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Proline metabolism by germinating *Lilium longiflorum* pollen. I. Labelling of cytoplasmic, wall and culture medium molecules

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Abstract

Radioactivity occurs in trichloroacetic acid (TCA)-soluble and precipitable, cytoplasm and salt-washed walls following germination of *Lilium longiflorum, cv.* 'Ace' pollen in medium containing [^{14}C]-proline (Pro). Sephadex gel filtration on G-25 through G-100 was employed to determine whether radioactivity in cytoplasm, wall and growth medium from pollen fed [^{14}C]-Pro or [^{3}H]-Pro plus [^{14}C]-arabinose (Ara) was contained within molecules possessing molecular weights of 5,000 to 100,000 daltones or greater. G-25 elution profiles of a crude cytoplasmic fraction (15,000 × g supernatant) from [^{14}C]-Pro labelled pollen yielded a radioctive void volume peak and a retarded peak. The void volume peak contained hydroxyproline (Hyp), and exhibited a coincidence of [^{3}H]-Pro and [^{14}C]-Ara labelling when pollen was double labelled with the two isotopes. This peak also contained radioactivity when pollen was germinated in 2-[^{3}H]-myo-inositol. Germination in medium supplemented with 100 μM 2,2'-dipyridyl eliminated radioactivity from 2-[^{3}H]-myo-inositol or [^{14}C]-Pro in the peak. Filtration on G-25 of a TCA-soluble fraction of a salt-extract of walls from [^{14}C]-Pro labelled pollen resulted in void volume and two retarded peaks. Void volume and two retarded peaks were also obtained upon G-25 filtration of a cellulase--digest of walls from [^{3}H]-Pro labeled pollen. The void volume peak contained Hyp, Lys, Gly, Ala, Ser, Glu and Asp acids, Val, Tyr, Leu or Ileu and Pro. Sephadex G-50, 75, and 100 elution profiles of cellulase--digests of walls from [^{3}H]-Pro and [^{14}C]-Ara labelled pollen yielded radioactive retarded and Hyp-containing void volume peaks with a coincidence of [^{3}H] and [^{14}C] labelling. Label in the void volume was obtained when either rhozyme P11- or pepsin-digests of walls from [^{41}C]-Pro labelled pollen were gel filtered on G-50. Paper electrophoresis coupled with paper chromatography of acid hydrolyzates of salt-washed wall fractions demonstrated 15 of the common amino acids. Gel filtration on G-25 of growth

medium in which pollen was germinated resulted in two peaks, one of which eluted in the void volume, contained Hyp and excluded during subsequent gel filtration on G-100.

INTRODUCTION

Ungerminated, freshly-harvested *Lilium longiflorum*, cv. 'Ace' pollen contains 0.14% soluble and 0.35% protein-bound Pro on a dry weight basis (Dashek, 1966; Dashek, Harwood, 1974). In contrast, these Pro levels were 0.10% (soluble)and 0.99% (protein-bound) for pollen stored 4 months at 4-6° C. Time-dependent changes in colorimetrically--detectable (Dashek Harwood, 1974; Harwood, 1970) as well as radioactive Pro and Hyp following germination of pollen in medium containing [^{14}C]-Pro (Dashek et al., 1971; Dashek, Horwood, 1974; Dashek, Mills, 1980)revealed unlabelled and labelled Pro and Hyp in TCA-precipitable and -soluble, cytoplasmic fractions. Both unlabelled and labelled Pro and Hyp also occurred in a salt-washed wall fraction.

The present communication reports an attempt to isolated and approximate the molecular weights of cytoplasmic, wall and growth medium molecules resulting from germination of pollen in culture medium supplemented with [^{14}C]- or [^{3}H]-Pro.

MATERIAL AND METHODS

Germination conditions

Lilium longiflorum, cv. 'Ace' pollen (freshly-harvested or stored at 4° C for 1-2 months) was germinated for various times at 25±2° C in Petri dishes containing sterile 10% sucrose and 10 ppm boric acid (Rosen et al., 1964) or Dickinson's (1965) media. To the media was added either [^{3}H]-Pro (specific activity, 403 mCi/mmol, New England Nuclear) or [^{14}C]-Pro (specific activity, 1.5 Ci/mmol or 205-214 mCi/mmol, New England Nuclear) or 2-[^{3}H]-myo-inositol (MI, specific activity 1.27 Ci/mmol, New England Nuclear). In some cases double labeling of germinating pollen with [^{3}H]-Pro and L-1-[^{14}C]-Ara (specific activity 10 mCi/mmol, New England Nuclear) was carried out. In other cases, pollen was incubated in culture medium containing both 100 μM 2,2'--dipyridyl and [^{14}C]-Pro. The procedures for preparing cytoplasm, salt-extracts and enzymic-digests of walls and culture medium for gel filtration on Sephadex are given in the figure legends.

