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Electrophoretic Protein Patterns of Sugar Beet Tissue Lines

By

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With 3 figures

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Summary

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Cellular and extracellular proteins accumulating in normal (hormone dependent), habituated and crown gall tumour callus lines of sugar beet (*Beta vulgaris* L. var. *altissima*) were studied. Proteins were separated by SDS-polyacrylamide gel electrophoresis and silver stained. Glycoproteins with D-manose in their glycan component were detected on protein blots by concanavalin A-peroxidase staining. A few tissue specific cellular proteins (16, 17, 18, 19, 24, 37 kDa) were detected. Patterns of proteins secreted in the nutrient medium of suspension cultured cells were distinct for each tissue line. Nonorganogenic habituated tissue contained more protein bands in the range between 25 and 66 kDa than the other lines. Among glycosilated extracellular proteins, the 68 kDa protein was common for all tissues, whereas the 45 kDa was distinct for both habituated lines. The

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nonorganogenic habituated tissue was deficient in the 34 kDa polypeptide, and the crown gall tumour in the 23 kDa one.

Zusammenfassung

KRSNIK-RASOL M., ČIPČIĆ H., POLJUHA D. & HAGÈGE D. 2001. Elektrophoresemuster von Proteinen verschiedener Kalluslinien von Zuckerrüben. – Phyton (Horn, Austria) 41 (1): 13–20, with 3 figures. – Englisch mit deutscher Zusammenfassung.

Zelluläre und extrazelluläre Proteine wurden von normalen (hormonabhängigen), angepassten und Wurzelhalstumor-Kalluslinien von Zuckerrüben (*Beta vulgaris* L. var. *altissima*) untersucht. Die Proteine wurden mittels SDS-Polyacrylamidgel-Electrophorese getrennt und mit Silber gefärbt. Die Glykoproteine mit D-Manose in ihrem Glykan wurden auf dem Proteinblot mittels ConcanavalinA-Peroxidase-Färbung identifiziert. Es konnten einige gewebespezifische Zellproteine (16,17,18,19,24,37 kDa) bestimmt werden. Die elektrophoretischen Muster von extrazellulären Proteinen aus dem Nährmedium von Zellsuspensionskulturen waren für jede Gewebelinie charakteristisch. Die angepasste Linie, in der keine Organogenese vorkommt, sezernierte mehr Proteine zwischen 25 und 66 kDa als die anderen Linien; das 34 kDa Protein fehlte in dieser Linie. Das 68 kDa Glykoprotein wurde in allen Linien gefunden und das von 45 kDa war für die beiden angepassten Linien charakteristisch. Das 23kDa Protein wurde im Nährmedium von Wurzelhalstumorkulturen nicht gefunden.

Introduction

In vitro culture of cells and tissues has a notable value in providing ways to study cell differentiation and development in plants. In particular, habituated and crown-gall tumour tissues are helpful due to their ability for autonomous growth. Normal (N), fully habituated nonorganogenic (HNO) and habituated organogenic (HO) callus lines of sugar beet (*Beta vulgaris* L. var. *alitissima*) were intensively investigated (DE GREEF & JACOBS 1979, CRÈVECOEUR & al. 1987, HAGÈGE 1993, GASPAR 1995, KEVERS & al. 1999) and recommended as a model in plant development studies. To better understand a process of habituation, we have established a crown gall tumour line (T) of sugar beet, as a positive control to habituated callus. The HNO callus with disturbed metabolism of nitrogen, sugar and tetrapyrrole compounds (LE DILY & al. 1993, BISBIS & al. 1993, HAGÈGE & al. 1992) has several traits common to animal cancers. Compared to normal calli the habituated ones are vitrified containing less lignin and cellulose in cell walls (LE DILY & al. 1993). They also show a reduced ethylene production (HAGÈGE & al. 1994).

Proteins as direct gene products should be useful to identify a gene expression program characteristic for different callus lines. To better understand the effect of habituation on gene expression, accumulation of mRNAs was studied in perwinkle cultures (DROUAL & al. 1998). Habituation

and 2,4-D treatment changed the polypeptide profiles of the microsomal membranes in periwinkle cell suspensions (MERILLON & al. 1995). Changes in glycan components of arabinogalactans may be involved in the development of plant anatomy (KNOX 1995).

The aim of this work was to compare the normal, habituated and crown gall tumour callus lines regarding their protein profiles and to identify some developmentally specific cellular and extracellular protein markers.

Materials and Methods

Tissue lines

Four lines of sugar beet (*Beta vulgaris* L. var. *altissima*) tissue: a normal line (N), two habituated lines, one organogenic (HO) and one nonorganogenic (HNO), and one crown gall tumour line (T) were analysed. Tissue cultures were maintained on the PG basal medium (NEGRUTIU & al. 1975) at 25 °C and 16 h photoperiod. Normal tissue required an addition of plant hormones 2,4-D and BA (0.1 µg/L 2,4-dichlorophenoxyacetic acid and 0.1 µg/L benzylaminopurine) for its growth. The other lines, being hormone independent, were cultivated on the hormone-free medium. Primary crown-gall tumours were induced by *Agrobacterium tumefaciens* strain B6S3.

