussed later, the inhibition of formation of the subunit-type complex in this instance is likely due to the binding of Bchl by the PufX core segments, thus lowering the effective concentration for formation of the subunit complex.

Since the synthetic polypeptides reproducing the core region of PufX exhibited less specific inhibitory activity than the native PufX proteins, it was of interest to extend these assays to a *Rhodospirillum rubrum* reconstitution system. It was previously reported that the native PufX protein did not inhibit reconstitution of the subunit or LH1 complex formation in *Rs. rubrum* (*20*). As shown in Figure 8, modest inhibition by the *Rb. sphaeroides* 39mer core segment was found in reconstitution of *Rs. rubrum* subunit and LH1 complexes when this core segment was present in an approximately 5:1 concentration ratio relative to the *Rs. rubrum* α-polypeptide which was 4 $\mu$M. Only weak inhibition was exhibited in reconstitution of the *Rs. rubrum* subunit and LH1 complexes when the core segment 43mer of *Rb. capsulatus* was tested (data not shown).

*CD Measurements.* Measurements of the UV CD spectra of PufX of *Rb. sphaeroides* and *Rb. capsulatus* in OG had indicated a low α-helical content (*20*). The CD spectra of the synthetic segments were examined in TFE since they readily dissolved in this solvent, and it is often thought to provide an environment similar to that of membranes. The 39mer core segment, the N-terminal fragment, and the C-terminal fragment of *Rb. sphaeroides* displayed 47, 39, and 34% α-helix, respectively, in TFE (data not shown). Lower percentage α-helices were observed for OG solutions of these segments, but the samples appeared to contain aggregates based on evidence for scattering observed in the UV absorption spectra. Importantly, the CD spectra of the 43mer of *Rb. capsulatus* gave 59% α-helix in TFE and 55% in OG (Figure 9). All data in TFE and this latter set of data in OG were thought to be most reliable because the solutions were clear and little, if any, aggregation was present as indicated by a lack of sample scattering in the UV absorption spectra.

## DISCUSSION

On the basis of the nucleotide sequences of the *pufX* genes of *Rb. sphaeroides* (*32*) and *Rb. capsulatus* (*31*), it has been suggested that the secondary structure of this molecule is similar to that of the LH1 α- and $\beta$-polypeptides each of which contain a polar N-terminal segment, a nonpolar

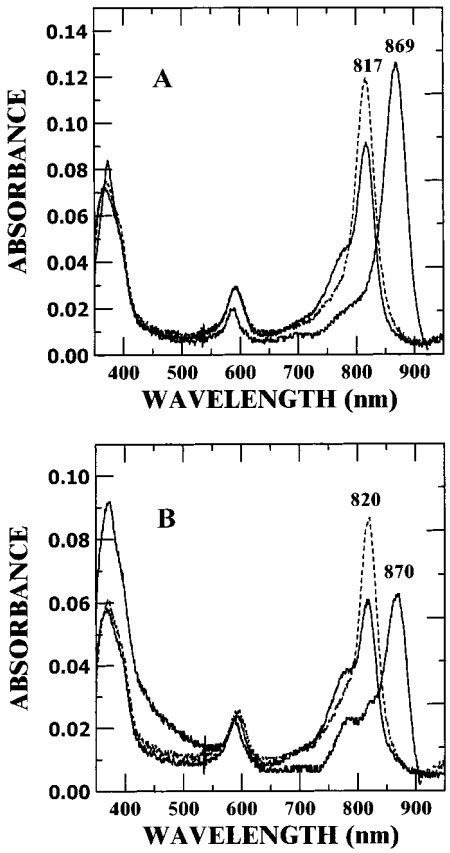


FIGURE 8: Inhibition of *Rs. rubrum* LH1 formation by the 39mer core segment of *Rb. sphaeroides* PufX. (A) Control reconstitution assay with LH1 α- and β-polypeptides of *Rs. rubrum* and Bchl. (B) Same reconstitution assay as panel A but in the presence of PufX 39mer. Spectra were recorded at 0.90% OG (solid curves with maxima at 817 nm (A) and 820 nm (B), 0.75% OG (dashed curves) and 0.66% OG after standing overnight at 4 °C (solid curve with maxima at 869 nm (A) and 870 nm (B). Concentrations at 0.90% OG were α = 4.1 μM, β = 2.9 μM, Bchl = 1.3 μM (A) and 1.0 μM (B) and *Rb. sphaeroides* 39mer = 6× the α-concentration.

