Technical communication

Two dimensional electrophoresis of thylakoid membrane proteins and its application to microsequencing

Shi-Gui Yu, Hreinn Stefansson, Elzbieta Romanowska & Per-Åke Albertsson*
Department of Biochemistry, University of Lund, PO. Box 124, S-221 00 Lund, Sweden; Present address: Department of Plant Physiology II, University of Warsaw, 00 927 Warsaw; * Author for correspondence

Received 27 September 1993; accepted in revised form 18 July 1994

Key words: isoelectric focusing, Photosystem I, Photosystem II, Rieske iron-sulfur protein, thylakoid membrane organization, two-dimensional electrophoresis

Abstract

A procedure of two-dimensional gel electrophoresis adapted for application on membrane proteins from the thylakoids is described. It involves isoelectric focusing in the first dimension and size dependent electrophoresis in the second dimension. About 100 polypeptides are clearly separated with relatively little streaking. About 20 polypeptides are identified by immunoblotting or location in the gel. They are the polypeptides of the PSI core, the 64 kDa protein, the α and β subunits of CF1 ATPase, cytochrome f, Rieske iron-sulfur protein, the 23 kDa and 33 kDa polypeptides of the oxygen evolving complexes, CP29, CP24, CP27 and CP25 (last two proteins belong to LHCII). Some proteins give rise to two or more separate spots indicating a separation of different isoforms of these proteins. Among them, the LHCII polypeptides (27 kDa and 25 kDa) were each resolved into at least three spots in the pH range 4.75–5.90; the Rieske FeS protein, as published elsewhere (Yu et al. 1994), was separated into two forms having different isoelectric points (pI 5.1 and 5.4), each of them was also microsequenced; the 64 kDa protein claimed to be a LHCII-kinase was found to be multiple forms appearing in at least two isoforms with pI 6.2 (K1) and 6.0 (K2) respectively, furthermore, K1 can be resolved into two subpopulations.

The lateral distribution of these proteins in the thylakoid membrane was determined by analysing the vesicles originating from different parts of the thylakoids. The data obtained from this analysis can be partially used as markers for different thylakoid domains.

This procedure for sample solubilization and 2-D electrophoresis is useful for the analysis of the polypeptide composition of vesicles originating from the thylakoid membrane and for microsequences of individual polypeptides isolated from the 2-D gel.

Abbreviations: ATP – adenosine triphosphate; BCIP – 5-bromo-4-chloro-3-indolyl phosphate, 50 mg/ml in 70% dimethylformamide; LHCII – Light harvesting complex II; NADPH – reduced form of nicotinamide adenine dinucleotide phosphate; NBT – nitro blue tetrazolium, 50 mg/ml in 70% dimethylformamide; NONIDET (Shell Trademark) P40 – octylphenol ethylene oxide condensate, non-ionic surfactant; PSI and PS II – Photosystem I and II; SDS-PAGE – sodium dodecylsulphate polyacrylamide gel electrophoresis

Introduction

During the last decades powerful techniques have been devised for the separation and characterization of proteins. Among them the high resolution two-dimensional (2-D) gel electrophoresis has proved to be a versatile technique for protein identification and purification (Celis and Bravo 1984). This technique which was originally developed by O’Farrell in 1975 (O’Farrell 1975), is becoming an ever more important and popular approach in the area of protein analyses. In particular, significant achievements have been made
in comprehensive research on human, animal and bacteria proteins. Today, through the use of 2-D and its associated satellite techniques, the possibility exists for the identification, purification and microsequence determination of a large majority of polypeptides, the data of which could bolster existing protein databases used for projects such as various genome research programmes (Celis 1989).

