JOURNAL OF BACTERIOLOGY, Oct. 1986, p. 167–172
0021-9193/86/100167-06$02.00/0

Vol. 168, No. 1

Effect of Uncoupler on Assembly Pathway for Pigment-Binding Protein of Bacterial Photosynthetic Membranes

ROLAND DIERSTEIN* AND GERHART DREWS

Institute of Biology 2, Microbiology, Albert-Ludwigs University, D-7800 Freiburg, Federal Republic of Germany

Received 31 March 1986/Accepted 22 May 1986

The uncoupler carbonylcyanide *m*-chlorophenylhydrazone (CCCP) was used to investigate membrane protein assembly in the phototrophic bacterium *Rhodobacter capsulatus*. As found for *Escherichia coli* (T. Date, G. Zwizinsky, S. Ludmerer, and W. Wickner, Proc. Natl. Acad. Sci. 77:827–831, 1980) and mitochondrial proteins (N. Nelson and G. Schatz, Proc. Natl. Acad. Sci. USA 76:4365–4369, 1979), assembly across the bacterial photosynthetic membranes was sensitive to CCCP. At uncoupler concentrations which were sufficient to block the export of the periplasmic cytochrome c_2 and an outer membrane protein, the integration of pigment-binding protein into the photosynthetic apparatus was abolished. The unassembled protein was detected on the inner surface of the intracytoplasmic membrane. After inactivation of CCCP, accumulated protein continued insertion into the membrane. The data suggest that after binding to the cytoplasmic face of the membrane (i), translocation of protein into a transmembrane orientation takes place (ii), which is a prerequisite for the formation of a functional pigment-protein complex (iii).

Assembly of protein into and across biological membranes has been studied in various organisms, organelles, and in vitro systems (for a recent review, see reference 37). Multiple mechanisms were found when either eucaryotic or procaryotic membranes were examined (reviewed in references 30 and 37). In spite of the differences, three basic themes for a protein's insertion across membranes became evident: (i) membrane recognition, (ii) translocation through the hydrophobic core, and (iii) maturation. Particularly with *Escherichia coli*, it was firmly established that the maintenance of proton motive force is crucial for the translocation step in protein export (6, 7, 17, 41).

In *E. coli*, proton motive force can direct not only the translocation of exported proteins but also trans-bilayer polypeptides of the cytoplasmic membrane, such as M13 major coat protein and leader peptidase (38, 41). Little information exists for other bacteria. Here we present data for a rather distantly related group, the gram-negative purple bacteria (22). These organisms have both the photosynthetic and respiratory energy-conserving machinery in a system of cytoplasmic and intracytoplasmic membranes (reviewed in reference 29). This higher degree of compartmentation compared with other gram-negative bacteria and the versatility of the purple bacteria in potential energy conservation make these organisms interesting candidates for the study of membrane assembly.

The special intracytoplasmic membranes are the domain of the bacterial photosynthetic apparatus (29). They are strikingly enriched in pigment-binding proteins (PBP), which are intrinsic proteins in a transmembrane orientation (8, 15, 25). In a fixed stoichiometry, usually two different polypeptide subunits bind noncovalently the photopigments bacteriochlorophyll and carotenoids to form a specific pigment-protein complex of the photosynthetic machinery (15). The DNA and protein sequences are already known for most of the polypeptide subunits (34, 39, 40). They are synthesized without a cleaved amino-terminal leader (signal) sequence. Individual steps of their membrane assembly have not been demonstrated.

* Corresponding author.

The biosynthesis of one prominent PBP, the α subunit of the large antenna complex B800-850 of *Rhodobacter capsulatus* has recently been studied in vitro (10). This protein is a 60-residue polypeptide with a central hydrophobic stretch flanked on either side by polar domains (34). Its characteristic transmembrane orientation (28, 33) suggests the requirement for a specific translocation event. In vivo, its assembly into complexes was found to be dependent on continuing protein synthesis (11). However, the nature of the synthesis coupling was unknown. In the present study we have examined the integration of the protein into the membrane and its assembly into functional antenna complexes when carbonylcyanide *m*-chlorophenylhydrazone (CCCP) was employed to lower the proton motive force (26). Translocation of the B800-850 α subunit and of two exported proteins is blocked by the uncoupler. This block also prevents the assembly of a functional pigment-protein complex and suggests an order for these membrane assembly events.

MATERIALS AND METHODS

Materials. Proteinase K was from Merck, Darmstadt. M_r standard proteins (Combithek) were from Boehringer Mannheim. Phenylmethylsulfonyl fluoride (PMSF) and insoluble protein A were from Sigma, Munich. [^{35}S]methionine (1 Ci/μmol) and the fluorographic reagent Amplify were purchased from Amersham-Buchler, Braunschweig, F.R.G.

