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FUNCTION OF GOLGI VESICLES IN RELATION TO CELL WALL SYNTHESIS IN GERMINATING PETUNIA POLLEN. II. CHEMICAL COMPOSITION OF GOLGI VESICLES AND POLLEN TUBE WALL

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SUMMARY

Golgi vesicles and pollen tube walls isolated from germinating *Petunia* pollen were chemically analysed. The protein: lipid ratio of the Golgi vesicles was 70:30. The total carbohydrate contents of the Golgi vesicles and pollen tube wall were found to be 7.2% and 61% of the dry weights respectively. The alkali insoluble material of the pollen tube wall contained 6% of the total carbohydrate; this material has been found to be cellulosic in nature. The sugar composition of the polysaccharides extracted from the Golgi vesicles and the tube wall is presented and discussed.

1. INTRODUCTION

It is possible to distuinguish three main groups of polysaccharides by means of acidic and alkaline extractions of primary cell walls viz. cellulose, hemicellulose, and pectin. Since it is questionable whether always a complete separation of these polysaccharides is obtained by these procedures (ALBERSHEIM et al. 1967, BLAKE & RICHARDS 1971, HERTH et al. 1972, NANCE 1973, VAN DER WOUDE et al. 1971), quantitative results are to be considered only as rough estimates.

In *Lilium longiflorum* Thunb. the hot water extracts of pollen tube walls and Golgi vesicles isolated from the pollen tubes have a similar carbohydrate composition (VAN DER WOUDE et al. 1971). Polysaccharides are found in the Golgi vesicles of *Lilium* pollen tubes (DASHEK & ROSEN 1966, VAN DER WOUDE et al. 1971) and of wheat seedlings (*Triticum vulgare*) (PICKETT-HEAPS 1968). The transport of polysaccharides by means of Golgi vesicles to the plasma membrane and the release of the vesicle contents outside the cytoplasm has been observed by NORTHCOTE & PICKETT-HEAPS (1966) and VAN DER WOUDE & MORRÉ (1968).

Cellulose has not been found as a component of the Golgi vesicles in plant materials, except in the alga *Pleurochrysis scherffelii* (BROWN & FRANKE 1971). The cell wall of this alga is composed of scales synthesised in the Golgi vesicles (HERTH et al. 1972, BROWN & FRANKE 1971). These results indicate an involvement of Golgi vesicles in cell wall synthesis at least in algae.

In a preceding paper (ENGELS 1973) a method was described for isolating Golgi vesicles in large quantities. In this article the carbohydrate composition of Golgi vesicles and the pollen tube wall is given. The results are discussed in

connection with the question whether the polysaccharide composition of Golgi vesicles is similar to that of the tube wall and whether the Golgi vesicles contain the machinery for the synthesis of the pollen tube wall polysaccharides.

2. MATERIALS AND METHODS

2.1. Isolation of Golgi vesicles

The isolation of Golgi vesicles (GV) has been described previously by ENGELS (1973). The GV were stored at −20 °C prior to analysis.

2.2. Isolation of the pollen tube wall

Pollen from *Petunia hybrida*, strain W 166K, were grown for 15 hr under conditions as described previously (ENGELS 1973). Germinated pollen were centrifuged and washed. Pollen grains with large tubes were separated from those with small tubes and ungerminated ones by the use of a fine screen sieve. The water adhering to the material was removed with filter paper and the germinated pollen were placed in a mortar. Liquid nitrogen was added and pollen tubes were broken at several places by light grinding while the pollen grains remained intact. After thawing the homogenate was placed on a 20 μm sieve and small tube pieces were washed through the sieve with water. The adhering cytoplasm was removed by a short ultrasonic treatment and subsequent washing. The pollen tube walls (PTW) prepared in this manner were stored at −20 °C prior to analysis.

