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ISOLATION AND CHARACTERIZATION OF THE STORAGE PROTEIN OF YAM TUBERS (*DIOSCOREA ROTUNDATA*)

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Key Word Index—*Dioscorea rotundata*; Dioscoreaceae; yam; storage protein; amino acid composition; subunits.

Abstract—The major proteins of the yam tuber, which were identified as storage proteins by virtue of their abundance (*ca* 85% of the total protein content), amino acid composition (high in amide content) and cellular location within the tuber, were isolated by ion-exchange chromatography and characterized using, in particular, polyacrylamide gel techniques. They consist principally of subunits of one size, apparent MW 31 000, and *N*-terminal amino acid glutamine/glutamic acid, of which there are a number of charge isomers; these usually contain one intra-chain disulphide bond. The subunits associated into polymers depending on the protein concentration, pH value and ionic strength of the milieu and, therefore, a value for the MW of the native protein(s) is not given. The storage proteins are not glycoproteins. They are intracellularly located as protein 'aggregates' within cellular protein vacuoles, and also within the cytoplasm.

INTRODUCTION

Yam tubers are of considerable importance in world agriculture, principally as a source of calories, but in areas where they are used as dietary staples, they may also supply a substantial proportion of the total protein consumed. Yams contain *ca* 1–3% crude protein as eaten [1], although when the crude protein content is measured on a dry wt basis, the values obtained (*ca* 6–13%) [2–6] approach the levels found in cereal grains.

Studies of the protein in yams, however, have been directed primarily to the determination of the protein content and its amino acid balance with emphasis on nutritional and agronomic aspects [2–6] and, apart from the potato, the proteins of which have been studied in more detail [7–10], very little is known about the constituent proteins of the root and tuber storage organs. These tissues, in addition to containing the widely distributed so-called house-keeping enzymes, also have specialized 'storage' proteins which occur in large amount, and it is these proteins in yam which are the subject of this study. The major yam storage protein has been isolated, characterized and compared with the storage protein(s) of the potato tuber.

RESULTS

Extractions and isolation of the storage protein

The use of alkaline buffers (borate or Tris, pH 8.3) enabled extraction of *ca* 85% of the total protein content of yam meals as determined from quantitative amino acid analysis of extracts compared to those of ethanol-extracted meals (protein *in situ*). Precipitated protein in the buffer extracts was also analysed by the Lowry procedure and a similar figure of 89% exraction was obtained. Sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis (PAGE) of the extracted protein indicated that there was one major protein polypeptide in the preparation, which represented *ca* 85% of the total protein extracted, and attempts were directed towards the further purification of this subunit. Both ammonium sulphate precipitation and molecular sieve chromatography proved ineffective, but a satisfactory purification (> 98%) was achieved with the use of anion-exchange chromatography. Even so, the purified protein preparation contained, in addition to the major subunit, three smaller subunits in small amounts (2% of the total protein) (Fig. 1). Two of these were shown by two-dimensional techniques to be artefacts produced *in vitro* upon reduction of a small fraction of the storage protein (data not presented). The third was considered most probably to represent a genetic variant of the major subunit since it had a mobility in SDS polyacrylamide gels which corresponded to that of the unreduced major subunit as well as having a similar isoelectric point.

Characterization

A chemical analysis of the purified storage protein is given in Table 1. Under apparent non-dissociating conditions, a single *N*-terminal amino acid, glutamine/glutamic acid, was identified, but after pre-treatment with SDS, several additional *N*-terminal amino acids were also found. SDS, however, appeared to aggravate the breakdown of the protein, as indicated by the appearance of lower MW fragments in the subunit profile of the storage protein after prolonged incubation with SDS at room temperature (1 hr or more). Several different smaller MW fragments could also be generated from the major subunit after SDS incubation for 6 min or more at 100°. Both these effects were increased in the presence of 2-mercaptoethanol, but could be eliminated by the incorporation of urea into the incubation medium, or by prior treatment with guanidine–HCl, followed by reduction and alkylation.

