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SEQUENCE HOMOLOGY AND STRUCTURAL SIMILARITY AMONG B 870 (B 890) POLYPEPTIDES OF PURPLE PHOTOSYNTHETIC BACTERIA AND THE MODE OF BACTERIOCHLOROPHYLL BINDING

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Interspecies comparison of B 870 (B 890) bacteriochlorophyll proteins of several purple photosynthetic bacteria revealed homology in the amino acid sequences. The hydrophobic segment of the protein was predicted to span a membrane in an α-helical structure, where one histidine residue (the candidate of a BChl binding site) exists.

The light-harvesting bacteriochlorophyll (BChl)-protein which absorbs around 870 (890) nm is called a B 870 (B 890) complex. Its minimum unit consists of two polypeptides (called α and β), two BChl's and one carotenoid.[1] The interaction of the BChl with the apoprotein is of vital importance in the primary process of photosynthesis. However, no direct evidence concerning the BChl binding has been obtained. Recently the amino acid sequences of the B 870 polypeptides have been found from direct protein sequence analyses[2-4] and DNA sequences of the encoding genes.[5,6] Interspecies comparison of these sequences revealed characteristic features among them. In this communication we wish to present the analysis of the secondary structures of the B 870 (B 890) polypeptides, and the binding mode of the BChl's with the proteins, along with the newly found sequences of amino(N-) terminal residues of polypeptides from Rhodopseudomonas (Rp.) sphaeroides, and Chromatium (Ch.) vinosum.

Rh. sphaeroides and Ch. vinosum were cultured phototrophically and the B 870 (B 890) BChl protein complexes were prepared as described previously.[7-11] B 870 (B 890) polypeptides were extracted with a mixture of chloroform and methanol (C/M) (1:1, v/v) from the freeze dried B 870 (B 890) complexes with the method of Zuber et al.[2 — 4] Sequence analysis was carried out by automated Edman degradation with a Beckman Model 890 M and an Applied Biosystems Model 470 A. Deblocking for the N-terminal blocked B 870 (B 890) polypeptides was achieved with 5% HCl in C/M (1:1, v/v).[3] Circular dichroism (CD) spectra of B 870 (B 890) complexes were measured with a JASCO J-500C spectrodichrometer with a 0.061 mm cell.

The hydrophobicity of the amino acid sequence was examined for potential membrane-spanning regions by the method of Kyte and Doolittle.[12] In this method, each amino acid is rated on a hydropathy scale, which ranges from 4.5 for

isoleucine, judged to be the most hydrophobic amino acid, to -4.5 for arginine, the most hydrophilic residue. The average hydropathy value of a moving segment (9 residues) is calculated over the entire sequence. The secondary structure of proteins is predicted by Chou and Fasman method[13] by calculating the conformation parameters P_α, and P_β, that is, the average potentials for any protein segment to be in α-helix, and β-sheet, respectively. The secondary structure is also predicted by classification of residues into strong helix former (H_α), helix former (h_α), weak helix former (I_α), helix indifferent (i_α), helix breaker (b_α), and strong helix breaker (B_α). Similar classification is applied to the β-sheet structure. Further the secondary structure is checked by calculating the conformational parameters of helical boundary residues, $P_{\alpha N}$, $P_{\alpha C}$, $P_{n\alpha N}$, and $P_{n\alpha C}$. Where, $P_{\alpha N}$ and $P_{\alpha C}$ denote the normalized frequency of residues in the N-terminal and C-terminal helix regions, and $P_{n\alpha N}$, and $P_{n\alpha C}$ represent the normalized frequency of residues in the N-terminal and C-terminal nonhelical regions. Similar values for the β-sheet structure are also calculated.

For Rp. capsulata the amino acid sequences of both B 870 polypeptides have been determined from their DNA sequences.[5] The B 870 α-polypeptide has been determined for Rhodospirillum (Rs.) rubrum by diret protein sequence analysis.[3,4] The N-terminal amino acid sequences of the B 890 α-polypeptide of Ch. vinosum and the B 870 α-polypeptide of Rp. sphaeroides were firstly determined by us. All of these results are shown in Table 1.