2.3. Extraction of GV and PTW

GV and PTW were pre-extracted with a series of 80, 90, and 100% ethanol (ETOH) solutions, respectively. The extracts were combined and evaporated to dryness. The residues after ETOH extraction were extracted 1) twice with 100% diethylether, 2) 1 hr with boiling water, 3) 1 hr with 1N HCl at 100 °C, 4) 15 hr with 2N KOH at room temperature. The supernatants of 1) to 3) were evaporated directly to dryness. The supernatant of 4) was first passed through a Dowex-50 (H^+) column and then evaporated to dryness. The residues of the GV and PTW after KOH extraction were dried. All fractions obtained by this procedure were hydrolysed in 80% (V/V) trifluoro-acetic acid for 48 hr at 120 °C. The hydrolysates were evaporated and the acid was removed by repeated addition of water and evaporation.

2.4. Separation of neutral and acidic sugars

The neutral and acidic sugars present in the various extracts were separated and chromatographed according to the methods used by KROH (1973). The ETOH extracts of the GV and the PTW were either directly chromatographed or hydrolysed prior to chromatography.

2.5. Measurements

The protein content of the ETOH soluble and ETOH insoluble fractions of the

GV and PTW were measured according to LOWRY et al. (1951). The ETOH extract of the GV was therefore further separated by the chloroform-methanol-water method of BLIGH & DYER (1959).

Lipid estimations were done gravimetrically as well as colorimetrically according to RENKONEN (1961).

Carbohydrate estimations on ETOH soluble and insoluble fractions of the GV and PTW and on the alkali insoluble fraction of the PTW were carried out colorimetrically by means of the phenol-sulfuric acid method (DUBOIS et al. 1956).

3. RESULTS

3.1. Composition of the GV and PTW

The components of the GV and PTW are divided into ETOH soluble and insoluble fractions. The ETOH soluble fraction of the GV is divided into protein, lipid, and carbohydrate fractions *(table 1)*.

Table 1. The amount of carbohydrate, protein, and lipid in ETOH soluble and ETOH insoluble fractions as percentage of the original dry weights.

	ETOH soluble		ETOH insoluble	
	GV	PTW	GV	PTW
Carbohydrate	4.0	3.5	3.2	57.5
Protein	4.4	0.0	40.0	13.3
Lipid	5.0	0.0	13.5	0.0
Unrecovered	28.6	0.0	1.3	25.7
Total	42.0	3.5	58.0	96.5

The ETOH extraction removes 42.0% of the total dry weight of the purified GV. In addition to carbohydrates (4.0%) distinct amounts of proteins (4.4%) and lipids (5.0%) are present. In contrast to GV only 3.5% of the tube wall material is soluble in ETOH. This 3.5% represents exclusively carbohydrates. The ETOH insoluble material of the GV fraction contains mostly protein (40.0%) and lipid (13.5%) and only a small amount of carbohydrate (3.2%), while in the fraction of the PTW carbohydrates (57.7%) and protein (13.3%) are the main constituents. Lipid could not be found in the ETOH insoluble fraction of PTW.

3.2. Neutral sugars

Separation by thin-layer chromatography (TLC) of the neutral sugars from the hydrolysates of the various extracts gives the monosaccharides commonly found in plant cell walls *(table 2)*.

The ETOH fraction of the GV contains xylose but lacks arabinose whereas the reverse is true for the PTW. Quantitative differences are found in mannose and glucose. Both ETOH fractions reveal a reducing carbohydrate (X) on

Tabel 2. Monosaccharides detected in the different fractions after TFA hydrolysis. The presence or absence of each monosaccharide is indicated by the symbols + and −, respectively, as revealed by comparison with reference sugars. The symbol ⊗ is used when a sugar could be barely detected, the symbol ++ when a sugar gives a very intensive spot.

	Fractions									
	ETOH		H_2O		HCl		KOH		TFA	
Sugars	GV	PTW	GV	PTW	GV	PTW	GV	PTW	GV	PTW
rha	+	++	+	+	++	+	++	++	−	−
fuc	+	+	+	+	+	+	+	−	−	−
xyl	+	−	+	−	⊗	⊗	+	−	−	−
ara	−	+	−	−	+	−	+	−	−	−
man	++	+	++	+	++	+	+	+	−	−
glu	+	++	+	++	+	++	+	+	+	+
gal	−	−	⊗	+	⊗	−	⊗	+	−	−
X	+	+	−	−	−	−	−	−	−	−
glu UA	−	−	+	⊗	+	+	−	−	−	−
XX UA	+	−	−	−	−	−	−	−	−	−
gal UA	−	−	+	⊗	+	+	−	−	−	−

chromatographs located between the origin and galactose both before and after TFA hydrolysis. The nature of this compound remains unknown. The only difference between the unhydrolysed and hydrolysed ETOH fraction is noted at the origin which is cleaned up after hydrolysis.

