Conformation of a Purified “Spontaneously” Inserting Thylakoid Membrane Protein Precursor in Aqueous Solvent and Detergent Micelles*

Received for publication, October 20, 2000, and in revised form, December 18, 2000
Published, JBC Papers in Press, January 16, 2001, DOI 10.1074/jbc.M009600200

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Subunit W of photosystem II (PsbW) is a single-span thylakoid membrane protein that is synthesized with a cleavable hydrophobic signal peptide and integrated into the thylakoid membrane by an apparently spontaneous mechanism. In this study, we have analyzed the secondary structure of the pre-protein at early stages of the insertion pathway, using purified recombinant pre-PsbW. We show that the protein remains soluble in Tris buffer after removal of detergent. Under these conditions pre-PsbW contains no detectable α-helix, whereas substantial α-helical structure is present in SDS micelles. In aqueous buffer, the tryptophan fluorescence emission characteristics are intermediate between those of solvent-exposed and hydrophobic environments, suggesting the formation of a partially folded structure. If denaturants are excluded from the purification protocol, pre-PsbW purifies instead as a 180-kDa oligomer with substantial α-helical structure. Mature-size PsbW was prepared by removal of the precursor, and we show that this protein also contains α-helix in detergent but in lower quantities than the pre-protein. We therefore propose that pre-PsbW contains α-helical structure in both the mature protein and the signal peptide in nonpolar environments. We propose that pre-PsbW acquires its α-helical structure only during the later, membrane-bound stages of the insertion pathway, after which it forms a “helical hairpin”-type loop intermediate in the thylakoid membrane.

A substantial proportion of newly synthesized proteins are inserted into membrane bilayers, and studies on a variety of systems have shown that this process can occur co- or post-translationally. However, the post-translational targeting of hydrophobic membrane proteins is potentially problematic because these proteins are by nature prone to aggregation in polar environments. Hence, considerable attention has centered on the mechanisms by which these proteins are initially maintained in a soluble form, and how the hydrophobic regions are then transferred from an aqueous milieu into the apolar regions of the membrane bilayer. The majority of chloroplast and mitochondrial membrane proteins, and probably some bacterial membrane proteins, are inserted post-translationally, and studies on these systems have demonstrated two broad categories of insertion mechanism, which can be regarded as “assisted” and “unassisted” according to whether protein translocation machinery is utilized (reviewed in Ref. 1).

In bacteria, the secretory (Sec) apparatus, is used for the translocation of proteins into the periplasm, and the same apparatus is also required for the insertion of some membrane proteins. Additional factors are also used for the targeting of membrane proteins, particularly signal recognition particle (SRP) and FtsY (2–7). A similar assisted pathway operates in plant chloroplasts for the targeting of some cytosolically synthesized thylakoid membrane proteins. After import across the chloroplast envelope, a subset of thylakoid membrane proteins, exemplified by the major light-harvesting chlorophyll-binding protein, Lhcb1, are targeted by a pathway that requires GTP, a stromal form of SRP and FtsY (1, 8–10). This pathway is thus similar in many respects to bacterial SRP-dependent pathways and is presumably inherited from the cyanobacterial-type progenitors of plant chloroplasts.

Other thylakoid membrane proteins are targeted by a second pathway that exhibits very different characteristics. Subunit II of the integral CF$_{0}$ component of the ATP synthase (CF$_{0}$,II), and subunits X and W of photosystem II (PsbX, PsbW) are synthesized with bipartite presequences in which the stroma-targeting peptide is followed by a hydrophobic “signal” peptide. These signal peptides are similar in overall terms to Sec-type signal peptides that direct the targeting of lumenal proteins across the thylakoid membrane by the SecYEG complex. However, these proteins integrate into the thylakoid membrane in the absence of stromal factors, nucleoside triphosphates, or a ΔpH (11, 12). Furthermore, proteolysis of thylakoids, which blocks Sec-, SRP- and the lumen-targeting ΔpH dependent mechanisms, has no effect on the insertion of these proteins (13). In the absence of any identifiable proteinaceous targeting factor, it has been proposed that they may insert spontaneously into the thylakoid membrane.

