Analysis of thylakoid membrane protein complexes from maize by Blue-Native Gel Electrophoresis and Tandem Mass Spectrometry[1]

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Introduction

Blue-Native Polyacrylamide Gel Electrophoresis (BN-PAGE) is a powerful technique to separate and analyse protein complexes from biological membranes. The technique was developped to separate protein complexes from mitochondria (Schägger and Von Jagow, 1991) but has been extended to the analysis of proteins of different membrane systems (Heinemeyer et al., 2007). Regarding a research project aimed in the study of the function of maize chloroplastidial transglutaminase (chlTGase) and the characterisation of protein post-translational modifications (PTM's) mediated by this enzyme (Villalobos et al., 2004; Carvajal-Vallejos et al., 2007; Santos et al., 2007) we undertaken a work to optimize the solubilisation of thylakoid protein complexes from maize and characterize the complexes by BN-PAGE and mass spectrometry (MS). Using the non-ionic detergent n-dodecyl-β-D-maltoside (DDM), protein complexes could be efficiently solubilised from thylakoid extracts and separated by BN-PAGE. Protein bands were subjected to trypsin digestion and analysed by micro LC-MS/MS. Thylakoid proteins could be identified and the different complexes separated by BN-PAGE inferred. We regard this approach of relevant analytical power to study the maize thylakoid membrane complexes and modifications occurring in the subunits of each complex upon environmental stimulus. To the best of our knowledge this work represents the first characterization of maize thylakoid protein complexes by BN-PAGE and MS.

Materials and methods

Leaves from 20 day greenhouse growth maize seedlings were collected and thylakoid enriched fractions were obtained (Berthold et al., 1981). Protein concentration was determined by Bradford method. A first experiment was performed in which thylakoid protein complexes were solubilized with non-ionic detergent n-dodecyl-β-D-maltoside (DDM) at 1.3% (w/v) (Heinemeyer et al., 2007), to protein concentrations of 2, 3 and 6 mg /ml. Efficiency of protein solubilization was assessed by BN-PAGE (Heinemeyer et al., 2007). Results are presented in terms of the ratio between detergent and protein weight in the sample. A second experiment was made to verify the effects of detergent concentration (0.95%-1.8%) on BN-PAGE protein patterns, taking as reference the most suitable solubilization condition considered in the first experiment. Protein bands from BN-PAGE gels were isolated, digested according to Pandey et al. (2000) and analyzed by micro LC-MS/MS (LTQ mass spectrometer, Thermo Finnigan). Peptides were concentrated in a Peptrap (ThermoElectron) and separated in a reversed-phase column (ThermoElectron) with a linear gradient of 5% to 80% acetonitrile with 0.1% formic acid at flow rate of 2μL/min. All product ion spectra were searched using TurboSEQUEST (Bioworks 3.3.1, Thermo Finnigan, San Jose, CA, USA) against Uniprot database (downloaded from NCBI website, 5128948 protein entries). Peptide recognition take in account a peptide probability superior to 0,01 and Xcorr correlation with charge state of 1.5, 2.0, 2.5 and 3.0 for 1+, 2+, 3+ and 4+ charged peptides.

Results

Proteins from thylakoid membranes were solubilized with different ratios of detergent to protein

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and separated by BN-PAGE (Fig. 1). The lowest ratio tested that allowed obtaining a clear BN-PAGE pattern was 5:1. This BN-PAGE pattern is consistent with the BN-PAGE patterns reported for other species thylakoid membranes, which lead us to consider this detergent:protein ratio appropriated for the solubilization of thylakoid membrane complexes from maize. Further increase in detergent to protein ratio lead to changes in BN-PAGE pattern resulting in the increase of the complex with approximately 242 kDa and the decrease of the high MW complexes (Fig.1). In order to identify the complexes being separated by BN-PAGE, protein bands were isolated, subjected to in-gel trypsin digestion and the resulting peptide mixtures analyzed by LC-MS/MS.

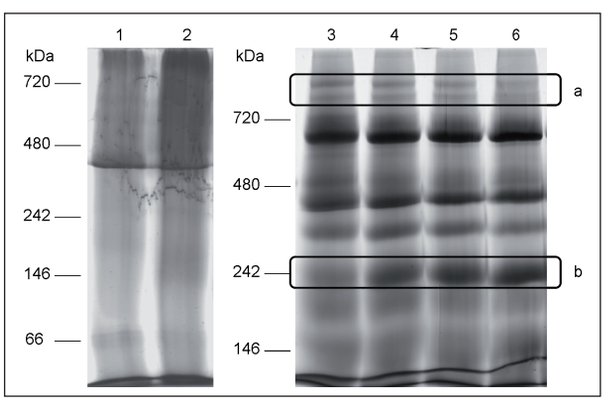
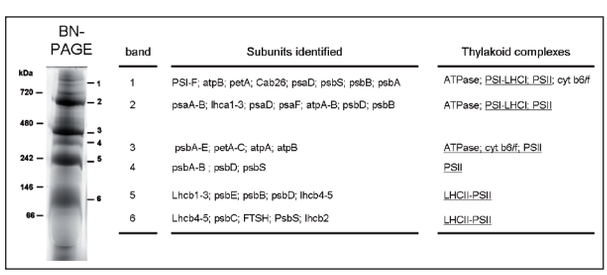