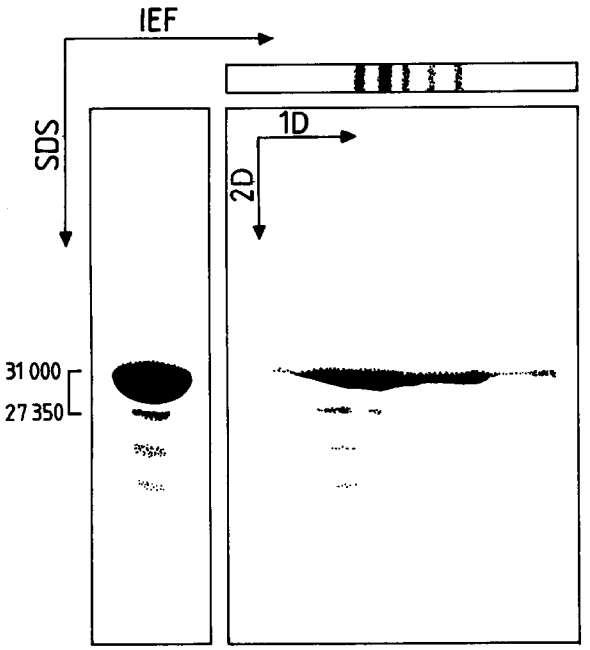

Fig. 1. Analysis of the yam storage protein using 2D-polyacrylamide gel techniques. (1D) Separation by gel isoelectric focussing (IEF), pH range 3–10. (2D) Separation, after pre-treatment with SDS and 2-mercaptoethanol, by SDS gel electrophoresis using the discontinuous SDS buffer system. The profile obtained after 1D-isoelectric focussing is shown above the 2D gel, and that obtained after 1D-SDS gel electrophoresis to the left of the 2D gel.

The storage protein was soluble in all buffers investigated in the pH range 6–9, both at room temperature and at 4°, irrespective of the ionic strength of buffer or concentration of protein. It was also soluble in buffer of pH 4.7–5 at high ionic strength (0.01–0.02 M acetate containing 0.3 M sodium chloride) but the effect of decreased ionic strength at this pH value (0.01–0.02 M acetate) caused a fraction (*ca* 14%) of the protein to precipitate. The remainder of the protein formed a stable solution in the low ionic strength buffer at room temperature, cryoprecipitated at 4° and redissolved on warming. By contrast, the protein was completely soluble in buffer of pH 2.5 at low ionic strength (0.02 M glycine–HCl ± 0.05 M sodium chloride), both at room temperature and at 4°, but with increased ionic strength (addition of 0.3 M sodium chloride), the total protein fraction precipitated, gradually redissolving in the original low ionic strength glycine–HCl buffer.

Subunit structure

The minimal MW of the protein, based on one cysteic residue per molecule, was 10 390 ± 280. A different value of 31 000 was obtained by SDS gel electrophoresis for the MW of the major subunit. The supposition from these results taken in conjunction, namely that there may be three cysteine residues per subunit, was strengthened from data obtained after SDS-PAGE in the absence of prior reduction of the storage protein. Under these conditions, the major subunit, apart from a small fraction which remained unaffected by this treatment, showed an increased mobility and apparent MW of 27 350, behaviour consistent with the presence of one intra-chain disulphide bond within each subunit. The subunit profile also contained one new band with decreased apparent mobility and MW of 55 000, i.e. it is probable that some degree of dimer formation between subunits could be effected through a third cysteine residue to form inter-chain disulphide bridges.

Table 1. Chemical analysis of the storage protein of *Dioscorea rotundata* cv Nwapoko

Amino acid*	Grams of amino acid residue per 100 g protein (d.b.)†
Aspartic acid†	13.47 ± 0.36
Threonine§	3.80
Serine§	5.76
Glutamic acid‡	18.36 ± 0.42
Proline‡	3.85 ± 0.16
Glycine‡	2.81 ± 0.08
Alanine‡	3.84 ± 0.08
Valine‖	4.93 ± 0.35
Methionine‡	2.05 ± 0.08
Isoleucine¶	4.35 ± 0.10
Leucine‡	7.91 ± 0.25
Tyrosine§	4.69
Phenylalanine‡	7.06 ± 0.54
Histidine‡	2.17 ± 0.11
Lysine‡	5.03 ± 0.45
Arginine§	8.98
Cysteic acid‡	0.99 ± 0.10
Total	100.07
Protein content**	101.3 ± 2.5
Kjeldahl N	16.03 ± 0.16
Carbohydrate	Negative
N-Terminal amino acid	Glutamic acid/glutamine
Lectin activity	Negative

*Tryptophan and amide contents not determined.

†Values obtained from six hydrolysates, two at each of 22, 48 and 72 hr duration of hydrolysis.

‡Mean of 22, 48 and 72 hr hydrolysates ± s.d.

§Extrapolation to zero time [11].

‖Mean of 72 hr hydrolysates ± s.d.

¶Mean of 48 and 72 hr hydrolysates ± s.d.

**Determined by the Lowry method with bovine serum albumin as reference.

Size heterogeneity of the undenatured protein

A series of molecular sieve experiments using Bio-gel P-150 with various buffers revealed that a dynamic association–dissociation reaction, dependent on protein concentration, pH value and ionic strength of the milieu, existed between protein subunits (monomers), dimers, tetramers and higher polymers (a representative sample of these results in which protein concentration was varied is shown in Fig. 2). Association occurred with increasing protein concentration and, at pH8, with decreasing ionic strength of the milieu. Both monomer and polymers were distinguishable in acetate buffer, pH 4.7, containing 0.3 M sodium chloride, but with decreasing ionic strength protein gradually precipitated. Only monomer, and a small amount of dimer, were identified in glycine–HCl

