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CHARACTERIZATION OF MAJOR PROTEINS IN SWEET POTATO TUBEROUS ROOTS

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Key Word Index—*Ipomoea batatas*; Convolvulaceae; sweet potato; storage protein; electrophoresis.

Abstract—The tuberous roots, but not other organs, of sweet potato contained large quantities of two proteins which accounted for more than 80% of the total proteins. The two proteins, tentatively named sporamins A and B, were monomeric forms with similar M_rs (25 000). They were separated from each other by electrophoresis on polyacrylamide gels in a non-denaturing buffer or a buffer containing sodium dodecyl sulphate without being reduced by dithiothreitol. They were very similar to each other with respect to amino acid composition, peptide map and immunological properties. These proteins decreased in preference to other proteins during sprouting. The amino acid sequencing of the amino terminal part of sporamin A suggested that it consists of at least two molecular species with different combinations of a few amino acids.

INTRODUCTION

For more than a century, plant seed storage proteins have attracted the attention of scientists because of their importance as a major source of protein for humans and livestock as well as of nitrogen and amino acids for embryonic axes during seed germination. Recent interest in the biochemistry of these proteins has been directed to cloning and analysis of their genes since they are synthesized in preference to other proteins and in large quantities during seed development (see ref. [1] for a review).

In recent years major proteins, probably storage proteins, in plant tuberous organs have been recognized. A group of immunologically identical glycoproteins with apparent M_rs of *ca* 40 000 have been shown to exist in large amounts (40–45% of the soluble proteins) in potato tubers but not, or only in trace amounts, in other organs of the same plant [2–4]. These proteins, given the trivial name 'patatin' [2] (once they were called tuberin [5, 6]), seem to be preferentially synthesized during tuber development. Thus they have been thought to be a useful biochemical tool for cloning and analysing plant nuclear genes and for studying the process of tissue differentiation, just like seed storage protein [4]. Recently yam tubers were also shown to contain a family of major proteins (about 85% of the soluble proteins) which consisted principally of subunits of one size (M_r *ca* 31 000) and were recognized as the storage proteins because of their exclusive localization in organelles, similar to seed protein bodies [7].

In the present work, we have characterized the major proteins in sweet potato tuberous roots and prepared the antibody against one of these proteins to make them accessible for cloning and analysis of their genes and for biochemical studies of tissue differentiation. We report here the presence in the tuberous roots of two major proteins having similar molecular structures. We tentatively call these proteins 'sporamin A' and 'sporamin B'.

RESULTS AND DISCUSSION

When soluble proteins from sweet potato tuberous roots were analysed by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis, a major protein band was observed at the position corresponding to M_r of 25 000 (Fig. 1, lane 2). The major protein, which we gave the trivial name 'sporamin', was purified by ammonium sulphate fractionation, and Sephadex G-75 gel filtration. In the last purification step, sporamin was eluted as a single peak at the position corresponding to an M_r of *ca* 26 000, which suggested that it was in a monomeric form. The partially purified sporamin preparation still contained other proteins with M_rs of 24 000 and 19 000, though in very small amounts (Fig. 1, lane 3).

Further analysis revealed that sporamin was not a single species of protein. When the partially purified sporamin preparation was subjected to SDS-polyacrylamide gel electrophoresis without undergoing reduction with dithiothreitol (DTT), two sporamin bands appeared at positions corresponding to M_rs of 31 000(A) and 22 000(B) (Fig. 2, lane 1). After elution from the gel, both proteins again migrated to positions corresponding to their apparent M_rs on the SDS-polyacrylamide gel (Fig. 2, lanes 2 and 3). However, they migrated to one position corresponding to M_r of 25 000 after being reduced by DTT (Fig. 2, lanes 4–6). Although no definite interpretation of this behaviour can yet be proposed, sporamin A and B may have the same M_r but different surface charges and stereo-structures when bound with SDS in the presence of disulphide bonds. Sporamin A and B could also be separated from each other when the partially purified sporamin preparation was electrophoresed in a non-denaturing buffer on a polyacrylamide gel. The slow-migrating protein was identified as sporamin A, and the other as sporamin B. When eluted from the gel, they also migrated at different rates on the gel with isoelectric points of 5.2 and 5.1, respectively.

