Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6748–6752, September 1990
Cell Biology

Metal-ion-center assembly of ferredoxin and plastocyanin in isolated chloroplasts

(precursor/import/assembly localization/protein conformation)

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Communicated by Eric E. Conn, June 25, 1990

ABSTRACT **Most chloroplastic proteins are cytosolically synthesized and posttranslationally transported to their proper locations. Two examples of this group of proteins are ferredoxin and plastocyanin, both of which are metal-containing components of the photosynthetic electron-transport chain. The import process for these two proteins includes the insertion of the metal ions to produce the holo forms of the proteins. We show here that *in vitro* translated precursor proteins of ferredoxin and plastocyanin are synthesized as apo forms and are assembled into their respective holo forms after being imported into isolated chloroplasts. We also provide evidence that only mature-sized proteins are competent to be assembled into holo forms.**

Most chloroplastic proteins are encoded in the nucleus and synthesized in the cytosol as higher molecular weight precursors with amino-terminal extensions called transit peptides. They are imported into chloroplasts posttranslationally. The import process is usually divided into the following steps: (*i*) binding of precursors to the outer envelope membrane; (*ii*) translocation of polypeptides across the outer and inner envelope membranes; (*iii*) removal of transit peptides by the stromal processing protease; and (*iv*) depending on the protein, either further sorting into other chloroplastic compartments and/or assembly into their respective complexes (1). Most import studies have been performed using an *in vitro* reconstituted system in which radiolabeled precursors are synthesized by *in vitro* transcription and translation from cloned genes and are subsequently imported into isolated chloroplasts.

Precursors to ferredoxin (prFD) and plastocyanin (prPC) are two proteins that have been examined in transport studies (2). The mature forms of these proteins are metalloproteins that function in photosynthetic electron transport. One unsolved aspect of their biogenesis concerns assembly of their metal-ion prosthetic groups. Although these two proteins follow the general steps of import described above, current evidence is inadequate to determine when during the import process prosthetic groups are added. An understanding of when the metal-ion centers are assembled will have important implications for possible import mechanisms. For example, if the metal-ion centers are added in the cytosol, the precursors would then have to carry the metal-ion centers across the envelope during import. If the metal-ion centers are assembled inside chloroplasts, this raises questions about whether assembly occurs before or after proteolytic processing. Imported precursors and mature-sized proteins may have different conformations and only one of them may be competent for prosthetic group assembly.

Higher plant ferredoxin (FD) contains a 2Fe-2S-type iron-sulfur center and functions exclusively in the stroma of chloroplasts. A pathway for introducing the iron-sulfur center into spinach FD in isolated chloroplasts has been described (3). Isolated chloroplasts were incubated with exogenous cysteine and the sulfur atoms derived from the cysteine were later found to be incorporated into the iron-sulfur center of endogenous FD. This demonstrates that isolated chloroplasts are capable of assembling the iron-sulfur center into FD. The authors suggested that iron-sulfur center assembly occurs after imported precursors are processed to their mature size.

Plastocyanin (PC) contains a copper ion as its prosthetic group, and functions in the thylakoid lumen. PC transport requires two steps: import into the stroma and subsequent translocation into the thylakoid lumen where it is processed to its mature size (2). A soluble, intermediate-sized PC (iPC) has been observed in the stroma in import experiments and has been suggested to be a pathway intermediate during the import of PC (2). It is not known whether the copper center is assembled in the stroma or in the lumen, or whether it is assembled in the cytosol when precursor is first synthesized. There is also little information on the mechanism of copper-center assembly. Assembly can be achieved *in vitro* simply by adding copper ion to a solution containing apo-PC (4). It has been suggested that holo-PC in chloroplasts results from chelating of copper ion by apo-PC (5) rather than from an enzyme-mediated assembly process.

In the work reported here, we examined the assembly of prosthetic groups into FD and PC imported into isolated pea chloroplasts. Owing to the limited amount of protein that could be synthesized with the *in vitro* translation system, a native gel electrophoresis system, rather than absorption spectra analysis and other methods, was used to distinguish holo and apo forms of both proteins. We provide evidence that both FD and PC were synthesized as apo precursors and assembled into holo forms after import. Mature apo-FD, but not prFD, was competent to be reconstituted *in vitro* into the holo form by rhodanese. An organelle-generated mature PC was assembled into the holo form in stroma. Mature PC generated by an *in vitro* reconstituted thylakoid translocation system was also assembled into the holo form without the presence of stromal components.