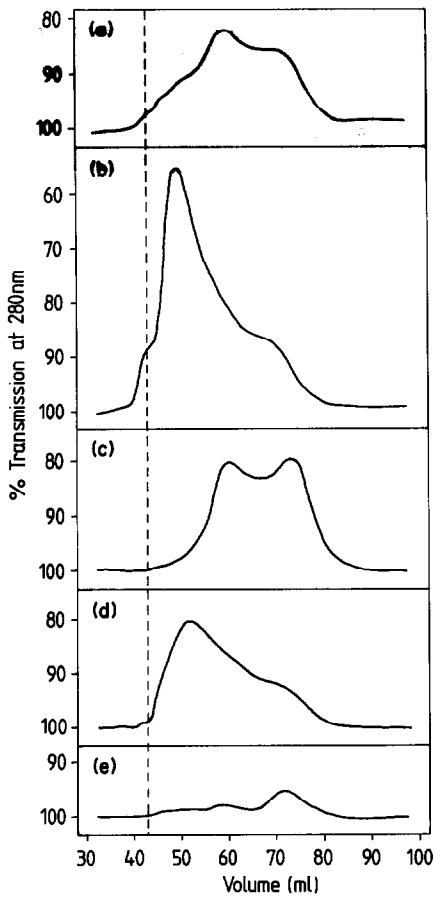

Fig. 2. Elution profiles obtained after molecular sieve chromatography of the yam storage protein under conditions of varying initial protein concentration, but constant sample volume. The column, Bio-gel P. 150 (45 × 2 cm), was operated at a constant flow rate of 4.8 ml/hr·cm² using the eluant 0.05 M Tris–HCl, 0.15 M NaCl, pH 8.3. (a) and (b) Storage protein, as prepared by ion-exchange chromatography, at initial concentrations of 7.7 and 13.8 mg/ml, respectively. (c) Fractions of the monomer, collected from several molecular sieve chromatographic analyses, which were pooled, concentrated to 9.0 mg/ml, and further chromatographed at that protein concentration. (d) Fractions of the tetramer, collected from the same chromatographic analyses as for (c), which were pooled, concentrated to 7.2 mg/ml and further chromatographed at that protein concentration (e) Pooled fractions of the tetramer as for (d), but without concentration, which were further chromatographed; the protein concentration was 1.0 mg/ml.

buffer, pH 2.5, but at this pH value protein precipitated with increased ionic strength. The association–dissociation equilibrium also depended on the nature of the starting subunits, since individual samples of monomer, tetramer and the initial protein preparation gave different equilibrium positions to one another when chromatographed under the same conditions with regard to protein concentration and ionic strength and pH value of buffer (Fig. 2). Chromatography in the presence and absence of dithiothreitol (DTT), under conditions normally favouring association, yielded the same result, indicating that the principal mechanism of interaction between subunits did not involve inter-chain disulphide bond formation.

Charge heterogeneity of the protein and its constituent subunits

Five charge isomers of the storage protein were distinguished after gel isoelectric focussing (Fig. 1). The p*I* values of the isomers ranged between 5.2 and 6.8.

Further confirmation of charge and size heterogeneity of the protein

Ferguson plots constructed for the storage protein (Fig. 3) confirmed that the multiplicity of bands observed after electrophoresis using the alkaline Ornstein–Davis type buffer system (data not presented) arose from both charge and size heterogeneity within the preparation, i.e. using alkaline conditions, such plots enabled the identification of monomer, dimer and tetramer together with their respective charge isomers. In the acidic buffer system (data not presented) much simpler patterns were obtained because the protein components were separated primarily on the basis of size, i.e. charge differences within size groups were minimal.