Chromatographic separation of amino acids

For determination of the amino acid profile of walls, 0.5 g fresh weight lots of 4° C stored (14 months, 10% viability or 5 days, 50% viability) pollen were germinated in 10% sucrose and 10 ppm H_3BO_3 for 3-4 h. Pollen was harvested by gentle centrifugation and ruptured 3-5 min with glass beds in a water-cooled cell mill. The homogenate was centrifuged 15 min at 15,000 × g. The 15,000 × g plus 800 × g pellets constituted the wall fraction which was washed with 3-5 ml aliquots of 0.1, 0.5 and 1.0 M NaCl, twice with each salt concentration. Salt-treated walls were washed twice with H_2O and then hydrolyzed 18 h in 6N HCl at 105° C. The hydrolyzate was filtered, evaporated to dryness, the residue taken up in H_2O and layered onto a column of Dowex AG 50W-X4, 200—400 mesh, H^+-form. The column was eluted with 2N NH_4OH and the eluate evaporated to dryness. The residue was reconstituted in H_2O and following spotting on Whatman No. 3 MM paper, amino acids were separated in one direction by paper electrophoresis for 80 min in a pH 1.9 buffer consisting of 2.5% v/v acetic acid, 8.7% v/v formic acid and H_2O. Following drying, the paper was rotated 90° and subjected to paper chromatography for 18 h in 1 : 1 tertiary amyl alcohol:buffer. The buffer consisted of 50% v/v pyridine and 0.6% n ethyl morpholine adjusted to pH 8.2 with acetic acid. Amino acids were detected, eluted and quantitated according to Heilman et al. (1957).

RESULTS

Gel filtration of cytoplasm

Filtration of cytoplasm from pollen germinated in medium containing [^{14}C]-Pro on Sephadex G-25 (exclusion > 5,000 molecular weight) yielded 2 labelled peaks, the first of which eluted in the void volume (Fig. 1). The void volume also contained [^{3}H] when germinating pollen was labelled with 2-[^{3}H]-myo-inositol and its cytoplasm gel filtrated on G-25. Germination of pollen in medium containing either [^{14}C]-Pro or 2-[^{3}H]-myo-inositol with 100 μM 2,2′--dipyridyl followed by gel filtration of a cytoplasmic fraction on G-25 resulted in elimination of the void volume peak (Fig. 2) with a concomitant shift of radioactivity to lower molecular weight constituents. When pollen was germinated in medium containing both [^{3}H]-Pro and [^{14}C]-Ara, a coincidence of labelling between [^{3}H] and [^{14}C] was observed for both void volume and retarded peaks. Both types of peaks contained colorimetrically detectable Hyp.

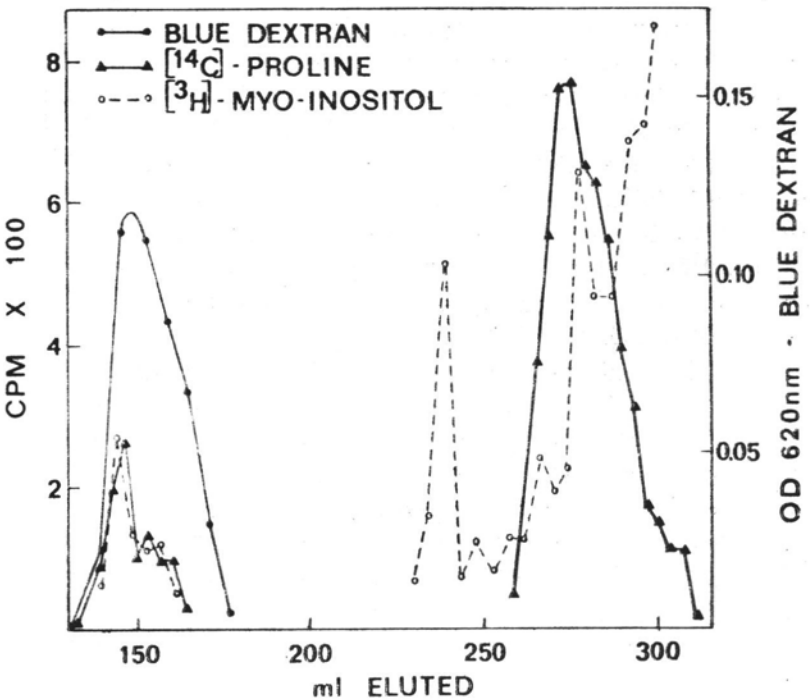

Fig. 1. Sephadex G-25 elution profile of cytoplasmic fractions obtained from pollen germinated in either labelled Pro or MI

Twenty mg fresh weight pollen germinated 18 h in 10% sucrose, 10 ppm boric acid medium containing 10 μCi [^{14}C]-Pro (specific activity, 205 mCi/mmol, New England Nuclear) or 20 mg fresh weight pollen germinated 18 h in the same medium but containing 10 μCi [^{3}H]-MI (specific activity 1.27 Ci/mmol, New England Nuclear); cytoplasmic fraction obtained through centrifugation (500 × g, 5-10 min) of pollen ruptured by grainding with a mortar and pestle; 500 × g supernatant centrifuged at 15,000 × g supernatant lyopholized and residue re-constituted in 6ml 0.1N acetic; dissolved lyopholizate centrifuged at 15,000 × g and supernant concentrated to 1 ml rotary flash evaporation; concentrate layered onto a Sephadex G-25 column eluted with 0.1N acetic acid.