MATERIALS AND METHODS

Percoll, authentic FD (from spinach), and rhodanese (thiosulfate:cyanide sulfurtransferase, EC 2.8.1.1; from bovine liver) were obtained from Sigma. [^{3}H]Leucine (≈140 Ci/mmol; 1 Ci = 37 GBq) was from DuPont/New England

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Abbreviations: FD, ferredoxin; prFD, precursor to FD; PC, plastocyanin; prPC, precursor to PC; iPC, intermediate-sized PC.

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Nuclear. Wheat germ was a gift from General Mills. Authentic holo-PC (from spinach) was a generous gift of D. R. McMillin (Purdue University). All other chemicals were reagent grade.

Intact chloroplasts were isolated from 10- to 15-day pea (*Pisum sativum* cv. Little Marvel) seedlings as described (6). Isolated chloroplasts were resuspended in import buffer (330 mM sorbitol/50 mM Hepes/KOH, pH 8.0) at a concentration of 1 mg of chlorophyll per ml and kept on ice in the dark until use.

Tritium-labeled precursor proteins were synthesized as described (2). Import experiments were performed in import buffer containing chloroplasts at 0.33 mg of chlorophyll per ml, 5 mM ATP, and precursors from a protein translation mixture (2×10^6 dpm in a 150-μl reaction mixture). Reactions were conducted at room temperature in the light for 20 min. After the reaction, intact chloroplasts were reisolated by centrifugation through a 40% (vol/vol) Percoll cushion in import buffer (7). Recovered chloroplasts were subjected to further fractionation. This was accomplished by suspension of reisolated chloroplasts in 3 mM $MgCl_2$, incubation on ice for 5 min, and centrifugation in an Eppendorf microcentrifuge at 10,000 × *g* for 2 min. The supernatant (stromal fraction) was mixed with an equal volume of 2× SDS-containing sample buffer, for SDS/PAGE, or with 2× sample buffer lacking SDS and 2-mercaptoethanol, for native gel analysis. The pellets were resuspended in 3 mM $MgCl_2$ containing 0.1% (vol/vol) Triton X-100, incubated on ice for 5 min to release the lumenal contents, and spun in a microcentrifuge at 10,000 × *g* for 2 min to pellet the thylakoid membranes from the lumenal fractions. The lumenal fraction was mixed with sample buffers for SDS/PAGE or native gel electrophoresis as described above. After electrophoresis, the gels were fluorographed.

Native gel electrophoresis was performed using the same buffer system as described by Laemmli (8), except SDS was omitted from all solutions. All gels contained 15% polyacrylamide.

The conversions between apo-FD and holo-FD were accomplished as described (9). In brief, apo-FD was prepared by precipitating holo-FD with 10% (wt/vol) trichloroacetic acid to remove the iron-sulfur center and then washing with 1% trichloroacetic acid. The final pellet was resuspended in 0.15 M Tris/HCl (pH 7.3). Reconstitution was accomplished by incubating 60 μM apo-FD with 20 mM DL-dihydrolipoate, 1 mM sodium thiosulfate, 1 mM ferric citrate, and 3 μM rhodanese for 60 min at 0°C. When prFD was used, a volume equal to half that of apo-FD was added to the reaction mixture. The final concentration of prFD in the mixture was usually about 0.73 pM.

Copper removal from holo-PC and other PC-related proteins was performed as described (4). In brief, proteins were reduced by adding ascorbate and the solutions were dialyzed against 25 mM Tris/HCl buffer (pH 8.0) containing 0.01 M KCN for 2 hr at 4°C to remove the Cu^+. Excess CN^- was removed by dialyzing the samples against the same Tris buffer without KCN for another 3 hr.