The composition of the hot water soluble fraction is the same in GV and PTW, with one exception. Xylose is present only in the GV fraction. On chromatographs of the GV fraction mannose stains more intensily, i.e. is predominant over glucose. In the PTW fraction, however, glucose is predominant over mannose. Since the staining is less sensitive for mannose than for glucose, this indicates a large difference in the concentration of the two sugars. The HCl extract also shows a large difference between GV and PTW with respect to the relative concentrations of mannose and glucose. The KOH extract of the PTW lacks the sugars fucose, xylose, and arabinose that are present in the KOH extract of the GV. After TFA hydrolysis of the KOH residue of the GV and the PTW fraction a clear spot appears which corresponds to glucose.

The sugar compositions of the ETOH, H_2O, HCl, and KOH extracts of the GV differ only slightly; galactose appears as an additional sugar in the H_2O-extract and this extract lacks the unknown sugar (X). The HCl-extract reveals the presence of arabinose and galactose which are absent from the ETOH-extracts. The only distinction between the HCl and H_2O extracts is the presence of arabinose in the HCl extract. The sugar composition of the KOH extract is nearly the same as that of the HCl extract.

The differences in sugar composition of the H_2O and HCl extract of the PTW fractions are small. The HCl extract reveals no galactose and the presence of xylose is doubtful. The KOH and HCl extractions differ in the presence or absence of fucose and galactose. The KOH extract contains galactose and lacks fucose while the reverse is found in the HCl extract.

3.3. Acidic sugars

The GV fraction contains an acidic carbohydrate with a mobility between galacturonic acid (gal UA) and glucuronic acid (glu UA). In the ETOH-extract of the GV and PTW no galacturonic acid and glucuronic acid could be detected. Glucuronic acid and galacturonic acid are found in the hot water and HCl fractions of both the GV and PTW. In the hot water fraction of the PTW the uronic acids are barely detectable. In addition to these uronic acids there are traces of acidic carbohydrates with higher mobilities than galacturonic acid and glucuronic acid.

3.4. Nature of the alkali insoluble material

Analysis of the alkali insoluble material of PTW preparations reveals that the carbohydrate content of this material accounts for 6% of the total dry weight. Table 2 shows that this carbohydrate material consists exclusively of glucose. Preliminary investigation by X-ray diffraction of this material from PTW reveals its cellulosic nature. The nature of the alkali insoluble material of the GV is under study.

4. DISCUSSION

The protein and lipid content of the GV in the ETOH soluble fraction is 4.4% and 5.0% of the dry weight, respectively. Small amounts of carbohydrates are found in the lipid as well as in the protein fraction. Is is not clear whether a part of the protein and lipid materials is possibly bound to the ETOH soluble carbohydrates. Some evidence has been presented indicating that such carbohydrate complexes with proteins and lipids may be precursors in polysaccharide synthesis (ANDERSON et al. 1965, DATTA et al. 1973, KNEE 1973). TALMADGE et al. (1973) found that 2% of the dry weight of sycamore primary cell walls was soluble in chloroform-methanol. This material was found to be hydroxyproline rich glycoprotein. In the ETOH soluble fraction of the *Petunia* pollen tube no protein has been detected.

The protein: lipid ratio in the GV of *Petunia* pollen tubes is 70:30. In HeLa cells the protein:lipid ratio in plasmamembranes is 60:40 and in Golgi cisternal membranes 33:67 (BOSMANN et al. 1968). Thus it appears that the protein content of the GV of *Petunia* pollen tubes is quite high. One may, therefore, assume that not all protein isolated from GV represents structural protein of the unit membrane of the GV but that part of it derives from enzymes, possibly from those involved in polysaccharide biosynthesis. Some of these enzymes have been detected in GV (FRIEND 1969, HARRIS & NORTHCOTE 1971, HEYN 1971, NORTHCOTE & PICKETT-HEAPS 1966, YOUNG 1973).

