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Two dimensional analysis of thylakoid membrane polypeptides of a barley mutant

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Summary. One dimensional SDS-PAGE and two dimensional electrophoresis of the thylakoid membrane proteins of a barley mutant and its original variety were carried out. It was found that (1) approximately 20 bands could be identified. The mutant lacked one band at 23 kd, and two bands at 17.5 nd 18.3 kd were fainter when compared with the original variety which served as the standard. (2) In two dimensional patterns the 23 kd band was resolved into two faint bands which were composed of three peptide spots having a pH of 6.35, 6.46 and 6.50, respectively. The band in the 17–18 kd region was resolved into five heavy peptide spots with varying pH values of 6.35–6.62.

Key words: Thylakoid – Membrane – Proteins – Two dimensional electrophoresis – Barley

Introduction

In chloroplast mutants changes in chlorophyll protein complexes and other thylakoid membrane proteins always occur in addition to the changes in leaf color. Mutations of chlorophyll protein complexes I and II were first observed by Herrmann in *Antirrhinum majus* and *Pelargonium zonale* (Herrmann 1971, 1972, 1974). Anderson et al. (1978) observed a deficiency of some thylakoid membrane polypeptides in both barley and spinach mutants. Members of the Carlsberg Laboratory in Copenhagen have done a great deal of research in this area (von Wettstein 1981, 1982; Machold 1979; Høyer-Hansen et al. 1982). Two types of barley mutants have been obtained and investigated by them. One is the mutant associated with a deficiency of PS-I; another is associated with a deficiency of PS-II. In addition, other mutants are associated with a deficiency of subunits of the coupling factor (CF_1) and/or iron-sulphur proteins. Different kinds of cytochromes have also been observed.

Nearly 30 to 40 bands of thylakoid membrane polypeptides may be observed by one dimensional SDS polyacrylamide electrophoresis (PAGE). Some of them are not stable and may change under the conditions of the separating procedures. As for the other more stable bands we can not know whether they are unique or complex compositions based on the results obtained by SDS-PAGE. It is therefore difficult to understand the functional identification of the membrane proteins. It is for this reason we performed the following study with two dimensional electrophoresis.

Materials and methods

Barley mutant 'Yellow-green 1832C' and its original cultivated variety 'Hedist' were used in this experiment. Characteristics of these materials have been described previously (Li 1983).

Isolation of thylakoid membrane proteins and the SDS-PAGE

The methods of isolation and purification of the thylakoid membrane proteins were based on the modified procedure of Machold (1979). System II of Machold was followed in gel electrophoresis.

Two dimensional electrophoresis

The procedure used is essentially the same as that described by O'Farrell (1975) and Remy (1983). The first dimension is the isoelectric focusing (IEF). For eight gel tubes (120×3 mm inner diameter) 10 ml of the gel mixture were prepared: 5.5 g urea, 2 ml 10% NP-40, 1.97 ml distilled water and 1.70 ml 30% acrylamide solution (28.38% acrylamide and 1.62% Bis in water). The mixture was heated ans shaken gently to solubilize the urea. After cooling, 0.4 ml ampholine, pH 5–8, and 0.1 ml pH 3.5–10, 20 µl 1% persulfate solution and 15 µl TEMED

were added. The glass tubes were filled with the above solution and overlayed with 8 M urea. After polymerization the urea solution was removed and replaced by 20 µl lysis buffer (9.5 M urea, 2% NP-40.2% ampholine and 5% β-mercaptoethanol) and overlayed with 0.02 M NaOH. The tubes were then put into a disc electrophoretic apparatus. 0.02 M NaOH was used as upper reservoir buffer and 0.01 M H_3PO_4 was used as the lower reservoir buffer. Pre-electrophoresis was carried out before pooling out the upper reservoir solution and the overlayed mixture and 20–30 µl sample solution was loaded on the top of each gel and overlayed with 10 µl of a solution containing 10% glycerol and 1% ampholine. The upper reservoir buffer was poured in electrophoresis was carried out at 400 v for 12 h, and then at 800 v for 1 h.

After electrophoresis the gel strips were taken out and one of them was used to measure the pH gradient.