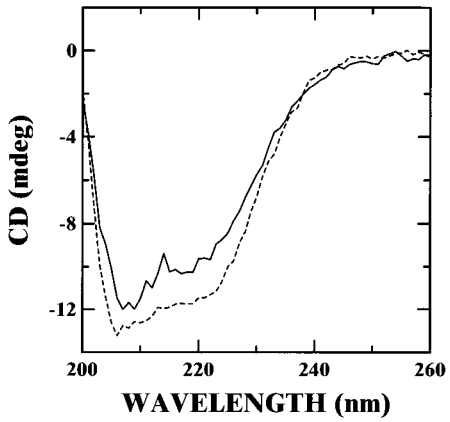


FIGURE 9: CD spectra of the 43mer core segment of PufX of *Rb. capsulatus* in trifluoroethanol (TFE) (dashed curve) and 0.80% OG at pH 7.5 (solid curve). Concentration of the 43mer was 20 μM. 1-mm cuvette.

α-helical, membrane-spanning segment, and a polar C-terminal segment (*18*, *19*). As with the LH1 polypeptides, the N-terminus of *Rb. sphaeroides* PufX has been reported to be exposed to the cytoplasmic side of the membrane (*33*). However, to date the C-terminal portion has not been located. From data presented in this paper, 12 C-terminal amino acids coded for by the *Rb. sphaeroides pufX* gene and nine C-terminal amino acids coded for by the *Rb. capsulatus pufX* gene were absent in the isolated protein (Figure 1). These C-terminal amino acids were apparently removed in processing, upon insertion of PufX into the membrane, or possibly by protease activity in vivo. It is unlikely that the C-terminal amino acids were removed during isolation because the same polypeptide was isolated by $CHCl_3/CH_3OH$ extraction of *Rb. sphaeroides* lyophilized whole cells in which there was presumably no opportunity for protease cleavage in the organic solvent. A shortened C-terminus may be inconsistent with the results of Francia et al. (*21*) who incorporated a His-tag into the 3′ end of the *pufX* gene and used the tag to isolate PufX in an *E. coli* expression system as well as to evaluate the quantity of PufX in ICM from *Rb. sphaeroides*. It appears that the C-terminal end of PufX was not removed by post-translational modification in those studies and that the core complex developed normally. Perhaps the difference in the mutants used in the two studies is an important consideration. While PufX was isolated from an LH2(−)-LH1(−) mutant for the current studies, an LH2(−) mutant was used in the work of Francia et al. (*21*). It is possible that part of the PufX protein is more susceptible to protease activity in vivo when LH1 is not present. However, the C-terminus of PufX from *Rb. capsulatus* also appears to be shortened, and it was isolated from cells containing LH1.

From the results with the chemically synthesized segments of PufX, the polar N- and C-terminal synthetic fragments *Rb. sphaeroides* PufX were without effect on the reconstitution of LH1. The two core synthetic segments, however, were quite effective in inhibiting LH1 formation. Also effective in causing inhibition of reconstitution of LH1 was the synthetic core segment of *Rb. capsulatus* which, in this case, contained all of the C-terminal end of the presumed mature protein except for the last two amino acids which are not conserved between *Rb. sphaeroides* and *Rb. capsulatus* PufX.

A comparison of the amino acid sequences predicted by the gene sequences of *pufX* from *Rb. sphaeroides* and *Rb. capsulatus*, indicates only 23% identity. This low percent identity is to be contrasted with over 75% identity for LH1 α- and β-polypeptides (*36*, *37*) and the L-, M-, and H-polypeptides of the RC (*14*, *38*) of the same two organisms. If one compares the core regions (from residue 21 through 67 of the *Rb. sphaeroides* and from residue 20 through 66 of *Rb. capsulatus* PufX), 38% identity is found. If conservative changes are also considered, the two sequences show 59% similarity. This higher percent identity and similarity in the core regions is consistent with the finding that it is the core region that specifically inhibits reconstitution of LH1. On the basis of homology model building (*39*), this degree of similarity might be used to predict that the two core regions would have very similar three-dimensional structures. A very similar structure would also be predicted on the basis of their parallel role in vivo (*31*, *32*, *34*, *35*) and their similar in vitro inhibition of LH1 formation (*20*). From measurements of the CD spectra of the core segments (Figure 9), it is expected that this segment would contain between 50 and 60% α-helical structure in a membrane environment.