The total carbohydrate content of the PTW is 61% and the protein content 13.5% of the dry weight. These data agree very well with the values reported for the hyphal walls of *Ceratocystis* (HARRIS & FABER 1973) and the cell walls of sycamore cell suspension cultures (TALMADGE et al. 1973). The total carbohydrates of the PTW contain 6% alkali insoluble material with glucose as the only

monosaccharide after TFA hydrolysis. This alkali insoluble material proves to be cellulosic in nature as will be published in detail later. The amount of this cellulosic material in the PTW is in good agreement with the cellulose content (7%) in *Lilium* pollen tubes (VAN DER WOUDE et al. 1971).

Chromatographs of hot water fractions of the GV reveal the monosaccharides rhamnose, fucose, mannose, xylose, glucose, and galactose; the PTW gives the following monosaccharides: rhamnose, fucose, mannose, glucose, and galactose. With the exception of arabinose in the GV and xylose and arabinose in the PTW, these sugars are the same as those found in the GV and PTW from *Lilium* (VAN DER WOUDE et al. 1971).

The sugar composition of the ETOH and hot water extracts of the GV is nearly identical with the exception of galactose and sugar X *(table 2)*. In the ETOH fraction galactose is absent, sugar X is present, while the reverse is the case in the hot water extract.

The corresponding extracts of the PTW show the same behaviour with respect to these two sugars. Arabinose is found only in the ETOH extract of the PTW. Hot water extraction of the GV and the PTW, without ETOH prior to it, results in a sugar composition that is found when the separated extracts of ETOH and hot water are combined. The differences in sugar composition found in the subsequent extraction with ETOH and hot water stresses the importance of ETOH as starting extraction medium.

The ETOH extracts of the GV and the PTW contain probably different kinds of components. Some of these are monosaccharides visible on TLC plates prior to TFA hydrolysis. It is known that free monosaccharides may occur in plant material, although mostly glycosides are found (SCHAFFER 1972). After TFA hydrolysis a clean origin is obtained on TLC plates. However, no additional sugars are found. This could mean that ETOH has extracted besides monosaccharides oligosaccharides or carbohydrates attached to proteins and lipids. These last substances were also found in the ETOH extract and could be hydrolysed by TFA.

When the extracts of the GV are compared in the subsequent steps of extraction with respect to their sugar composition, it is observed that the ETOH and hot water fractions resemble one another. The same observation was made with respect to the HCl and KOH extracts. The differences in the monosaccharide composition of the various extracts of the PTW are more pronounced than those of the GV, especially with respect to the monosaccharides xylose, fucose, arabinose, and galactose.

The ETOH fraction of the GV reveals an acidic sugar with a mobility between galacturonic acid and glucuronic acid. Apart from these no other uronic acids are found in the ETOH fraction of both the GV and PTW. Glucuronic acid and galacturonic acid are found in the H_2O and HCl fractions of the GV and the PTW. The presence of galacturonic acid and glucuronic acid in pollen tube walls from *Lilium* was reported by LABARCA & LOEWUS (1972). However, VAN DER WOUDE et al. (1971) found only galacturonic acid in Lilium pollen tube walls. Galacturonic acid and glucuronic acid are commonly found in pectin and

hemicellulose respectively (ASPINALL 1970, BAUER et al. 1973, DANISHEFSKY et al. 1970, TALMADGE et al. 1973).