This particular insertion mechanism is unusual because it involves the use of cleavable signal peptides for unassisted membrane insertion (at the very least, SRP, FtsY, and Sec involvement can be ruled out). Only one other protein, M13 procuct, has been shown to be synthesized with an N-terminal signal peptide yet inserted into the Escherichia coli plasma membrane by a Sec/SRP-independent mechanism (for review, see Ref. 14).

* This work was supported by an Engineering and Physical Sciences Research Council studentship (to C.W.), EPSRC Biosciences Interface Network Grant GR/M91105 (to A.R. and C.R.), and by Biotechnology and Biological Sciences Research Council Grant C07900 (to C.R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: Sec, secretory; SRP, signal recognition particle; PsbW, subunit W of photosystem II; DTT, dithiothreitol; GuHCl, guanidinium hydrochloride.
although procoat insertion does require the activity of a recently identified membrane-bound component, YidC (15).

Irrespective of whether unidentified membrane-bound machinery is required for the insertion of the above thylakoid membrane proteins, these proteins represent simple and attractive model systems for the analysis of the early stages of the insertion process. The final stages of the pathway have been characterized to some extent: the two hydrophobic regions (one in the presequence and the other in the mature protein) insert into the membrane, flipping the central acidic region across the membrane and thereby forming a loop intermediate structure. Cleavage by thylakoidal processing peptidase on the trans side of the membrane releases the presequence, leaving the N terminus of the mature protein in the thylakoid lumen. The formation of the loop structure has been confirmed (16), but the early stages of the insertion pathway are very poorly understood and the same applies to most other membrane proteins. The basic problem is identical in nearly every case: membrane proteins are inherently difficult to prepare and characterize in aqueous solvents and must normally be stabilized by detergents or chaotropic agents.

The relatively simple insertion mechanism used by the PsbW-type proteins, together with the notable insertion-competence of the in vitro translation products, suggested that these proteins may be inherently stable in aqueous phases and hence good model systems for the study of membrane proteins in general. We describe here the purification of pre-PsbW from an E. coli overexpression system in high yields and its characterization in aqueous solution and membrane-mimicking environments.

**EXPERIMENTAL PROCEDURES**

Overexpression and Purification of Pre-PsbW—The coding sequence for spinach pre-PsbW (17) was cloned into the pMW172 vector for overexpression in the C41 strain of E. coli (18). Expression was induced by the addition of 1 mM isopropyl-β-D-thiogalactoside, and the cells were cultured overnight. Cells were harvested, washed with 50 mM Tris-HCl, pH 8, and resuspended in 20% sucrose, 50 mM Tris-HCl, pH 8.0. The cells were lysed by passing them twice through a French press at 1000 p.s.i. The inclusion bodies were collected by centrifugation at 18,000 g for 10 min through a 40% sucrose pad (wash A), and the pellet was washed with buffers B, C, and D (buffer B = 0.4 M NaCl, 1 mM dithiothreitol (DTT), 0.5% Nonidet P-40, 10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA; buffer C = 50 mM NaCl, 1 mM DTT, 0.5% Nonidet P-40, 10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA; buffer D = 50 mM NaCl, 1 mM DTT, 10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). Finally, the pellet was resuspended in 8 M urea, 30 mM Hepes/KOH, pH 8.0, 1 mM DTT, 0.1 mM EDTA and left to rotate slowly overnight at 10 °C. Inclusion bodies were collected by centrifugation at 18,000 × g, resuspended in the same buffer, and left rotating at 10 °C for at least 2 h. The preparation was centrifuged again at 18,000 × g and resuspended in PGEDS buffer (50 mM sodium phosphate buffer, pH 8.0, 5% (w/v) glycerol, 1 mM EDTA, 1 mM DTT, 50 mM NaCl) and left rotating at 10 °C for 1 h. The purified inclusion bodies were collected by centrifugation at 18,000 × g and solubilized in PEGEDS buffer including Sarkosyl (2% v/v) and left to rotate slowly overnight at 10 °C. Purified solubilized protein was dialyzed against 20 mM Tris-HCl, pH 8.0, 1 mM DTT to remove free Sarkosyl. Overexpression and purification were analyzed by Tricine SDS-polyacrylamide gel electrophoresis (19) and Western blotting.

