

TISSUE DISTRIBUTION AND DEPOSITION PATTERN OF A CELLULOSIC PARENCHYMA-SPECIFIC PROTEIN FROM CASSAVA ROOTS

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ABSTRACT

A protein with a molecular mass of 22kDa was purified from the cellulosic parenchyma of cassava roots. The amino acid composition of the protein was determined and antibodies generated against the purified protein were used to show that the concentration of the protein remains unchanged during root "tuber" formation. By using a tissue printing technique, as well as western blot, it was shown that the cellulosic parenchyma was the only root tissue in which the protein was deposited.

Key words: *Manihot esculenta*; mandioca; proteínas vegetais; cassava; plant protein.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is widely cultivated in South America, South-East Asia and Africa, where it is a very important source of carbohydrates (COCK, 1985). The application of the new biotechnological tools to the improvement of agronomic traits of this crop, such as nutritional quality, disease resistance, starch quality, etc., is being hampered by the lack of basic knowledge about the biochemistry of the cassava plant (SCHOPKE *et al.*, 1996). For example, it is widely accepted that one of the priorities in cassava biotechnology should be the improvement in the quantitative and qualitative protein profile in the roots (THRO *et al.*, 1995), yet no major proteins from any of the root tissues were isolated and characterised in any detail (SHEWRY *et al.*, 1992). We are studying the root proteins of cassava, trying to identify proteins with a specific spatial and temporal pattern of deposition (MACIEL *et al.*, 1995), to identify tissue-specific promoters that could be used in cassava biotechnology. In the present paper, we present data on the characterisation and amino acid composition of a major albumin

isolated from the cellulosic parenchyma of cassava roots, together with its pattern of deposition during root development.

MATERIAL AND METHODS

Plant material. Cassava (cv. Tapicínea) roots were obtained from plants grown at the experimental station of the Centro Nacional de Agroindústria Tropical (CNPAT/EMBRAPA) at Pacajús, Ceará, Brazil.

Protein extraction and characterisation. After the removal of the periderm, the peel and cellulosic parenchyma were separated, dipped into liquid nitrogen, freeze-dried and finely powdered. Proteins were extracted (1:5, w/v) using 0.1M Tris/HCl, NaCl 1%, pH 8.0 for two hours at 4°C. Polyvinylpyrrolidone (2%, w/v) was added for the removal of phenolics. After centrifugation (10,000g, 4°C), the supernatant was extensively dialysed against distilled water and albumin fraction was collected, freeze-dried and stored at -20°C. Protein purification was performed by the fractionation of freeze-dried albumin fractions by ion-exchange chromatography and reverse-phase HPLC as previously described (QUIRINO *et al.*, 1993). For antibodies generation, purified protein was subjected to SDS-PAGE (LAEMMLI, 1970), stained with coomassie blue and protein band of 22kDa was cut out of the gel, sonicated and applied into a female New Zealand White rabbit. The primary immunisation dose contained approximately 100 micrograms of the protein. The IgG fraction was purified from whole serum by affinity chromatography on protein A beads (HARLOW & LANE, 1988).

Protein deposition during root development. Young roots were collected and separated according to its diameter (cm): 0.3 to 0.6 (stage 1), 0.6 to 1.0 (stage 2), 1.0 to 1.5 (stage 3), 1.5 to 2.0 (stage 4), 2.0 to 2.5 (stage 5), 2.5 to 3.0 (stage 6) and 3.0 to 3.5 (stage 7). The albumin fractions from each one of these root groups were prepared as described above. After PAGE-SDS of β -mercaptoethanol treated samples, proteins were transferred to a nitrocellulose membrane as described by (TOWBIN *et al.*, 1979), probed against polyclonal antibodies raised against the protein and detected with alkaline phosphatase-conjugated anti-goat immunoglobulins antibodies. The distribution of the 22kDa protein within the root tissues was visualised by western blot and by a tissue imprinting technique (CASSAB, 1993). Fully mature

roots were transversally cut with a sharp blade and gently pressed against a nitrocellulose membrane. The proteins bound to the membrane were then probed against polyclonal antibodies raised against the protein and detected with alkaline phosphatase-conjugated anti-goat immunoglobulins antibodies. For protein staining, the membranes were dipped for two minutes in a solution of the Ponceau reagent (HARLOW & LANE, 1988).

