Ultrastructural localization of polysaccharides and N-acetyl-d-galactosamine in the secretory pathway of green algae (Desmidiaceae)

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Abstract

In order to characterize the secretory pathway leading to multipolar tip growth in green algae of the family Desmidiaceae, different general polysaccharide stainings, such as the periodic acid–silver hexamine method and the periodic acid–silver proteinate method as well as different lectins specific to defined sugar residues have been employed. General polysaccharide stainings label different kinds of secretory vesicles starting from the onset of vesicle production up to their delivery into the primary cell wall, however, the discrimination of Golgi products is possible using lectins. Both gold-labelled lectins from Helix pomatia and from Glycine max with affinity to N-acetyl-d-galactosamine only produce labelling of primary wall material containing ‘dark vesicles’ on ultrathin sections of high-pressure frozen and freeze-substituted Micrasterias cells, whereas other vesicle types remain unstained. ‘Dark vesicles’ are labelled when still attached to trans-Golgi compartments, when distributed throughout the cytoplasm or when fusing with the plasma membrane with the same staining intensity which indicates that the sugars detected by the methods used are present from the onset of visible vesicle production. Gold-labelling of N-acetyl-d-galactosamine is also observed in the primary cell wall. In control experiments the staining vanishes when ultrathin sections are pre-incubated with N-acetyl-d-galactosamine. Various other lectins with affinity to different sugar residues than N-acetyl-d-galactosamine do not produce any staining of the cell wall nor of any kind of secretory vesicles. As N-acetyl-d-galactosamine is usually not present in N-linked polysaccharides the results point towards the presence of O-linked-glycoproteins in the primary cell wall of desmids.

Key words: Golgi, lectins, Micrasterias, N-acetyl-d-galactosamine, secretion, vesicles.

Introduction

Dictyosomes of plant cells are known to be responsible for the synthesis of glycoproteins and complex polysaccharides and for their packaging into secretory vesicles. Transport from the endomembrane system through the cytoplasm to the plasma membrane and fusion of secretory vesicles with the plasma membrane leads to growth of both the plasma membrane itself and the cell wall.

In growing desmids the ER delivers secretory products to the cis-cisternae of dictyosomes via transition vesicles, whereas different kinds of secretory vesicles are released from the trans-side depending on the stage of development (for details, see Kiermayer, 1981; Url and Kiermayer, 1990; Höftberger and Meindl, 1993). Actin-driven cytoplasmic streaming is responsible for distribution of Golgi products in the cytoplasm. Localized fusion of wall material containing vesicles at particular areas of the plasma membrane contributes to the establishment of the extra-ordinary cell pattern of, for example, a Micrasterias cell (for summary, see Meindl, 1993). Insight into the composition of vesicle contents which are responsible for primary wall formation may thus yield important information on the physical and chemical basis for cell shaping and growth.

Two vesicle populations appear to be involved in primary wall formation, the ‘dark vesicles’ (DV; Kiermayer, 1981) which are formed at medial Golgi cisternae and are forwarded towards the trans-region and another smaller type of vesicles released from the trans Golgi network (A-vesicles; Meindl et al., 1992). A third kind of secretory vesicles synthesized during the whole
cell cycle and also during primary wall formation are the mucilage containing ‘large vesicles’ (LV; Kiermayer, 1981). Earlier cytochemical studies by Tutumi and Ueda (1975) on *Micrasterias americana* indicated that polysaccharides like starch, cell wall and slime produced electron dense precipitates with silver hexamine. Three different types of vesicles, two populations of LVs and the primary wall forming DVs also seemed to contain polysaccharides. Studies by Menge (1976) on *Micrasterias denticulata* revealed that DVs and LVs contain acidic polysaccharides. Menge (1976) reported a gradual increase in the staining intensity of DVs and LVs during vesicle maturation, starting from vesicles attached to medial dictyosomal cisternae up to vesicles already released from the dictyosomes. This finding corresponds to observations by Meindl et al. (1992) who found in high-pressure frozen *Micrasterias* cells that DVs appear to be forwarded from one cisterna to the next while reaching their final size as well as that LVs are smaller in diameter when still connected to the dictyosome than when delivered into the cytoplasm.

Studies with tunicamycin, which inhibits the N-linked glycosylation of proteins (Elbein, 1987), and with cyclopaizonic acid an inhibitor of Ca²⁺-dependent ATPase in the ER (Goeger and Riley, 1989), both revealed an influence on the secretory pathway in *Micrasterias denticulata* leading to a cessation in cell growth (Höftberger et al., 1995a). Höftberger et al. (1995a) found by electron microscopical investigations that cyclopiazonic acid prevented dictyosomal supply with ER products and caused dilution of ER cisternae and reduction in the number of Golgi cisternae, combined with a loss of dictyosomal activity, whereas tunicamycin treatment resulted in a disintegration of the dictyosomes into small vesicles. Disturbed cell wall formation under the influence of tunicamycin indicates that N-linked glycosylation is required for normal cell growth in *Micrasterias*. Possible changes in cytoplasmic free calcium concentration caused by ATPase inhibition in the ER (Tornquist, 1993) during treatment with cyclopiazonic acid, may inhibit wall material secretion by disrupting the normal ER-dictyosome association (Höftberger et al., 1995a). Recently it has been demonstrated that the influence of Brefeldin A (BFA), which among others inhibits the synthesis of O-linked oligosaccharide chains of membrane glycoproteins (Kimura et al., 1993) is different to the effects of tunicamycin (inhibitor of N-linked glycoproteins) treatment in *Micrasterias* at an ultrastructural level. The effects caused by BFA on *Micrasterias* (Salomon and Meindl, 1996) are, besides morphological and structural changes of dictyosomes, especially a gradual decrease in the number of dictyosomal cisternae which leads to a dramatic reduction of Golgi stacks. BFA is the only drug applied to *Micrasterias* so far which induces a complete and reversible dissociation of dictyosomes in growing cells. In addition to the results described above, the ionophores monensin and nigericin have been demonstrated to produce marked changes in dictyosomal function and morphology during development of desmid cells (Höftberger et al., 1993, 1995b).