Size Exclusion Chromatography of Purified Pre-PsbW—A Superose 6 gel filtration column (HR 10/30, Amersham Pharmacia Biotech) was equilibrated in 20 mM Tris-HCl, pH 8.0, 1 mM DTT. 200 μl of dialyzed pre-PsbW was loaded onto the column, which was then run at 0.2 ml/min. Fractions were analyzed by Tricine SDS-polyacrylamide gel electrophoresis. Using identical conditions, the same column was cali-
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RESULTS

Overexpression and Purification of Pre-PsbW—The goal of this study was to analyze the structures of pre-PsbW and mature PsbW to obtain information on key points of the overall insertion pathway. The single most important objective was to prepare the precursor protein in aqueous solution because the early stages of membrane protein insertion are so poorly understood. Here it is important to point out that, although the first “envelope transit” signal in the presequence is probably removed prior to insertion into thylakoids, the full precursor protein is fully competent for thylakoid insertion and has been routinely used as a substrate in the in vitro insertion studies carried out to date (12, 16). This form of PsbW was chosen for biophysical analysis because the hydrophilic nature of the envelope transit domain was predicted to help maintain the protein in a buffer-soluble form. In addition, data on the full precursor protein are valuable because they provide insights into the structure of the protein prior to transport into the chloroplast.

Spinach pre-PsbW (molecular mass 14 kDa) was over-expressed in E. coli as detailed under “Experimental Procedures” and the protein was observed to form inclusion bodies, which were purified through several wash steps (Fig. 1A, lane IB). These inclusion bodies were found to be insoluble in urea, and the pre-PsbW was effectively purified to near homogeneity by simply washing the inclusion bodies twice in urea and then once in buffer, thereby solubilizing and removing the majority of contaminating proteins. Pre-PsbW remained in the insoluble pellet fraction and was then solubilized using the detergent Sarkosyl; Fig. 1B shows that the majority of protein is found in the supernatant fraction when this suspension is centrifuged at high speed. This procedure yields pre-PsbW in a purified form and the identity of the protein was confirmed by Western blotting using antibodies raised against a C-terminal region of spinach PsbW (Fig. 1, A and B, lower panels).

The Sarkosyl-solubilized pre-PsbW was extensively dialyzed against Tris buffer/DTT to remove free detergent, and the sample was subsequently spun at 100,000 × g to remove any insoluble aggregates. Unexpectedly, the majority of the pre-PsbW remained fully soluble in Tris buffer, and we characterized this protein using calibrated gel filtration chromatography (using Superose 6). The elution profile (Fig. 2, dotted line) shows that the protein elutes in fractions 15–30, indicative of high molecular mass multimers (average size calculated to be 180 kDa). However, these fractions were combined, concentrated, and unfolded in the presence of GuHCl and then re-run on the gel filtration column. Under these conditions the protein elutes primarily as a sharp peak (fraction 39) corresponding to a molecular mass of about 25–30 kDa (Fig. 2, solid line). The column was pre-equilibrated and eluted in Tris buffer containing DTT, and the GuHCl was found to elute much later (fractions 42–45; not shown). Thus, pre-PsbW can be prepared in a Tris-soluble form in a size indicative of a monomer or dimer.

Fractions containing pre-PsbW were pooled and concentrated using Amicon centrifprep concentrators with a YM3 filter. The sample was incubated in 4 m guanidinium hydrochloride (GuHCl) containing 1 mM DTT and applied to the Superose 6 column (pre-equilibrated and eluted with 20 mM Tris-HCl, pH 8.0, 1 mM DTT). Fractions were analyzed using Tricine SDS-polyacrylamide gel electrophoresis. For studies in detergent micelles, SDS was added to the protein sample to a final concentration of 0.5% (w/v). The mix was left at room temperature to equilibrate for 30 min before analysis.

Circular Dichroism Measurements—Far UV circular dichroism spectra were acquired for pre-PsbW at a concentration of 10 μM. Experiments were performed on a Perkin-Elmer LS50 luminescence spectrometer. Tryptophan fluorescence was measured using an excitation wavelength of 280 nm. Excitation and emission bandwidths were both 4 nm. Typically, 16 scans were averaged for each spectrum. All spectra had the background obtained with buffer alone subtracted from them. Structure fitting was carried out using the cdstr program as described in Refs. 20 and 21.