Antibody raised against sporamin A formed a pre-

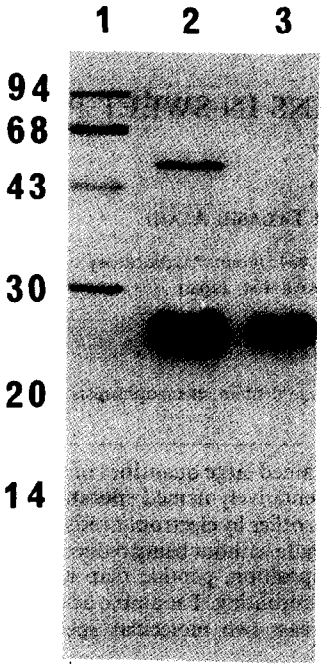

Fig. 1. SDS-polyacrylamide gel electrophoresis of the crude extract (lane 2) and partially purified sporamin preparation (lane 3) from sweet potato tuberous roots. Lane 1: M_r markers, of which the M_rs $\times 10^{-3}$ are shown on the left side.

cipitin line with sporamin B as well as with sporamin A, but a spur was formed between two precipitin lines with sporamins A and B (Fig. 3), indicating that the two proteins have very similar, but not identical, immunological properties. The amino acid compositions of sporamins A and B also resembled each other but were not the same (Table 1). The one-dimensional maps of peptides produced by digestion of the proteins with *Staphylococcus* V8 protease differed from each other, but a major peptide with M_r of 10 500 was present in both maps (Fig. 4), which again suggests that the structures of sporamins A and B are similar but not identical to each other.

Patatin and yam storage proteins appear as more than five species of proteins [3, 4, 7, 8], whereas sporamin exists in only two species, sporamins A and B. However, the possibility that neither sporamin A nor B is a single molecular species has been raised from the amino acid sequencing of the amino terminal part of sporamin A. The sequence was as follows (X is an unidentified amino acid).

1 2 3 4 5 6 7 8 9 10 11

Ser-X-Glu-Thr-Pro-Val-Leu-Asp-$\begin{matrix}\text{Val}\\ \text{Ile}\end{matrix}$-Asn-Gly

Both valine and isoleucine were found in about equal amounts at position 9, which suggests that sporamin A consists of at least two molecular species with essentially the same amino acid sequence but differences in a few amino acids. Nevertheless, we expect that sporamin will be a better tool for biochemical studies on plant genes and

