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Effect of low temperature on the protein content of the thylakoid lumen of *Arabidopsis thaliana*

M. Schubert[1, 2], M. Wirén[2], T. Kieselbach[1,] and W.P. Schröder[2]

[1]*Department of Medical Nutrition, Karolinska Institute, Novum, SE- 14186 Huddinge, Sweden. Thomas.Kieselbach@mednut.ki.se*
[2]*Department of Natural Science, Södertörns University College, Bipontus, Box 4101, SE-14104 Huddinge, Sweden. maria.bystedt@sh.se (M. Schubert), wolfgang.schroder@sh.se*

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Introduction

The completion of the *Arabidopsis thaliana* genome-sequencing project by the end of 2000 [The Arabidopsis Genome Initiative, 2000] started a new era in plant research. To apply the knowledge of the Arabidopsis genome to an investigation into the chloroplast lumen, we designed a purification method for the lumenal proteins from Arabidopsis chloroplasts and studied them systematically by proteomics [Schubert *et al*., 2001]. Based on these and other recent results, a new picture of the thylakoid lumen of higher plant chloroplasts is emerging. The lumenal compartment is no longer only a space for accumulating protons and ensuring charge balance during ATP production, but seems to play an important role in regulating the stability, function and assembly of the different photosynthetic complexes. In this study we applied our 2D-maps to analyse, if the lumen proteome changes upon cold treatment. Our preliminary data indicate that the proteins expressed in the thylakoid lumen indeed changed when Arabidopsis plants were grown in low temperature.

Materials and methods

A. thaliana accession *Colombia* was grown hydroponically in nutrient solution [Siegenthaler and Depéry, 1976].The daytime was set to 8 h and the light intensity to approximately 100 µmole photons/ m^2/s. The temperature was 20 °C during light hours and 18 °C during the dark period. After 11 weeks half of the plants were transferred to a coldroom with a temperature of 6 °C and kept there for two weeks meanwhile the control plants stayed in 20 °C. The thylakoid lumen fraction from the cold treated and control plants were isolated in parallel essentially as described in [Kieselbach *et al*., 1998] but with the chloroplast preparation medium according to [Norén *et al*., 1999]. The lumen fraction was concentrated using Centriprep YM-3 and protein quantification was carried out according to [Bradford, 1976].

1D-SDS-PAGE was performed on slab gels containing 18% polyacrylamide and 2M urea essentially as in [Laemmli, 1970] and 2D-gel electrophoresis, image analysis and protein identification as described in [Kieselbach *et al*, 2000]. Both 1D- and 2D-gels were silver stained according to[Bjellqvist *et al*, 1993]. SyproRuby staining (MolecularProbe) was performed according to the manufactures description.

Results and discussion

The cold treated plants did not show any obvious phenotype or signs of stress when grown in 6° C for two weeks, in contrary they seemed to gain biomass. No drastic changes could be seen between cold treated and control plants when the total chloroplast, the thylakoid and stroma fractions were analysed with 1D-SDS-PAGE. However, some differences were observed in the lumen fraction (data not shown).

As the resolving power of 1D SDS-PAGE was too limited to perform a detailed analysis of the cold induced changes in the protein content of the chloroplast lumen, we performed 2D- electrophoresis. Figure 1 shows a 2D-gel (pH-range 4-7)with the lumen fractions from control plants (A) and cold treated plants (B). In general the

