IDENTIFICATION OF P700–CHLOROPHYLL *a*–PROTEIN COMPLEX AS A PRODUCT OF CHLOROPLAST PROTEIN SYNTHESIS

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1. Introduction

Isolated chloroplasts incorporate labelled amino acids into ⩾10 discrete polypeptides, distinguishable by SDS–polyacrylamide gel electrophoresis, and are therefore capable of synthesizing certain of their own proteins [1,2]. The labelled polypeptides were found to be distributed among the soluble, the thylakoid and the envelope membrane proteins [3]. The identities of most of these polypeptides remain unknown.

Here, we have examined the possible synthesis of P700–chl *a*–protein complex by isolated mesophyll chloroplasts of *Sorghum vulgare*, a C_4 tropical plant. Contrary to [4], our results show that 2 of the 3 subunits of this complex are made in the mesophyll chloroplasts on endogenous templates. The mesophyll chloroplasts were preincubated in light for 1 h at 25°C to deplete the endogenous templates and when the chloroplasts were allowed to translate the exogenously added *Sorghum* total leaf RNA, all 3 subunits of light-harvesting chl *a*/*b*–protein complex and the 3 subunits of P700–chl *a*–protein complex were found to be synthesized. This indicates the possible role of nuclear genome in the synthesis of all the polypeptides of light-harvesting chl *a*/*b*–protein complex and 1 of the 3 polypeptides of P700–chl *a*–protein complex.

2. Materials and methods

2.1. *Preparation of photosynthetic membranes*

Chloroplasts from greenhouse grown *Sorghum vulgare* were isolated by centrifugation as in [5] in 0.06 M Tris–HCl buffer (pH 6.8) without osmoticum and resuspended in the same buffer. The suspension was then centrifuged at 10 000 × *g* for 10 min and the resulting pellet was resuspended and washed twice with the chloroplast resuspension buffer containing 0.1 M NaCl. The suspension was then centrifuged at 10 000 × *g* for 10 min and the resultant pellet was subsequently washed twice with resuspension buffer containing 1 mM EDTA and the final membrane pellet was resuspended in a minimal volume of the same buffer without EDTA, stored below 0°C and used for further analysis.

2.2. *SDS–polyacrylamide gel electrophoresis*

The photosynthetic membranes were solubilized in 0.06 M Tris–HCl buffer (pH 6.8) with final conc. SDS:chl = 10:1 (w/w). The membranes were further solubilized by applying 3 strokes in a Teflon hand homogenizer. The supernatant containing essentially all the chlorophyll was resolved by SDS–polyacrylamide gel electrophoresis [6] in 6 × 6 cm gel tubes without boiling, using a 10% polyacrylamide separation gel and 4% stacking gel.

2.3. *Other methods*

Absorption spectrum: The P700–chl *a*–protein and the light-harvesting chl *a*/*b*–protein complexes were eluted out of the gel by repeated extraction of gel slices in a minimal volume of 80% acetone. The absorption spectra were monitored in a Gilford spectrophotometer model 250.

The conditions for the incorporation of amino acids into hot trichloroacetic acid-insoluble polypeptides by isolated chloroplasts and for the electrophoretic analysis of the apoprotein compositions of the complexes on SDS–polyacrylamide gels were as in [5]. *Chlorella* [^{14}C]protein hydrolysate (spec. act. 27–42 mCi/m atom carbon) was obtained from Bhabha Atomic Research Centre, Bombay.

Abbreviations: Chl, chlorophyll; EDTA, ethylene diamine tetracetic acid; SDS, sodium dodecyl sulphate

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2.4. *The light-driven protein synthesis with the exogenous templates*

The mesophyll chloroplasts were prepared as in [5]. The freshly isolated chloroplasts at 500 μg/ml chl equiv., were incubated in light at 20 000 lux at 25°C without labelled amino acids. With the preincubated chloroplasts, the reaction was initiated with 50 μg *Sorghum* leaf RNA or 30 μg *Sorghum* chloroplast RNA and 0.5 μCi ^{14}C-labelled amino acids in 100 μl total vol. Appropriate dark and light controls of the reaction mixture without the exogenous templates were maintained. After desired time intervals, known aliquots were transferred to Whatman no. 3 filter paper discs and dried quickly. Further processing of the discs was done as in [5].

Leaf RNA from *Sorghum vulgare* was extracted by the modified hot phenol method [7]. *Sorghum* chloroplast RNA was extracted by the method in [8] as modified [9], except that all operations were performed in the cold.