The monosaccharide composition of the polysaccharides extracted from the GV and the PTW are very similar. A number of data such as the enzymes found in Golgi vesicles (FRIEND 1969, HARRIS & NORTHCOTE 1971, HEYN 1971, NORTHCOTE & PICKETT-HEAPS 1966, YOUNG 1973), the presence of polysaccharides in Golgi vesicles (DASHEK & ROSEN 1966, VAN DER WOUDE et al. 1971), the increasing stainability of the Golgi vesicles on their way from Golgi cisternae up to the cell wall (VAN DER WOUDE et al. 1971), the resemblance in monosaccharide composition of the polysaccharides of the GV and the PTW, the fusion of Golgi vesicles with the plasmalemma (SIEVERS 1963, SASSEN 1964, CRANG & MILES 1969, ROSEN & GAWLIK 1965, VAN DER WOUDE & MORRÉ 1968, VAN DER WOUDE et al. 1971) strongly support the idea that Golgi vesicles are involved in the synthesis of the cell wall material. It can, however, at present not be excluded that besides Golgi vesicles also other organelles contribute to the cell wall synthesis. However, it must be mentioned that differences in monosaccharide composition of the GV and the PTW are observed. Pollen tube preparations contain a relatively low amount of pollen tube tips (light microscopic observation). Golgi vesicles fuse with the plasmalemma at the tip of the growing pollen tube. It could be that synthesis of cell wall material is continued after this fusion process and that sugars may be brought to the cell wall not only by Golgi vesicles but perhaps also by an other system. Perhaps the particles derived from the endoplasmatic reticulum, which were observed by VAN DER WOUDE et al. (1971) in *Lilium* represent such a system.

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REFERENCES

ALBERSHEIM, P., D. J. NEVINS, P. D. ENGLISH & A. KARR (1967): A method for analysis of sugars in plant cell wall polysaccharides by gas-liquid chromatography. *Carbohyd. Res.* **5**: 340–345.

ANDERSON, J. S., M. MATSUHASKI, M. A. HASHIN & J. L. STROWINGER (1965): Lipid-phosphoacetylmuranyl-pentapeptide and lipid-phosphotrisaccharide-pentapeptide: presumed membrane transport intermediates in cell wall synthesis. *Proc. Nat. Acad. Sci.* **53**: 881–889.

ASPINALL, G. O. (1970): Pectins, plant gums and other plant polysaccharides. In: W. PIGMAN & D. HORTON, eds., *The Carbohydrates*, Vol. **11B**. Academic Press, New York. pp. 515–536.

BAUER, W. D., K. W. TALMADGE, K. KEEGSTRA & P. ALBERSHEIM (1973): The structure of plant cell walls. II: The hemicellulose of the walls of suspension-cultured Sycamore cells. *Plant Physiol.* **51**: 174–187.

BLAKE, J. D. & G. N. RICHARDS (1971): An examination of some methods for fractionation of plant hemicelluloses. *Carbohyd. Res.* **17**: 253–268.

BLIGH, E. G. & W. J. DYER (1959): A rapid method of total lipid extraction and purification. *Can. J. Biochemical Physiol.* **37**: 911–917.

BOSMANN, H. B., A. NAGOPIAN & E. H. EYLAR (1968): Cellular membranes: The isolation and characterisation of the plasma and smooth membranes of HeLa cells. *Arch. Biochem. & Biophys.* **128**: 51–69.

BROWN, R. M. & W. W. FRANKE (1971): A microtubular crystal associated with the Golgi field of Pleurochrysis scherffelii. *Planta* **96**: 354–363.

CRANG, R. E. & G. B. MILES (1969): An electron microscope study of germinating Lychnis alba pollen. *Amer. J. Bot.* **56**: 398–405.

DANISHEFSKY, I., R. L. WHISTLER & F. A. BETTELHEIM (1970): Introduction to polysaccharide chemistry: In: W. PIGMAN & D. HORTON, eds., *The Carbohydrates*, Vol. **11A**. Academic Press, New York. pp. 375–413.

DASHEK, W. V. & W. G. ROSEN (1966): Electronmicroscopic localization of chemical components in the growth zone of Lily pollen tubes. *Protoplasma* **61**: 192–204.

DATTA, A., R. D. CAMERINI-OTERO, S. N. BRAUNSTEIN & R. M. FRANKLIN (1973): Proteins of the cell envelope of a marine Pseudomonad Ps. Bal-31. *Biochim. Biophys. Acta* **311**: 163–172.

DUBOIS, M., K. A. GILLES, J. K. HAMILTON, P. A. REBERS & F. SMITH (1956): Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**: 350–356.

ENGELS, F. M. (1973): Function of Golgi vesicles in relation to cell wall synthesis in germinating Petunia pollen. 1. Isolation of Golgi vesicles. *Acta Bot. Neerl.* **22**: 6–13.