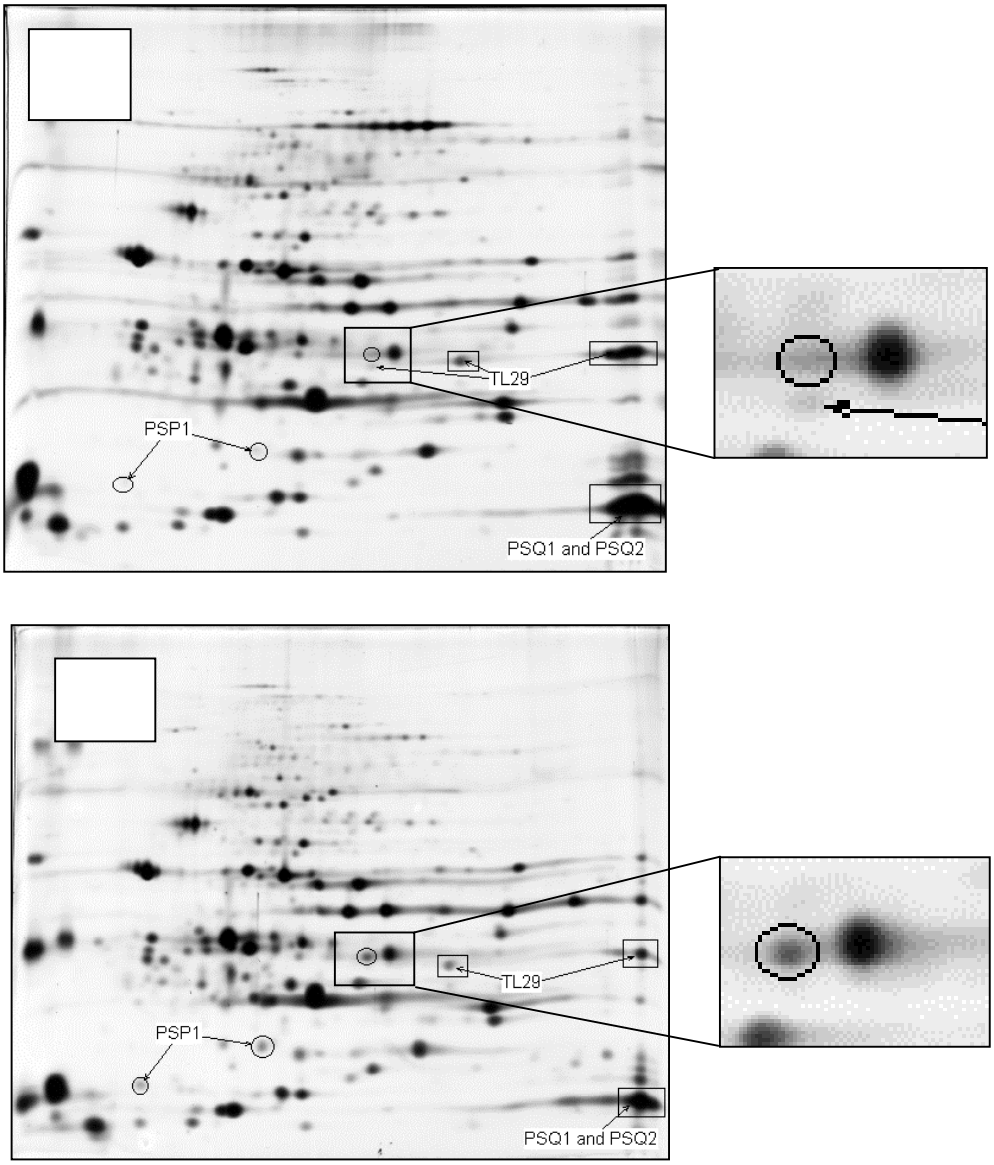

Fig. 1. Silver stained 2D-gels with the soluble lumen fraction from control (A) and cold treated (B) plants. The circled protein spots showed a stronger intensity in the cold treated plants. Protein spots with lower intensity in the cold treated plants have been squared. The close up picture show the 24.3 kDa protein with changed intensity in the different gels.

pattern looked very similar with an average of 300 spots detected in both gels. One problem with this type of analysis is the variations in staining using the silver technique. Thus reliable quantification needs more quantitative staining methods, such as fluorescent dyes. Therefore we also stained our 2D-gels with SyproRuby shown in figure 2. The sensitivity of the SyproRuby staining were not as good as with silver but the same differences were seen in these gels as compared to the silver stained gels. Three protein spots (circled) show a stronger intensity in the lumen map from cold stressed plants. Two of these corresponds to the degradation product of PsbP1,and one correspond to a protein with a molecular weight of 24.3 kDa that have not yet been identified. On the other hand three spots (squared) showed a weaker intensity in the lumen map from cold treated Arabidopsis plants.