Thylakoid membranes for *in vitro* reconstituted translocation experiments were prepared as follows: chloroplasts were hypotonically lysed at a concentration of 0.1 mg of chlorophyll per ml in 10 mM Hepes, pH 8.0/1 mM $MgCl_2$ (lysis buffer) for 5 min on ice. Membranes were isolated by centrifugation at 4000 × *g* for 6 min and subsequently washed in lysis buffer at the same concentration. The final pellet was resuspended to a concentration of 1 mg of chlorophyll per ml in import buffer and kept dark on ice until use. This preparation was routinely assayed for complete lysis by the following method. A portion was loaded on top of a 40% Percoll cushion and centrifuged. The absence of any pellet underneath the cushion indicated that no intact chloroplasts contaminated the preparation.

prPC from *Arabidopsis thaliana* (10) was used in the *in vitro* reconstituted translocation experiments. In a typical reaction, resuspended thylakoids were incubated with prPC from a translation mixture and 10 mM ATP at 30°C in the dark for 30 min. Reactions were stopped by pelleting the membranes for 3 min at 7000 × *g* in a tabletop centrifuge. An aliquot of the supernatant was mixed with same buffer for either SDS/PAGE or native gel electrophoresis for the analysis of untranslocated soluble reaction products. Membranes were washed once in import buffer and protease-treated by adding 1/10th volume of a 100 μg/ml freshly prepared solution of trypsin/chymotrypsin and incubating for 10 min on ice. Proteolysis was terminated by adding a 10-fold excess of soybean trypsin inhibitor. The protease-treated membranes were washed again in import buffer and solubilized with 0.2% Triton in import buffer. The samples were centrifuged at 10,000 × *g* for 10 min. An aliquot of the supernatant was mixed with sample buffers for analysis of the translocated soluble products.

RESULTS

prFD Was Synthesized as an Apo Form and Holo FD Was Assembled after Import. The holo and apo states of authentic FD can be distinguished in a native gel system (Fig. 1*A*, lanes 1 and 2). The results were similar to those described by Takahashi *et al.* (3). Holo-FD, owing to its compact conformation, ran as a single band very close to the dye front. Apo-FD ran as several slowly migrating bands because of the random formation of intra- and intermolecular disulfide bonds (3). We used this system to investigate the status of FD after it was imported into isolated chloroplasts. Only mature-sized FD accumulated inside chloroplasts, indicating that prFD was processed after import (Fig. 1*B*, lane 2). In a native gel system, some of the imported protein ran with the same mobility as authentic holo-FD (Fig. 1*A*, lanes 2 and 4). This

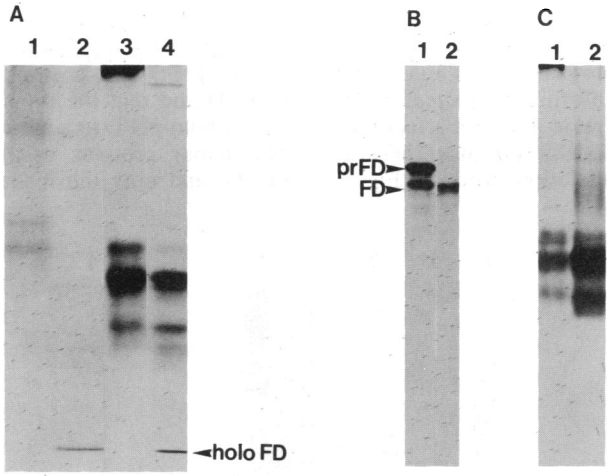

FIG. 1. Analysis of the assembly status of prFD and imported FD. (*A*) Discrimination of authentic holo-FD from authentic apo-FD on a native gel and the migration patterns of prFD and imported FD on the same gel. Lanes: 1, authentic apo-FD prepared from holo-FD; 2, authentic holo-FD; 3, prFD; 4, imported FD from the stromal fraction of the reisolated chloroplasts. Proteins in lanes 1 and 2 were revealed by Coomassie blue staining, and those in lanes 3 and 4 by fluorography. The position of holo-FD is indicated. (*B*) Samples from the same experiment as shown in *A* but analyzed by SDS/PAGE and fluorography. Lanes: 1, prFD; 2, imported FD from the stromal fraction. Positions of prFD and FD are indicated. (*C*) Trichloroacetic acid treatment of prFD analyzed on a native gel. Lanes 1 and 2, before and after treatment, respectively.

indicated that at least some of the imported protein was assembled into the holo form.

prFD produced a novel migration pattern on a native gel. This was probably due to the presence of the transit peptide and/or to the size heterogeneity of proteins synthesized from the *in vitro* translation system (Fig. 1*B*, lane 1). As a result, the assembly status of prFD could not be determined by comparing the migration patterns with the authentic holo- and apoproteins. We tried to resolve this problem by using conditions that would remove the iron-sulfur center. The rationale for doing this was that if there was an iron-sulfur center in the precursor, its depletion should cause some changes in the migration pattern of prFD on a native gel. As shown in Fig. 1*C*, the migration pattern of prFD remained essentially the same before and after treatment, whereas the migration pattern of holo-FD was altered by the same treatment (Fig. 1*A*). We thus conclude that prFD synthesized in the wheat germ *in vitro* translation system was in an apo form.