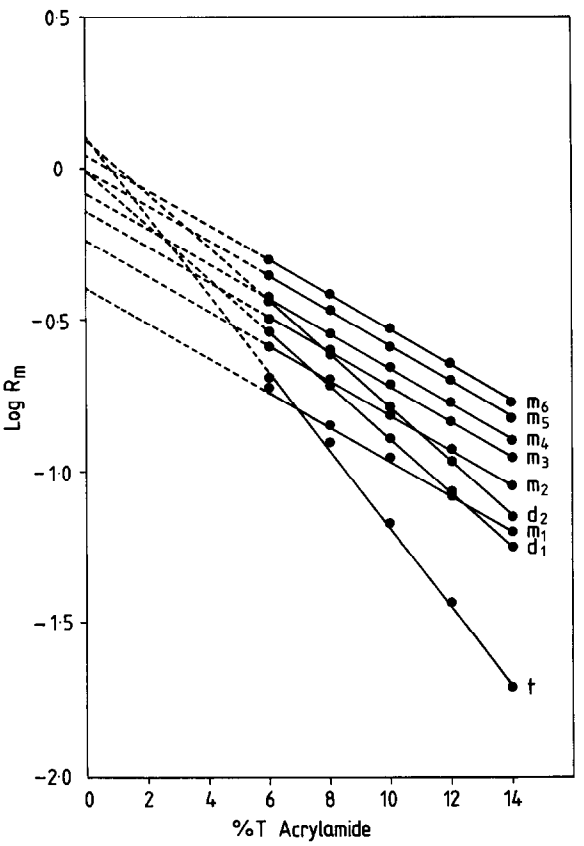

Fig. 3. Ferguson plots constructed by regression analysis for the main protein components of the storage protein separated by non-dissociating polyacrylamide gel electrophoresis, using alkaline buffer conditions. R_m values were obtained from analysis of a series of densitometric profiles which depicted the separation of the protein components obtained after electrophoresis on gels of varying total (%*T*) acrylamide concentration but constant crosslinker (5%C) concentration. m_1–m_6, Monomer components of the storage protein; d_1, d_2, dimer components of the storage protein; t, tetramer.

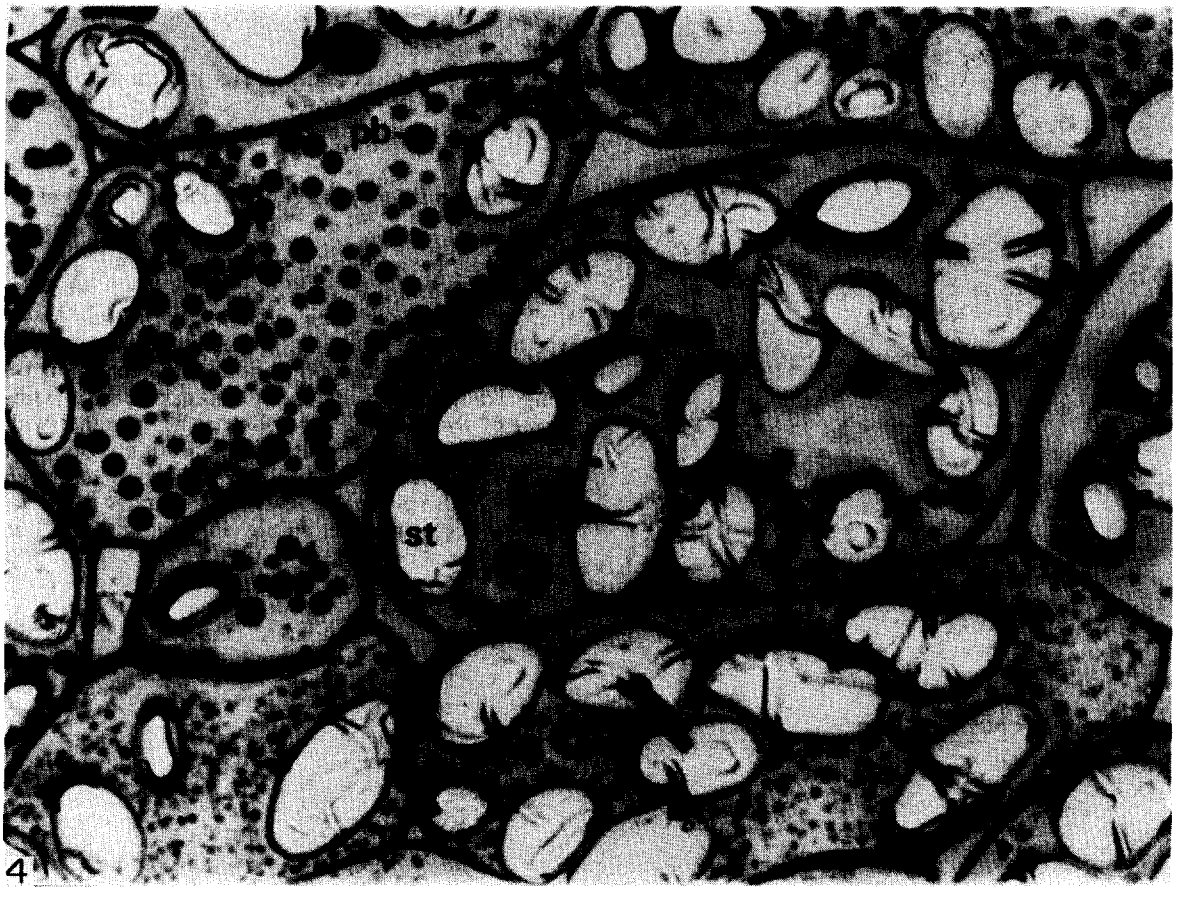

Fig. 4. A section, examined by light microscopy, of a mature dormant yam tuber illustrating the intracellular distribution of protein 'bodies'. Magnification, × 228; pb, protein 'body'; st, starch grain.

Protein distribution within the tuber

Sections from the storage tissue of a mature dormant tuber, examined by light microscopy, showed a uniform intracellular distribution of, for the most part spherical, protein 'bodies' of various sizes within the storage cells (Fig. 4). Electron micrographs showed that the protein was deposited in protein vacuoles (Fig. 5a), and also as cytoplasmic protein aggregates (Fig. 5b); the latter may originally have been vesicular but, in the mature storage organ, membranes were not visible.