Table 1. Comparisons for Amino Acid Sequences of B 870 (B 890) Polypeptides

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                          10        20        30        40
                 12345678901234567890123456789012345678901234567890123456789
Rp. capsulata β:[5)] MADKAPLSFTGLTDEQAQELHAVYMSGLSAFIAVAVLAHLAVMIWRPTF
                                   10        20        30        40        50
                          123456789012345678901234567890123456789012345678901234
Rs. rubrum α:[3,4)]          MWRIWQLFDPRQALVGLATFLFVLALLIHFILLSTERFNWLEGASTKPVQTS
                                 10        20        30        40        50
                          12345678901234567890123456789012345678901234567890123456
Rp. capsulata α:[5)]         MSKFYKIWLVFDPRRVFVAQGVFLFLLAVLIHLILLSTPAFNWLTVATAKHGYVAAQ
                                 10        20        30        40        50
                          12345678901234567890123456789012345678901234567890123456
Rp. sphaeroides α:           MSKFYKIRMIFIPRRVF----------------------------------[a)]
                                 10        20        30        40        50
                          12345678901234567890123456789012345678901234567890123456
Ch. vinosum α:               M-AYL-KIWLLVWPWR----------------------------------[a)]
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a) The symbol - indicates an amino acid which has not been determined yet.

The B 870 α-polypeptides of Rp. capsulata and of Rs. rubrum show an essential homology in their amino acid sequences. The two polypeptides share the common amino acid sequences (PheAspProArg) from the 11th to the 14th in Rp. capsulata α- and from the 8th to the 11th in Rs. rubrum α-polypeptides. They also shows a homology (PheLeuPhe( )LeuAla( )LeuIleHis( )IleLeuLeuSerThr) from the 23rd to the 38th in Rp. capsulata α-, and from the 20th to the 35th in Rs. rubrum α-polypeptides. In these sequences it is characteristic that His (which is a potential candidate for the BChl binding) is surrounded by hydrophobic amino acids. A similar disposition can be found from the B 870 β-polypeptide of Rp. capsulata.

Thus, the comparison of amino acid after positioning the His residue of Rp. capsulata B 870 β-polypeptide at the same position to those in the B 870 α-polypeptides of Rp. capsulata and Rs. rubrum, reveals the common amino acid sequences among them.

Though only the N-terminal sequences were determined for the B 890 α-polypeptide of Ch. vinosum and the B 870 α-polypeptide of Rp. sphaeroides, they show common features to those described above. The N-terminal amino acid sequence of the B 870 α-polypeptide of Rp. sphaeroides demonstrates the same amino acid sequence to that of Rp. capsulata α-polypeptide (MetSerLysPheTyrLysIle( )( )( )Phe( )ProArgArgValPhe). This implies that the rest of the sequence would be similar. Also the N-terminal amino acid sequence of Ch. vinosum contains that of LysIleTrpLeu( )( )( )Pro( )Arg, which is similar to that of the Rp. capsulata α-polypeptide. Again the N-terminal amino acid sequence similarity suggests the rest of amino acids would be similar. It should be noted here that the N-terminal amino acids contain more positively charged amino acids (Lys, Arg) in the α-polypeptide and negatively charged amino acids (Asp, Glu) in the β-polypeptides. This may imply some interactions between them.

According to the Kyte and Doolittle method,[12] the hydropathy index (H_Y) was calculated from the sequence shown in Table 1. The distribution of hydrophobic residues along the polypeptide chain with a sampling span of 9 residues, indicates one membrane-spanning region for each B 870 polypeptide. By defining Hy > 1 as a hydrophobic region, the amino acids belong to this region are those from the 19th to the 38th in Rp. capsulata α-polypeptide, those from the 16th to the 32nd in Rs. rubrum α-polypeptide, and those from the 23rd to the 42nd in Rp. capsulata β-polypeptide. These segments are predicted to exist in a hydrophobic portion of the phospholipid bilayer. The Chou-Fasman method[13] predicts both the α-helical and β-sheet structures from Arg-14 to Thr-38 in Rp. capsulata α-polypeptide. Though the conformational parameter values (P_β = 1.23, P_α = 1.07) prefer the β-sheet structure, this segment has $(H_9h_{10}I_1i_4b_0B_1)_\alpha$ as compared to $(H_6h_{12}I_0i_5b_2B_0)_\beta$. (Where subscripts represent the numbers of amino acids which belong to each classification.) Therefore the α-helical structure can be predominant in these sequences. The conformational parameters of boundary residues of N- and C-terminal amino acids also reinforce this conclusion. Thus, N-terminal α-helical and β-sheet boundary residues AspPro has $P_{\alpha N} + P_{n\alpha N}$ of 2.99, while $P_{\beta N} + P_{n\beta N}$ of 1.99. The α-helical C-terminal boundary residues, ThrPro, have $P_{\alpha C} + P_{n\alpha C}$ of 2.23, while the β-sheet C-terminal boundary residues, ProAla, have $P_{\beta C} + P_{n\beta C}$ of 1.14. The predicted secondary structures are consistent with the experimental result for the B 890 complex of Ch. vinosum which showed the CD spectrum ascribable to the α-helical structure (Fig. 1).