Unfortunately, some unique difficulties with respect to chloroplast membrane proteins arose during the application of 2-D (Roscoe and Ellis 1982). As can be seen from the previous work (Novak-Hofer and Siegenthaler 1977; Boscetti et al. 1978; Gilbert and Buetow 1981; Masojidek et al. 1987), there were obvious difficulties regarding the resolution of the 2-D (see Results and discussion in this text). 2-D electrophoresis in photosynthesis research has therefore been employed much less than 1-D SDS-PAGE, even though the former technique can offer much more information. With recent progress in the research on the thylakoid membrane organization, more sensitive and comprehensive methods such as 2-D and its associated satellite techniques for membrane protein separation and identification are required beside polyacrylamide gel electrophoresis (1-D), since the 2-D approach permits the determination of not only molecular weight, but also isoelectric points of proteins with extreme precision (in principle, a single charge difference in polypeptides can be detectable). By the 2-D method, isofoms of proteins can also be distinguished. These isofoms have various biological significance (Bolwell et al. 1985; Lamb et al. 1989). One may fail in the sequence analysis using only 1-D for the isolation and the purification of the polypeptide due to either the contamination by other proteins having the same molecular weight, or the existence of some isofoms of the protein of interest as a result of genetic heterogeneity.

In this work we present a modified technique which can be employed for the identification and isolation of polypeptides of the thylakoid membrane and for its direct application to microsequencing analysis. Using the above-technique combined with mechanical fragmentation and aqueous two phase partitioning, we provide additional evidence for heterogeneity of the thylakoid membrane organization at the molecular level. We also show that several proteins, the α and β subunits of CF1 ATPase, polypeptides of LHClI, the Rieske iron-sulfur protein (Yu et al. 1994) and the 64 kDa protein (claimed to be a kinase), display two or three different spots in the 2-D gel which might be due to isofoms of these proteins.

Materials and methods

Spinach thylakoids were prepared as described earlier (Albertsson and Yu 1988).

Fragmentation and isolation of the thylakoid membrane fractions

Vesicles originating from grana (inside-out vesicles or B3) were obtained by a batch partitioning procedure in three steps after fractionation treatment either by passing the thylakoids suspended in 100 mM sucrose, 10 mM sodium phosphate buffer pH 7.4, 5 mM MgCl₂ and 5 mM NaCl twice through a Yeda press at nitrogen gas pressure of 10 MPa or by sonicating the thylakoids suspended in an aqueous two phase system comprised of 5.7% (w/w) Dextran 500 (Pharmacia, Uppsala, Sweden), 5.7% (w/w) PEG 4000 (Carbovax PEG 3350, obtained from Union Carbide, New York, NY), 20 mM sucrose, 10 mM sodium phosphate buffer pH 7.4, 3 mM NaCl and 1 mM MgCl₂.

Vesicles originating from stroma lamellae (named Y100) were obtained by passing the thylakoid suspension twice through the Yeda press as mentioned above, then centrifuging at 40 000 × g for 30 min. The vesicles representing the Y100 in the supernatant were recovered by centrifugation at 100 000 × g for 90 min (Albertsson and Yu 1988).

Different thylakoid membrane fractions were prepared by a multistage sonication - aqueous two phase partition procedure principally as described earlier (Albertsson and Yu 1988; Svensson and Albertsson 1989). The inside-out vesicles suspended in the same phase system as above were sonicated for 6 × 30 sec in a sonicator (Sonic & Materials inc. Danbury, Connecticut, U.S.A.) equipped with 1/2 inch horn. The interval between two sonication steps was 1 min. The ultrasonic exposure was adjusted to an intensity output of 7 with 20% duty pulses and a sample aluminium container was surrounded by ice-water. No increase of the temperature of the sample container was observed. After sonication, the phase system was thoroughly mixed at 3°C and separated by low-speed centrifugation (1000 × g for 3 min) The upper phase named 180s was collected and a fresh upper phase with an equal volume was added to the remaining lower phase. This completes one sonication-phase partitioning cycle, which was then repeated three times. The upper phases gave fractions 180s, 360s, 540s and 720s respectively, while the remaining fraction of the last lower phase was named BS. The fractions 180s and