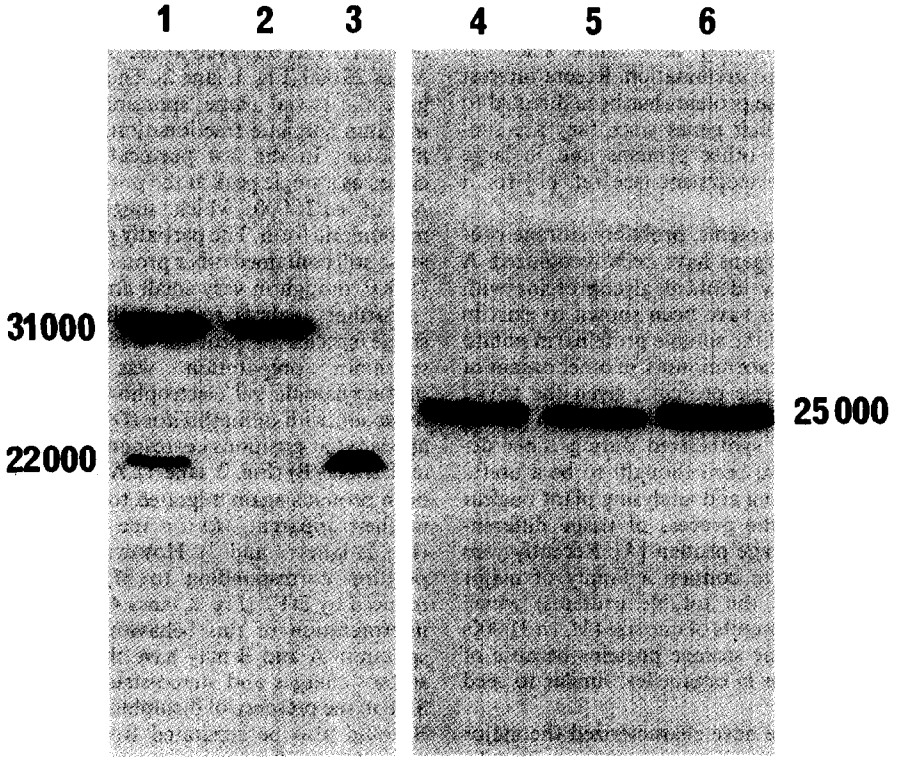

Fig. 2. SDS-polyacrylamide gel electrophoresis of sporamins A and B. The protein samples for lanes 1–3 were heated at 70° in the presence of SDS before electrophoresis, whereas those for lanes 4–6 were heated in the presence of SDS and DTT. Lane 1, the partially purified sporamin preparation (14 μg protein); lane 2 and 4, sporamin A (7 μg); lanes 3 and 5, sporamin B (6 μg); lane 6, a mixture of sporamins A and B (7 and 6 μg, respectively). Sporamins A and B were isolated by eluting them electrophoretically from the gel, on which the partially purified sporamin preparation had been electrophoresed under the same conditions as those for lane 1, without staining for protein.

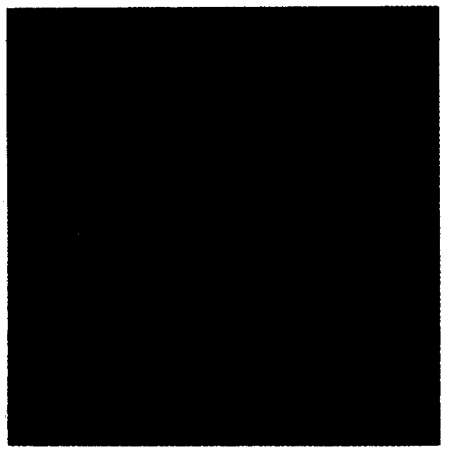

Fig. 3. Double immunodiffusion tests of sporamins A and B with antibody against sporamin A that was isolated as described for Fig. 2. Centre well, anti-sporamin A immunoglobulin G; well 1, sporamin A (1 μg); well 2, sporamin B (1 μg); well 3, a contaminant (1 μg) with M_r of 19000 in the partially purified sporamin preparation (cf. Fig. 1, lane 2); well 4, the crude extract (1 μg protein). Sporamins A and B were separated from each other as described in Fig. 2.

development of tuberous organs than patatin and yam storage proteins because sporamin seems to be composed of simpler constituents.

Patatin, but not yam storage proteins, is composed of a group of glycoproteins. Sporamin A and/or B did not seem to be glycoproteins according to our present findings; that is, they were not adsorbed on a concanavalin A–Sepharose column or stained by periodic acid–Schiff staining of the gels.

Table 1. Amino acid compositions of sporamins A and B

	Sporamin A		Sporamin B	
	mol %	number*	mol %	number*
Cys	2.1	5	1.9	4
Asp	16.1	37	16.2	37
Thr	7.0	16	6.8	15
Ser	8.3	19	8.8	20
Glu	6.0	14	7.9	18
Pro	3.6	8	3.3	8
Gly	8.0	18	7.7	18
Ala	6.9	16	6.1	14
Val	8.5	20	9.1	21
Met	2.2	5	1.2	3
Ile	4.8	11	5.9	13
Leu	7.0	16	5.6	13
Tyr	3.6	8	3.8	9
Phe	5.3	12	5.7	13
His	1.9	4	0.7	2
Lys	4.2	10	4.1	9
Arg	3.6	8	4.5	10
Trp	0.9	2	0.7	2
Total	100.0	229	100.0	229
M_r†	24952		25150	

Sporamins A and B were isolated by eluting them electrophoretically from polyacrylamide gel.

*The numbers of amino acids were calculated on the assumption that the M_rs of both proteins were about 25000.

†Calculated M_rs: namely the sums of the M_rs of the constituent amino acids.

From the viewpoint of food biochemistry, patatin has been noted as a family of proteins with a good amino acid balance [6, 9]. Examining sporamins A and B in the same way (Table 1) shows that the chemical score of sporamin A is 77% with lysine as the limiting amino acid while that of sporamin B is 55% with methionine. Thus sporamin A has rather a good balance of amino acids.