DISCUSSION

The results presented in this paper show that the yam tuber contains a principal protein which represents *ca* 85% of the total protein of the storage tissue. By virtue of its preponderance over other proteins in a storage organ it can be considered a 'storage' protein, the more so since it is deposited as aggregates in both vacuoles and in the cytoplasm of the storage cells of the tuber.

Plant storage proteins typically have a high amide content (high relative percentage nitrogen), a feature presumed to reflect their role in nitrogen storage. In common with these proteins, the yam storage protein can be assumed to be highly amidated from inspection of the amino acid composition in relation to the pH-mobility curves. Thus, pH-mobility curves for the isomers were mostly united at pH values of *ca* 4 and less, but above this pH, they ran essentially parallel with one another through to alkaline pH values. This pattern was the same under both dissociating and non-dissociating conditions. The overall shape of these curves reflects larger relative amounts of basic as compared to acidic amino acids, i.e. the greater proportion of aspartic and glutamic acid residues in the protein must, therefore, be amidated.

Subunits of the yam storage protein have a common MW of *ca* 31 000, and a common *N*-terminal amino acid, glutamine/glutamic acid. They are, however, heterogeneous with respect to charge. The main mechanism of interaction between subunits to yield dimers, tetramers and higher polymers is through non-covalent, i.e. electrostatic, hydrogen, or hydrophobic, bonding, although a small fraction of the protein appears to be capable of inter-molecular disulphide bond formation to give dimers, as indicated from results of SDS electrophoresis in the absence of a reducing agent.