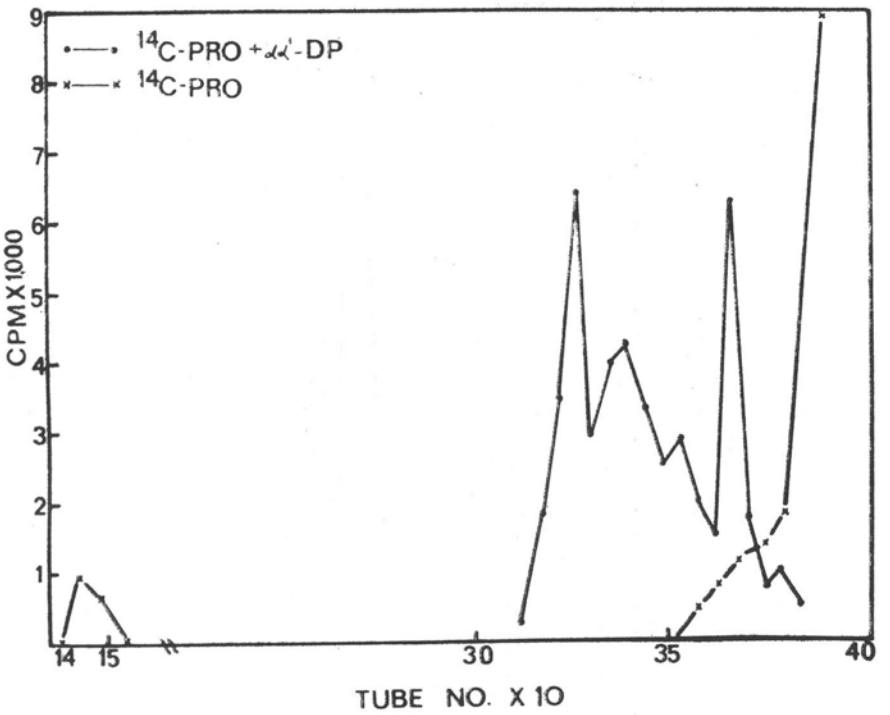

Fig. 2. Elution profile from Sephadex G-25 of a cytoplasmic fraction from pollen incubated in medium containing both [^{14}C]-Pro and 2,2′-dipyridyl

Twenty mg fresh weight of freshly-harvested pollen incubated 18 h at 25 ± 2°C in Dickinson's (1965) medium without phosphate and tetracycyline but containing 10 μCi [^{14}C]-Pro (specific activity, 1.5 Ci/mmol, New England Nuclear) and 100 μM 2,2′-dipyridyl; harvested pollen was ruptured by grinding with a mortar and pestle; grindate centrifuged for 15 min; 500 × g supernatant lyopholized; residue taken up in H_2O and gel filtrated on a G-25 column (void volume 144 ml); column eluted with 0.1N acetic acid; control — same treatment except minus 2,2′-dipyridyl.

Gel filtration of a salt-extract of a wall fraction

Filtration on Sephadex G-25 of a TCA-soluble fraction of a salt--extract of walls obtained from germinating pollen labelled with [^{14}C]--Pro resulted in 3 radioactive peaks (Fig. 3). The first peak eluted in the void volume.

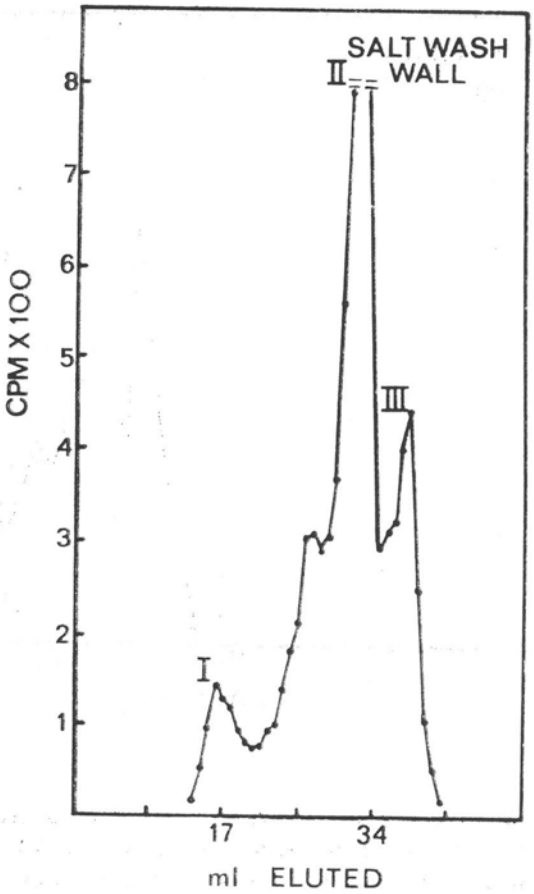

Fig. 3. Sephadex G-25 elution profile of a soluble fraction of a salt-extract of wall derived from germinated pollen

Ten pollen lots of 20 mg fresh weight each germinated in Petri dishes containing 10 ml aliquots Dickinson's (1965) medium without KH_2PO_4 and tetracycline but with 0.2 µCi [^{14}C]-Pro (specific activity, 214 mCi/mmol) 6 h at 25 ± 2°C; at 6 h tube elongation terminated by transferring Petri dishes to 4°C; germinated pollen harvested by gentle vacuum filtration onto Whatman No. 1 filter paper; harvested pollen washed 44 ml Dickinson's (1965) medium lacking [^{14}C]-Pro; washed pollen transferred to 10 ml H_2O and sonicated with a Biosonik III (Bronwill Scientific) sonicator 5 min at setting 80 with a flat probe; sonicated pollen centrifuged 3-5 min at 500 × g to pellet walls; walls washed 10 times with 1M NaCl and salt-washes lyopholized; residue extracted 30 min with 5% TCA and centrifuged 15 min at 30,000 × g supernatant lyopholized; residue suspended in 0.1N acetic acid and gel filtrated on a Sephadex G-25 column having a void volume betwen 15 and 25 ml; column eluted with 0.1N acetic acid; procedures modified after those of Brysk and Chrispels (1972).