The chemically synthesized core segments of *Rb. sphaeroides* and *Rb. capsulatus* PufX (39mer and 43mer, respectively) exhibit less specificity than the native PufX polypeptides.

This is consistent with the absence of 17 and 16 amino acids at their N-termini, respectively, which exhibit little amino acid sequence identity. These N-terminal segments also presumably contribute to the greater effectiveness of inhibition exhibited by native PufX. The inhibitory activity of the synthetic core segments was also observed in reconstitution of the LH1 complex of *Rs. rubrum* (Figure 8). This latter result is of special interest because a *PufX* gene is not present in *Rs. rubrum* (*40*, *41*). It might be tentatively concluded that structural features conserved in the core region of the LH1 α- and β-polypeptides which are responsible for formation of the subunit and LH1 complexes are also involved in recognition of PufX.

The inhibition of the formation of homologous (ββ) subunit-type complexes are of particular interest and provide new insights. The observation that subunit formation is much more strongly inhibited in the case when the β-polypeptide of *Rb. capsulatus* is used rather than that of *Rb. sphaeroides* presumably reflects the fact that the association constant is about 50× smaller for forming a homologous (ββ) subunit-type complex with the β-polypeptide of *Rb. capsulatus* as compared to that of *Rb. sphaeroides* (*42*). This result suggests that the PufX core segment polypeptides bind Bchl. Also consistent with the expectation that the PufX core segments bind Bchl is the pattern of inhibition shown with the *Rb. sphaeroides* β-polypeptide system where modest inhibition is observed in 0.9% OG, but the inhibition decreases and becomes insignificant at 0.75% OG and 0.66% OG (data not shown). Because the multicomponent equilibrium associated with subunit formation should have a steeper dependence on OG concentration than that of a bimolecular reaction between PufX and Bchl, B820 formation should be more favorable at lower percent OG. Preliminary experiments using a fluorescence probe covalently attached to the synthetic core segment of *Rb. sphaeroides* (39mer) indicate that Bchl does indeed bind to this polypeptide (unpublished results).

Several earlier experimental results pointed to the likelihood that PufX interacts specifically with the LH1 α-polypeptide (*20*). In one of these experiments, it was observed that the LH1 α-polypeptide and PufX tended to migrate together during gel filtration on a Sephadex LH60 column as well as upon HPLC (*20*). Another observation was that there appeared to be no interaction between the LH1 β-polypeptide and PufX as witnessed by the lack of effect of mature PufX on the reconstitution of homologous subunit complexes containing only the β-polypeptide and Bchl (*20*). The data on LH1 inhibition reported here imply that the core segments of PufX not only bind Bchl, but also interact with the α-polypeptide, perhaps upon oligomerization. Thus, in systems in which formation of a subunit-type complex is not inhibited, but LH1 formation is (Figures 4A, 6, and 8 and ref *20*), such inhibition is not explained simply by the binding of Bchl by the PufX polypeptides. This is because, in these systems, subunit formation becomes optimized and overcomes inhibition by PufX core segments upon lowering the OG concentration from 0.90 to 0.66%, but substantial inhibition of LH1 formation is still observed. It follows that PufX interaction in LH1 may involve binding of PufX both to Bchl and to the α-polypeptide in an oligomerized state. Because the in vivo ratio of PufX to the RC has been estimated to be 1:1 (*21*), and the ratio of the LH1 α-polypeptide to the RC is approximately 12:1 (*4*, *43*, *44*), interaction by PufX would be expected to affect the size of LH1 formed by oligomerization of the heterodimeric subunit complex and perhaps limit it to approximately 12 αβ pairs. The purpose of such "capping" or "interruption" of LH1 is presumably to provide a path for the reducing equivalents contained in $Q_BH_2$ to be made available to the bc1 complex (*34*, *35*). The fact that the α-polypeptide is located on the inside of the oligomeric LH2 ring structures (*10*−*12*) would make it ideal for interaction with PufX which, in turn, may interact with the RC, assuming LH1 exhibits a similar oligomerization pattern in vivo. A role for PufX in binding both to LH1 and the RC has also been suggested by Pugh et al. (*33*), Lilburn et al. (*45*), Francia et al. (*21*), and Frese et al. (*23*).