Labeling cells. *Rhodobacter capsulatus* (= *Rhodopseudomonas capsulata*) strain 37b4 (DSM 938; German Collection of Microorganisms, Göttingen) was grown chemotrophically at low aeration in a malate-mineral medium (RÄ) (12) to a density of 5×10^8 cells per ml. Cell suspensions (2 ml) were briefly centrifuged in a microcentrifuge, and the sedimented bacteria were suspended in 2 ml of fresh RÄ medium. This suspension was transferred to a warmed conical vessel with a magnetic stirrer and an electrode for dissolved oxygen as described earlier (12). After 10 min for equilibration at a temperature of 32°C and an oxygen partial pressure of 700 Pa, CCCP, dissolved in dimethyl sulfoxide (DMSO), or DMSO alone was added to the culture at 50 μM. After 2 min, [^{35}S]methionine (50 μCi, 1 Ci/μmol) was added, and the incubation was continued for 1 min. Pulse-labeling was

stopped by chilling the cells for 5 min at 0°C in the presence of 2 mM unlabeled methionine. Cells were collected by sedimentation (5 min, 4°C in a microfuge), suspended in buffer (1 ml of 30 mM Tris chloride, 5 mM EDTA, pH 7.5, 0°C), and again collected by sedimentation.

Preparation of membranes and high-speed supernatant. Cells were suspended in one culture volume of ice-cold buffer (30 mM Tris chloride, 5 mM EDTA, pH 8.0). DNase was added (to 10 μg/ml), cells were sonicated, and membranes were prepared as described previously (12). After centrifugation at 100,000 × *g* for 90 min, the clear supernatant (S100) was withdrawn for analysis of soluble components.

Preparation of saline extracts. Cells (1-ml samples) were harvested by centrifugation and kept on ice in 0.1 ml of a solution of 150 mM NaCl for 2 h with occasional shaking. The cells were sedimented in a microfuge for 15 min at 4°C. The supernatant was withdrawn and centrifuged again. To this supernatant an equal volume of 50% (wt/vol) trichloroacetic acid was added. The sample was kept on ice for 60 min and then centrifuged for 5 min. The pellet was washed in 0.2 ml of ice-cold acetone and dried.

Protease treatment. Membrane vesicles were treated with 0.1 mg of proteinase K at 25°C in a buffer containing 30 mM Tris hydrochloride and 5 mM EDTA, pH 7.5, for various times. Digestion was stopped by chilling the samples and adding an equal volume of 50% (wt/vol) ice-cold trichloroacetic acid. The samples were left on ice for 30 min and then centrifuged in a microfuge for 5 min. Pellets were washed once in ice-cold acetone. The dried samples were solubilized by boiling for 5 min in 62.5 mM Tris hydrochloride–sodium dodecyl sulfate (SDS)–1 mM PMSF, pH 6.8. After cooling, PMSF was added again to 1 mM.

Immunoprecipitation and gel electrophoresis. PBP and cytochrome c_2 were recovered by immunoprecipitation as described previously (14) with a 5% (wt/vol) suspension of insoluble *Staphylococcus aureus* protein A. The antisera were raised in rabbits (10, 12). Proteins were released by boiling for 5 min in SDS sample buffer (24) and then analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) with polyacrylamide gradient gels (10) in the Laemmli system (24). The same system was used for membrane protein patterns and standard proteins. To solubilize protein under denaturing conditions, samples were heated for 30 min at 70°C in SDS sample buffer (24). For electrophoresis of pigment-protein complexes under nondenaturing conditions, the method of Broglie et al. (3) was used, with 9% (wt/vol) acrylamide and 0.24% bisacrylamide. For fluorography, gels were kept for 45 min in a fixing solution of 50% (vol/vol) methanol and 10% (vol/vol) acetic acid. They were then soaked in Amplify for 15 min, dried, and exposed to Kodak X-Omat films at −70°C. Staining was performed with Coomasie brilliant blue R250.

RESULTS

Synthesis of photosynthetic membrane proteins. Uncouplers on the photosynthetic membrane cause a parallel decrease in the membrane potential and the proton gradient (26). In our study we used a 2-min treatment with the uncoupler CCCP to lower proton motive force in exponentially growing cells of *R. capsulatus*. In all experiments, the cells were incubated under low aeration to allow the full expression of photosynthetic proteins. The cells were pulse-labeled with radioactive methionine and then incubated in the presence of excess unlabeled methionine (chase). After 5, 10, and 30 min, samples were taken and membranes were isolated (Materials and Methods). The membrane proteins were analyzed by SDS-PAGE.