After protein synthesis in vitro with either *Sorghum* total leaf or chloroplast RNA, the chloroplasts were extensively dialyzed against 0.06 M Tris–HCl buffer (pH 6.8) for 18 h and the photosynthetic membranes were prepared as in section 2.2.

After gel electrophoresis, the ^{14}C-labelled P700–chl *a*–protein and light-harvesting chl *a*/*b*–protein complexes were eluted out from the cut regions of the gels by grinding the finely divided gel slices for a prolonged time in 0.06 M Tris–HCl buffer (pH 6.8) to which the respective carrier proteins were added and the extracts were precipitated with 10% (w/v) trichloroacetic acid. The trichloroacetic acid precipitate was extensively dialyzed against the same buffer and analyzed by electrophoresis on SDS–polyacrylamide gels by the method in [10]. The molecular weights of the unknown proteins were determined following [11].

For measuring radioactivity, the gels were cut into 1 mm slices in a Mickle Gel Slicer and solubilized by incubation with 0.3 ml 30% H_2O_2 in a boiling water bath for 3 h. After cooling, 0.1 ml aliquots were transferred to Whatman no. 3 filter paper discs, dried and the radioactivity was measured in a liquid scintillation counter at 70% efficiency.

Protein was estimated by the method in [12] and total chl according to [13].

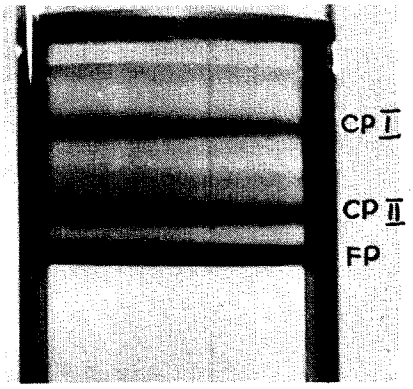

Fig.1. Analysis of labelled, unboiled SDS extract of chloroplast membranes of *Sorghum vulgare* by SDS–polyacrylamide gel electrophoresis: CPI, P700–chl *a*–protein complex; CPII, light-harvesting chl *a*/*b*–protein; FP, free pigment zone.

3. Results

Analysis of SDS extract of *Sorghum vulgare* chloroplast membrane products synthesized in vitro on endogenous templates in light showed 3 pigmented zones upon polyacrylamide gel electrophoresis (fig.1). The bluish green zone of lowest electrophoretic mobility corresponds to the P700–chl *a*–protein complex, the yellowish green zone of intermediate mobility, the light-harvesting chl *a*/*b*–protein and the green zone of highest mobility, the free pigment complexed with the detergent.

Fig.2 shows the room temperature absorption

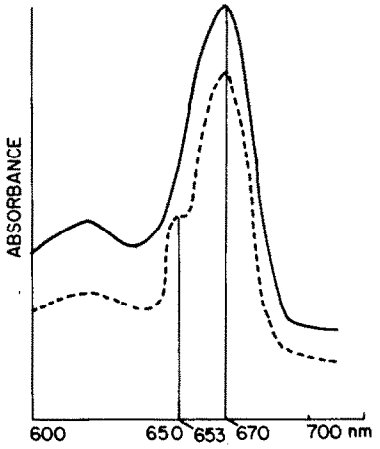

Fig.2. Room temperature absorption spectra of P700–chl *a*–protein complex and the light-harvesting chl *a*/*b*–protein: (——) P700–chl *a*–protein complex; (– – –) light-harvesting chl *a*/*b*–protein.

Table 1
Distribution of radioactivity in the chl–protein complexes (cpm/band)

Products	P700–chl *a*–protein complex	Light-harvesting chl *a*/*b*–protein
Endogenous	21 671	–
Sorghum chloroplast RNA translated products	23 802	–
Sorghum leaf cellular RNA translated products	21 542	19 436

spectra of the P700–chl *a*–protein and the light-harvesting chl *a*/*b*–protein eluted out from the gels. The P700–chl *a*–protein has A_{max} at 670 nm whereas the light-harvesting chl *a*/*b*–protein had two maxima at 653 and 670 nm. The most probable pigments among the known constituents of the complex that can be assigned to these peaks are chl *a* (670 nm) and chl *b* (653 nm).