Focusing on the core region (residues 21 through 67 for *Rb. sphaeroides* and 20 through 66 for *Rb. capsulatus*; see Figure 1) of the PufX proteins, the amino acid sequences are striking in containing 7 Gly and 3 Pro. Two of the Gly and all three of the Pro are conserved between *Rb. sphaeroides* and *Rb. capsulatus* PufX. Several more of the Gly might be viewed as conserved with the insertion of appropriate gaps. If a major portion of the interacting region of the core regions of the PufX proteins are assumed to have an α-helical structure, the multiple Gly could allow a close approach to the LH1 α-polypeptide to optimize van der Waals' interactions, similar to the dimerization of the membrane spanning portion of glycophorin (*46*) and as generally noted for membrane-spanning α-helices of integral membrane proteins (*47*). In this regard, a PHD prediction (Burkhard Rost (*48*−*50*)) of the secondary structure of this core segment suggested an α-helical membrane-spanning segment from Gly34 to Gly51 for *Rb. sphaeroides* PufX and for Ala29 to Gly50 for *Rb. capsulatus* PufX. The expected accuracy was about 65%, somewhat low because there were no homologous sequences found in the current version of Swissprot. This amount of α-helix is consistent with that obtained in CD measurements (Figure 9).

*Relationship of LH1 and PufX to the RC.* Assuming that the role of PufX in *Rb. sphaeroides* and *Rb. capsulatus* is to "cap" or "interupt" oligomerization of LH1 subunits so they do not completely encircle the RC, it seems implicit that such an interaction should occur adjacent to the $Q_BH_2$ site of the RC. This suggests that a further interaction of PufX would likely involve the exposed isoprenoid tail of $Q_B$ and/or the first membrane-spanning segment of the M-polypeptide of the RC, the latter of which forms the binding site for the first half of the $Q_B$ tail (the part nearest to the quinone ring) (Figure 10A−C). If one examines the amino acid sequence (*38*) and the crystal structure (*5*−*9*) of the *Rb. sphaeroides* RC, it seems significant that the first membrane-spanning segment (span A) of the M-polypeptide has many Gly residues (Figure 10D). Because of the 2-fold symmetry in the RC, the first membrane-spanning segment of the L-polypeptide also has many Gly, but the single membrane spanning segment of the H-polypeptide covers much of the $Q_A$ binding site. It is possible that the external surface of the membrane-spanning A segment of the M-polypeptide allows extensive matching of van der Waals surfaces to those of the PufX protein. Thus, the in vivo LH1 might appear as a "horseshoe" about the RC if capping or interuption occurs at the first membrane-spanning segment of the M-polypeptide.