Apo-FD, But Not prFD, Could Be Reconstituted into Holoprotein by Rhodanese. We were interested in knowing whether prFD would still be import-competent if it were reconstituted into holo form. As a control, we first tried to reconstitute the iron-sulfur center into authentic apo-FD by using rhodanese (9). When treated FD was run on a native gel, the rapidly migrating band corresponding to holo-FD reappeared (Fig. 2*A*, lane 3). When reconstitution was performed using ^{35}S-labeled sodium thiosulfate, the holo form was the only protein labeled with ^{35}S (data not shown). These results confirmed that the reconstitution reaction resulted in reinsertion of the iron-sulfur center into the apoprotein using the sulfur provided.

We then tried the same reconstitution procedure on prFD. Analysis by native gel electrophoresis revealed a band with the same mobility as authentic holo-FD (Fig. 2*A*, lane 5, asterisk). The other changes in the gel pattern were caused by reagents in the reconstitution reaction. When the reaction mixture was filtered through a Sephadex G-25 column to remove small molecules before being loaded onto the polyacrylamide gel, the gel patterns of prFD and reconstituted prFD were identical except for the band comigrating with holo-FD (data not shown). If this new band represented holo-prFD, it would mean that holo-prFD has a similar conformation to mature-sized holo-FD and that the transit peptide does not affect the mobility of holo-prFD in a native gel. But because there were two major proteins in the translation product (Fig. 1*B*, lane 1), and only the higher molecular weight one was prFD, the new band on the native gel could have resulted from reconstitution of the iron-sulfur center into the lower molecular weight protein, which is similar to mature FD (see below). This lower molecular weight protein consistently appeared in our translation products. Because it was neither bound nor imported by chloroplasts, we conclude that it lacked a functional transit peptide. This lower molecular weight protein most likely resulted from internal initiation of translation at a methionine that is present as the last residue of the transit peptide (11). If so, this protein is identical to mature FD except that it contains one additional residue, a methionine, at its amino terminus. In order to determine which protein(s) was reconstituted into holo form in our experiment, the region of the gel containing the new band was excised and the protein was rerun into a denaturing gel (Fig. 2*B*). The reconstituted holo protein was composed entirely of the lower molecular weight protein.

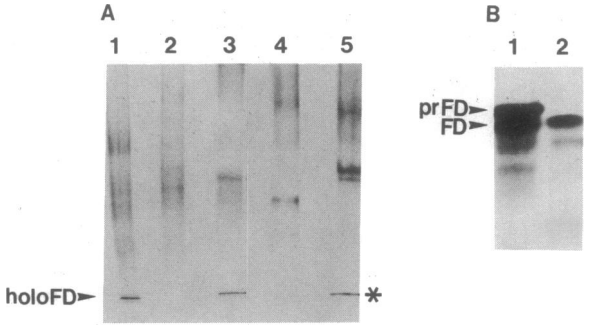

FIG. 2. Reconstitution of apo-FD and prFD by rhodanese and analysis of the reconstituted protein. (*A*) Analysis of reconstitution on a native gel. Lanes: 1, authentic holo-FD; 2, apo-FD prepared from holo-FD; 3, apo-FD after the reconstitution reaction; 4, prFD; 5, prFD after the reconstitution reaction. Proteins in lanes 1–3 were revealed by Coomassie blue staining. Lanes 4 and 5 show a fluorogram of the other half of the same gel. (*B*) Size analysis by SDS/PAGE of the protein indicated by the asterisk in *A*. Lanes: 1, prFD from a translation mixture; 2, excised gel slice containing the asterisk-indicated band.

prPC Was Synthesized in an Apo Form; Mature PC Was in the Holo Form After Completion of Translocation. We were also able to distinguish holo- and apo-PC with the same native gel system. Authentic holo-PC ran primarily as a single band with high mobility (Fig. 3*A*, lane 1). When converted into the apo form (see *Materials and Methods*), the high-mobility species was largely depleted. Instead, several bands with slower migration rates appeared on the gel (Fig. 3*A*, lane 2). The reason that the apo form migrated more slowly than the holo form remains unexplained.