When the unboiled thylakoid membrane components synthesized in vitro on the endogenous templates or by the preincubated chloroplasts primed with *Sorghum* chloroplast specific RNA were analyzed on preparative SDS–polyacrylamide gels, considerable amount of radioactivity was found to comigrate with the P700–chl *a*–protein complex as shown in table 1; the region corresponding to light-harvesting chl *a*/*b*–protein complex was not labelled. This might indicate that CPI is coded either totally or partially by the chloroplast genome and CPII by the extraplastidic genome.

The labelled CPI band on the preparative gels was eluted out to which the carrier CPI protein was added and the protein was precipitated with 10% trichloroacetic acid. The precipitated protein after dialysis was boiled with SDS and analyzed on 10% SDS–polyacrylamide gels. Two polypeptides with mol. wt ~70 000 and ~58 000 were found to be labelled. These two proteins appear to be synthesized by isolated chloroplasts with either endogenous or chloroplast RNA as template using light as the sole source of energy (fig.3).

Similarly when the thylakoid membrane components synthesized by *Sorghum* leaf cellular RNA in the preincubated chloroplasts were analyzed on SDS–polyacrylamide gels, it was observed that appreciable amount of radioactivity comigrated with region corresponding to both CPI and CPII as shown in table 1.

The labelled CPI and CPII bands were eluted out to which the corresponding carrier proteins were added

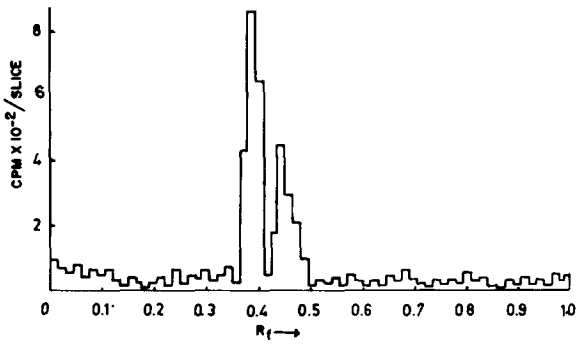

Fig.3. SDS–polyacrylamide gel electrophoretic profile of the labelled P700–chl *a*–protein complex synthesized in vitro on endogenous templates in the isolated mesophyll chloroplasts of *Sorghum vulgare* incubated with ^{14}C-labelled amino acids. The peaks correspond to the polypeptides of mol. wt 70 000 and 58 000.

and the boiled samples of the dialyzed 10% trichloroacetic acid precipitates of the proteins were analyzed separately on SDS–polyacrylamide gels to determine the polypeptide composition of both the complexes. In the case of CPI, there was one more additional

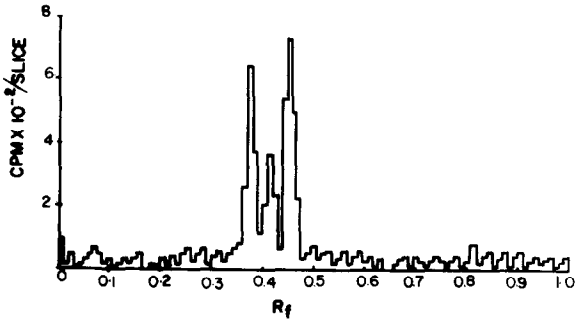

Fig.4. SDS–polyacrylamide gel electrophoretic profile of the labelled P700–chl *a*–protein complex synthesized in vitro in the preincubated chloroplasts of *Sorghum vulgare* primed with *Sorghum* leaf cellular RNA. The peaks correspond to the polypeptides of mol. wt 70 000, 65 000 and 58 000.

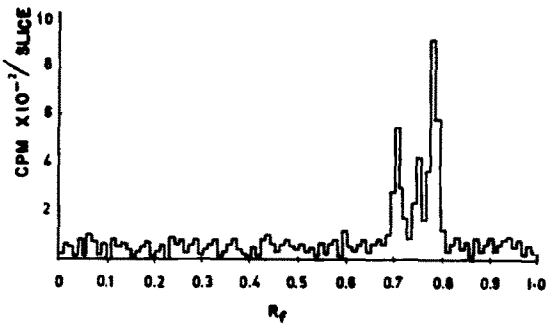

Fig.5. SDS–polyacrylamide gel electrophoretic profile of the labelled light-harvesting chl *a*/*b*–protein synthesized in vitro in the preincubated chloroplasts of *Sorghum vulgare* primed with *Sorghum* leaf cellular RNA. The peaks correspond to the polypeptides of mol. wt 27 500, 25 000 and 23 000.

minor polypeptide of mol. wt 65 000 labelled besides the 2 major polypeptides obtained with endogenous templates translated products (fig.4). In the case of CPII, 3 polypeptides of mol. wt 27 500, 25 000 and 23 000 were found to be labelled (fig.5).