Fluorescence Spectroscopy—Fluorescence emission spectra were obtained for pre-PsbW at a concentration of 3 μM. Experiments were performed on a Perkin-Elmer LS50 luminescence spectrometer. Tryptophan fluorescence was measured using an excitation wavelength of 280 nm. Excitation and emission bandwidths were both 4 nm. Typically, 16 scans were averaged for each spectrum. All spectra had the background obtained with buffer alone subtracted.

Purification of Near Mature-size PsbW—Samples of 20 μM pre-PsbW in SDS were passed through a NAP-10 column (Amersham Pharmacia Biotech) containing 20 mM Tris-HCl, pH 8.0, 1 mM DTT, 5% n-octyl-β-D-glucopyranoside, and the eluate was mixed with 50 units of immobilized trypsin (Pierce) in the same buffer. The digestion mix was rotated slowly at room temperature for 3 h, spun to remove the trypsin beads, and the mixture applied to a mono-Q column (Amersham Pharmacia Biotech). The column was washed using 100 μl of 20 mM Tris-HCl, pH 8.0, 1 mM DTT, 2% n-octyl-β-D-glucopyranoside and eluted in the same buffer with a gradient of 0–500 mM NaCl. Elution of the PsbW degradation product was monitored by immunoblotting and peak fractions containing the protein were exchanged from n-octyl-β-D-glucopyranoside into SDS by passing through a NAP-10 column pre-equilibrated in 20 mM Tris-HCl, pH 8.0, 1 mM DTT, 0.5% SDS. Protein concentrations were determined by measurement of the A280 and A260 after subtracting the background of the obtained with buffer alone. Protein concentration was obtained using a correction formula (+t): 1.55 × A280 − 0.76 × A260 = mg of protein/ml (22).

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The protein is purified to homogeneity as judged by silver-staining of polyacrylamide gels (not shown).

Biophysical Analysis of Pre-PsbW—The formation of α-helices is a key event during membrane protein insertion. Energetic considerations strongly suggest that these must form prior to full insertion (for review, see Ref. 23), but the precise timing of α-helix formation is unclear in most cases. In principle, these structures may form in solution or they may form preferentially in the membrane interface region where competition for H-bond formation by water molecules may be reduced. In most cases this problem is extremely difficult to address because of the insoluble nature of recombinant membrane proteins in aqueous buffer. The Tris-soluble forms of pre-PsbW were therefore subjected to biophysical analysis to probe their structures in more detail.

The far UV circular dichroism spectrum of the initial, multimeric Tris-soluble form of pre-PsbW (average size 180 kDa) indicates a high proportion of α-helical structure characterized
**Fig. 5. Generation of mature-size PsbW by trypsin digestion.** A, pre-PsbW was incubated with immobilized trypsin for times indicated above the lanes (in min), and fractions were analyzed by Coomassie staining of polyacrylamide gels (top panel) or immunoblotting with PsbW-specific antibodies (bottom panel). Degradation product evident in immunoblot is denoted PsbW. B, a sample of pre-PsbW was digested with trypsin for 3 h (pre-column lane) and then chromatographed on a mono-Q column. Fractions 1–5 represent column washes (see “Experimental Procedures”) after which the protein was eluted with a 0–500 mM NaCl gradient (fractions 6–24). Elution of PsbW was monitored using immunoblotting.

**Fig. 6. Comparison of the CD spectra of pre-PsbW and trypsin-generated mature PsbW.** Pre-PsbW was prepared as shown in Figs. 2 and 3, and mature PsbW as in Fig. 5, and CD spectra were recorded in 0.5% SDS. Pre-PsbW, dotted line; PsbW, solid line.

by negative maxima at 208 and 222 nm. This secondary structure is predictably lost upon unfolding in the presence of GuHCl (Fig. 3A). The second Superose eluate (monomer/dimer) exhibits, by comparison, a substantial loss of secondary structure producing a relatively flat spectrum, which indicates the presence of very little conserved secondary structure (Fig. 3B). We deduce from these data that Sarkosyl only partially solubilizes the inclusion bodies containing pre-PsbW, forming multimeric structures that the GuHCl is then able to unfold. In contrast, the fluorescence emission profile of the monomeric/dimeric protein is not as tightly folded as the multimer, but they also suggest that the tryptophan residues are not fully exposed to aqueous solvent and that the protein may therefore be folded to some extent, despite lacking conserved secondary structure.