Gel filtration of enzymic-digests of a salt-washed wall fraction

Filtration on Sephadex G-25 of a cellulase-digest of walls from germinating pollen labelled with]^{3}H[-Pro yielded 3 labelled peaks (Fig. 4) was previously reported (Dashek et al., 1971; Dashek, Harwood, 1974). Peak I, which eluted in the void volume, contained Hyp. Peak III was retarded and contained labelled free Hyp. Peak II was also retarded but in contrast to Peaks I and III lacked labelled Hyp. In addition, Peak II did not consistently occur. Paper electrophoresis followed by paper chromatography of 6N HCl hydrolyzates of Peak I revealed Lys, Gly, Ala, Ser, Glu, Val, Tyr, either Leu or Ileu and Pro in addition to Hyp. In contrast, Peak III contained the same amino acids as Peak II plus Val and Pro. Filtration on Sephadex G-25, G-50 or G-75 of enzymic-digests of walls of [^{3}H]-Pro and [^{14}C]-Ara labelled pollen, produced a coincidence of [^{3}H] and [^{14}C] labelling. A single, symmetrical peak

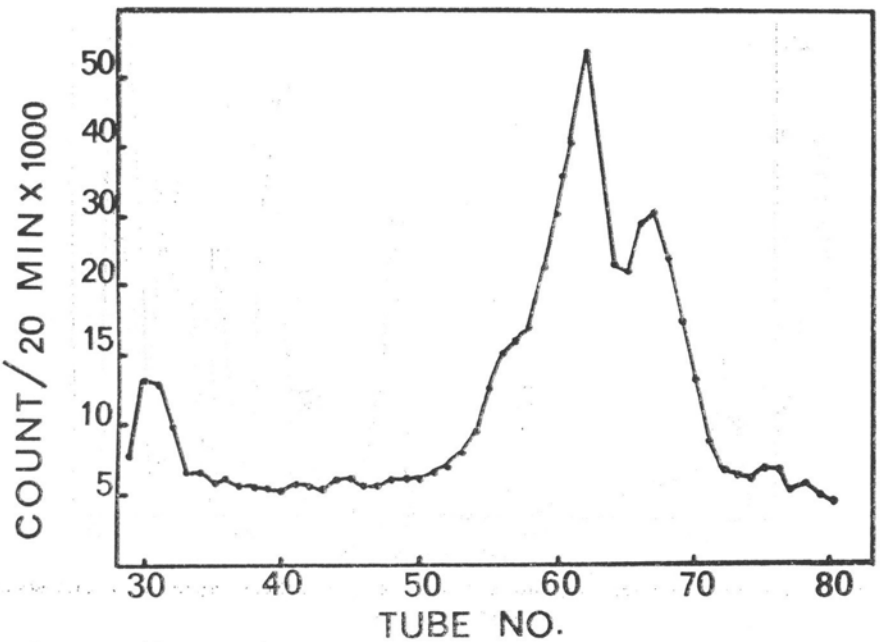

Fig. 4. Sephadex G-25 elution profile a cellulase-digest of a salt-washed wall fraction

One half-gram fresh weight pollen germinated 6 h in 100 μCi uniformly labelled [^{3}H]-Pro (specific activity, 403 mCi/mmol); pollen ruptured as in Fig. 1; homogenate centrifuged at 500 × g 5-10 min; 500 × g sediment washed 5-10 times with 1M NaCl and then 3-5 times with H_2O; walls refluxed 18 h and treated with cellulase (10 mg/g dry weight wall) at 37°C pH 4.5; digest contrifuged 10,000 × g 20 min; supernatant evaporated to 1 ml and gel filtrated on Sephadex G-25 (column void volume ~ 30 ml); a modification of this figure appears in Dashek and Harwood (1974).

exhibiting [^{3}H] and [^{14}C] labelling coincidence was noted for both G-25 and G-50. In contrast, gel filtration on G-75 (exclusion > 70,000 molecular weight) yielded an asymmetrically-shaped peak eluting partially in the void volume. This asymmetry suggested the presence of more than one labelled void volume macromolecule. This suggestion was confirmed by gel filtration on G-100 (exclusion > 150,000 molecular weight) of cellulase-digests of walls from [^{3}H]-Pro and [^{14}C]-Ara labelled germinating pollen. The G-100 elution profiles revealed a void volume (I) and a variety of retarded peaks (III, V, VI and VIII) showing a coincidence of [^{3}H] and [^{14}C] labelling (Fig. 5). Acid hydorolysis followed by colori-