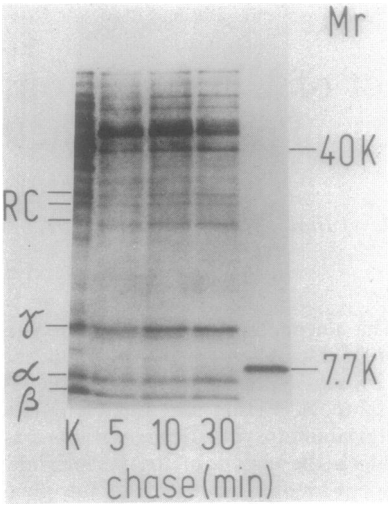

FIG. 1. Synthesis of membrane protein in the presence of uncoupler. Exponentially growing cells were treated with CCCP in DMSO and pulse-labeled with [^{35}S]methionine (Materials and Methods). Unlabeled methionine (2 mM) was added, and the incubation was continued (chase). At the times indicated, samples were withdrawn. The cells were chilled on ice and harvested by centrifugation, and membranes were isolated (Materials and Methods). Newly synthesized membrane protein was analyzed by SDS-PAGE followed by fluorography (Materials and Methods). Lane K, membranes of cells which were not treated with uncoupler; right lane, M13 procoat as membrane protein M_r standard. RC, Triad of reaction center polypeptides. α, β, and γ subunits of the B800-850 antenna complex are indicated.

Figure 1 depicts the synthesis pattern of membrane proteins in a pulse-chase experiment. As a comparison, a membrane sample from untreated cells was coelectrophoresed (Fig. 1, lane K). As in the untreated cells, the PBPs of the photochemical reaction center and the major antenna complex B800-850 were synthesized and membrane-bound in the CCCP-treated cells. The binding resisted ultrasonication in the presence of EDTA during membrane preparation (Material and Methods); this indicates a rather strong membrane association. The polypeptides were very stable during the chase period; see, for example, the B800-850 α subunit with an M_r of 7,300 (7.3K) (34), which migrates slightly faster than the M13 membrane marker protein of 7.7K (Fig. 1, right lane). Overall, the synthesis patterns of PBPs in CCCP-treated and untreated cells look very similar. Striking differences are evident in the upper M_r region. The dominant band at 40K in untreated cells disappeared, and a band with a somewhat higher M_r emerged. The 40K band can be related to the protein of cosedimenting outer membrane (Materials and Methods) (Fig. 2).

Translocation across the lipid bilayer. To monitor the effect of CCCP on protein translocation across membrane lipid bilayers, we followed the appearance of the saline-extractable outer membrane protein (36) and the periplasmic cytochrome c_2 (21) in the target compartments. Figure 2A depicts the outer membrane protein (apparent M_r of 40,000) as the major component in the saline extract of both untreated and CCCP-treated cells. Water-soluble standard proteins and isolated cytochrome c_2, which runs slightly faster than horse heart cytochrome *c* (12.5K), were coelectropho-

resed (Fig. 2A). When cells were pulse-labeled with methionine, newly synthesized outer membrane protein was detected in the saline extract, and cytochrome c_2 was immunoprecipitated from the water-soluble S100 fraction (see Materials and Methods) (Fig. 2B). However, in CCCP-treated cells no labeled newly synthesized outer membrane protein and mature cytochrome c_2 were found in the saline extract or the immunoprecipitate (Fig. 2B). This demonstrates a complete block of protein export by CCCP at a concentration of 50 μM, the concentration used throughout the study.

E. coli cells were treated with CCCP to accumulate the proteins to be translocated at the inner face of the membrane and then protein synthesis was inhibited and the uncoupler was inactivated with excess 2-mercaptoethanol. After further incubation, the accumulated proteins were translocated across the lipid bilayer (14, 37, 38). We followed the same strategy to investigate the assembly of PBP. Membranes of CCCP-treated cells were isolated and incubated with protease to test the accessibility of PBP at the inner face of the membrane. We chose proteinase K which digests native proteins unspecifically and effectively (16).