4. Discussion

Our results indicate that *Sorghum* chloroplasts are capable of synthesizing certain portions of P700–chl *a*–protein complex. This apparently indicates that 2 out of 3 subunits are synthesized on chloroplast ribosomes and the third subunit is coded for by nuclear genome and synthesized in the cytoplasm in vivo. This is analogous to the situation with RuBP carboxylase, chloroplast specific coupling factor 1 and chloroplast ribosomal proteins [3,14,15]. This conclusion has been excluded [4] in work with isolated mature intact pea chloroplasts where none of the major chl–proteins was synthesized. The chl–proteins or a necessary part of them are made on cytoplasmic ribosomes, but it was pointed out that less mature chloroplasts might synthesize one or both of these complexes [4].

The *en:alba-1* mutant of *Antirrhinum majus* does not synthesize the P700–chl *a*–protein [16]. Since this plant is reported to be a plastome mutant, the site of coding of this component is construed to be in the chloroplast. The site of synthesis of the chl–proteins was studied using the incorporation of labelled amino acids into the 2 complexes during antibiotic inhibition of protein synthesis [17] and it was concluded that at least a main component of P700–chl *a*–protein was synthesized in the cytoplasm. With photoactive preparations of photosystem I from *Chlamydomonas reinhardi* Y-1, obtained by treatment with lipid micelles and deoxycholate, it was shown to contain two polypeptides of chloroplastic and one of cytoplasmic origin [18].

The mode of inheritance of the primary structure of the light-harvesting chl *a*/*b*–protein by interspecific, reciprocal hybrids was used in [19] to deduce that nuclear DNA codes for this protein, which has been further confirmed in [17]. Our observations with *Sorghum* leaf cellular RNA translated products constitute the most direct evidence for the site of synthesis of this protein and are in consonance with the earlier observations by others indicating the nucleo-cytoplasmic synthesis of this complex. However, this is in contrast to the conclusions in [18] that the photosystem II preparation contains 3 major polypeptides of cytoplasmic origin and 2 of chloroplastic origin. Conclusive evidence for the nucleo–cytoplasmic synthesis of CP II has also been presented [20,21].

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References

[1] Bottomley, W., Spencer, D. and Whitfeld, P. R. (1974) Arch. Biochem. Biophys. 164, 106–117.
[2] Morgenthaler, J. J. and Mendiola-Morgenthaler, L. M. (1976) Arch. Biochem. Biophys. 172, 51–58.
[3] Blair, G. E. and Ellis, R. J. (1973) Biochim. Biophys. Acta 319, 223–234.
[4] Eaglesham, A. R. and Ellis, R. J. (1974) Biochim. Biophys. Acta 335, 396–407.
[5] Geetha, V. and Gnanam, A. (1980) J. Biol. Chem. in press.
[6] Kan, K. S. and Thornber, J. P. (1976) Plant Physiol. 57, 47–52.
[7] Girard, M. (1967) Methods Enzymol. 12A, 581–584.
[8] Parish, J. H. and Kirby, K. S. (1966) Biochim. Biophys. Acta 129, 554–562.
[9] Hartley, M. R. and Ellis, R. J. (1973) Biochem. J. 134, 249–262.

[10] Laemmli, U. K. (1970) Nature 227, 680–685.
[11] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[12] Lowry, O. H., Rosebrough, N., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[13] Arnon, D. I. (1949) Plant Physiol. 24, 1–15.
[14] Mendiola-Morgenthaler, L. R., Morgenthaler, J. J. and Price, C. A. (1976) FEBS Lett. 62, 96–100.
[15] Bogorad, L. (1975) Science 188, 891–893.
[16] Herrmann, F. (1971) FEBS Lett. 19, 267–269.
[17] Machold, O. and Aurich, O. (1972) Biochim. Biophys. Acta 281, 103–112.
[18] Bar-Nun, S. and Ohad, I. (1977) Plant Physiol. 59, 161–166.
[19] Kung, S. D., Thornber, J. P. and Wildman, S. G. (1972) FEBS Lett. 24, 185–188.
[20] Chua, N.-H. and Gillham, N. W. (1977) J. Cell Biol. 74, 441–452.
[21] Cashmore, A. R. (1976) J. Biol. Chem. 251, 2848–2852.