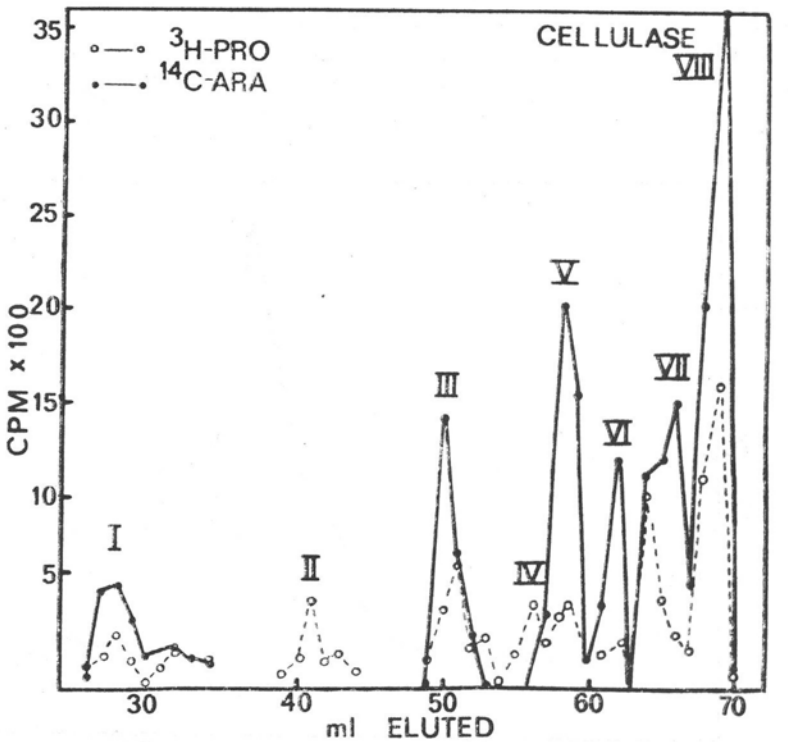

Fig. 5. Elution profile from Sephadex G-100 of a cellulase-digest of a salt-washed wall fraction

One half-gram fresh weight stored pollen germinated 6 h in 100 µCi uniformly labelled [^{3}H]-Pro (specific activity, 403 mCi/mmol); and 10 µCi L-1-[^{14}C]-Ara (specific activity, 10 mCi/mmol); wall fraction washed 5-10 times with 1M NaCl and then 3-5 times with H_2O; refluxed (18 h) wall treated with cellulase 10 mg/mg dry weight wall) at 37°C, pH 4.5; digest centrifuged (10,000 × g, 20 min); 10,000 × g supernatant concentrated to 1 ml and gel filtrated on a Sephadex G-75 column with a void volume of ~ 28 ml; the resultant asymmetrically shaped void volume peak exhibiting a coincidence of labelling from [^{3}H]-Pro and [^{14}C]-Ara layered onto a G-100 column with a void volume of ~ 28 ml; procedures adapted with modification from Lamport (1969); a modification of this figure appears in Dashek and Harwood (1974).

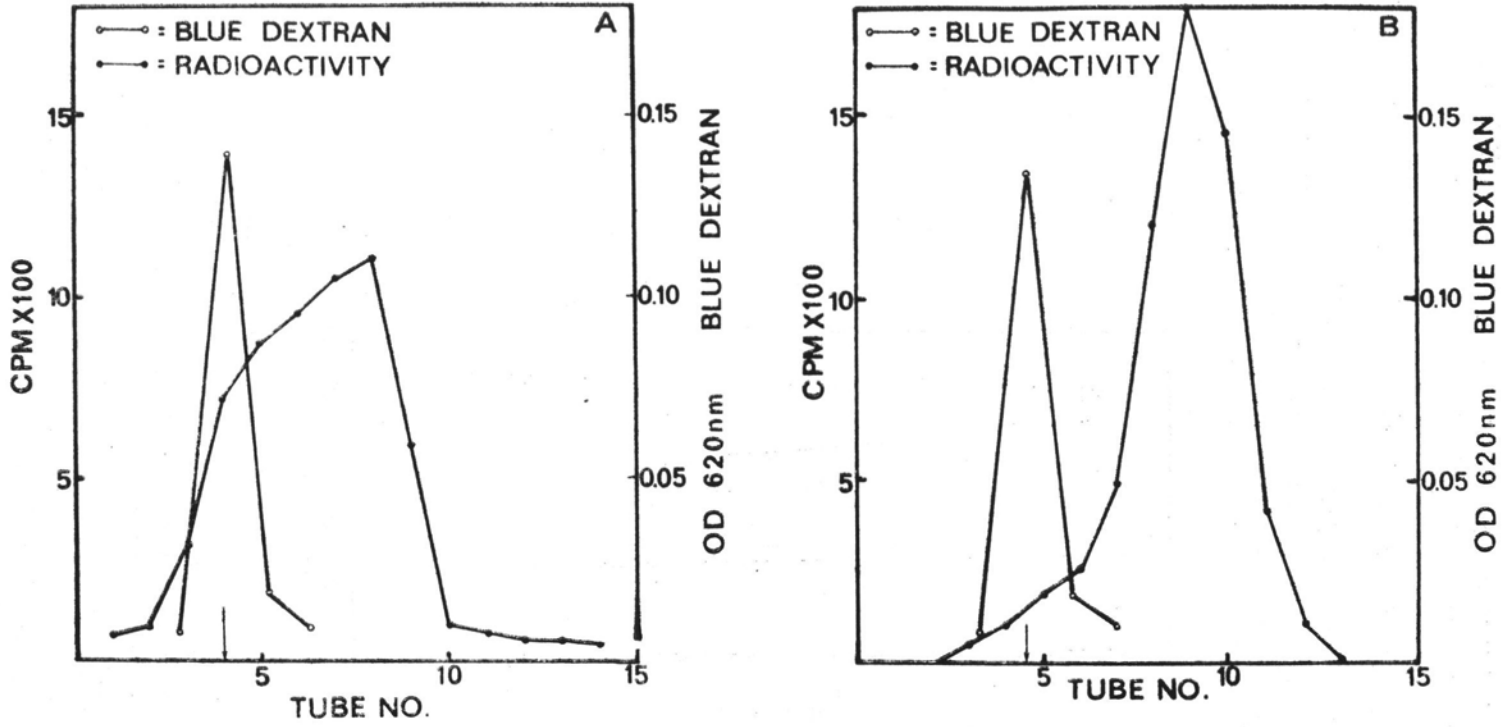

Fig. 6a, b. Gel filtration on G-50 of rhozyme P11 (A) and pepsin (B) digests of salt-washed wall fractions obtained from pollen germinated 18 h in medium containing [^{14}C]-Pro