The second dimension was SDS-PAGE. The procedure used was similar to those described by Laemmli (1970). The gel strips were equilibrated in a solution containing 2.3% SDS, 10% glycerol, and 5% β-mercaptoethanol in 0.625 M Tris-HCl for 2 h at room temperature. A 12.5% acrylamide solution was used as the separating gel, set between two glass plates (20×20×0.15 cm) and overlayed with isobutanol. After polymerization, the iso-butanol was removed, the gel surface was washed with distilled water several times, and finally washed with a few drops of stacking gel mixture. The stacking gel with 4.75% acrylamide was poured to within 2 mm of the top of the notched plates. After polymerization, the equilibrated gel strip was placed on the stacking gel surface and fixed into place with melted 1% agarose gel dissolved in equilibrated solution. Electrode buffer 0.025 M Tris and 0.192 M glycine 0.1% SDS was added to the upper reservoir buffer. Electrophoresis was carried out at 18 mA for 10 h.

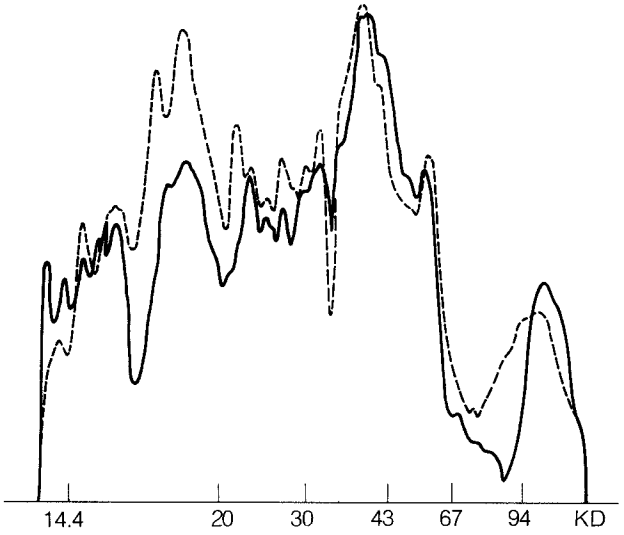

Fig. 1. Photodensitometric scanning map of SDS-PAGE of the membrane polypeptides of a barley mutant and standard. —— mutant, – – – standard

Results

The barley mutant used in this experiment was known by our previous work to be deficient in CPII (Li Jigeng et al. 1983). Results obtained by SDS-PAGE indicated that about 20 bands of thylakoid membrane polypeptides separated out both in the mutant and in its original variety, which was the standard. In comparison to the standard, one 23 kd band was missing in the mutant. The peak response to the 23 kd band was also missing in the photo-densitometric scanning map. In addition, two other peaks of 17.5 and 18.3 kd were significantly fainter in the mutant than in the standard (Figs. 1–3).

Results obtained by two dimensional electrophoresis revealed that the basic separated patterns of membrane polypeptides from the mutant and standard varieties were compatible. Significant differences were as follows.

(1) In the standard, two faint bands near 23 kd and one heavy band near 17 kd were observed. The pH of their location are in the range of 6.3–6.7; all these bands were absent in the mutant.

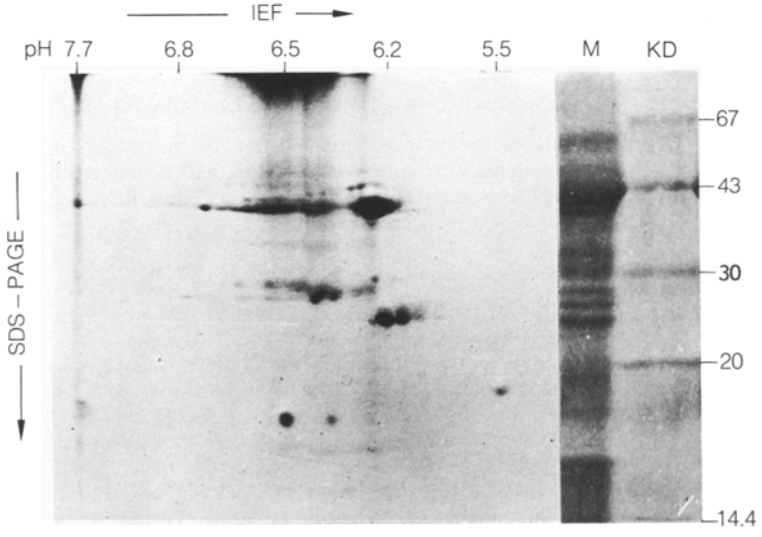

Fig. 2. Two dimensional separation of the membrane polypeptides of a barley mutant (*M*)