Figure 3 (panel 1) shows the effect of protease treatment on the protein pattern of isolated photosynthetic membranes (chromatophores). Only the low-M_r region is depicted. The bands were visualized by Coomassie staining and represent the majority of chemical amounts of polypeptides which were synthesized prior to the uncoupler treatment. The time course of digestion demonstrates that the α subunit is in a more protease-resistant orientation than the γ subunit. The observation is in agreement with recent topographic studies which established that the α subunit is transmembrane and the γ subunit is exposed on the cytoplasmic face (19, 28, 33). This suggests that the orientation of already assembled PBP was not disturbed in the presence of the uncoupler. Fluorography of the same gel reveals the protein which was newly synthesized during the pulse after the uncoupler had dissipated the proton motive force (Fig. 3, panel 2). Under these conditions, the α subunit was extremely susceptible to the added protease (after 10 min, more than 90% of the band had disappeared). This indicates that the molecule was exposed to protease at the cytoplasmic face of the membrane. However, when the CCCP-treated cells were further incubated in the presence of 2-mercaptoethanol to inactivate the uncoupler and allow the maintenance of proton motive force, the α subunit became protease resistant. This is demonstrated by the coelectrophoresis of the immunoprecipitates with specific antibodies (Fig. 3, panel 3). The accumulated PBP was now translocated into a protease-resistant transmembrane orientation. This step was blocked by CCCP, and it occurred after completion of the polypeptide (posttranslationally), as chloramphenicol at high concentration was present during the period after inactivation of the uncoupler.

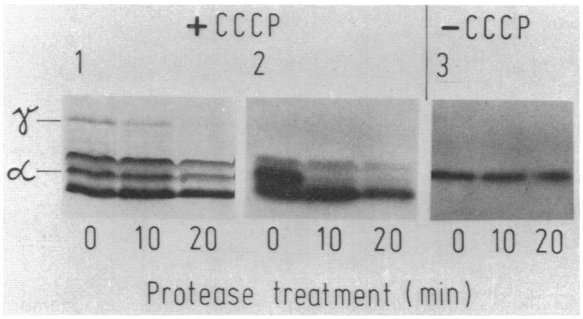

FIG. 3. Localization of the B800-850 α subunit before and after translocation. Cells were treated with CCCP in DMSO and then pulse-labeled (Materials and Methods). Methionine (2 mM) was added, and incubation was continued for 5 min. Chloramphenicol (1 mg/ml) was added, and half of the cells were withdrawn. 2-Mercaptoethanol was added to the rest of the cell suspension to a final concentration of 40 mM, and incubation was continued for 20 min. Cells were chilled on ice and harvested by centrifugation, and membranes were isolated and treated with proteinase K (Materials and Methods). Portions of the samples from cells treated with CCCP alone were immediately subjected SDS-PAGE, followed by staining (panel 1) and fluorography (panel 2). Samples from cells which were additionally treated with 2-mercaptoethanol were subjected to immunoprecipitation in the presence of 1 mM PMSF. Portions of the precipitates were analyzed by SDS-PAGE and fluorography (panel 3). Only the lower part of the gels is shown. Panels: 1, Coomassie-stained proteins; 2, fluorography of the samples shown in 1; 3, fluorography of immunoprecipitates with antibodies directed against the B800-850 protein. The α and γ subunits of theB800-850 complex are indicated.

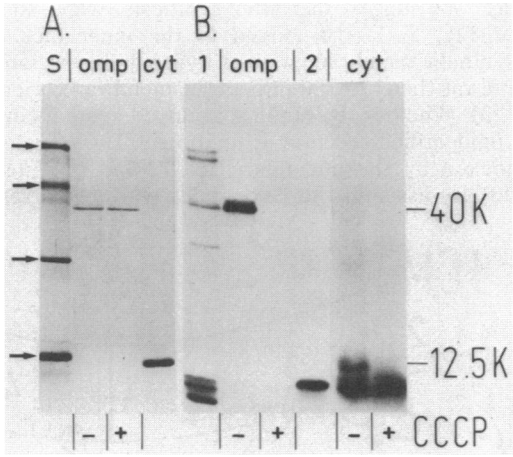

FIG. 2. Inhibition of protein export to periplasmic space and outer membrane. Cells were treated with CCCP in DMSO or solvent without CCCP and then pulse-labeled (Materials and Methods). The fate of cytochrome c_2 (cyt) and outer membrane protein (omp) was followed by testing their appearance in the target compartments. Cytochrome c_2 was immunoprecipitated from the water-soluble S100 fraction, and outer membrane protein was saline-extracted from whole cells (36) as described in Materials and Methods. The results of SDS-PAGE runs are shown. (A) Coomassie staining reveals one dominant outer membrane protein of 40K (omp) in the saline extracts of CCCP-treated (+) and untreated (−) cells. Lane S, Arrowheads point to the protein standards (from top to bottom: bovine serum albumin [68K], ovalbumin [45K], chymotrypsinogen [25K], cytochrome *c* [12.5K]). cyt, Isolated cytochrome c_2 (12.8K). (B) Fluorography detects the newly synthesized outer membrane proteins and cytochrome c_2 only in the saline extract and water-soluble fraction, respectively, of untreated cells. Lane 1, Pulse-labeled membrane fraction; lane 2, M_r standard M13 procoat of 7.7K.