When prPC was imported into isolated chloroplasts, it was processed to its mature size in the lumen (ref. 2; also Fig. 3*B*, lane 3). When this imported mature PC was run on a native gel, it yielded a pattern identical to that of the authentic holo-PC (Fig. 3*A*, lane 5). After removal of the copper by treatment with ascorbate and cyanide, however, the migration pattern of the imported protein matched that of authentic apo-PC (Fig. 3*A*, lane 6). It was thus clear that the imported lumenal PC was present as the holo form.

The same rationale described for determining the status of prFD was used to determine whether prPC was present as an apo or holo form. Treatment of prPC with ascorbate and cyanide to remove copper did not cause a change in the migration pattern on a native gel (Fig. 3*A*, lanes 3 and 4). Accordingly, we conclude that prPC was synthesized in a copper-free (apo) form by the *in vitro* translation system and was assembled into the holo form during or after translocation.

Stromally Localized PC Was Assembled into the Holo Form. A chimeric protein referred to as FD–PC was described previously (2). It was constructed by fusing the transit peptide of prFD and the first six amino acids of mature FD with the last two amino acids of the prPC transit peptide followed by the entire mature protein of PC. When this protein was imported into isolated chloroplasts, the transit peptide of prFD was cleaved off, leaving a modified PC in the stroma (2). This protein is slightly larger than authentic PC because of the eight extra amino acids (Fig. 3*B*, compare lanes 3 and 4).

We took advantage of the mislocalization of this modified PC to ask whether conversion to the holo form could occur in the stroma. FD–PC was imported into chloroplasts and the stromal fraction was analyzed on a native gel. Modified PC from the stroma produced a band pattern similar to the mature holo-PC from the lumen, but with lower mobility (Fig. 3*A*, lane 9). This alteration of mobility might have been caused by the extra amino acids present in the modified PC. To determine whether this protein contained copper, part of the sample was treated with ascorbate and cyanide and then analyzed on a native gel. This treatment resulted in a change in the migration pattern (Fig. 3*A*, lanes 9 and 10) similar to that observed when copper was depleted from the lumenal holo-PC (Fig. 3*A*, lanes 5 and 6). On the other hand, the

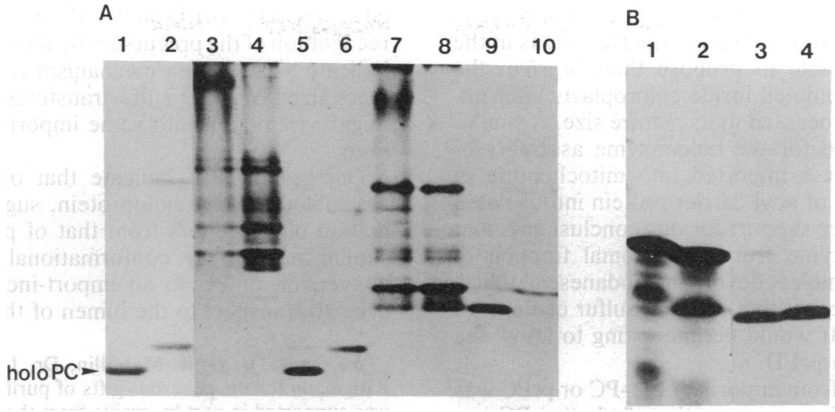

FIG. 3. Import and assembly of prPC and FD–PC. (*A*) Analysis of imported proteins on a native gel. Each sample was also subjected to copper removal as described in *Materials and Methods* and run in the lane next to the untreated sample. Authentic holo-PC was subjected to the same reaction at the same time to serve as a positive control. Lanes 1, 3, 5, 7, and 9 are protein samples before copper removal. Lanes 2, 4, 6, 8, and 10 are the respective proteins after copper removal. Lanes: 1 and 2, authentic PC; 3 and 4, prPC; 5 and 6, imported PC from the lumenal fraction; 7 and 8, FD–PC; 9 and 10, the modified PC from the stromal fraction of FD–PC import. Proteins in lanes 1 and 2 were revealed by Coomassie blue staining. Lanes 3–10 show a fluorogram of the other half of the same gel. (*B*) Samples from the same experiment as in *A*, but analyzed by SDS/PAGE. Lanes: 1, prPC; 2, FD–PC; 3, imported PC from the lumenal fraction; 4, the modified PC from the stromal fraction of FD–PC import.