Amino acid analysis. Protein samples were resuspended in 500 µl of constant-boiling 6N HCl containing 0.1% phenol. Ten per cent of each sample was brought up to a final volume of 150 µl of constant-boiling 6N HCl plus 0.1% phenol. Hydrolysis was carried out in evacuated, sealed, thick-walled borosilicate glass tubes for 24 hours at 120°C. Cooled samples were opened, dried under vacuum and stored at -20°C until derivatized. Samples were derivatized with PITC (Pierce) according to BIDDLEMEYER *et al.* (1984). The protein hydrolysates were re-dried twice with ethanol:water:triethylamine (2:2:1, v/v), and derivatized with ethanol:water:triethylamine:PITC (7:2:2:1, v/v). Samples were analysed by reverse-phase HPLC on a Waters system equipped with a Waters 712 WISP and a Waters system interface module linking the HPLC to a Baseline 810 workstation. A Pico-Tag column for hydrolysate amino acid analysis (Waters) maintained at 46°C by a Waters temperature-control module was used in accordance with the manufacturer's recommended procedure. Hydrolysate amino acid standards (Sigma) were used for peak identification and subsequent calculations. Lysozyme was also analysed and the results were compared with published values to ensure the accuracy of the technique.

RESULTS AND DISCUSSION

We have purified a protein with a molecular mass of 22kDa from cassava root parenchyma to a very homogeneous state (Figure 1). The amino acid composition of this protein is shown in Table I. The protein is devoid of threonine and isoleucine and has relatively high levels of methionine, an amino acid which is generally present at very low levels in cassava roots (EGGUM, 1970). An amino acid composition search on the ExPASy world wide web server (<http://www.expasy.ch/ch2d/aacompi.html>) showed that the amino acid composition of this protein has no significant degree of similarity with any known plant protein.

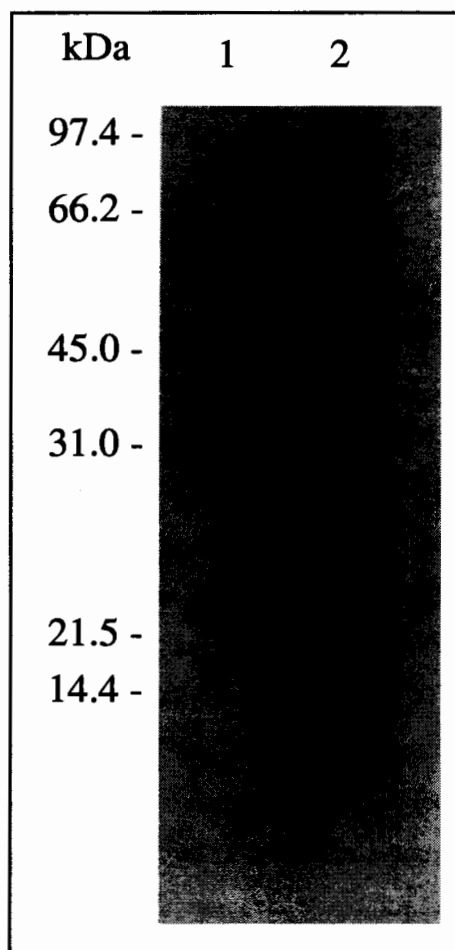


FIGURE 1- SDS-PAGE of purified albumin from cassava roots. Lane 1, 0.5 μ g of purified albumin; lane 2, 40 μ g of total water-soluble proteins. The molecular mass of the protein standards is indicated on the left.

TABLE I - Amino acid composition of the 22kDa protein isolated from the cellulosic parenchyma of cassava roots. Composition is expressed in numbers of residues per mole of protein, based on a molecular mass of 22kDa. The values presented are an average of three independent analysis. The values for cysteine and tryptophan were not determined (nd).

