BIOSYNTHESIS OF PLASTOCYANIN: IDENTIFICATION OF PRECURSORS

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1. Introduction

Chloroplasts are capable of nucleic-acid and protein synthesis [1,2]. They are, however, not autonomous, because many proteins are synthesized initially on cytoplasmatic ribosomes. These proteins have to be transported from the cytoplasma into the chloroplast through membranes, e.g., the chloroplast envelope. It has been shown for the small subunit of the ribulose-1,5-bisphosphate carboxylase [1,3], the chlorophyll *a*/*b*-binding protein [4], and ferredoxin [5] that these plastidic proteins are synthesized as precursors having an M_r ~4000 larger than the authentic protein. This extra sequence, called 'transit peptide', is believed to ensure the specific transport of cytoplasmatically synthesized proteins into the chloroplast. The transport itself is thought to be a post-translational event [1,2].

The green alga *Scenedesmus acutus* synthesizes the copper protein plastocyanin in response to the cupric-ion content of the growth medium [6,7]. Under copper deficiency, plastocyanin is replaced by cytochrome *c*-553. Plastocyanin and cytochrome *c*-553 form a mutually exchangeable pair functional in redox activity both in 'in vivo' and 'in vitro' assays [7,8].

In an attempt to quantitatively determine this variable plastocyanin content in *Scenedesmus* cells, it was found that immunological methods yielded consistently higher values than spectroscopic methods (optical, EPR), which only register the holoprotein [9]. We assumed that the antiserum against plastocyanin cross-reacted with apoplastocyanin and possibly with other copper-free precursors. Therefore, the cross-reacting material was further analyzed on sodium dodecylsulfate (SDS)–polyacrylamide electrophoresis. It could be shown that *Scenedesmus* cells grown in a copper-free medium, thus containing large amounts of plastidic cytochrome *c*-553, contain in addition two precursors of plastocyanin with M_r-values of 10 500 and 14 000, respectively.

2. Materials and methods

The growth of the alga *Scenedesmus acutus* (strain 276-3a), the isolation of plastocyanin, and the properties of the antiserum were as in [6,9]. Copper-deficient algae were obtained by continuous growth in a copper-free medium ($<10^{-8}$ M Cu^{2+}) for several months; plastocyanin was detectable neither by optical nor EPR spectroscopy.

2.1. *Immunoprecipitation*

The plastocyanin antiserum was purified by ammonium sulfate precipitation (50%) and concentrated 3-fold. A 100 μl aliquot of the cell extracts (or of pure protein solution) was mixed with 50 μl antiserum and incubated for 72 h at 4°C. The precipitates were centrifuged (5 min, 8000 × *g*), the supernatant carefully removed by aspiration, and the pellet resuspended in 10–20 μl distilled water.

2.2. *Scenedesmus cell extracts*

Algae (3–4 g) were broken with glass beads [8] in a medium containing 0.2 M Tris–HCl (hydroxymethyl aminomethane) (pH 8.0). The homogenate was centrifuged for 1 h at 200 000 × *g*. The clear supernatant was subjected to a 100% ammonium sulfate precipitation; the precipitate was dissolved in 5 ml 20 mM Tris–HCl (pH 8.0). It was dialyzed against the same buffer and placed on a DE-52 column (1 × 5 cm) equilibrated with 20 mM Tris–HCl (pH 8.0) plus 0.06 M NaCl. After washing the column with the same buffer, the cytochrome *c*-553 or plastocyanin-containing fractions were eluted with 0.2 M

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NaCl. Plastocyanin precursors copurified with each of the proteins. The eluate was again concentrated by ammonium-sulfate precipitation (100%), dissolved in 1 ml distilled water, and dialyzed overnight. SDS–polyacrylamide electrophoresis with 15% gels was as in [10]. The protein solution to be analyzed was diluted 1:1 by a buffer solution containing 4% SDS, 2% dithiothreitol, 20% glycerol, and 60 mM Tris–HCl (pH 6.8). The mixture was incubated for 1 h at 60°C and then subjected to electrophoresis.

3. Results

When *Scenedesmus* cells are grown in a copper-free medium, values of 2.5–3.7 nmol cytochrome *c*-553/μmol chlorophyll are found. This is comparable to the highest plastocyanin content which can be obtained when algae are grown in the presence of 0.5–10 μM $CuSO_4$ [6,8,9,11]. In copper-free grown algae, plastocyanin is not detectable spectroscopically (detection limit: 1 plastocyanin/5000 chlorophylls). Nevertheless, these algae contain proteins cross-reacting with plastocyanin antiserum.

As shown in fig.1A, the immunoprecipitate analyzed on SDS–polyacrylamide gels contains two proteins with different M_r-values (marked by arrows, no. 1–4) in addition to the antiserum bands. The calibration curve reveals apparent M_r-values of 10 500 and 14 000, respectively (fig.2). Densitometric scans of immunoprecipitates of fig.1A, recording only proteins with an $M_r < 20\,000$, reveal the following (fig.1B): Extracts from cells grown in the presence of $CuSO_4$ exhibit only the 10 500 M_r species, probably consisting of holo- and apoplastocyanin (trace 1, arrow b). In copper-depleted algae, the larger M_r com-

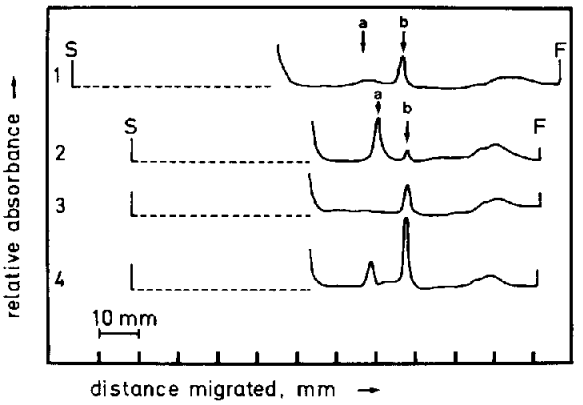

Fig.1B. Densitometric scans of the immunoprecipitates shown in fig.1A. Only proteins with $M_r < 20\,000$ were recorded; (a,b) denote immuno-reactive proteins with M_r-values of 14 000 and 10 500, respectively: S, start; F, front.