Many of the properties of the storage protein from the

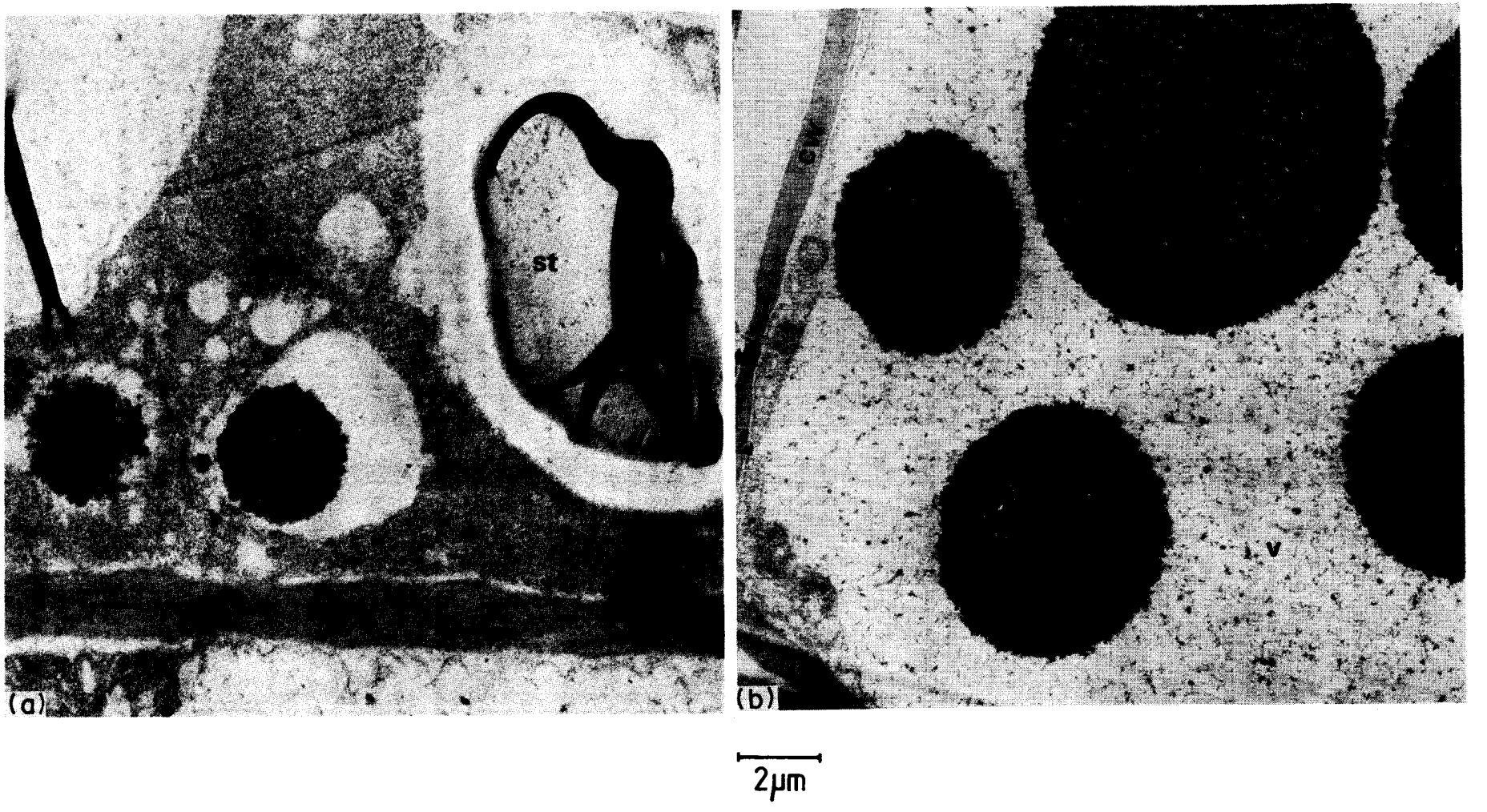

Fig. 5. Electron micrographs of a mature dormant yam tuber illustrating protein 'bodies' within both cellular protein vacuoles (a), and as cytoplasmic protein aggregates (b). (a) Magnification × 8550; (b) Magnification × 5700; pb, protein 'body'; st, starch grain; cw, cell wall.

yam, a monocotyledonous plant, are also shared with the storage protein from the potato, a dicotyledonous plant. The latter protein, which represents *ca* 70–80% of the total extractable protein of potato tubers [12], exhibits similar solubility characteristics, i.e. only limited solubility in water [13]. The undenatured potato storage protein also exhibits considerable charge heterogeneity, with each charge isomer having the same subunit structure consisting in this instance of three principal subunits with MWs 16 800, 18 000 and 19 500, together with a lesser amount of disulphide-linked dimers of these subunits [7]. Subunits of the potato storage protein, however, do not contain intra-molecular disulphide bridges, unlike the majority of those of the yam storage protein and, in addition, reversible association–dissociation behaviour between subunits or between the undenatured charge isomers has not been demonstrated. [7]

The yam protein shares many characteristics, e.g. solubility, multimeric nature, association–dissociation behaviour and high amide content with the legume seed storage proteins [14]. Clearly, these properties which distinguish this class of protein from the enzymes are related to their storage function, i.e. deposition and subsequent utilization.

EXPERIMENTAL

Plant material. Tubers of *Dioscorea rotundata* cv Nwapoko were peeled, cut into thin slices, lyophilized, then ground to pass a 423 μm sieve. These meals were prepared at the International Institute of Tropical Agriculture, Ibadan, Nigeria, and air-freighted in sealed containers to Durham.

Preparation of the storage protein. Yam meals (1:20 w/v) were stirred for 4 hr at 4° in pre-chilled 0.05 M Na borate buffer, pH 8.3, containing 10 mM 2-mercaptoethanol, or in 0.05 M Tris–HCl, pH 8.3, containing 10 mM 2-mercaptoethanol, then clarified by centrifugation at 2400 *g* then 30 000 *g*. Clarified extracts (400 ml) were loaded onto columns (12.5 × 2.5 cm) of DE-52 equilibrated with 0.05 M Tris–HCl, pH 8.3, and the storage protein was eluted with 0.15 M NaCl in 0.05 M Tris–HCl, pH 8.3.

Estimation of protein in situ. Yam meals were extracted for 19 hr in 70% EtOH (1 : 100 w/v) on a Soxhlet apparatus, dried in a thermostatic oven at 60° and analysed for amino acids and total N content.

Total N *content.* Determined as the indophenol-blue complex after digestion by a micro-Kjeldahl technique [15].

Protein determination. Protein in soln was ppted with 10% TCA, dissolved in NaOH then analysed according to ref. [16] with bovine serum albumin as the reference protein.

Amino acid analysis. Performed on a Locarte automatic-loading amino acid analyser, on duplicate 22, 48 and 72 hr acid hydrolysates prepared according to ref. [11]. Cysteine (50% cystine) was determined after performic acid oxidation as cysteic acid [17].

N-*Terminal amino acids.* Determined by the dansylation procedure of ref. [18], and after performic acid oxidation and treatment with SDS by the method of ref. [19].

Carbohydrate content. Measured, after TCA precipitation of the protein, by GC according to ref. [20]. SDS gel electropherograms were also tested for carbohydrate content using the periodic acid–Schiff technique of ref. [21], and the fluorescent method of ref. [22].

Polyacrylamide gel electrophoresis. For routine analysis, vertical slabs (17.6 × 0.45) were prepared as described in ref. [23], using the discontinuous SDS buffer system of ref. [24]. Protein samples were denatured prior to electrophoresis by incubation for 2 min at 100° in 2% SDS ± 5% 2-mercaptoethanol ± 6 M urea; they were also prepared by the method in ref. [25] for denaturation with guanidine–HCl followed by alkylation. Electrophoresis was conducted at 15 mA (constant current) for the stacking gel, 25 mA for the separating gel.