**Secondary Structure Analysis of the Mature Protein**—The above studies indicate that pre-PsbW adopts an α-helical structure within SDS micelles, but these data do not indicate whether both hydrophobic regions contribute to the observed spectrum. To gauge the structure of the detergent-solubilized precursor more precisely, we sought to compare it with that of the mature protein, which lacks the signal peptide. A near mature-size protein can be generated from pre-PsbW by trypsin digestion because the mature protein contains only a single basic residue, arginine, at position 5. Fig. 5A shows that trypsin cleaves the majority of pre-PsbW to this “mature” size after 30 min of digestion. Most of the pre-PsbW disappears by this time point although mature-size PsbW is not evident on this gel because it does not stain with Coomassie Blue. Nevertheless, the appearance of the degradation product can be monitored by immunoblotting (lower panel) and this smaller protein was purified as shown in Fig. 5B. This procedure generates near mature-size PsbW that is purified to homogeneity as judged by silver-staining of polyacrylamide gels (data not shown) and the protein is hereafter referred to as “mature PsbW” for simplicity.

Fig. 6 compares the CD spectra of pre-PsbW and mature PsbW in SDS micelles, with both proteins present at 10 μM concentration. The data show that the mature protein also adopts an α-helical conformation, and this almost certainly
Fig. 7. Secondary structure content of pre-PsbW and PsbW in SDS micelles. A, the data from Fig. 6 were quantified using the cdstr program (20) and are shown expressed in terms of residues per protein molecule. B, schematic illustration of the structure of pre-PsbW. The bipartite presequence contains an N-terminal envelope transit sequence that is removed by stromal processing peptidase (SPP) in vivo (although insertion into thylakoid membranes normally if this domain is present), followed by a hydrophobic signal peptide. Removal of the signal peptide is carried out by thylakoidal processing peptidase (TPP) on the trans side of the thylakoid membrane after the formation of a loop intermediate. The signal peptide is capable of forming a 20-residue transmembrane helix according to the TopPred 2 program (28), and 24 hydrophobic residues are present in the transmembrane span of the mature protein.

represents the formation of the predicted transmembrane α-helix in the mature protein. Importantly, the mature protein contains significantly less α-helical structure than the precursor protein, and we conclude from this finding that the signal peptide in the pre-PsbW presequence forms an additional α-helix in hydrophobic environments. These data were quantitated using the cdstr program (20) and are shown in Fig. 7 together with a diagram of the basic structure of pre-PsbW. The CD data predict a total of 44 α-helical residues within the precursor protein and 18 in the mature protein, which is in reasonable agreement with the overall structures of the two proteins (Fig. 7B). The two hydrophobic regions within pre-PsbW account for ~44 residues (of a 137 total), of which 24 are present within the mature protein (17) and about 20 in the signal peptide. Further analysis is required to characterize the structure of the loop intermediate in authentic membranes, but we propose from these data that the precursor protein does indeed form a two-helix structure in nonpolar environments and therefore forms a helical hairpin following insertion into the thylakoid membrane.

Discussion

The early events surrounding post-translational membrane protein biogenesis have remained unclear in the vast majority of cases. In vivo, proteins such as pre-PsbW are exposed to two distinct aqueous environments in route to the thylakoid membrane: the cytoplasm (prior to chloroplast import) and the stroma after import. As yet, we do not understand how they avoid aggregation or whether, for example, chaperone molecules help to maintain the proteins in an insertion-competent form. The primary technical problems are that membrane proteins tend to require stabilization with detergents after extraction from membranes, and the in vitro assays used to study protein insertion pathways do not provide any information on the conformation of the membrane proteins at any stage prior to membrane insertion. We have therefore sought to prepare chemical amounts of pre-PsbW to initiate studies into this area.