Assembly into pigment-protein complexes. CCCP-treatment allowed the detection of an intermediate conformation of PBP at the membrane before the translocation step. Insertion into the membrane occurred independently of protein synthesis (Fig. 3). For membrane insertion, the B800-850 α subunit seems to use the same CCCP-sensitive assembly pathway as the exported proteins. However, the destination for the PBP is not simply the membrane but the B800-850 antenna complex in particular. To follow the fate

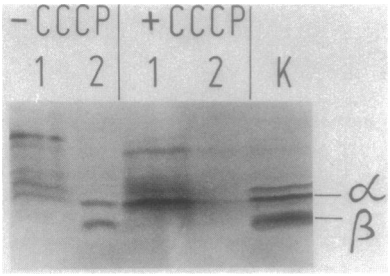

FIG. 4. Assembly into pigment-protein complexes. Membranes isolated from cells treated with CCCP and untreated control cells were subjected to LDS-PAGE under nondenaturing conditions at 4°C (3) (first run). After the run the bands with the characteristic absorption at 800 and 850 nm (B800-850 complex) were spectroscopically identified. Gel strips were excised from the position of the pigmented complex and from the low-M_r region near the gel front, heated in SDS sample buffer (Materials and Methods), and analyzed by SDS-PAGE (second run). The fluorograph of the second run is shown. Lanes 1, Analysis of the low-M_r regions; lanes 2, B800-850 complexes from treated (+CCCP) and untreated (−CCCP) cells. Lane K, Coelectrophoresis of membranes of untreated cells. Only the lower part of the gel is shown. The positions of the α and β subunits of the B800-850 complex are indicated.

of newly synthesized subunits, we analyzed the B800-850 antenna complexes isolated from untreated cells and cells treated with uncoupler.

After pulse-labeling of the cells, membranes were isolated, solubilized by mild detergent treatment, and subjected to lithium dodecyl sulfate (LDS)-PAGE at low temperature (3). This method allows separation of intact pigment-protein complexes. The band of the B800-850 complex was identified spectroscopically in the gel by its characteristic near-infrared absorption at 800 and 850 nm and then excised. Its polypeptide composition was analyzed by SDS-PAGE after solubilization under denaturing conditions (Materials and Methods). To detect unassembled protein, gel strips were excised just above and at the pigmented front in the low-temperature LDS-PAGE and coelectrophoresed on denaturing SDS-PAGE. The result of an SDS-PAGE run followed by fluorography is shown in Fig. 4. In untreated cells, newly synthesized α subunit was detected in the B800-850 complex together with the pigment-binding β subunit (Fig. 4, lane 2). In CCCP-treated cells, the α subunit was not assembled in a complex with the β subunit, but a band at the position of the standard emerged in the fraction of unassembled polypeptides (Fig. 4, lane 1). In parallel, the β subunit band was detected in the front of the LDS-PAGE gel (not shown). This demonstrates that the incorporation of PBP into functional pigment-protein complexes was inhibited by the uncoupler. It strongly suggests that complex assembly depends on translocation of its polypeptide subunits.

DISCUSSION

By use of the uncoupler CCCP, we obtained experimental evidence for an assembly pathway for PBP of the bacterial photosynthetic apparatus. Individual steps and their requirements fit the existing models of bacterial membrane assembly and export (reviewed in reference 37). These are (i) binding to the cytoplasmic face of the photosynthetic membrane and (ii) insertion, which is proton motive force dependent and does not require continuing protein synthesis. The third step, maturation with covalent modification by proteolytic cleavage, is inapplicable to the PBPs, as they are made without a cleavable leader sequence, as are the majority of inner membrane proteins (2, 4, 10, 38, 39). In addition, the concomitant block of protein export to the periplasmic space and the outer membrane (Fig. 2) strongly suggests that individual steps of a common pathway were shared by the proteins with different destinations in this phototrophic bacterium.

A characteristic feature for the assembly pathway is that PBP not only inserts into membranes but also assembles into functional pigment-protein complexes. Our study demonstrates that CCCP treatment blocks integration of the PBP, which is already membrane-bound, into pigment-protein complexes. Apparently, formation of a complex is not an obligatory intermediate step for translocation. Rather, the subunits are inserted into the membrane to form complexes. Complex assembly might be a step analogous to the maturation step of other polypeptides, with the exception that modification is not covalent. This order of the individual steps is in agreement with recent in vitro studies with plant chloroplasts, in which the assembly of chlorophyll-protein complexes in the thylakoid membrane occurred after insertion of the apoprotein (31).