Amino acid	Residues
Asx	19.1
Glx	14.6
Ser	8.0
Gly	9.6
His	20.4
Arg	7.6
Thr	0.0
Ala	4.6
Pro	20.7
Tyr	21.2
Val	11.4
Met	11.9
Cys	nd
Trp	nd
Ile	0.0
Leu	4.4
Phe	5.0
Lys	4.9

Figure 2 shows western blot analysis of the tissue distribution of the 22kDa protein. Apparently, the antibodies failed to recognize any protein from the peel, reacting only with protein extract obtained from the cellulosic parenchyma, thus indicating the tissue-specificity of deposition of this protein. Our tissue print analysis also corroborated with this conclusion: Despite the fact the peel has a protein concentration, as indicated by staining with the Ponceau reagent, much higher than the cellulosic parenchyma, the antibodies reacted only with protein in the zone corresponding to the cellulosic parenchyma (Figure 3).

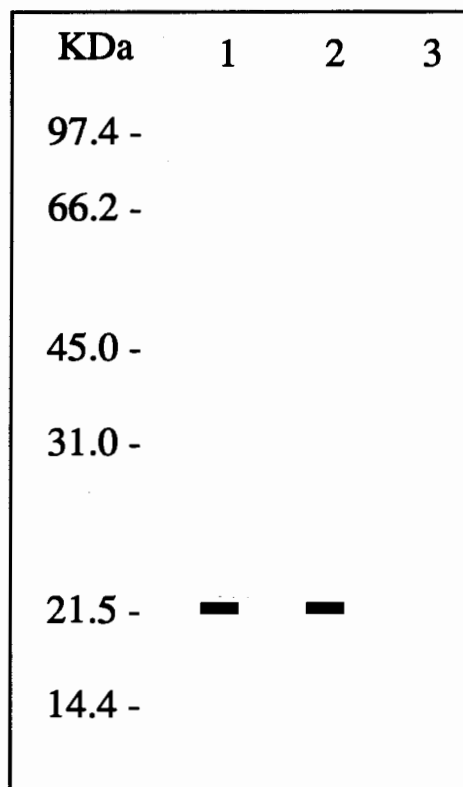


FIGURE 2 - Schematic drawing of a western blot analysis of total water-soluble proteins from the cellulosic parenchyma (lane 1), purified 22kDa protein (lane 2) and total water-soluble protein from the peel (lane 3). The quantities applied to the gel were 0.1, 50 and 100 μ g, respectively. The print was reacted with polyclonal antibodies (diluted 1:2500) against the 22kDa protein and detected with alkaline phosphatase-conjugated anti-goat immunoglobulins antibodies. The molecular mass of the protein standards is indicated on the left.