copper-depletion process did not have any influence on the migration pattern of precursor FD–PC (Fig. 3*A*, lanes 7 and 8). We thus conclude that the modified PC was assembled into the holo form in the stroma.

PC Translocated into Isolated Thylakoids Was Assembled into the Holo Form in the Absence of Stroma. Although we were able to show that if it is stranded in the stroma a near-mature-sized PC can be assembled into the holo form, the naturally occurring form of PC in the stroma is not PC, but iPC. However, it is not yet possible to block the second step of PC import (from the stroma to the thylakoid lumen) *in organello*. It is thus difficult to obtain sufficient amounts of organelle-generated pathway intermediates from the stroma to investigate whether they are already converted into the holo form. However, an *in vitro* reconstituted system for translocation of prPC into isolated thylakoids was recently developed (C.M.B. and K.K., unpublished work). In this system, prPC is translocated into the thylakoid lumen and processed to mature size in an energy- and temperature-dependent fashion. Stromal extracts are not required for translocation. Thus it is possible to generate mature lumenal PC in the absence of stromal components. If assembly of holo-PC occurs only in the stroma with iPC, then mature lumenal PC translocated in the absence of stroma should not be assembled into the holo form. On the other hand, if holo-PC assembly occurs in the thylakoid lumen with mature PC, then *in vitro* translocated PC should be assembled into the holo form.

When mature lumenal PC from an *in vitro* translocation experiment (Fig. 4*B*, lane 3) was run on a native gel, it gave a band pattern exactly the same as mature holo-PC (Fig. 4*A*, lane 3). This indicated that lumenal PC was assembled into the holo form in the absence of stroma. The supernatant from this experiment contained mostly prPC, with some PC that was probably generated by inverted or broken thylakoid membranes. As a result, the migration pattern of proteins from the supernatant was a combination of those of prPC and PC (Fig. 4*A*, lane 2). Interestingly, most PC in the supernatant seemed to be in the apo form (compare Fig. 4*A*, lane 2, with Fig. 3*A*, lanes 1 and 2). This would be expected if holo PC assembly occurs only inside intact thylakoids.

DISCUSSION

Several chloroplastic proteins have been shown to be assembled into their respective enzymatic or macromolecular complexes after being imported into isolated chloroplasts (12, 13). This indicates that the *in vitro* reconstituted system is competent not only in the translocation step but also in the assembly step. Here, we extend these observations on assembly by showing that both FD and PC are assembled into their holo forms after import into isolated chloroplasts, thereby demonstrating that prosthetic-group assembly into metalloproteins can occur in the *in vitro* reconstituted import system.

Authentic apo-FD could be reconstituted into the holo form *in vitro* by rhodanese with high efficiency. Moreover, in the translation products, containing a mixture of prFD and a smaller protein that is almost identical to FD, the smaller protein was the only species that was competent to be reconstituted. This argues that prFD and FD must have different conformations and that rhodanese can act only on the latter. This may not give information about the assembly status of prFD *in vivo*, since the role of rhodanese in the assembly of the iron-sulfur center into FD is still uncertain