Twenty mg freshly-harvested *L. longiflorum*, cv. 'Ace' pollen germinated 18 h at 25 ± 2°C in 5 ml Dickinson's (1965) medium minus KH_2PO_4 and tetracycline but containing 750,000 cpm [^{14}C]-Pro (specific activity, 1,5 Ci/mmol, New England Nuclear); harvested pollen ruptured by grinding with a mortar and pestle in H_2O; walls obtained by centrifugation at 500 × g 3-5 min and the pellet washed 5-10 times with 1M NaCl and 5 times with H_2O; washed walls refluxed in H_2O 18 h and then treated 18 h with either pepsin (3 mg/ml of refluxed wall suspension) at pH 1.9 and 37°C or rhozyme P11 (Rohm and Hass, 25 mg/ml of refluxed wall suspension) at pH 6.0 and 25°C; aliquots of digests layered onto a G-50 column (void volume 42 ml) and eluted with 0.1N acetic acid; free Pro eluted at tube no. 8.

metry revealed the presence of Hyp in Peaks I, III, V and VIII with Peaks I and III being richest in Hyp.

Gel filtration of protease-digests of salt-washed wall fractions

The G-50 elution profiles for pepsin and rhozyme P11-diagests of wall fractions from pollen germinated in medium containing [^{14}C]-Pro are shown in Figs 6a, b. A broad peak of labelled constituents was released by rhozyme P11. Ten % of the peak eluted in the void volume

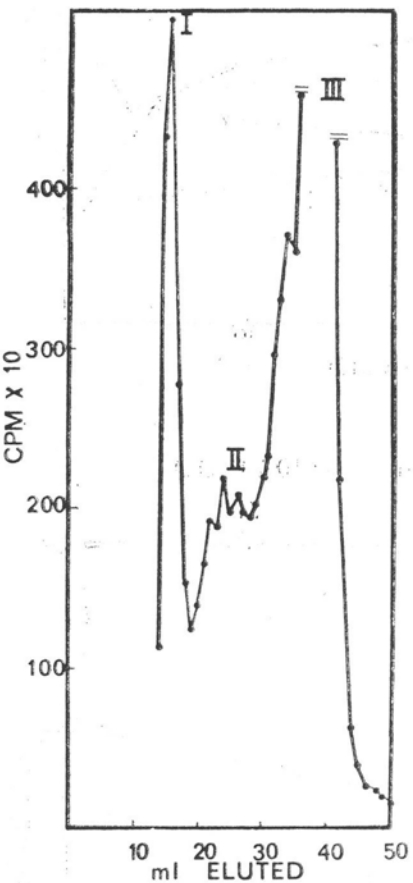

Fig. 7. Elution profile from Sephadex G-100 of pollen culture medium

Ten pollen lots of 20 mg fresh weight germinated in 10 ml aliquots Dickinson's (1965) medium without KH_2PO_4 and tetracycline containing 0.2 µCi [^{14}C]-Pro (specific activity, 214 mCi/mmol) 6 h at 25 ±2° C; pollen separated from growth medium by gentle vacuum filtration onto Whatman No. 1 filter paper; collected medium concentrated to 1-2 ml by lyopholization; concentrate gel filtrated on Sephadex G-25 (column void volume between 20 and 30 ml); fractions composing the void volume peak from G-25 were pooled and gel filtrated on Sephadex G-100 (column void volume between 10 and 20 ml).

(arrow Fig. 6a). Whereas the majority of the labelled substances released by pepsin was retarded, a radioactive shoulder eluted in the void volume.

Gel filtration of culture medium

Filtration on Sephadex G-25 of culture medium in which pollen was germinated yielded two peaks. Whereas Peak I eluted in the void volume and contained colorimetrically-detectable Hyp, Peak II was retarded, lacked Hyp and probably represented small peptides or free amino acids. Subsequent filtration of culture medium, G-25 Peak I on Sephadex G--100 resulted in 3 peaks, one of which eluted in the void volume (Fig. 7).

Amino acid profile of walls

The amino acid contents of salt-washed wall fractions from germinated pollen possessing 10 and 50% viabilities are presented in Table 1. With the exception of Phe, Thr and Lys, the amino acid contents of the wall appear to be similar for pollen with either viability.