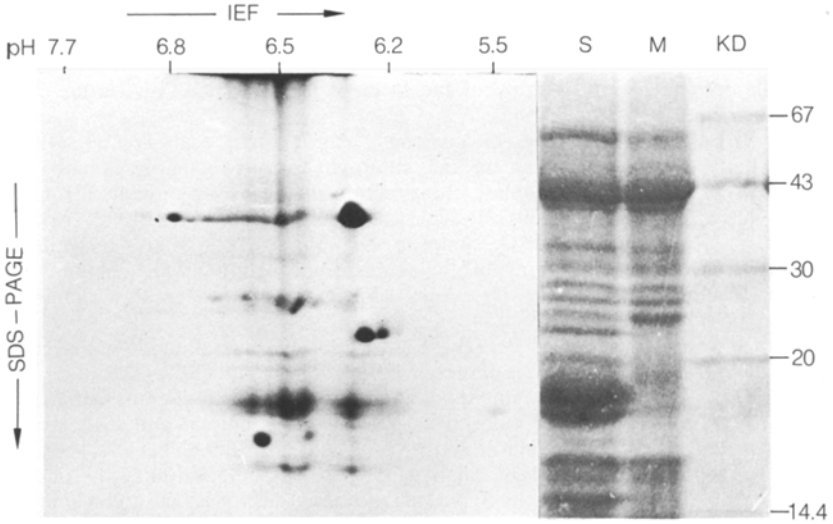

Fig. 3. Two dimensional separation of the membrane polypeptides of a barley variety used as standard (*S*)

(2) Two faint bands near 23 kd in the two dimensional gel may be separated from the 23 kd band in one dimensional SDS-PAGE.

(3) Two faint bands were re-separated into three peptide spots with pH values of 6.35, 6.46 and 6.5 respectively and other two minor components in two dimensional gel.

(4) The heavy band near 17 kd was re-separated into five heavy peptide spots in the pH range of 6.35–6.62.

(5) One spot in the region of 17 kd near pH 5.5 migrated to the more alkaline side than its counterpart in the standard.

Discussion

1 Location of the membrane polypeptides specific for CPII

The content of Chl b in CPII is so much higher than in CPI, that the mutant deficiency in CPII used to be ascribed to a deficiency of Chl b. Thornber and Highkin (1974) were the first to report the absence of a major component in an electrophoretic pattern of chloroplast membrane proteins from a barley Chl b-less mutant. They showed that this component comprised the protein complex of the PSII chlorophyll-proteins. Genge et al. (1974); Anderson et al. (1978) and Apel (1978) obtained similar results with barley mutants deficient in Chl b.

As for the location of membrane peptides specific for CPII, Henriques et al. (1975 a) considered that the 23 kd polypeptides was the major location of Chl b in spinach. But in barley they found that the most noticeable difference between the mutant and wild type is the total absence of 25 kd and two other minor components of 27.5 and 20 kd (1975 b). Similar results obtained by von Wettstein et al. (1982); Machold et al. (1979) and Høyer-Henson et al. (1982) indicated that in Chl b deficient barley mutants, CPII is completely absent. The major component of CPII is the band at 25 kd and two other minor bands at 26 kd and 24 kd.

Based on the identification of chlorophyll-protein complexes, the barley mutant used in this experiment in a CPII deficient mutant. In the SDS-PAGE of the membrane polypeptides the 23 kd polypeptide was totally absent. The scanned peaks of 17.5 kd and 18.3 kd bands were significantly lower than that of standard. Thus, we considered it as a Chl b deficient mutant. This result is agreeable with those obtained by the others mentioned above. Some discrepency among them my be related to differences of varieties and analytical procedures used in experiments.

2 Species of membrane polypeptides specific for CPII

No matter whether the 23 kd or 25 kd band is the major component specific for CPII, the component composition of this band can not be determined only by one dimensional SDS-PAGE. However, this limitation can be overcome by two dimensional electrophoresis. Our present data fully demonstrate that the 23 kd polypeptide actually consisted of at least three peptides with the same molecular weight but different amino acid composition. The 17 kd polypeptide was also composed of five peptides with the same molecular weight but different amino acid compositions. These results are valuable and helpful for further understanding of the amino acid composition of thylakoid membrane polypeptides.

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