TWO DIMENSIONAL SEPARATION OF THYLAKOID MEMBRANE POLYPEPTIDES FROM WILD TYPE AND CHLOROPHYILL DEFICIENT MUTANTS OF PISUM SATIVUM L

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In algae and higher plants chlorophyll is always associated with specific thylakoid membrane proteins to form chlorophyll protein complexes. Some of these complexes contain chlorophyll a and b, both being present in approximately equal amounts. The main function of chlorophyll a/b protein complexes is light harvesting; therefore, they are called light-harvesting chlorophyll a/b protein complexes (LHGP). The protein moieties are called apoproteins.

By investigating chlorophyll b-deficient mutants it might be possible to elucidate more details concerning the role of chlorophyll b, its binding to proteins, and its role in photosynthetic functions. For this reason two chlorophyll mutants obtained from Dr. Gottscha1k's collection were analyzed using morphological and biochemical methods.

The mutants exhibit a similar phenotype caused by a total chlorophyll reduction of about 65%. One mutant, 130A, lacks chlorophyll b altogether, whereas the other mutant, chlorotica-29, possesses 24% chlorophyll b in relation to the initial line, cv 'Dippes Gelbe Viktoria'. Besides large differences in the structural development of thylakoid membrane systems, both mutants are characterized by differences in thylakoid membrane polypeptide composition as demonstrated by polyacrylamide gel electrophoresis (1). This polypeptide pattern was reinvestigated by two-dimensional gel electrophoresis.

Proteins were isolated from thylakoid membrane fractions by mild treatment with sodium dodecyl sulfate (SDS), and separated on polyacrylamide slab gels using Laemmli's Tris-glycine buffer system. separation gels of the first dimension (10-20% acylamide concentration) contained 0.1% SDS. Unstained lanes were cut out and polymerized in the stacking gel (1.5 mm thick) for second dimension gels containing 0.1% SDS and 5M urea. As a control, protein samples isolated from the different genotypes were one-dimensional1y electrophoresed on both gel systems with and without 5M urea. The polypeptide pattern became visible by staining gels with Coomassie brilliant blue G-230.

The different electrophoregrams of the thylakoid membnane polypeptides isolated from the three genotypes are presented in Fig. 1. The relative molecular mass range between 20 and 35 kD is shown because differences are located in this range. In one-dimensional separation of wild type (WT) the apoprotein band of LHCP from photosystem 11 is predominant (arrows, Fig. 1 A, B). This band is drastically reduced in chlorotica-29, and can only be detected as a very thin bind in chlorophyll b-deficient mutant 130A (arrows, Fig. 1, A,B). In the SDS gel (Fig. IB) two additional faint bands are visible in the lane containing 130A (arrowheads). In the gel with 5M urea (Fig. 1A) the polypeptide pattern is slightly different; resolution is not as good as in the SDS gel but the reduction of the apoprotein of LHCP is avident in lanes containing mutants.

Two-dimensional gel electrophoresis (Fig. IC) results in a better resolution compared with the one-dimensional gels. The reduction of LHCP apoprotein is well demonstrated (arrows, Fig. 1C). The staining intensity of. the spots in gels of mutants is more pronounced. This phenomenon is caused by the marked decrease in apoprotein. Applying the same protein concentration on gels this reduction alters the relative concentration of each polypeptide. To identify the spots with lower

concentrations on two-dimensional gels it might be more useful to stain the gels by the silver staining procedure. The question whether the second spot below the LHCP apoprotein (arrowhead, Fig. 1C) is also an apoprotein moiety of LHCP as discussed in the literature cannot be answered before using protein blotting and immunodetection methods.

A correlation between the concentration of chlorophyll b and the amount of the apoprotein of light-harvesting chlorophyll a/b protein complex from photosystem II can be observed in chloroplasts of the two mutants. This decreased apoprotein content might be caused by the instability of the complex. At present, it cannot be decided whether this instability depends on the deficiency of chlorophy11.b or on the altered apoprotein itself.