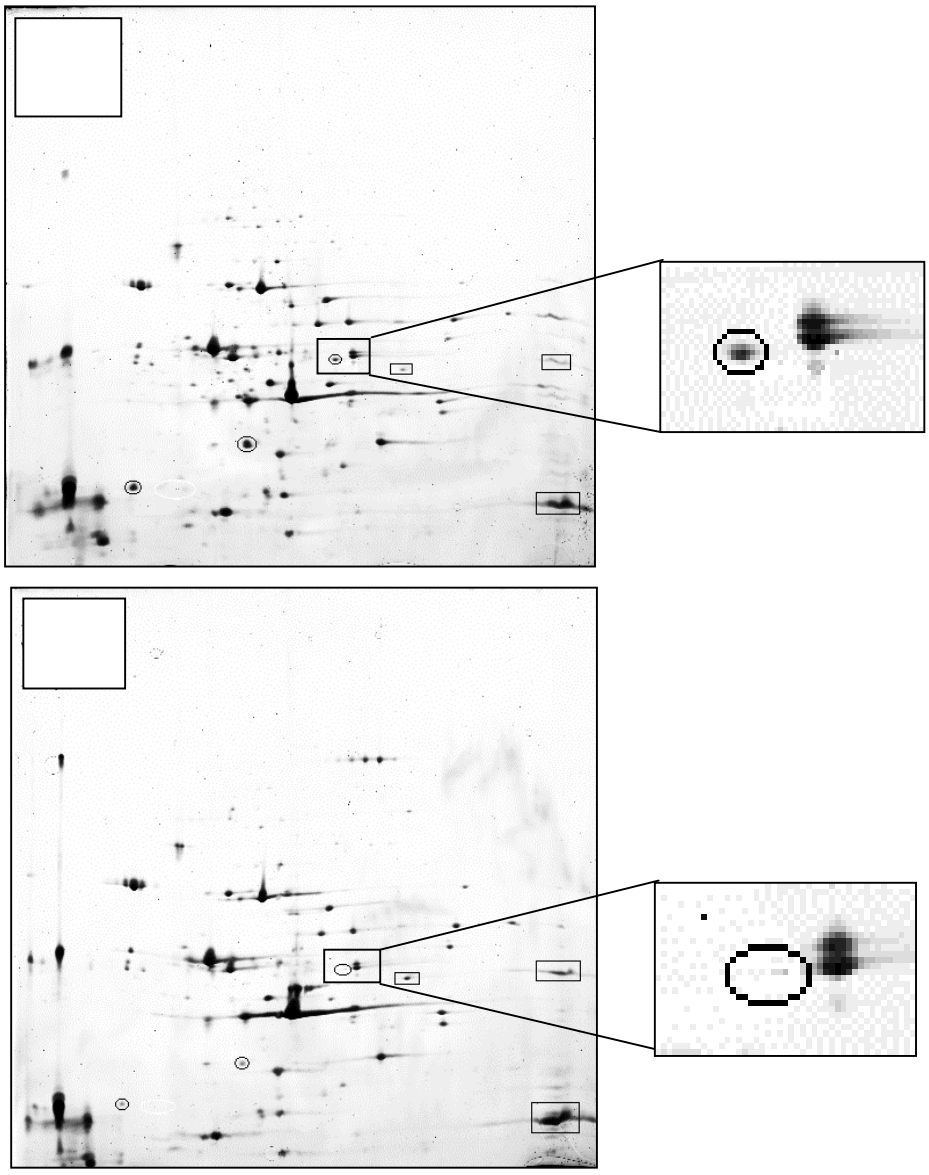

Fig.2. SyproRuby stained 2D-gels with the soluble lumen fraction from control (A) and cold treated (B) plants. The same differences were seen between these gels as compared to the silver stained gels. The circled protein spots showed a stronger intensity in the cold treated plants. Protein spots with lower intensity in the cold treated plants have been squared.

Interestingly, two of the weaker spots correspond to the putative ascorbate peroxidase TL29 and the third spot matches the extrinsic proteins PsbQ1 and PsbQ2 [Kieselbach *et al*., 2000]. This observation indicates a down regulation of these protein during cold treatment and a possible role of these proteins in the adaptation of the chloroplast to low temperature. However, these data are yet preliminary and further experiments are required to confirm a participation of these proteins in cold adaptation.

Taken together these results indicate that the intensity differences seen between the gels were not only due to variations in the staining but indeed reflected on the differences in the protein expressed in the thylakoid lumen under different environmental conditions.

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