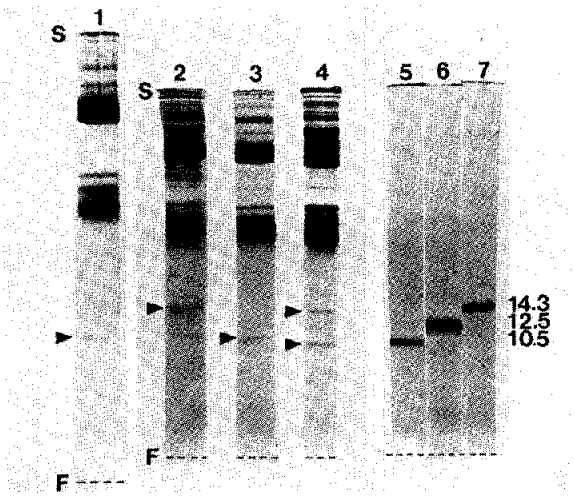

Fig.1A. SDS–polyacrylamide gel electrophoresis of immunoprecipitates obtained with a plastocyanin antiserum. The immunoprecipitates were formed by incubation of antiserum with: (1) extracts from *Scenedesmus* grown in the presence of 1 μM $CuSO_4$; (2) absence of cupric ions; (3) purified *Scenedesmus* plastocyanin; (4) with a sample of partially purified plastidic cytochrome *c*-553 isolated from *Scenedesmus* grown in the presence of 0.02 μM $CuSO_4$. No. 5–7 are reference proteins run on the same gel: (5) plastocyanin; (6) cytochrome *c*-550 (horse-heart); (7) lysozyme.

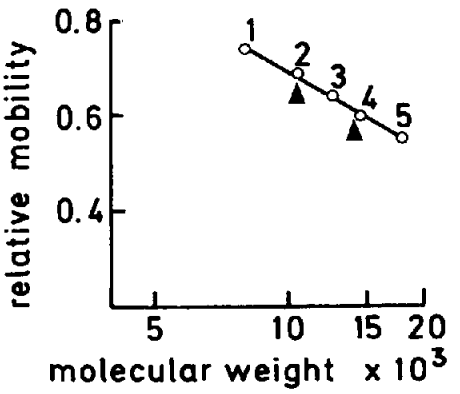

Fig.2. Determination of M_r of immunoreactive proteins by SDS–polyacrylamide electrophoresis (15%). The protein markers are (M_r): (1) *Pseudomonas* cytochrome *c*-551 (8000); (2) plastocyanin (10 500); (3) cytochrome *c*-550 (horse-heart, 12 500); lysozyme (14 300); (5) myoglobin (17 800). Arrows denote the relative mobilities of proteins obtained by immunoprecipitation of extracts from *Scenedesmus* grown in the absence and presence of $CuSO_4$.

ponent (a) is more prominent (trace 2). It is important in this respect to note that the immunoprecipitate formed with authentic plastocyanin gives rise to only one band at $< 20\,000\ M_r$ (trace 3). This plastocyanin band matches with the 10 500 M_r precursor (b). Thus, the antibody proteins are not interfering with the detection of plastocyanin precursors under our conditions.

When algae are grown in standard nutrient solution containing 0.02 μM $CuCO_4$ only, up to 1.5 molecules cytochrome *c*-553/1000 chlorophylls *a* and *b* are formed; plastocyanin is not detectable by absorption spectroscopy. As shown in trace 4 of fig.1B, both plastocyanin precursors can be isolated and enriched by immunoprecipitation from cell-free extracts, since they copurify with cytochrome *c*-553.

4. Discussion

Plastocyanin is a low M_r copper protein, mediating electron transport between cytochrome *f* and the photosystem-I reaction center in the photosynthetic electron transport chain. It is thought to be localized inside the thylakoid lumen [12]. Cycloheximide, but not chloramphenicol, inhibits plastocyanin biosynthesis in higher plants and *Scenedesmus* [13,14]. These data indicate that plastocyanin is synthesized in the cytoplasm by nuclear DNA and, as shown here, as a high M_r precursor (14 000). One might speculate that this precursor is processed within the chloroplast yielding the 10 500 M_r apoprotein. In the presence of cupric ions, authentic plastocyanin is formed. Synthesis of plastocyanin is dependent on light and suppressed by the uncoupler carbonylcyanide-*p*-trifluoromethoxy phenylhydrazone (FCCP) [11]. Our data also show that plastocyanin precursors accumulate in significant amounts when formation of the holoprotein is blocked by the absence of copper. Even in the presence of copper, when *Scenedesmus* cells contain levels of 2–3 plastocyanin/1000 chlorophylls, approximately one additional immuno-reactive protein molecule (mainly the 10 500 M_r species) is found by quantitative immunoelectrophoresis, based on a calibration curve with pure plastocyanin [9]. Among the cytoplasmatically-synthesized chloroplast proteins so far investigated, plastocyanin differs in one important aspect: it has to cross the chloroplast envelope and the thylakoid membrane to reach its final localization.

Acknowledgements

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