Protein samples

Total soluble proteins were extracted by grinding 0.5-1.0 g of fresh tissues in 2 ml of 0.1 M Tris/HCl buffer, pH 8.0 at 4 °C. Homogenates were centrifuged at 20 000 × g and 4 °C for 15 minutes. The supernatants were centrifuged again at 40 000 × g (4 °C) for 50 minutes. Protein content of supernatants was determined according to BRADFORD 1976. Samples were denatured using 0.125 M Tris buffer (pH 6.8), containing 5% (v/v) β-mercaptoethanol and 2% (w/v) SDS (sodium dodecyl sulphate). For the SDS-PAGE approximately the same amount of protein (5-8 µg) per sample was loaded onto the gel.

Extracellular proteins were obtained from a liquid nutrient medium of 5-day old suspension cultures. The medium was decanted and passed through a mash filter to remove the cell debris. The extracellular proteins were precipitated by the addition of 2 volumes of cold acetone (-20 °C). After an incubation at - 20 °C for minimum two hours, proteins were collected by centrifugation at 10 000 g at 4 °C for 20 min and resuspended in SDS sample buffer. The other way to concentrate the extracelluar proteins was by reducing the filtrate volume (approximately 5 times) with a Sephadex G-25.

Electrophoresis and electroblotting

Both intracellular and extracellular proteins were analysed by SDS electrophoresis in 12% T (2.67% C) polyacrylamide gels or in 8-18% T (2.67% C) gradient gels, with the buffer system of LAEMMLI 1970. The protein bands were visualised by silver staining (BLUM & al. 1987). Extracellular proteins were transferred to a nitrocellulose membrane in the vertical tank apparatus for electroblotting. Glycoproteins with D-manose in their glycan component were detected on nitrocellulose membrane by reaction with concanavalin A. The bands were visualised by peroxidase reaction using 4-chloro-1-naphtol as a substrate (HAWKES & al. 1982).

Results

A morphology of normal (N), habituated nonorganogenic (HNO), habituated organogenic (HO) and crown gall tumour (T) callus lines of sugar beet (*Beta vulgaris* L. var. *altissima*) is shown in the Fig. 1. Electrophoretic patterns of cellular proteins were similar and very stable for all lines during many years of tissue culture. The addition of hormones into the nutrient medium of hormone independent callus lines had no influence on the protein-banding pattern. The polypeptides of 16 and 17 kDa (Fig. 2A, line 1, arrows) were characteristic for the N and T tissue. Both tissue lines are nonorganogenic, the N is green and the T is yellowish with randomly dispersed violet spots of cells containing β-cyanin. The 37 kDa polypeptide (Fig. 2A, line 4, arrow) was more highly expressed in the T than in the N tissue and it was not detected in the HNO and HO tissue lines. The bands of 18 and 19 kDa were constantly expressed in the HNO line while in the HO and T line they were hardly detectable. The polypeptide of 24 kDa (Fig. 2A, line 3,arrow) was the most prominent in the HO line, which continuously produced shoots with hyperhydric leaves.

Extracellular protein patterns (Fig. 2B) exhibited more significant differences between callus lines. A liquid medium of the N cell suspension culture contained four quantitatively dominant polypeptides of 34, 33, 28 and 22 kDa (Fig. 2B, line 1, arrows). In the medium of HNO cell suspension culture, a lot of faint bands (25 to 66 kDa) and two intense ones (37 and 12 kDa, line 2, arrows) were detected. The HO medium contained three quantitatively dominant bands (Fig. 2B, line 3, arrows). The T cells did not excrete any tumour specific protein. Some protein bands were common to the N and T lines (Fig. 2B, arrows). The polypeptide of 25 kDa was excreted in higher amount by the HO and T cells than by the N and HNO cells.

Reaction of protein blots with concanavalin A (Fig. 3) showed glycosilated extracellular proteins with D-manose in their glycan component. The 68 kDa protein was present in the nutrient media of all cell suspension lines. The band of 45 kDa was characteristic for both habituated tissue lines and the one of 43 kDa was detected in the HNO only. The protein of 34 kDa was present in all lines except the HNO. In the medium of the T cell suspension only two bands of 68 and 34 kDa were highly expressed. The polypeptide of 23 kDa was abundant in the N and HNO, faint in HO and it was missing in the T.

Discussion

A comparison of the intracellular protein profiles of four sugar beet callus lines showed minor differences and only a few specific bands were detected. Repeated electrophoretic analysis over the period of five years, demonstrated that the 18 and 19 kDa proteins were continuously ex-