Figure 5 summarizes our data and their interpretation. The polar and hydrophobic domains in the polypeptide chains are indicated by lines and boxes, respectively, according to the analysis of their hydropathic character (15, 23). The orientation of complex-assembled subunits, with the carboxy-terminal end on the periplasmic face and the amino-terminal end exposed at the cytoplasmic face, is according to recent results of topographic studies with different bacterial species (15, 25, 33). No attempt was made to predict a secondary structure. We suggest that after synthesis, water-soluble α subunit (Fig. 5, 1) (10) bound to the inner face of the photosynthetic membrane with its hydrophobic regions (Fig. 5, 2) and was then brought into a transmembrane orientation (Fig. 5, 3). Whether the binding occurred spontaneously or via an unidentified receptor is unknown. The translocation was blocked by the uncoupler CCCP, and this step was necessary for assembly into a complex with the correspond-

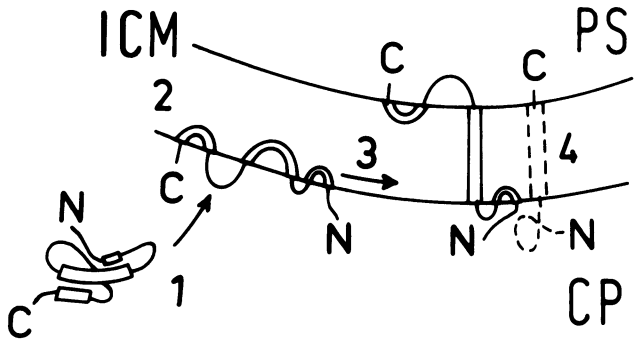

FIG. 5. Hypothetical scheme for the insertion of PBP into the bacterial photosynthetic apparatus. Step 1: Water-soluble B800-850 α subunit (10) reaches the photosynthetic membrane (ICM). Step 2: The polypeptide is bound at the cytoplasmic (CP) face of the membrane. It is accessible to proteinase K when the enzyme is added to the membrane vesicle suspension. Step 3: Translocation of C-terminal polar regions across the hydrophobic core causes a transmembrane orientation of the polypeptide. This step is blocked by the uncoupler CCCP. Step 4: Assembly of the polypeptide into a pigment-protein complex together with the β subunit (dashed lines) and the pigments (not drawn) depends on its translocation. Hydrophilic (———) and hydrophobic (□) regions of the subunit are indicated according to its hydropathy plot (15). PS, Periplasmic space.

ing β subunit and the pigments (Fig. 5, 4). Presumably, the translocation of the polar domain near the carboxy terminus across the lipid bilayer was promoted by proton motive force (35). The amino-terminal portion with two basic (charges not drawn) residues flanking a short hydrophobic stretch remains at the cytoplasmic face. The additional apolar region near the C terminus suggests a mechanism which would require two hydrophobic domains on either side of the polar region to be translocated, as proposed by the helical hairpin and the loop hypotheses (18, 20). Alternatively, the hydrophobic stretch of the β subunit might serve to facilitate a simultaneous trans-bilayer insertion of both polypeptides.

The β subunit is structurally different (15). A simpler pathway could be assumed for this protein: its hydropathy plot reveals a strongly hydrophobic domain in the C-terminal portion. The rest of the chain is polar. It resembles proteins, such as cytochrome b_5 which are anchored to the membrane by hydrophobic residues at the C terminus and insert spontaneously into the endoplasmic reticulum (1). Nevertheless, like the α subunit, the β subunit was not assembled into pigment-protein complexes in the presence of uncoupler (Fig. 4).

How does PBP recognize its insertion site? Although we did not specifically address this question, we made an interesting observation: unassembled PBP in bacteriochlorophyll-less or assembly-defective mutants is known to undergo rapid turnover (9, 13, 32); however, the unassembled PBP in CCCP-treated cells remained stable. If this difference was not simply due to a possible protease inactivation by ATP depletion (5), it could indicate protection from endogenous proteases by an early interaction with a molecule which was not available in the mutants. This could be a unique receptor for PBP. The recognition of a receptor early in assembly might be the key reaction in the postulated assembly control (2, 9, 10, 13, 40).

ACKNOWLEDGMENTS

We thank Nasser Gad'on for expert technical assistance. The stimulating discussions with Bernhard Dörge and Gabi Klug are gratefully acknowledged.

This work was supported by the Deutsche Forschungsgemeinschaft (Dr 29/30-1 and Di 298/3-1).