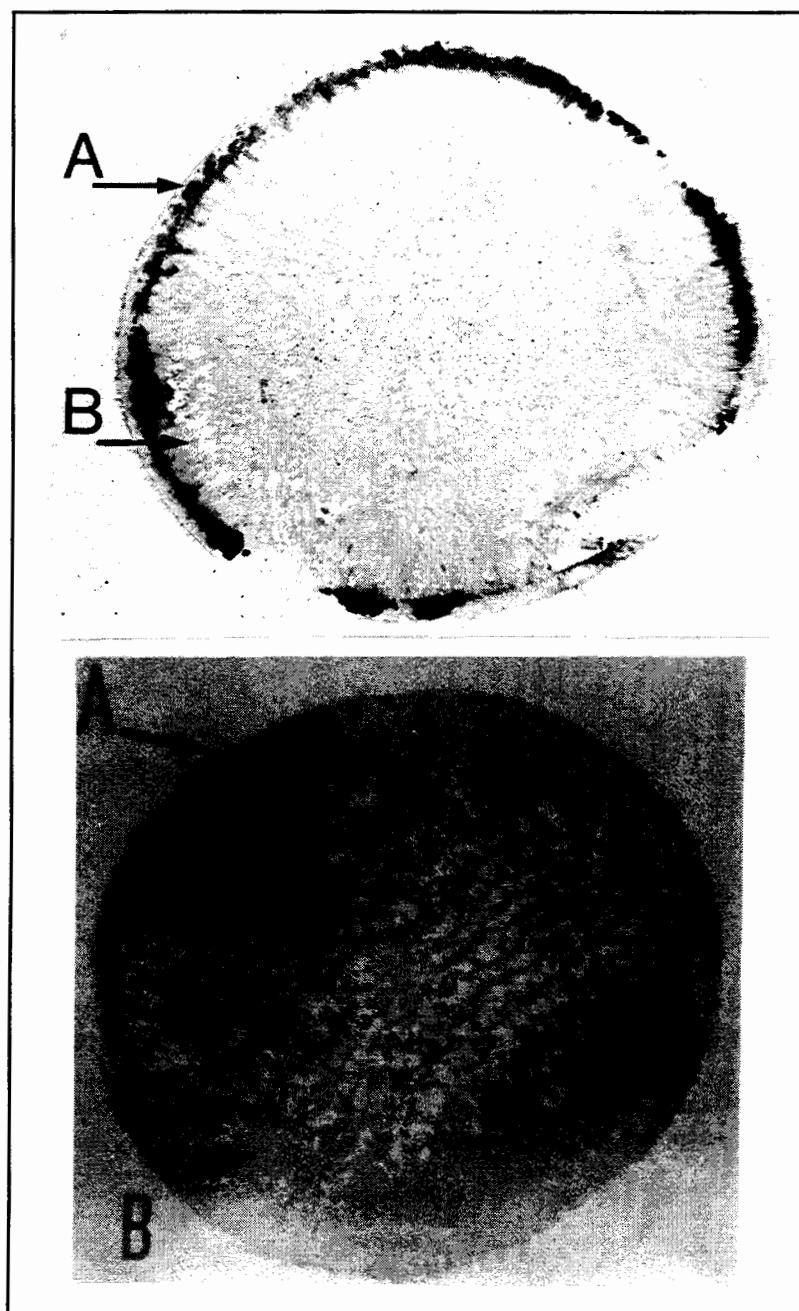


FIGURE 3 - Tissue-print of a cross-section of mature cassava root. **Top:** The print was stained for protein with the Ponceau reagent. **Bottom:** The print was reacted with polyclonal antibodies (diluted 1:1000) against the 22kDa protein and detected with alkaline phosphatase-conjugated anti-goat immunoglobulins antibodies. (A) peel; (B) cellulosic parenchyma.

As shown in Figure 4, the concentration of the 22kDa protein remained fairly constant in all of the developmental stages analysed. We could see by anatomical analysis of root sections that even in roots at stage 1 of development, the process of root "tuber" formation was well underway (data not shown). This indicated that this protein was not involved in any function related to root "tuber" differentiation.

We are currently attempting to obtain the complete amino acid sequence of this protein and determine its sub-cellular localisation within root parenchyma cells. We hope these informations will help to clarify its physiological role. Finally, as this protein seems to be specific to the cellulosic parenchyma, the antibodies we raised can be used as probes for the isolation of the gene of the 22kDa protein as well as its promoter.

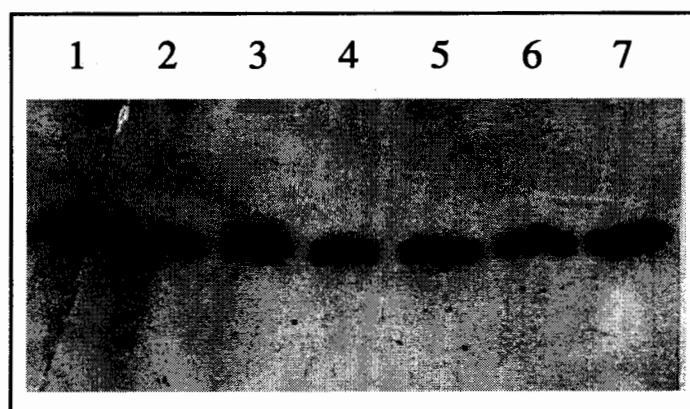


FIGURE 4 - Western blot analysis of accumulation of the 22kDa cassava protein during root "tuber" development. The print was reacted with polyclonal antibodies (diluted 1:1000) against the 22kDa protein and detected with alkaline phosphatase-conjugated anti-goat immunoglobulins antibodies. Lane 1, stage 1; lane 2, stage 2; lane 3, stage 3; lane 4, stage 4; lane 5, stage 5; lane 6, stage 6 and lane 7, stage 7. The amount of protein applied into each lane was 100 µg.

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