Of particular interest is the question of when and where the α-helices form in this type of membrane protein. The later stages of the pre-PsbW insertion pathway involve the formation of a loop intermediate that spans the thylakoid membrane twice with the N and C termini exposed to the cis face of the membrane and the N terminus of the mature protein in the lumen (16). The hydrophobic regions in the mature protein and signal peptide are predicted to form α-helices in this loop structure but this has not been tested experimentally, and we have no information on the timing or location of α-helix formation. Theoretical studies in this area have concluded that this process must occur before full insertion into the bilayer, and White and Wimley (23) have proposed that this type of protein could follow one of two basic insertion pathways: a “water path” in which the protein folds (i.e. forms α-helices) in the aqueous phase prior to insertion as a folded entity, or an “interface path” where the unfolded protein binds to the membrane interface where α-helix formation is promoted and insertion ensues. However, analyses of the water path are precluded in most cases because of the tendency of membrane proteins to aggregate in aqueous buffer. Apocytochrome c has been a popular model system because, despite being a hydrophilic protein, it has the unusual ability to insert spontaneously into membrane bilayers. Recent studies have shown that the protein is largely unstructured in aqueous solution but acquires a highly α-helical structure upon interaction with certain types of lipid, prior to full insertion (24). Apocytochrome c would thus appear to be a prime example of an interface path protein. Otherwise, the best studied cases are of membrane-interactive peptides such as classical signal peptides (25), relatively hydrophilic mitochondrial targeting signals (26), and melittin (27). In each of these cases, the peptide sequence exhibits a relatively extended conformation in solution but forms an α-helical structure in hydrophobic environments.

Pre-PsbW is by contrast a bona fide membrane protein and this study has provided information on its structure in both membrane-mimicking environments (SDS micelles) and aqueous solution. The CD spectrum of the trypsin-generated mature
protein in SDS indicates that, as predicted from the sequence (17), this protein forms a transmembrane α-helix in hydrophobic environments. The precursor protein contains an additional, hydrophobic signal peptide, and such peptides in other systems have been shown to form α-helices in membranes (26). Because pre-PsbW contains substantially greater α-helix in SDS than does the mature protein, we propose that its signal peptide does indeed form an α-helix and that the precursor therefore forms a helical hairpin once inserted into the membrane.

Pre-PsbW is also a rare example of a membrane protein that is amenable to analysis in aqueous environments, and on the basis of the data from this study, we propose that this protein is, like apocytochrome c, essentially an α-helical structure in the aqueous phase. The CD structure of the purified Tris-soluble pre-PsbW shows no detectable α-helix content, and the rapid appearance of this structure upon addition of SDS indicates that the hydrophobic regions are available to form α-helices in appropriate (i.e. more hydrophobic) environments. However, it is notable that the tryptophan fluorescence emission spectrum is not consistent with full exposure to solvent, raising the possibility that the protein may adopt a particular folding state(s) that helps to protect the hydrophobic regions. One distinct possibility is that the protein may adopt some form of non-helical hairpin structure in which the two hydrophobic regions interact with each other. One possibility is a loop structure that acquires helical structure once in an appropriate hydrophobic environment.

Although the emphasis in this study has been on the characteristics of the Tris-soluble monomer/dimer form of pre-PsbW, we should point out that the data do not rule out the possibility that the initial, 180-kDa multimeric form is physiologically relevant, because this assembly is also highly stable in Tris buffer. However, several factors suggest that this assembly is unlikely to form during the biogenesis of PsbW. First, proteins are imported into chloroplasts in a largely unfolded state, and monomeric pre-PsbW is therefore almost certainly released into the stroma. Second, pre-PsbW is transported into the thylakoid membrane very rapidly during in vitro assays, and it is unlikely that this complex form could be assembled under these conditions because (i) the targeting process is so rapid and (ii) cell-free translation systems generate very small quantities of protein, and hence relatively few molecules of pre-PsbW are imported into a given chloroplast.

Nevertheless, further studies are required to resolve these points, and it must be pointed out that it also remains to be confirmed whether the final preparation of purified protein is monomeric or not. Further studies are also required to determine whether the Tris-soluble protein is competent for insertion into thylakoid membranes, and insertion studies with such preparations will certainly help form a coherent picture of the biogenesis of this class of protein.