Non-dissociating gel electrophoresis was performed according to ref. [26] using buffer systems A and G of ref. [27] for separation at pH 9.45, ionic strength 0.0158, and at pH 3.51, ionic strength 0.0206, respectively. Separated proteins were stained with Coomassie Blue R250 (0.25% in MeOH–HOAc–H_2O, 5:1:5) or Procion Brilliant Blue R5 (1% in MeOH–HOAc–H_2O, 5:1:5), and destained by simple diffusion in the respective solvents. Densitometric analysis of stained gels was carried out with a chromoscan in transmission mode.

Quantitative evaluation of subunits. Subunit ratios were determined, after SDS electrophoresis of a dilution series (5–100 μg protein in 5 μl), from quantitative measurements of the amount of dye bound to each subunit, obtained by integrating the densitometric scans of gels.

Subunit MWs. Determined according to ref. [28] on gels of concn 17.6 × 0.45, 10.4 × 3.3, 10.2 × 1.8 and 10.0 × 0.9, (nomenclature of ref. [29]) prepared with the discontinuous SDS buffer system described [24], and on gels of concn 7.7 × 2.6 prepared with the continuous SDS buffer system of ref. [30].

Ferguson plots. Constructed according to ref. [27]. Rod gels (12 × 0.5 cm) of varying total (*T*) acrylamide concn but constant (5%) crosslinker concn were prepared using the alkaline and acidic non-dissociating buffer systems described. Optimum concn of polymerization catalysts [TEMED, $(NH_4)_2$ (S_2O_4)] were selected by prior expt, and polymerization was carried out at the temp. of electrophoresis (25°). For each expt, 12 gels representing each value of %*T* in duplicate were loaded with 20 μl protein soln and electrophoresed at 10 mA/cm². Internal standard proteins (β-lactoglobulin, ferritin ± myoglobin for the alkaline system, β-lactoglobulin, γ-globulin for the acidic system) were selected according to criteria set out in ref. [31] and electrophoresed together with the storage protein. On termination of electrophoresis, gels were sliced at the position of the tracking dye front, stained and destained, and R_m values determined from densitometric scans of the gels. Values for the slopes of the Ferguson plots (K_R values) were obtained through unweighted linear regression; relative measures of free electrophoretic mobility (Y_0 values) were obtained by extrapolation to 0% *T*. Approximate estimates of molecular radius, thence MW and *n*-mer order of the storage protein components were obtained by reference to calibration curves relating molecular radius with K_R values estimated for the standard proteins used in each system. The results (not presented) for the protein standards were the same as those obtained by Rodbard and Chrambach [27] using the same conditions.

Isoelectric focussing. This was carried out on non-dissociated protein samples using the method of ref. [32]. Rods of polyacrylamide (6.2 × 3.2) containing 2% carrier ampholytes pH range 3–10 were photopolymerized with 0.0005% riboflavin. Gels were prefocussed at 4 mA/cm² for 30 min, then 10 μl aliquots of protein soln containing 2% carrier ampholytes, pH range 3–10, were loaded and focussed at 0.7 W/cm² for 6 hr at room temp. Focussed proteins were detected using the procedure of ref. [33] and approximate p*I*s of stained bands were determined by reference to calibration curves relating distance along a focussed gel with pH value [32].

2*D-gel electrophoresis.* Protein samples were electrophoresed or isoelectrically focussed on rod gels (i.e. 0.25 cm) for the first dimension. The gels were then incubated for 45 min in 0.0625 M Tris–HCl, pH 6.8 containing 2% SDS and 10% 2-mercapto-

ethanol, then sealed to the surface on a SDS slab gel (17.5 × 0.45) with 2% agarose in the same buffer, before electrophoresis in the second dimension.

Molecular sieve chromatography. Columns of Bio-gel P-150 were equilibrated and operated with the following buffers: 0.05 M Tris–HCl, 0.15 M NaCl, pH 8.3; 0.05 M Tris–HCl 0.3 M NaCl, pH 8.3; 0.025 M Tris–HCl, pH 8.3; 0.025 M Tris–HCl, 0.01 M dithiothreitol, pH 8.3; 0.0125 M NaOAc, pH 4.7; 0.0125 M NaOAc, 0.3 M NaCl, pH 4.7; 0.02 M glycine; HCl, pH 2.5; 0.02 M glycine–HCl, 0.3 M NaCl, pH 2.5. Protein solns of constant vol. (1.5 ml) but varying concn were loaded onto the tops of columns and eluted at constant flow-rates of 4.8 ml/hr·cm^2: dilute protein solns were concd when necessary with a Minicon B15 concentrator. Approximate estimates of MWs were made by reference to calibration curves constructed according to ref. [34].