FRIEND, D. S. (1969): Cytochemical staining of multivesicular body and Golgi vesicles. *J. Cell Biol.* **41**: 269–280.

HARRIS, J. L. & W. A. FABER (1973): Compositional studies on the cell walls of the synnema and vegetative hyphae of Ceratocystis ulmi. *Can. J. Bot.* **51**: 1147–1153.

HARRIS, P. J. & D. H. NORTHCOTE (1971): Polysaccharide formation in plant Golgi bodies. *Biochim. Biophys. Acta* **237**: 56–64.

HERTH, W., W. W. FRANKE, J. STADLER, H. BITTIGER, C. KEILICH & R. M. BROWN Jr. (1972): Further characterization of the alkali stable material from the scales of Pleurochrysis scherffelii: A cellulosic glycoprotein. *Planta* **105**: 79–92.

HEYN, A. N. J. (1971): Observations on the exocytosis of secretory vesicles and their products in coleoptiles of Avena, *J. Ultrastructural Res.* **37**: 69–81.

KNEE, M. (1973): Polysaccharide changes in cell walls of ripening apples. *Phytochem.* **12**: 1543–1549.

KROH, M. (1973): Nature of the intercellular substance of stylar transmitting tissue. In: F. LOEWUS, ed., *Biogenesis of cell wall polysaccharides*. Academic Press, New York. pp. 195–206.

LABARCA, C. & F. LOEWUS (1972): The nutritional role of pistil exudate in pollen tube wall formation in Lilium longiflorum. I. Utilization of injected stigmatic exudate. *Plant Physiol.* **50**: 7–14.

LOWRY, O. H., N. J. ROSEBROUGH, L. FARR & R. J. RANDALL (1951): Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.

NANCE, J. F. (1973): Effects of calcium and kinetin on growth and cell wall composition of Pea epicotyls. *Plant Physiol.* **51**: 312–317.

NORTHCOTE, D. H. & J. D. PICKETT-HEAPS (1966): A function of the Golgi apparatus in polysaccharide synthesis and transport in the root-cap cells of wheat. *Biochem. J.* **98**: 159–167.

PICKETT-HEAPS, J. D. (1968): Further ultrastructural observations on polysaccharide localization in plant cells. *J. Cell Sci.* **3**: 55–64.

RENKONEN, O. (1961): A note on spectrophotometric determination of acyl ester groups in lipids. *Biochim. Biophys. Acta.* **54**: 361–362.

ROSEN, W. G. & S. R. GAWLIK (1965): Fine structure of the tips of Lilium longiflorum pollen tubes following growth in vivo. *J. Cell Biol.* **27**: 89A.

SASSEN, M. M. A. (1964): Fine structure of Petunia pollen grain and pollen tube. *Acta Bot. Neerl.* **13**: 175–181.

SCHAFFER, R. (1972): Occurrence, properties, and preparation of naturally occurring monosaccharides (including 6-deoxy sugars): In: W. PIGMAN & D. HORTON, eds., *The Carbohydrates*, Vol. **1A**. Academic Press, New York. pp. 69–113.

SIEVERS, A. (1963): Beteiligung des Golgi-Apparates bei der Bildung der Zellwand von Wurzelhaaren. *Protoplasma* **56**: 187–192.

TALMADGE, K. W., K. KEEGSTRA, W. D. BAUER & P. ALBERSHEIM (1973): The structure of plant cell walls I. The macromolecular components of the walls of suspension-cultured Sycamore cells with a detailed analysis of the pectic polysaccharides. *Plant Physiol.* **51**: 158–173.

VAN DER WOUDE, W. J. & D. J. MORRÉ (1968): Endoplasmic reticulum-dictyosome-secretory vesicle association in pollen tubes of Lilium longiflorum Thunb. *Proc. Indiana Acad. Sci* **77**: 164–170.

—, — & C. E. BRACKER (1971): Isolation and characterization of secretory vesicles in germinated pollen of Lilium longiflorum. *J. Cell Sci.* **8**: 331–351.

YOUNG, R. W. (1973): The role of the Golgi complex in sulphate metabolism. *J. Cell Biol.* **57**: 175–189.