Table 1

Amino acid profile of salt-washed wall fraction obtained from germinated pollen

Amino cid	10% Viability	50% Viability
	% μmol*	
Leu	9.80	9.14
Ile	4.20	4.10
Glu	11.55	10.68
Ala	9.97	9.31
Phe	4.03	2.76
Tyr	2.63	2.68
Thr	4.90	9.78
Lys	7.43	3.82
Arg	12.43	14.76
Val	5.60	4.28
Gly	10.15	7.65
Asp	10.50	12.96
Ser	6.83	8.07
Met	0.00	0.00
His	0.00	0.00

* Data were calculated as μmol for each amino acid/50 mg dry weight wall;

$$\% \ \mu\text{mol equals:} \frac{\mu\text{mol of each amino acid}}{\mu\text{mol of all amino acids}} \times 100.$$

DISCUSSION

Cytoplasm

The Sephadex G-25 void volume peak most likely consists of a variety of molecules which were labelled during germination in medium containing [^{14}C]-Pro. Some of these proteins may be retained intracellularly and their purification and characterization are in progress.

Previous colorimetric assay of Pro and Hyp and labelling with [^{14}C]--Pro followed by time-dependent changes in radioactive Pro and Hyp indicated that TCA-precipitable, cytoplasmic Hyp turned-over. Thus, it may be a precursor to wall-bound "extensin". Perhaps one of the labelled constituents of the G-25 void volume peak is the precursor. This is supported by: 1) the coincidence of labelling in a G-25 void volume peak between [^{3}H] and [^{14}C] derived from [^{3}H]-Pro and [^{14}C]-Ara; 2) the occurrence of radioactivity from 2-[^{3}H]-myoinositol, which can be converted to [^{3}H]-Ara (Chen, Loewus, 1977), in a G-25 void volume peak and 3) the elimination of the latter peak when pollen was incubated during germination with 2,2′-dipyridyl, a chelating agent which blocks Pro hydroxylation (Holleman, 1967).

Brysk and Chrispeels (1972) isolated and partially characterized a cytoplasmic precursor of "extensin" from carrot disks. The precursor was excluded by G-200 and contained Ser, Hyp, Lys, Gly and Ara. Thus, gel filtration of a Sephadex G-25 void volume peak on G-200 followed by establishment of the presence of Hyp in a resulting void volume peak would be required to provide minimal evidence that at least one of the labelled constituents in the void volume is an "extensin" precursor. Purification of a possible G-200 Hyp-containing void volume peak by standard protein purification procedures would be necessary to provide additional evidence for a cytoplasmic "extensin" precursor.

Cell wall

The G-25, 50, 75 and 100 elution profiles of cellulase-digests of tube plus grain wall fractions from pollen double labelled with [^{3}H]-Pro and [^{14}C]-Ara indicated that the tube and/or grain wall contains "extensin" or "extensin"-like molecules as previously suggested by Dashek and Harwood (1974). The observation that a G-100 void volume peak contained both Hyp and Ara suggests that pollen wall fractions may be a source of large fragments of "extensin". This attribute is coupled with a drawbeck. Wall fractions from germinated pollen possess low levels of Hyp (0.08% on a dry weight basis) compared with the 1-2% found in sycamore-maple and tomato-cell-suspension cultures (Lamport,

1969). Thus, batch cultures of pollen will be required for purification and characterization of presumed "extensin".

In addition to the G-25 through G-100 elution profiles of cellulase-digests of wall fractions, further supporting evidences for the occurrence of "extensin" in salt-washed and refluxed wall fractions include: 1) the presence of Ser, Tyr and Lys in G-25 Peak I (Fig. 4); 2) the elimination of G-25 Peak I upon gel filtration of a cytoplasmic fraction from pollen incubated in medium containing 2,2′-dipyridyl, and inhibitor of Pro hydroxylation (Holleman, 1967); 3) the release of proteases of a substance(s) which labels with [^{14}C]-Pro and which possesses a minimum molecular weight of 30,000 (Figs 6a, b and 4) the presence of amino acids in salt-washed wall fractions derived from germinated pollen.

Culture medium

The occurrence in the culture medium of a labelled Hyp-containing component which eluted in void volumes of both G-25 and G-100 columns suggests the presence of a Hyp-containing glycoprotein. While isolation, purification and characterization are required to confirm the occurrence of the glycoprotein in the medium, its presence is supported by the data of Olson et al. (1969) and Hori and Sato (1977) as well as Brysk and Chrispeels (1972), Burke et al. (1974), Moore (1973) and Pope (1977) who found Hyp-containing proteins (glycoproteins) in the culture medium of tobacco, carrot and cell suspension cultures of sugar-cane, rice, oat, soybean and sycamore-maple (Table 2). The origin of these medium Hyp-containing proteins is a matter of some controversy. It has been suggested that these proteins originate from the cell wall (Brysk, Chrispeels, 1972). Indeed, Moore (1973) demonstrated the presence of a Hyp-containing, extracellular mat of wall-derived material which floated on the surface of soybean suspension culture medium. However, Pope (1977) established that the glycosylation pattern of the Hyp-containing material in the culture medium differed from "extensin" indicating that the cell wall at least in sycamore-maple cell-suspension cultures is not the source of medium Hyp-containing proteins. No data are available from the present study to exclude this possibility for germinating lily pollen.