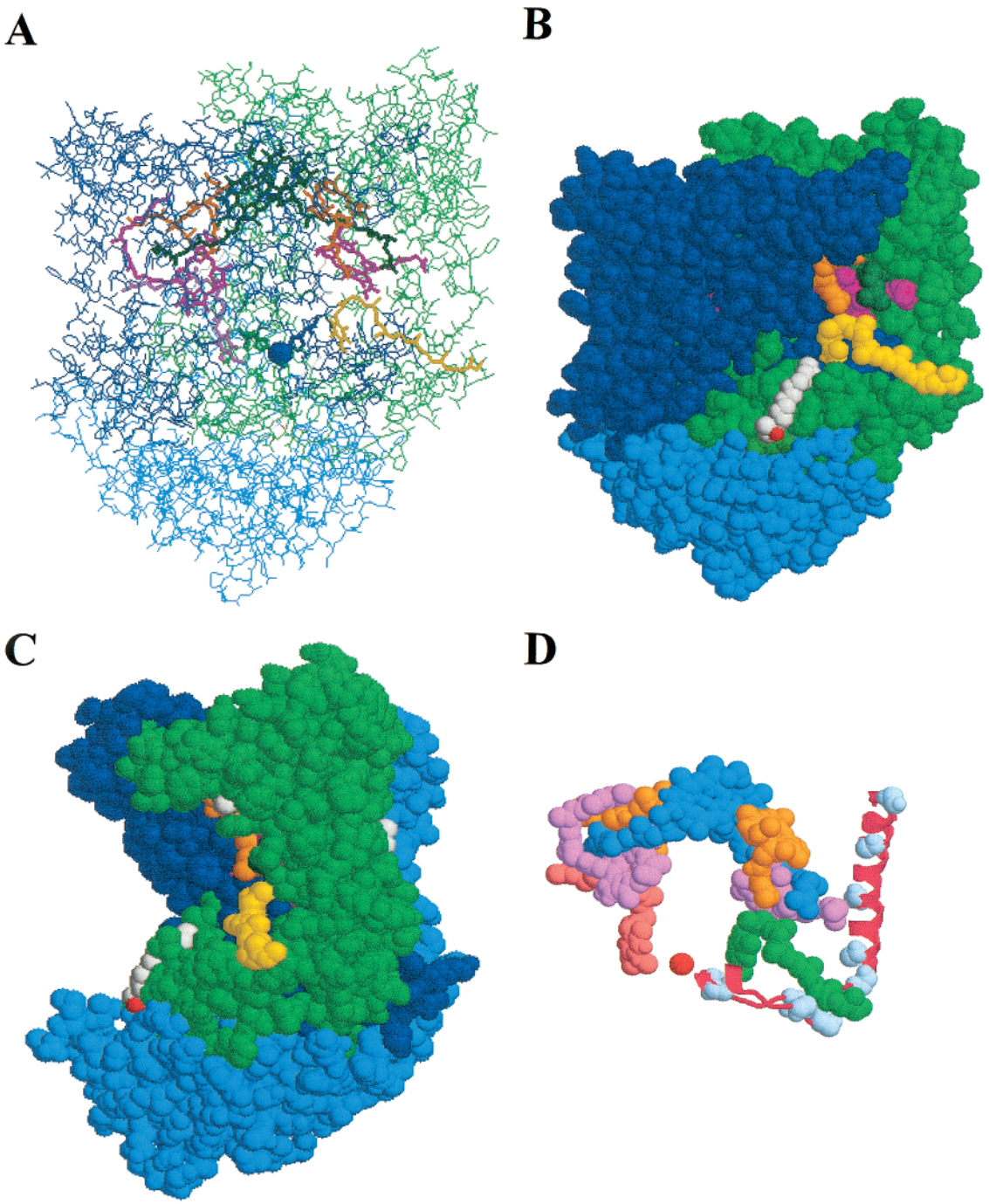


FIGURE 10: (A) Wireframe representation of the RC of *Rb. sphaeroides* (61) (Accession No. 1AIJ). The H-polypeptide is sky blue, the L-polypeptide is blue, and the M-polypeptide is sea green. Cofactors are represented in stick form with the donor Bchl in green-blue, the bridging Bchl in gold, the Bph in magenta, $Q_A$ in violet, iron as blue space-filled, $Q_B$ in yellow and detergent molecules are gray. The bottom of the structure is the cytoplasmic side. Rasmol was used to prepare the figure. (B) Same structure as shown in panel A except space-filled. (C) Same as panel B except rotated by 90°. (D) Same structure as A except that only the first TM segment of the M-polypeptide is shown in ribbon form along with an adjoining part of the N-terminus. Gly are indicated in light blue. Cofactors are shown as space-filled and are colored as blue (donor Bchl), gold (bridging Bchl), magenta (Bph), pink ($Q_A$), red (iron) and green ($Q_B$).

The idea of a "horseshoe" for the core LH structure is not consistent with models of LH1 based on cryoelectron microscopy of isolated LH1 (*13*) and core complexes (*14*) in which closed rings with 16 $\alpha\beta$ pairs are proposed. Because these preparations were obtained using detergent methodology, they may lack other possibly important polypeptide components. The low resolution so far achieved for the core complex (*14*) severely limits a positive identification of components. A 16-membered ring implies 32 Bchl/RC which is substantially greater than the value of 24 which has been consistently obtained by analytical assays (*43*, *44*). An earlier study by electron microscopy of *Rps. viridis* membranes was also interpreted to consist of RC surrounded by LH rings (*51*), but these data lacked sufficient resolution to be conclusive.

Finally, it is noteworthy that a dimeric structure for the bacterial RC has been suggested, first based on electrochemical and quinone extraction experiments (*52*, *53*), then on the

basis of cytochrome *c* coupling (*54*) and more recently on the basis of isolated complexes (*21*) and electron microscopy (*22*). It is perhaps significant to note in this regard that the PSII RC appears to be dimeric (*55*, *56*). A "horseshoe" arrangement, but not a closed circle, could be readily incorporated into a dimeric RC configuration.

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