LITERATURE CITED

1. **Anderson, D. J., K. E. Mostow, and G. Blobel.** 1983. Mechanisms of integration of *de-novo* synthesized polypeptides into membranes: signal recognition particle is required for integration into microsomal membranes of calcium ATPase and lens MP26 but not cytochrome b_5. Proc. Natl. Acad. Sci. USA **80**:7249–7253.
2. **Belanger, G., J. Berard, and G. Gingras.** 1985. Isolation and partial characterization of the messenger RNA encoding the B880 holochrome protein of *Rhodospirillum rubrum*. Eur. J. Biochem. **153**:477–484.
3. **Broglie, R. M., C. N. Hunter, P. Delepelaire, N.-H. Chua, and R. K. Clayton.** 1980. Isolation and characterization of pigment-protein complexes of *Rhodopseudomonas sphaeroides* by lithium dodecyl sulfate/polyacrylamide gel electrophoresis. Proc. Natl. Acad. Sci. USA **77**:87–91.
4. **Brusilow, W. S. A., R. P. Gunsalus, E. C. Hardeman, K. P. Decker, and R. D. Simoni.** 1981. *In vitro* synthesis of the F_0 and F_1 components of the proton translocating ATPase of *Escherichia coli*. J. Biol. Chem. **256**:3141–3144.
5. **Chung, C. H., and A. L. Goldberg.** 1981. The product of the lon (capR) gene in *Escherichia coli* is the ATP dependent protease La. Proc. Natl. Acad. Sci. USA **78**:4931–4935.
6. **Daniels, C. J., D. G. Bole, S. C. Quay, and D. L. Oxender.** 1981. Role for membrane potential in the secretion of protein into the periplasm of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78**:5396–5400.
7. **Date, T., G. Zwizinsky, S. Ludmerer, and W. Wickner.** 1980. Mechanisms of membrane assembly. The effects of energy poisons on the conversion of soluble M13 procoat to membrane bound coat protein. Proc. Natl. Acad. Sci. USA **77**:827–831.
8. **Deisenhofer, J., O. Epp, K. Miki, R. Huber, and H. Michel.** 1985. Structure of the protein subunits in the photosynthetic reaction center of *Rhodopseudomonas viridis* at 3 Å resolution. Nature (London) **318**:618–624.
9. **Dierstein, R.** 1983. Biosynthesis of pigment-protein complex polypeptides in bacteriochlorophyll-less mutant cells of *Rhodopseudomonas capsulata*. FEBS Lett. **160**:281–286.
10. **Dierstein, R.** 1984. Synthesis of pigment-binding protein in toluene-treated cells of *Rhodopseudomonas capsulata* and in cell-free systems. Eur. J. Biochem. **138**:509–518.
11. **Dierstein, R., and G. Drews.** 1982. Membrane differentiation and assembly of the pigment-protein complexes of the photosynthetic bacterium *Rhodopseudomonas capsulata*, p. 247–256. *In* G. Akoyuhoglou (ed.), Cell function and differentiation, part B. Alan R. Liss, Inc., New York.
12. **Dierstein, R., A. Schumacher, and G. Drews.** 1981. On insertion of pigment-associated polypeptides during membrane biogenesis in *Rhodopseudomonas capsulata*. Arch. Microbiol. **128**:376–383.
13. **Dierstein, R., M. H. Tadros, and G. Drews.** 1984. Turnover of pigment-binding protein in a mutant of *Rhodopseudomonas capsulata* which is defective in assembling reaction center and B870 into membranes. FEMS Microbiol. Lett. **24**:219–223.
14. **Dierstein, R., and W. Wickner.** 1985. The leader region of pre-maltose binding polypeptide binds amphiphiles. A model for self-assembly in protein export. J. Biol. Chem. **260**:15919–15924.
15. **Drews, G.** 1985. Structure and functional organization of light-harvesting complexes and photochemical reaction centers in membranes of phototrophic bacteria. Microbiol. Rev. **49**:59–70.
16. **Ebeling, W., N. Hennrich, M. Klockow, H. Metz, H.-D. Orth, and H. Lang.** 1974. Proteinase K from *Tritirachium album Limber*. Eur. J. Biochem. **47**:91–97.
17. **Enequist, H. G., T. R. Hirst, S. Harayama, S. J. S. Hardy, and L. L. Randall.** 1981. Energy is required for maturation of exported proteins in *Escherichia coli*. Eur. J. Biochem. **116**:227–233.
18. **Engelman, D. M., and T. A. Steitz.** 1981. The spontaneous insertion of proteins into and across membranes. The helical hairpin hypothesis. Cell **23**:411–422.
19. **Feick, R., and G. Drews.** 1979. Protein subunits of bacteriochlorophylls B802 and B855 of the light harvesting complex II of *Rhodopseudomonas capsulata*. Z. Naturforsch. **34c**:196–199.
20. **Halegoua, S., and M. Inouye.** 1979. Biosynthesis and assembly of the outer membrane proteins, p. 67–114. *In* M. Inouye (ed.), Bacterial outer membranes. John Wiley & Sons, Inc., New York.
21. **Hochmann, A., I. Fridberg, and C. Carmeli.** 1975. The location and function of cytochrome c_2 in *Rhodopseudomonas capsulata* membranes. Eur. J. Biochem. **58**:65–72.
22. **Imhoff, J. F., H. G. Trüper, and N. Pfennig.** 1984. Rearrangement of the species and genera of phototrophic "purple nonsulfur bacteria." Int. J. Syst. Bacteriol. **34**:340–343.
23. **Kyte, J., and R. F. Doolittle.** 1981. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
24. **Laemmli, U. K.** 1972. Cleavage of the structural protein during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
25. **Meister, H., R. Bachofen, G. Semenza, and J. Brunner.** 1985. Membrane topology of light-harvesting protein B870-a of *Rhodospirillum rubrum* G-9. J. Biol. Chem. **260**:16326–16331.
26. **Melandri, B. A., D. Zannoni, R. Casadio, and A. Baccarini-Melandri.** 1974. Energy conservation and transduction in photosynthesis and respiration of facultative photosynthetic bacteria, p. 1174–1162. *In* M. Avron (ed.), Proceedings of the 3rd

