GENETIC CONTROL OF PIGMENT–PROTEIN COMPLEXES I AND Ia OF THE PLASTID MUTANT EN:ALBA-1 OF *ANTIRRHINUM MAJUS**

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1. Introduction

Plastom mutants are well suitable for studying the function of genetic information of plastids, because the defects in mutant plastids are caused by mutations in the plastid DNA. The characterization and localization of the defects makes possible the detection of plastom-dependent reactions of plastids and gives information about the functions of plastid DNA [1]. From this point of view we recently investigated the plastom mutant en:alba-1 (extranuclear:alba-1) [2, 3]. The pale green mutated plastids are not capable of photosynthesis and have an impaired photosystem I [3].

The present study reports changes of SDS-solubilized lamellar proteins and pigment–protein complexes of mutated plastids. Gels from the plastom mutant en:alba-1 extracts do not show the bands corresponding to the chlorophyll–protein–detergent complexes I and Ia, nor are the corresponding protein bands present. Thus the lack of a functional photosystem I in this plastom mutant is due to absence of these chlorophyll–protein complexes. The defect of mutated plastids of the plastom mutant en:alba-1 is caused by a mutation of the plastid DNA; this indicates that the plastid DNA controls the formation of protein components of the pigment–complexes I and Ia.

Abbreviations:
Chl, chlorophyll.
SDS, sodium dodecyl sulphate.

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2. Material and methods

Plants of *Antirrhinum majus* L. var. 'Sippe 50' (green control) and the plastom mutant en:alba-1 were grown in a glass-house under dim light (about 2 klux). Under this condition the mutated plastids are pale green and the bleaching processes are slowed down. The mutated plastids are photosensitive, and under normal illumination they are white or pale yellow.

The isolation of stroma-freed chloroplasts of normal plants and of the plastom mutant of *Antirrhinum majus*, and the gel electrophoresis were carried out as described recently [8]. The chloroplast material is solubilized with a solution of SDS in 0.05 M sodium borate (pH 8.0) at a ratio of SDS/Chl = 15 (w/w). The SDS-solubilized lamellar proteins were separated by electrophoresis through a 9% polyacrylamide gel. The gels were stained with Coomassie Brilliant Blue R-250.

3. Results

The electrophoretic pattern obtained before and after staining the gels with Coomassie blue is shown in figs. 1 and 2. Coloured materials of SDS-solubilized wild-type chloroplasts of Antirrhinum were separated into three major pigment zones, which are designated as components I, II and III (fig. 1a). After staining the gels with Coomassie Blue, 15 protein bands of different intensity were detected (fig. 2a). The protein bands 2 and 13 have the highest intensity. They correspond to the pigment zones I and II,

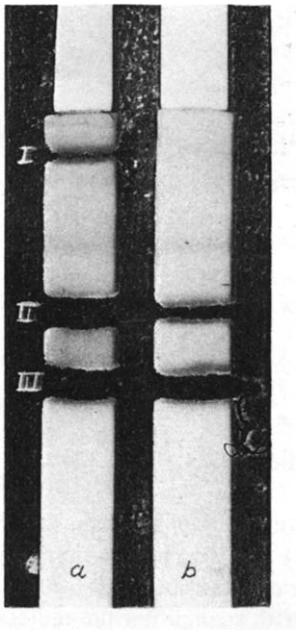

Fig. 1. Electrophoretic separation of pigment–protein complexes of wild type (a), and mutant en:alba-1 (b). Chloroplasts were from *Antirrhinum majus* after SDS-treatment (SDS/Chl ratio = 15).

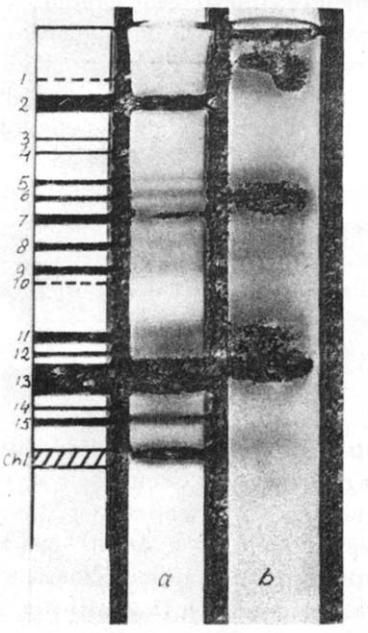

Fig. 2. Electrophorograms of chloroplast lamellar proteins of wild-type (a), and mutated (b) chloroplasts of plastom mutant en:alba-1 of *Antirrhinum* after staining the gels with Coomassie Blue.

which are associated with photosystems I and II, respectively. The pigment zone III only contains free chlorophyll released from the pigment–protein and does not contain protein complexes. (At the ratio SDS/Chl = 15 only the minor pigment–protein band IIc, corresponding to protein band 8, is green [8].)