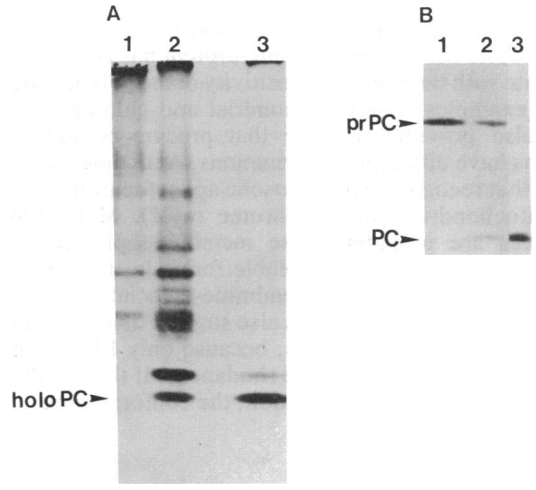

FIG. 4. Translocation of prPC into isolated thylakoids *in vitro*. Samples from an *in vitro* reconstituted thylakoid translocation experiment were analyzed by native gel electrophoresis (*A*) or SDS/PAGE (*B*). Lanes: 1, prPC before translocation; 2, untranslocated soluble reaction products; 3, soluble products recovered from the thylakoid lumen. A longer-exposure fluorogram of lane 3 in *B* is used to account for differential loading of the samples.

(14). However, together with the observation that *in vitro* translated prFD is in an apo form and imported FD is in the holo form, it is reasonable to propose that, *in vivo*, the iron-sulfur center is assembled inside chloroplasts after imported prFD has been processed to its mature size. A similar result has been reported for the holoenzyme assembly of aspartate aminotransferase imported into mitochondria *in vivo* (15) and the import of acyl carrier protein into isolated chloroplasts (16). Further support for our conclusion is that a partially purified enzyme from the stromal fraction of spinach chloroplasts, which is devoid of rhodanese activity, has been reported to reconstitute the iron-sulfur center into authentic apo-FD (17). It would be interesting to know the effect of this enzyme on prFD.

Mature PC generated from imports of FD–PC or prPC was assembled into the holo form, irrespective of whether PC was localized in the stroma or in the thylakoid lumen. Moreover, mature lumenal PC derived from translocation into isolated thylakoids in the absence of stroma was also assembled into the holo form. We have also observed that iPC generated by stromal extracts *in vitro* remained as an apo form even in the presence of thylakoids and a high concentration of copper (data not shown). Thus, even if some iPC is generated in our translocation system by residual stromal processing enzyme activity associated with the thylakoids, copper is not inserted into the iPC. These results suggest that copper insertion can occur either in the stroma or in the thylakoid lumen as long as the substrate is mature PC. We thus consider that, *in vivo*, copper is most probably assembled into PC in the thylakoid lumen since it is the place where mature PC naturally occurs.

Other authors have reported different conclusions about the conformational similarity between precursors and their mature proteins. The precursor of the chloroplastic enzyme 5-*enol*pyruvylshikimate-3-phosphate synthase has been shown to be enzymatically active and is similar to the mature enzyme in its sensitivity to the herbicide glyphosate (18). This indicates that the synthase precursor has a conformation similar to that of the mature enzyme. Similarly, precursor to the mitochondrial 4-aminobutyrate aminotransferase has been shown to be able to bind its cofactor and assemble into an active holoenzyme (19). On the other hand, several authors have reported conformational differences between precursors and mature proteins. For example, it has been proposed that, in bacterial export systems, polypeptides are export-competent only if they have not yet acquired their native conformations (20). The export competence of the precursor to the maltose-binding protein has been shown to correlate with the protease sensitivity of the polypeptide (21). Other examples with mitochondrial and chloroplastic proteins also provide evidence that precursors and mature proteins have different conformations. Antibodies have been raised that recognized the cytosolic apocytochrome *c* but not the mitochondrial holocytochrome *c* (22). More dramatic examples are probably those membrane proteins whose precursors are present as soluble forms in the cytosol but whose mature proteins are membrane-associated (13, 23, 24). Results from our experiments also suggest that prFD and FD have different conformations, because only FD was recognized by the sulfurtransferase rhodanese. If this result could be applied to the *in vivo* situation, the conformational difference between prFD and FD may not only facilitate the recognition of the precursors by the import apparatus but also indicate a protection mechanism to keep prFD from being recognized by other sulfurtransferases in the cytosol, which might assemble it into some import-incompetent conformations.

Our results also indicate that only mature-sized PC is assembled into the holoprotein, suggesting that the conformation of PC differs from that of prPC or iPC. Thus, in a similar manner, this conformational difference may prevent conversion of PC to an import-incompetent conformation prior to transport to the lumen of the thylakoid.

We thank Dr. D. R. McMillin, Dr. L. Morand, and Dr. D. W. Krogmann for the generous gifts of purified spinach PC. This work was supported in part by grants from the National Science Foundation and the Office of Basic Energy Sciences at the Department of Energy.

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