Microscopy. Samples of tuber tissue were fixed in 2.5% v/v glutaraldehyde, 1.5% v/v formaldehyde in 0.05 M sodium cacodylate buffer, pH 7.0, for 4 hr at room temp. then washed in buffer and post-fixed in 1% osmium tetroxide for 4 hr at 0°. After alcohol dehydration, the samples were embedded in Spurr's resin. Sections (1 μm) for light microscopy were stained with 1% Toluidine Blue in borax and examined and photographed in a Leitz-Ortholux microscope. Thin sections for electron microscopy were stained with aq. uranyl acetate, post-stained with Reynolds lead citrate and examined in a Philips EM400 electron microscope at 60 KV.

Lectin analysis. Performed using the haemagglutination methods of ref. [35], and also by diffusion in a supporting medium of agarose against the glycoproteins ovalbumin, thyroglobulin and fetuin, with concanavalin A as control; after diffusion, the agarose plates were dried, rinsed with 0.1 M NaCl, then stained with Coomassie Blue R250 (0.5% w/v in EtOH–HOAc–H_2O, 9:2:9) and destained by simple diffusion in the solvent.

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REFERENCES

1. Coursey, D. G. (1967) in *Yams: An Account of the Nature, Origins, Cultivation and Utilisation of the Useful Members of the Dioscoreaceae* (Coursey, D. G., ed.). Longman, London.
2. Splittstoesser, W. E. (1977) *Hort Sci.* **12**, 294.
3. Martin, F. W. and Thompson, A. E. (1971) *Hort Sci.* **6**, 545.
4. Martin, F. W. and Thompson, A. E. (1972) *J. Agric. Univ. P.R.* **57**, 78.
5. Francis, B. J., Halliday, D. and Robinson, J. M. (1975) *Trop. Sci.* **17**, 103.
6. Baquar, S. R. and Oke, O. L. (1976) *Nutr. Rep. Int.* **14**, 237.
7. Stegemann, H., Francksen, H. and Macko, V. (1973) *Z. Naturforsch. Teil C* **28**, 722.
8. Stegemann, H. (1978) in *Biochemical Aspects of New Protein Foods* (Adler-Nissen, J., Eggum, B., Munck, L. and Olsen, H. S., eds.) FEBS 11th Meeting, Copenhagen, 1977, Vol. 44, p. 11. Pergamon Press, Oxford.
9. Stegemann, H. (1979) in *The Biology and Taxonomy of the Solanaceae* (Hawkes, J. G., Lester, R. N. and Skelding, A. D., eds.) p. 279. Linnean Society of London and Academic Press, London.
10. Seibles, T. S. (1979) *Am. Potato J.* **56**, 415.
11. Moore, S. and Stein, W. H. (1963) *Methods Enzymol.* **VI**, 819.
12. Stegemann, H. (1975) in *The Chemistry and Biochemistry of Plant Proteins* (Harborne, J. B. and Van Sumere, C. F., eds.) Vol. II, p. 71. Academic Press, London.
13. Kapoor, A. C., Desborough, S. L. and Li, P. H. (1975) *Potato Res.* **18**, 469.
14. Derbyshire, E., Wright, D. J. and Boulter, D. (1976) *Phytochemistry* **15**, 3.
15. Varley, J. A. (1966) *Analyst* **91**, 119.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.
17. Moore, S. (1963) *J. Biol. Chem.* **238**, 235.
18. Gros, C. and Labouesse, B. (1969) *Eur. J. Biochem.* **7**, 463.
19. Gray, W. R. (1972) *Methods Enzymol.* **25**, 121.
20. Clamp, J. R., Bhattie, T. and Chambers, R. E. (1971) *Methods Biochem. Analysis* **19**, 229.
21. Glossmann, H. and Neville, D. M., Jr. (1971) *J. Biol. Chem.* **246**, 6339.
22. Eckhardt, A. E., Hayes, C. E. and Goldstein, I. J. (1976) *Analyt. Biochem.* **73**, 192.
23. Ames, G. F. L. (1974) *J. Biol. Chem.* **249**, 634.
24. Laemmli, U. K. (1970) *Nature (London)* **227**, 680.
25. Weber, K., Pringle, J. R. and Osborn, M. (1972) *Methods Enzymol.* **XXVI**, 3.
26. Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404.
27. Rodbard, D. and Chrambach, A. (1971) *Analyt. Biochem.* **40**, 95.
28. Weber, K. and Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406.
29. Hjerten, S. (1962) *Arch. Biochem. Biophys. (Suppl.)* **1**, 147.
30. Shapiro, A. L., Vinuela, E. and Miazel, J. V., Jr. (1967) *Biochem. Biophys. Res. Commun.* **28**, 815.
31. Johnson, G. (1979) *Prog. Nucleic Acid Res. Mol. Biol.* **22**, 293.
32. Wrigley, C. (1968) *Sci. Tools* **15**, 17.
33. Vesterberg, O. (1971) *Biochim. Biophys. Acta* **243**, 345.
34. Andrews, P. (1964) *Biochem. J.* **91**, 222.
35. Lis, H. and Sharon, N. (1973) *Annu. Rev. Biochem.* **42**, 541.