Sporamin accounted for more than 80% of the soluble proteins in sweet potato tuberous roots (Table 2), and the ratio of sporamin A to sporamin B content was about 2:1 judging from the intensity of gel staining (cf. Fig. 2, lane 1). During sprouting, sporamin decreased in preference to

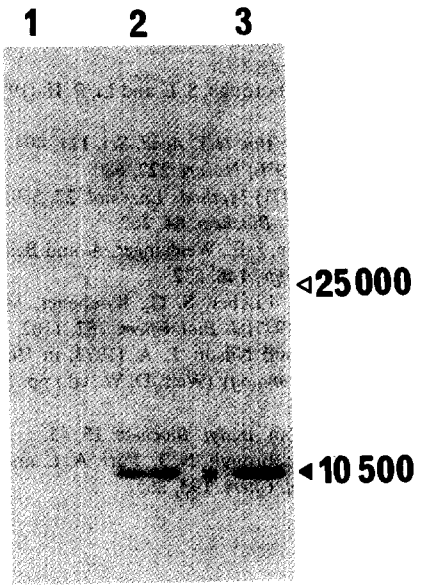

Fig. 4. Peptide maps of sporamins A and B. The proteins (7 μg each) were digested by 1 μg of *Staphylococcus* V8 protease, then the peptides produced were separated from one another by gel electrophoresis as described in Experimental. Lane 1, *Staphylococcus* V8 protease (1 μg); lane 2, digested sporamin A; lane 3, digested sporamin B. Asterisk indicates common peptide in both maps. Sporamins A and B were isolated given in Fig. 2.

Table 2. Sporamin content in various organs of sweet potato

Organ	Total protein (mg/g tissue)	Sporamin (mg/g tissue)
Tuberous root	5.33	4.41
Sprouting tuberous root	1.60	0.067
Stem	3.04	0.025
Petiole	2.10	<0.008
Leaf	4.28	<0.008

Crude extracts from the organs were assayed for the total sporamin A and B content by rocket immunoelectrophoresis.

other proteins: it accounted for only several percent of the proteins when the roots were allowed to sprout for 26 days (Table 2). A trace amount of sporamin was present in the stems but none was found in the leaves and petioles (Table 2). We thus conclude that just as reported for potato and yam [2–4, 7], the tuberous organ, but not the other organs, of sweet potato contains a large quantity of a family of proteins which have very similar structures and which seem to function in storage.

EXPERIMENTAL

Plant materials. Tuberous roots of sweet potato (*Ipomoea batatas*, Kokei No. 14) harvested in autumn were stored at 13–16° until use. The tuberous roots were allowed to imbibe H_2O from their distal sides at room temp for 26 days in a greenhouse in order to obtain leaves, petioles and stems. The tuberous roots after this cultivation were designated as sprouting tuberous roots.

Partial purification of sporamin. All the following procedures were performed at 0–4°. The parenchymatous tissue of tuberous roots was homogenized with cold buffer (50 mM Tris–acetate, pH 7.5, 1 mM EDTA, 1% w/v ascorbate, 0.5 M sucrose) at a final ratio of 2 vols/g of tissue and centrifuged at 10000 *g* for 20 min. The supernatant (crude extract) was fractionated with satd $(NH_4)_2SO_4$ soln (pH 7), and proteins precipitating between 35 and 45% satn were dissolved in 50 mM Tris–acetate buffer, pH 7.5. The soln was dialysed against 50 mM Tris–acetate buffer, pH 7.5, containing 1 mM EDTA, then applied to a DEAE–cellulose (DE-52) column (2.0 × 10 cm for 25 mg protein) pre-equilibrated with the buffer used for dialysis. The column was washed with three times the column vol. of 50 mM Tris–acetate buffer, pH 7.5, containing 1 mM EDTA and 0.1 M KCl, after which sporamin was eluted from the column with the column vol. of the same buffer as the above, using 0.2 M KCl in place of the 0.1 M KCl. The eluate was then fractionated with a Sephadex G-75 column (usually 5 ml of the eluate was applied to a 2.5 × 60 cm column) with 50 mM Tris–acetate buffer, pH 7.5, containing 1 mM EDTA and 0.1 M NaCl as the buffer for pre-equilibrating the column and eluting proteins.