Figure 1. *Solubilization of thylakoid protein complexes and separation by BN-PAGE. Solubilization performed at a DDM to protein weight ratio of 1:1 (1), 2:1 (2), 5:1 (3), 6.4:1 (4), 8:1 (5) and 10:1 (6). Decrease (a) and increase (b) in abundance of thylakoid complexes.*

Distinct proteins that are part of the composition of the major thylakoid protein complexes were identified (Fig. 2). With this information we could infer the presence of the several complexes separated in BN-PAGE and respective level of organization (Fig.2).



band	Subunits identified	Thylakoid complexes
1	PSI-F; atpB; petA; Cab26; psaD; psbS; psbB; psbA	ATPase; PSI-LHCI; PSII; cyt b6/f
2	psaA-B; lhca1-3; psaD; psaF; atpA-B; psbD; psbB	ATPase; PSI-LHCI; PSII
3	psbA-E; petA-C; atpA; atpB	ATPase; cyt b6/f; PSII
4	psbA-B ; psbD; psbS	PSII
5	Lhcb1-3; psbE; psbB; psbD; lhcb4-5	LHCII-PSII
6	Lhcb4-5; psbC; FTSH; PsbS; lhcb2	LHCII-PSII

Figure 2. *Protein identification by LC-MS/MS and assessment of thylakoid complexes separated by BN-PAGE. Underlined are the complexes that were reported also by other authors.*

A general BN-PAGE pattern for maize can be described that is in accordance with previous works of BN-PAGE from thylakoid membranes of higher plants. High similarity is verified with barley thylakoid protein complexes separated by BN-PAGE (Ciambella et al., 2005) consisting in the existence of LHCII-PSII and LHCI-PSI supercomplexes (> 600 kDa), LHCI-PSI complex and PSII dimeric core (band 2), Cyt b6/f dimer, ATPase and PSII monomer core complexes (bands 3 and 4) and the trimer and monomer LHCII (bands 5 and 6, respectively).

Conclusions

In this work we were able to establish a procedure to solubilize maize thylakoid protein complexes. Blue-native PAGE allowed the separation of complexes and further analysis by MS. Protein identification was achieved and sequence information of the several complex subunits obtained. This approach allows a level of fractionation of thylakoid membrane proteome suitable for a deeper characterization of the subunits of the complexes including LHCII proteins. Ongoing work consists in establish the second-dimension from BN-PAGE in denaturing conditions, the analysis of thylakoid proteins by MS in conditions of chloroplastidial transglutaminase activity and identification of post-translational modifications mediated by this enzyme.

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Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications[1]

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In order to improve the probability of detecting unintended side effects during maize gene manipulations by bombardment, proteomics was used as an analytical tool complementary to the existing safety assessment techniques. Since seed proteome is highly dynamic, depending on the species variability and environmental influence we analyzed the proteomic profiles of one transgenic maize variety (event MON 810) in T5 and T6 generation with their respective isogenic controls (F5 and F6). Thus, by comparing the proteomic profiles of F5 with F6 we could determine the environmental effects, while the comparison between F6 and T6 seeds from plants grown under controlled conditions allowed us to investigate the effects of DNA manipulation. Finally, by comparing T5 with T6 seed proteomes it was possible to get some indications about similarities and differences between the adaptations of transgenic and isogenic plants to the same strictly controlled growth environment. Approximately 100 total proteins resulted differentially modulated in the expression level as a consequence of the environmental influence (F6 *vs.* F5), whereas 43 proteins resulted up- or down-regulated in transgenic seeds with respect to their controls (T6 *vs.* F6), which could be specifically related to the insertion of a single gene into a maize genome by particle bombardment. Transgenic seeds responded differentially to the same environment as compared to their respective isogenic controls, as result of the genome rearrangement derived from gene insertion. To conclude, an exhaustive differential proteomic analysis allows to determine similarities and differences between traditional food and new products (substantial equivalence), and a case-by-case assessment of the new food should be carried out in order to have a wide knowledge of its features.

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