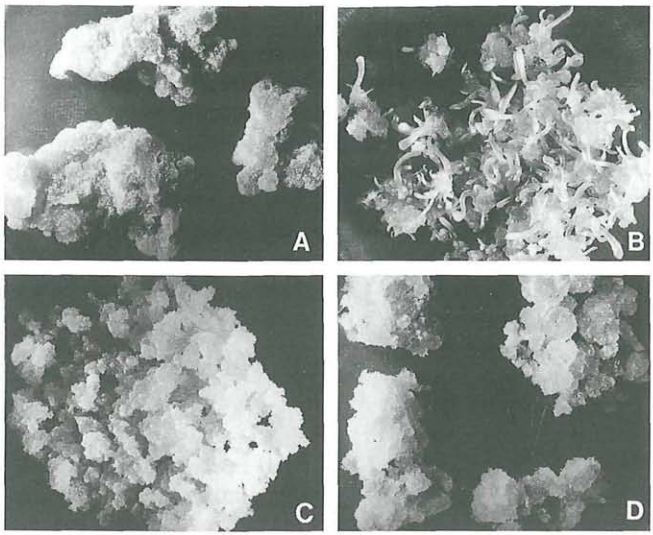

Fig. 1. Sugar beet (*Beta vulgaris* L. var. altissima) callus lines. A - normal hormone dependent tissue (N), B - organised habituated tissue (HO), C - unorganised habituated tissue (HNO), D - tumour tissue (T).

pressed in the HNO tissue line, while in the other lines they occasionally appeared only as very faint bands. The 24 kDa protein was constitutively expressed in the HO line. The N and HNO callus were studied also by two-dimensional gel electrophoresis (TACCHINI & al. 1995). Specific proteins for N and HNO calli were found. The expression of the proteins was found to be cell type specific and did not depend upon exogenous factors. Our experiments conformed that electrophoretic protein patterns did not change under the influence of exogenous hormones. MÉRILLON & al. 1995 reported that habituation, as well as treatment of the cells with 2,4-D, changed the polypeptide profiles of the microsomal membranes and that the membranes were targets for biochemical changes associated with habituation.

To establish more accurate protein markers correlating with developmental state of a callus line the extracellular proteins were analysed. Both methods for concentrating the proteins in nutrient media of suspension cultures gave the satisfactory results, but the sephadex G25 procedure (SAUL & DON 1984) was more rapid than the aceton precipitation, and it additionally purified the proteins avoiding the problem of pellet resolubilization. The cells of the HNO line produced a spectrum of protein bands in molecular weight range from 25 to 66 kDa which were not detected in nutrient media of the other lines. The HNO line is characterised by reduced cellulose and lignin deposition in cell walls and by a reduced cell to cell adhesion (TACCHINI & al. 1995).

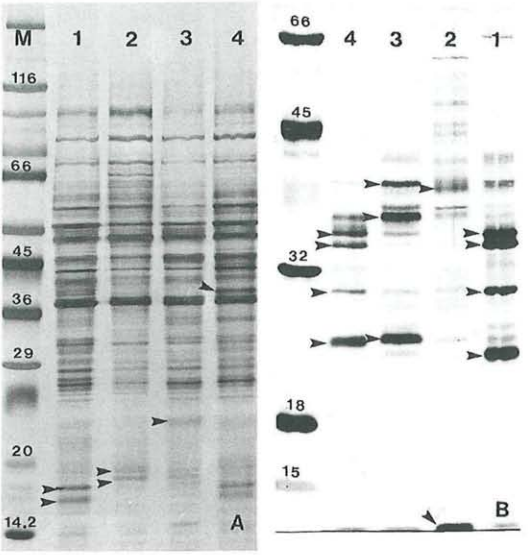

Fig. 2. Protein profiles of sugar beet callus lines. A – cellular proteins separated by SDS-polyacrylamide gel electrophoresis in 8–18 % gradient gel. B – extracellular proteins in 12 % SDS-polyacrylamide gel. Lane: 1, normal tissue line (N); 2, habituated unorganised line (HNO); 3, habituated organised line (HO); 4, crown gall tumour line (T); M, molecular mass markers. Arrows indicate bands mentioned in the text.

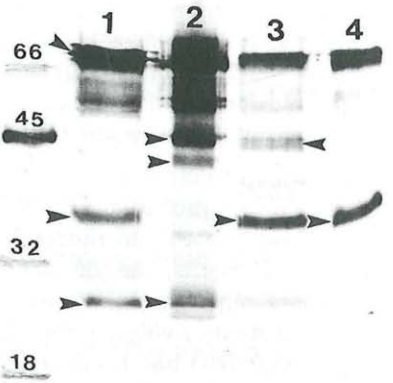

Fig. 3. Electro-blot of extracellular proteins of sugar beet cell suspension culture. Glycoproteins reacting with a lectine Concanavalin A were visualised by peroxidase reaction. Lanes description as in figure 2.

The expression of arabinogalctan-proteins (AGPs) is highly regulated during plant development and correlates with cell differentiation (DING & ZHU 1997). Soluble extracellular AGPs were studied in suspension-cultured plant cells and a developmentally regulated appearance of carbohydrate epitopes correlated with the formation of anatomical patterns (KNOX 1995). Concanavalin A staining of extracellular protein blots revealed the high mannose oligosaccharide chains of glycoproteins. The characteristic patterns of extracellular glycoproteins could be related to different phenotypes of sugar beet tissue lines. Further characterisation of the 34 kDa protein which is missing from the HNO medium or of the 45 kDa, which is common for both habituated lines, should contribute to better understanding of the habituated phenotype in tissue culture.

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