According to the hydropathy indexes and the predicted secondary structures, the folding patterns of the B 870 polypeptides can be depicted as shown in Fig. 2. Both B 870 α- and β-chains have α-helical regions in the membrane. The His residue exists close to the boundary in the hydrophobic segment. Presumably BChl's link to these chains one by one with the coordination bond between the imidazole nitrogen of His and the Mg(II) ion of BChl. This picture is consistent with the resonance Raman results which indicate the presence of five coordinated BChl's,[14] and also

with the CD results which indicate strong interactions between the BChl's.[11,12]

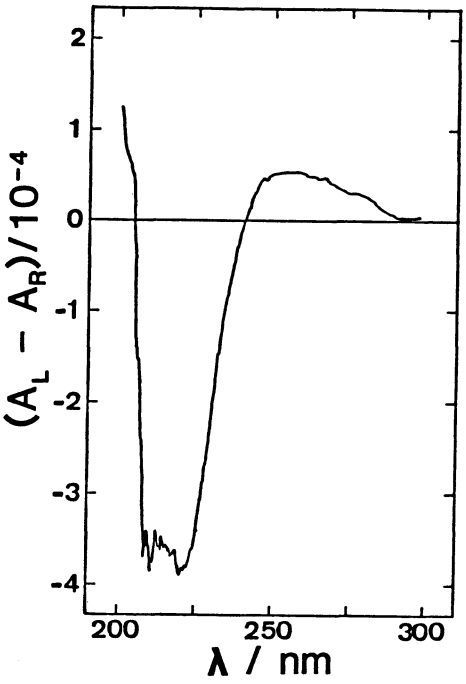

Fig. 1. The circular dichroism spectrum for the LH1 complex of Ch. vinosum in a Tris (tris(hydroxymethyl)aminomethane) buffer. The ordinate represents the differences in the absorbance for the left- and the right-circularly polarized light.

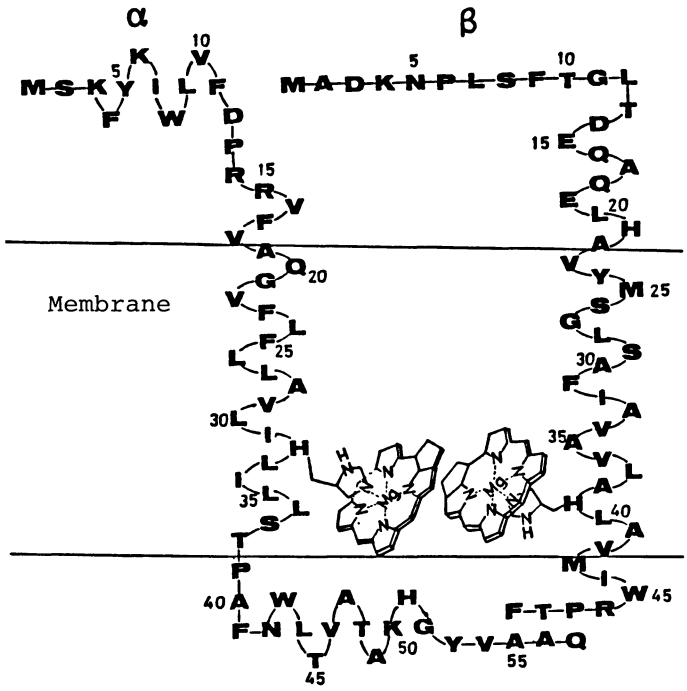

Fig. 2. A schematic model for the LH1 complex. The amino acid sequences were taken from those of Rp. capsulata α- and β-polypeptides in the reference 5. The secondary structures and the membrane spanning regions were determined as described in the text. The region between two horizontal lines indicates the hydrophobic part of the membrane.

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