Acknowledgments — We gratefully acknowledge help and advice from Sheena Radford and Teresa Pinheiro, and we thank Bruno Miroux for providing the C41 strain.

References

1. Dalbey, R. E., and Robinson, C. (1999) Trends Biochem. Sci. 24, 17–22
2. Gebert, J. F., Overhoff, B., Manson, M., and Boos, W. (1988) J. Biol. Chem. 263, 16652–16660
3. Traxler, B., and Murphy, C. (1996) J. Biol. Chem. 271, 12394–12400
4. De Gier, J. W. L., Mansournia, P., Val lent, Q. A., Phillips, G. J., Lui rink, J., and von Heijne, G. (1997) FEBS Letts. 399, 307–309
5. MacFarlane, J., and Müller, M. (1996) Eur. J. Biochem. 233, 766–771
6. Ulbricht, N. D., Newitt, J. A., and Bernstein, H. D. (1997) Cell 88, 187–196
7. Val lent, Q. A., de Gier, J. W. L., von Heijne, G., Kendall, D. A., ten Hagen-Jongman, C. M., Oudega, B., and Lui rink, J. (1997) Mol. Microbiol. 23, 55–64
8. Li, X., Hen ry, R., Y uan, J., Clí ne, K., and Hoff man, N. E. (1995) Proc. Nat l. Acad. Sci. U. S. A. 92, 3789–3793
9. Kogata, N., Nishio, K., Hirohashi, T., Kikuchi, S., and Nakai, M. (1999) FEBS Lett. 439, 329–333
10. Tu, C. J., Schuenemann, D., and Hoff man, N. E. (1999) J. Biol. Chem. 274, 27219–24
11. Michi, D., Robinson, C., Shackleton, J. B., Herrmann, R. G., and K lös gen, R. B. (1994) EMBO J. 13, 1310–1317
12. Kim, S. J., Robinson, C., and Mant, A. (1998) FEBS Lett. 424, 105–108
13. Robinson, D., Karnauchov, I., Herrmann, R. G., Klös gen, R. B., and Robinson, C. (1998) Plant J. 10, 149–155
14. Kuhn, A. (1995) FEMS Microbiol. Lett. 17, 185–190
15. Samuelson, J. C., Chen, M., Jiang, F., Moeller, I., Wiedmann, M., Phillips, G., and Dalbey, R. E. (2000) Nature (Lond) 406, 637–641
16. Thompson, S. J., Kim, S. J., and Robinson, C. (1998) J. Biol. Chem. 273, 18979–18983
17. Lorkovic, Z. J., Schroder, W. P., Pakrasi, H. B., Irrgang, K.-D., Herr mann, R. G., and Oelmüller, R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8930–8934
18. Miroux, B., and Walker, J. E. (1996) J. Mol. Biol. 260, 289–298
19. Schagger, H., and von Jagow, G. (1987) Anal. Bio chem. 166, 368–379
20. Johnson, W. C. (1999) Proteins Struct. Funct. Genet. 35, 307–312
21. Rodger, A., and Ismail, M. (2000) Spectrophotometry and Spectrofluorimetry, pp. 99–139, Oxford University Press, Oxford, UK
22. Harris, E. L. V. (1995) in Protein Purification Methods: A Practical Approach (Harris, E. L. V., and Angal, S., eds). IRL Press at Oxford University Press, Oxford, UK
23. White, S. H., and Wimley, W. C. (1999) Annu. Rev. Biophys. Biomol. Struct. 28, 319–365
24. Breyson, E. A., Rankin, S. E., Carey, M., Watts, A., and Pinheiro, T. J. T. (1999) Biochemistry 38, 9758–9767
25. Giersch, L. M. (1989) Biochem. J. 260, 923–930
26. Roise, D., Tomich, S. J., Richards, J. M., and Schatz, G. (1986) EMBO J. 5, 1527–1534
27. Ladoshin, A. S., and White, S. H. (1997) J. Mol. Biol. 265, 1363–1369
28. Claro, M. G., and von Heijne, G. (1994) Comput. Appl. Biosci. 10, 685–686
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J. Biol. Chem. 2001, 276:14607-14613.
doi: 10.1074/jbc.M009600200 originally published online January 16, 2001

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