International Congress on Photosynthesis. Elsevier, Amsterdam.
27. **Nelson, N., and G. Schatz.** 1979. Energy-dependent processing of cytoplasmatically made precursor to mitochondrial protein. Proc. Natl. Acad. Sci. USA **76**:4365–4369.
28. **Peters, J., and G. Drews.** 1983. The transverse membrane orientation of the light-harvesting and reaction center polypeptides of *Rhodopseudomonas capsulata*, investigated by surface iodination. FEBS Lett. **162**:57–60.
29. **Oelze, J., and G. Drews.** 1981. Membranes of phototrophic bacteria, p. 131–195. *In* B. K. Gosh (ed.), Organization of prokaryotic cell membranes, vol. 2. CRC Press, Boca Raton, Fla.
30. **Randall, L. L., and S. J. S. Hardy.** 1984. Export of protein in bacteria. Microbiol. Rev. **48**:290–298.
31. **Schmidt, G. W., S. G. Bartlett, A. R. Grossmann, A. R. Cashmore, and N.-H. Chua.** 1981. Biosynthetic pathways of two polypeptide subunits of the light-harvesting chlorophyll a/b protein complex. J. Cell. Biol. **91**:468–478.
32. **Tadros, M. H., R. Dierstein, and G. Drews.** 1984. Occurrence of the bacteriochlorophyll-binding polypeptides B870 α and B800-850 α in the mutant strains Y5 and A1a$^+$ of *Rhodopseudomonas capsulata*, which are defective in formation of the light-harvesting complexes B870 and B800-850, respectively. Z. Naturforsch. **39c**:922–926.
33. **Tadros, M. H., K. Frank, and G. Drews.** 1986. Localization of the exposed N-terminal region of light-harvesting complex B800-850 α and β polypeptides on the cytoplasmic surface of *Rhodopseudomonas capsulata* chromatophores. J. Bacteriol. **167**:96–100.
34. **Tadros, M. H., F. Suter, G. Drews, and H. Zuber.** 1983. The complete amino acid sequence of the large bacteriochlorophyll-binding polypeptide from the light-harvesting complex II (B800-850) of *Rhodopseudomonas capsulata*. Eur. J. Biochem. **129**:533–536.
35. **von Heijne, G., and C. Blomberg.** 1979. Trans-membrane translocation of proteins. The direct transfer model. Eur. J. Biochem. **97**:175–181.
36. **Weckesser, J., L. S. Zalman, and H. Nikaido.** 1984. Porin from *Rhodopseudomonas sphaeroides*. J. Bacteriol. **159**:199–205.
37. **Wickner, W. T., and H. F. Lodish.** 1985. Multiple mechanisms of protein insertion into and across membranes. Science **230**:400–407.
38. **Wolfe, P. B., and W. Wickner.** 1984. Bacterial leader peptidase, a membrane protein without a leader peptide, uses the same export pathway as pre-secretory proteins. Cell **36**:1067–1072.
39. **Youvan, D. C., E. J. Bylinda, M. Alberti, H. Begusch, and J. E. Hearst.** 1984. Nucleotide and deduced polypeptide sequence of the photosynthetic reaction center, B870 antenna and flanking polypeptides from *Rhodopseudomonas capsulata*. Cell **37**: 949–957.
40. **Youvan, D. C., and S. Ismail.** 1985. Light-harvesting II (B800-850) complex structural genes from *Rhodopseudomonas capsulata*. Proc. Natl. Acad. Sci. USA **82**:58–62.
41. **Zimmermann, R., C. Watts, and W. Wickner.** 1982. The biosynthesis of membrane-bound M13 coat protein. J. Biol. Chem. **257**:6529–6536.