Another source of a Hyp-containing macromolecule in the medium in which lily pollen was germinated could be secretion and/or leakage from the cytoplasm. This would require passage of an intact protein across both the plasmalemma and cell wall. While mechanisms exist which allow movement of intact proteins across the plasmalemma, e.g., paramural bodies (Dashek et al., 1971; Marchant, Robards, 1968),

Table 2

Summary of plant systems for which the occurrence of hydroxyproline-containing macromolecules in culture media have been reported

System	Investigator(s), year	Nature of macromolecule
Nicotiana tabacum, var. 'Xanthi'	Olson et al., 1969	Dialyzed material
Glycine max L. Merrill, var. 'Acme' suspension-cultured cells	Moore, 1973	Cell-free insoluble cell wall fraction containing 36% protein by weight
Sugarcane, rice and oat suspension-cultured cells	Burke et al., 1974	Ethanol insoluble
Acer pseudoplatanus suspension-cultured cells	Pope ,1977	Ethanol insoluble
Nicotiana tabacum suspension-cultured cells	Hori, Sato, 1977 Hori, 1978	Glycoprotein containing galactosamine
Lilium longiflorum, cv. 'Ace' pollen (germinated)	Dashek, Mills, present work	Unknown

the cell wall constitutes a barrier. Electron micrographs do not reveal the presence of plasmodesmata in *L. longiflorum* pollen tube walls (Rosen et al, 1964; Dashek Rosen, 1966; Rosen, Gawlik, 1966). However, elongation of lily pollen tubes occurs within the tip-most 3—5 μm (Rosen et al., 1964) and the wall in this region of the tube consists of compartments separated by membranes. Perhaps, flow of proteins between adjoining membranes of two adjacent compartments is possible. In addition, the wall at the growing tip differs from that of the non-growing region in that it lacks highly organized cellulose microfibrils (Dashek, 1966) and thus may be more plastic, possibly permitting movement of macromolecules to the extracellular milieu. In this connection, Dickinson (1965) reported that a β-fructofuranosidase can be found in the culture medium as lily pollen germination proceeds

Acknowledgments

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REFERENCES

Brysk M. M., Chrispeels M. J., 1972. Isolation and partial characterization of a hydroxyproline-rich cell wall glycoprotein and its cytoplasmic precursor. Biochim. Biophys. Acta 257: 421-432.

Burke D., Kaufman P., McNeil M., Albersheim P., 1974. The structure of plant cell walls. VI. A survey of the walls of suspension-cultured monocots. Plant Physiol. 54: 109-115.

Chen M., Loewus F., 1977. Myo-inositol metabolism in *Lilium longiflorum* pollen. Uptake and incorporation of myo-inositol-2-^{3}H. Plant Physiol. 59: 653-657.

Dashek W. V., 1966. The lily pollen tube: Aspects of chemistry and nutrition in relation to fine structure. Ph. D. Dissertation, Marquette University, Milwaukee, WI.

Dashek W. V., Harwood H. I., Rosen W. G., 1971. The significance of a wall-bound, hydroxyproline-containing glycopeptide in lily pollen tube elongation. In: J. Heslop-Harrison (Ed.), Pollen Development and Physiology, Butterworths, London, pp. 194-200.

Dashek W. V., Harwood H. I., 1974. Proline, hydroxyproline, and lily pollen tube elongation. Ann. Bot. 38: 947-959.

Dashek W. V., Mills R. R., 1980. Azetidine-2-carboxylic acid and lily pollen tube elongation. Ann. Bot. 45: 1-12.

Dashek W. V., Rosen W. G., 1966. Electron microscopical localization of chemical components in the growth zone of lily pollen tubes. Protoplasma 61: 192-204.

Dickinson D. B., 1965. Germination of lily pollen: Respiration and tube growth. Science 150: 1818-1819.

Harwood H. I., 1970. The significance of wall-bound, hydroxyproline-containing glycopeptides in plant cell elongation. Ph. D. Dissertation, Boston University, Boston, MA.

Heilman J., Barrollier J., Watzke E., 1957. Beitrag zur Aminosaurebestimmung auf Papierchromatogrammen. Physiol. Chem. 309: 219-220.

Holleman J., 1967. Direct incorporation of hydroxyproline into protein of sycamore cells incubated at growth-inhibitory levels of hydroxyproline. Proc. Natl. Acad. Sci. U. S. 57: 50-54.

Hori H., Sato S., 1977. Extracellular hydroxyproline-rich glycoprotein of suspension-cultured tobacco cells. Phytochem. 16: 1485-1487.

Hori H., 1978. The demonstration of galactosamine in extracellular hydroxyproline-rich macromolecule purified from the culture media of tobacco cells. Plant Cell Physiol. 19: 501-505.

Lamport D. T. A., 1969. The isolation and partial characterization of hydroxyproline-rich glycopeptides obtained by enzymic degradation of primary cell walls. Biochemistry 8: 1155-1163.

Marchant R., Robards A. W., 1968. Membrane systems associated with the plasmalemma of plant cells. Ann. Bot. 32: 457-471.

Moore T. S., Jr., 1973. An extracellular macromolecular complex from the surface of soybean suspension cultures. Plant Physiol. 51: 529-536.

Olson A. C., Evans J. J., Frederick D. P., Jansen E. F., 1969. Plant suspension culture media macromolecules-pectic substances, protein and peroxidase. Plant Physiol. 44: 1594-1600.

Pope D., 1977. Relationships between hydroxyproline-containing proteins secreted into the cell wall and medium by suspension-cultured *Acer pseudoplatanus* cells. Plant Physiol. 59: 849-900.

Rosen W. G., Gawlik S. R., Dashek W. V., Siegesmund K. A., 1964. Fine structure and cytochemistry of *Lilium* pollen tubes. Amer. J. Bot. 51: 61-71.

Rosen W. G., Gawlik S. R., 1966. Fine structure of lily pollen tube tips following various fixation and staining procedures. Protoplasma 61: 181-191.