The electrophoretic pattern of the separated lamellar proteins of mutated plastids shows only two green zones before staining the gels: the zone of pigment–protein complex II and the zone of free chlorophyll. The pigment–protein complex I is not detectable. Fig. 2 shows the gel after staining with Coomassie Blue. The protein bands 1 and 2 are absent. The bands 5 and 6 have a relatively higher intensity than those of the wild type chloroplasts. An additional protein band is present between the bands 8 and 9. The protein band 13, associated with pigment–protein complex II, has a relatively lower intensity than that of the control.

4. Discussion

Several authors have separated the SDS-solubilized chloroplasts lamellae as detergent complexes [4–8] by polyacrylamide electrophoresis. With this method a clear separation of pigment systems I and II is possible. This is shown by the significant differences in the ratio Chl *a*/*b* [4, 5] and the content of Chl *a* 680 and Chl *a* 670, respectively, in the two separated major chlorophyll–protein complexes I and II [8, 9].

The SDS-soluble chloroplast material of Antirrhinum was separated into two major pigment–protein complexes, 5 minor pigment–protein complexes and some colourless protein bands as we described recently [8]. The protein components 2 and 13 are identical with the pigment zones I and II, which are associated with photosystem I and II; the protein bands 1, 4, 6, 8 and 10 are assigned to the minor pigment–complexes Ia, IIa, IIb, IIc, and IId, respectively. (The pigment content of the minor complexes depends upon the SDS concentration used for solubilization-SDS/Chl ratio [8].) The minor complex Ia has similar spectroscopical properties as major complex I and therefore should be assigned to photosystem I [8].

The electrophoretic pattern of the mutated plastids of plastom mutant en:alba-1, which has an impaired photosystem I, demonstrates that the major pigment–

protein complex I and the minor pigment complex Ia, corresponding to photosystem I, are absent. An additional protein band appears between the band 8 and 9.

Recently Gregory et al. [10] reported that chlorophyll–protein complex I is also absent in detergent extracts of the mutant 8 of *Scenedesmus obliquus* which lacks activity in photosystem I. (It seems to be uncertain if mutant 8 is a gene or a plastom mutant.)

The defect of mutated plastids of the plastom mutant en:alba-1 is caused by a mutation of the plastid DNA. Therefore these results suggest that the plastid DNA controls the formation of protein components of the pigment–protein complexes I and Ia. The ability of the mutant plastids to become green under dim light – its chlorophyll content increases up to 38% of the control [3] – demonstrates that the chlorophyll synthesis in this plastom mutant is not impaired. The absence of the chlorophyll–protein complexes I and Ia obviously is not the result of the lack of chlorophyll. In fact, several studies on gene mutants of higher plants with lowered chlorophyll contents show that the chlorophyll synthesis is controlled by the nuclear genes [11]. Taking the results reported, we come to the conclusion that the genetic information of the plastid controls the formation of the structural proteins of pigment–complexes I and Ia to which the pigments are attached. The synthesis of pigments, however, is under control of nuclear genes.

The absence of the protein components of complexes Ia and I causes the defect in the photosystem I and the lack of photosynthetic activity. These changes in the lamellar proteins seem also to be responsible for the photooxidative bleaching of greenish mutated plastids.

The spectroscopic properties [8] of the complexes I and Ia and the absence of these complexes in the mutant en:alba-1 which lacks activity of photoreaction I suggest that these detergent–pigment–protein complexes are derived from a real and essential complex which is required *in vivo* for operation of photosystem I.

The appearance of a new protein band between bands 8 and 9 seems not to be specific for the defect in photosystem I, because such a protein is also found in the plastom mutant en:viridis-1, which has a defect in photosystem II. (In en:viridis-1 the chlorophyll protein complexes I and Ia are normally present.)

The plastom mutant en:alba-1 has also a slightly decreased activity of photosystem II [3]. The presence of an additional protein band between bands 8 and 9 and the lower intensity of band 13, associated with pigment–protein complex II, seem to be related to decreased activity of photosystem II.

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References

[1] R. Hagemann, Biol. Zbl. 90 (1971) 409.
[2] F. Hermann and G. Bauer-Stäb, Flora, Abt. A, 160 (1969) 391.
[3] F. Herrmann, Photosynthetica 5 (1971) in press.
[4] T. Ogawa, F. Obata and K. Shibata, Biochim. Biophys. Acta 112 (1966) 223.
[5] T.P. Thornber, R.P.F. Gregory, C.A. Smith and I.L. Bailey, Biochemistry 6 (1967) 391.
[6] O. Machold, Biochim. Biophys. Acta 238 (1971) 324.
[7] W. Menke and E. Schölzel, Z. Naturforsch. 26b (1971) 378.
[8] F. Herrmann and A. Meister, Photosynthetica, in press.
[9] O. Machold, A. Meister and K. Adler, Photosynthetica 5 (1971) 160.
[10] R.P.F. Gregory, S. Raps and W. Bertsch, Biochim. Biophys. Acta 234 (1971) 330.
[11] I.T.C. Kirk and R.A.E. Tilney-Bassett, The Plastids: Their Chemistry, Structure, Growth and Inheritance (W.M. Freeman and Co., London and San Francisco, 1967).