1. Schwartz, H. P. and K. Kloppstech. 1982. Planta 155:116-123.

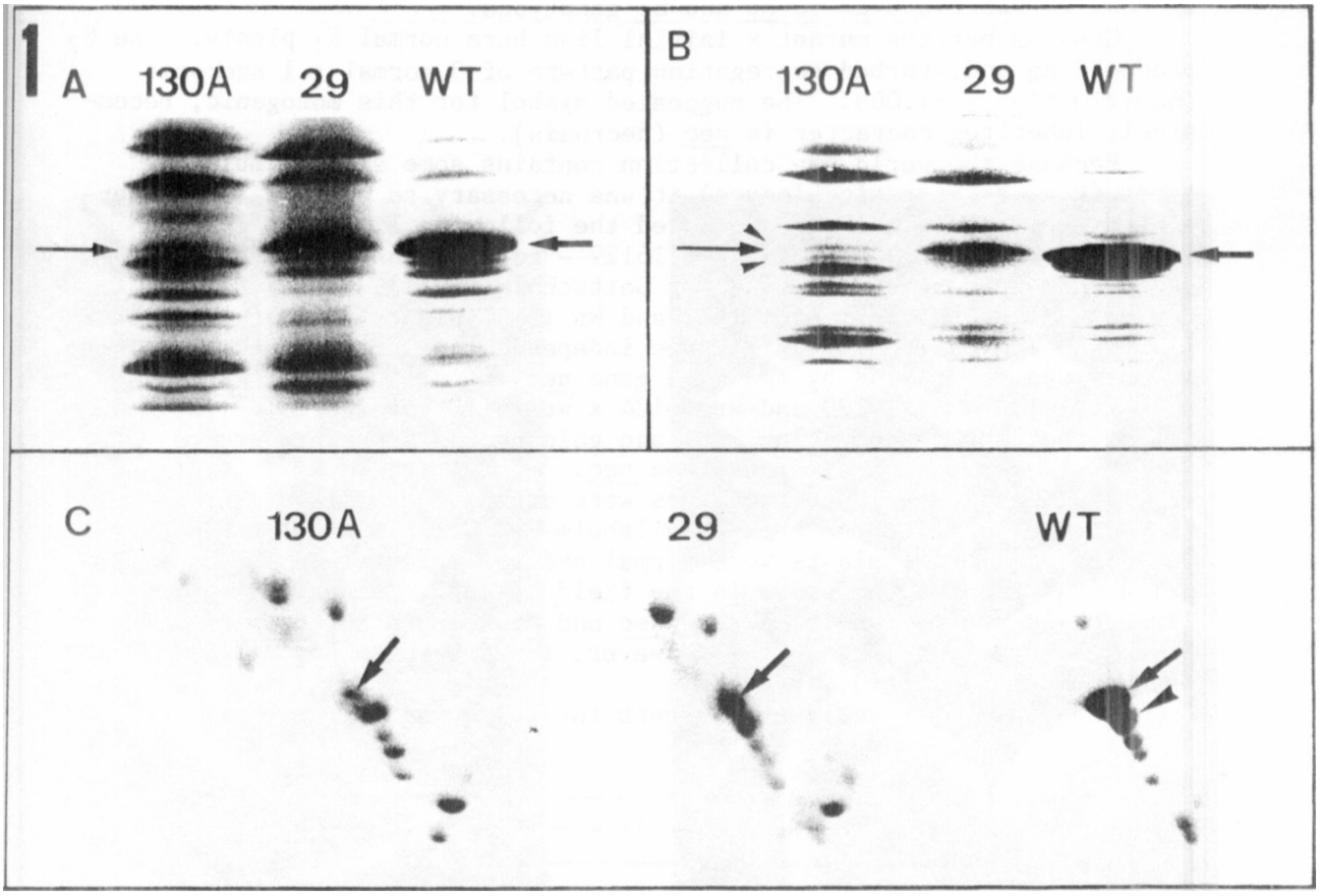

Fig. 1. Polyacrylamide gel electrophoresis of thylakoid membrane polypeptides.
Relative molecular mass range - 20-35 kilodaltons
WT - wild type, 29 - chlorotica-29,
130A - chlorophyll b-deficient mutant
A - Separation gel 10-15% acrylamide concentration containing 5M urea
B - Separation gel 10-20% acrylamide concentration
C - Two-dimensional gels, first dimension - 10-20% SDS-gel, second dimension - 10-15% SDS-gel containing 5M urea