Polyacrylamide gel electrophoresis. Gel electrophoresis in a non-denaturing buffer was performed at pH 8.9 on 7% polyacrylamide slab gels according to the method of ref. [10]. Gel electrophoresis in the presence of 0.1% SDS was done at pH 8.3 on 15% polyacrylamide slab gels by the method of ref. [11], after the samples had been heated at 70° for 20 min in the presence of 10% glycerol, 2% SDS and 2 mM DTT. After electrophoresis the gels were stained for proteins with Coomassie brilliant blue R.

Isoelectric focusing. Isoelectric focusing was carried out on 5% polyacrylamide disk gels containing 2% Ampholine (pH 3–10) and 6.5% glycerol by the method of ref. [12]. After electrophoresis at 200 V for 5 hr, the gels were stained for protein as described above.

Analysis of amino acid composition and sequence. Amino acid compositions of desalted and *S*-carboxymethylated proteins were analysed as described previously [13] with a JEOL JIC-8AH amino acid analyser. Sequential preparation of phenylthiohydantoin derivatives of amino acids from the amino-terminal part was performed with a JEOL JAS-47K sequence analyser according to the directions of the manufacturer. The derivatives produced were identified by HPLC as described in ref. [14].

Peptide mapping. Peptide mapping with *Staphylococcus* V8 protease on a 17% polyacrylamide slab gel was performed according to the method of ref. [15]. The gel was stained for protein as described above.

Immunological tests. Antibody against sporamin A was raised in a rabbit. The immunoglobulin G fraction was prepared from the antiserum by $(NH_4)_2SO_4$ fractionatin and DEAE–cellulose column chromatography. Double immunodiffusion tests were carried out by the method of ref. [16] on a 1% agar plate containing 20 mM Tris–HCl, pH 7.5, 0.7% NaCl and 0.01% NaN_3. Rocket immunoelectrophoresis was done by the method of ref. [17] on a 1% agarose plate containing 0.1 M Tris–HCl, pH 8.6, 0.4% Triton X-100 and 5% anti-sporamin A antiserum.

Protein determination. Protein was determined by method of ref. [18].

REFERENCES

1. Larkins, B. A. (1981) in *The Biochemistry of Plants* (Stumpf, P. K. and Conn, E. E., eds) Vol. 6, pp. 449–489. Academic Press, New York.
2. Racusen, D. and Foote, M. (1980) *J. Food Biochem.* **4**, 43.
3. Park, W. D., Blackwood, C., Migner, G. A., Hermodson, M. A. and Lister, R. M. (1983) *Plant Physiol.* **71**, 156.
4. Paiva, E., Lister, R. M. and Park, W. D. (1983) *Plant Physiol.* **71**, 161.
5. Osborne, T. B. and Campbell, G. F. (1896) *J. Am. Chem. Soc.* **18**, 575.
6. Kosier, T. and Desborough, S. L. (1981) *Plant Physiol.* **67**, S-92.
7. Harvery, P. J. and Boulter, D. (1983) *Phytochemistry* **22**, 1687.
8. Nakasone, K., Hayashi, R. and Hata, T. (1972) *J. Agric. Chem. Soc. Jpn.* **48**, 45.
9. Kapoor, A. C., Desborough, S. L. and Li, P. H. (1975) *Potato Res.* **18**, 469.
10. Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404.
11. Laemmli, U. K. (1970) *Nature* **227**, 680.
12. Wrigley, C. W. (1971) *Methods Enzymol.* **22**, 559.
13. Sasaki, T. (1978) *J. Biochem.* **84**, 267.
14. Bhown, A. S., Mole, J. E., Weidsinger, A. and Bennett, J. C. (1978) *J. Chromatogr.* **148**, 532.
15. Cleveland, D. W., Fischer, S. G., Kischener, M. W. and Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102.
16. Ouchterlony, O. and Nilson, L. A. (1973) in *Handbook of Experimental Immunology* (Weir, D. W., ed.) pp. 19.1–19.39. Blackwell, Oxford.
17. Laurell, C. B. (1966) *Analyt. Biochem.* **15**, 45.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.