The aim of the present study is to give more detailed information on the secretory pathway and to characterise secretory products in desmids, especially in *Micrasterias denticulata*, by means of general polysaccharide stainings and more specifically by different gold-labelled lectins.

### Material and methods

#### Cell cultivation

Cells of *Micrasterias denticulata* Brèb. and *Xanthidium armatum* were grown in a ‘desmid medium’ (Schlösser, 1982) at a constant temperature of 20°C and an illumination rhythm of 14 h light and 10 h darkness.

#### General polysaccharide stainings

For investigation of the secretory pathway in desmids the following general polysaccharide stainings were used on developing cells of *Micrasterias denticulata* and *Xanthidium armatum*:

1. a method using dialysed iron (Hardin and Spicer, 1971),
2. a ruthenium red-osmium tetroxide staining method (Luft, 1971),
3. periodic acid oxidation methods (PAS; for details see Lewis and Knight, 1992).

Usable results were obtained only with the PAS methods (periodic acid-silver hexamine method, periodic acid-silver proteinate method) which depend on the detection of aldehyde groups produced from oxidation by periodic acid. The oxidation step itself is thought to be relatively specific for sugar residues which carry a pair of free, unsubstituted hydroxy groups on adjacent carbon atoms (Lewis and Knight, 1992). More specific results can be obtained, in particular from the silver proteinate method when the cells are fixed without osmium tetroxide which may react unspecifically with thiosemicarbazide. Therefore, to begin with the material used here was only fixed with glutaraldehyde (1%) or formaldehyde (1% and 2%) solutions in cacodylate buffer. However, since the ultrastructure was not well preserved by only using aldehyde fixation, post-fixation with 2% osmium tetroxide was unavoidable. Subsequently the cells were dehydrated in increasing concentrations of ethanol, placed into propylene oxide, embedded in Epon 812, sectioned on an Ultracut (Reichert) and examined on a Philips EM 400 T at 60 kV. Ultrathin sections were stained according to the following procedures:

**Periodic acid-silver hexamine method:** staining protocols of Pickett-Heaps (1967), Tutumi and Ueda (1975), and Menge (1976) were used, but modified at the silver step. Sections, mounted on gold grids were treated with 1% periodic acid for 15 min at room temperature, and were then washed for 20 min with double-distilled water. For staining silver reducing sites, 0.1% silver nitrate in borate buffered 1% hexamine (pH 9.2) was used. This step was carried out in the dark at different temperatures and for different periods of time: 15, 30 and 45 min at 60°C, 60 and 120 min at room temperature. For treatment at 60°C the grids were placed on the silver nitrate solution, covered with a cover slip and vaseline and incubated in a pre-warmed oven. Thereafter the sections were washed
with double-distilled water for 20 min. For removing unreduced silver, the sections were treated with 2% thiosulphate solution for 2 min at room temperature. A final washing step with double-distilled water followed.

As a modification of this procedure, in order to block pre-existing aldehyde groups, the grids were placed on 2% sodium bisulphate solution for 15 min prior to the periodic acid step (Van der Woude et al., 1971). In this case the silver nitrate step lasted 30 min at 60°C.

Periodic acid-silver proteinate method: the staining protocol of Lewis and Knight (1992) was used, and modified at the silver step. Sections, mounted on gold grids were treated with 1% periodic acid for 15–20 min at room temperature, and rinsed several times with double-distilled water. Then the sections were floated for 30–45 min on 1% thiosemicarbazide in 10% acetic acid, which is used to detect the aldehydes produced by periodic acid oxidation (Hanker et al., 1964). After two rapid changes of 10% acetic acid the sections were washed 2 × 15 min in 10%, 5 min in 5%, 5 min in 1% acetic acid, and 2 × 5 min in double-distilled water. Treatment with 1% silver proteinate for the detection of thiosemicarbazide occurred in the dark at the following temperatures and time periods: 30 and 40 min at room temperature, 15 and 30 min at 60°C. After staining the sections were rinsed several times with double-distilled water.

In order to increase the intensity of the silver staining the silver enhancement procedure of Neiss (1988) described in Lewis and Knight (1992) was used.

Lectin stainings

For lectin-gold labelling experiments untreated young developing cells of *Micrasterias denticulata* were high pressure frozen with a Balzers HPM10 hyperbaric freezer in liquid nitrogen (for details see Meindl et al., 1992) and freeze-substituted in 0.1% tannic acid in acetone for 24 h at −80°C and in a mixture of 0.05% uranyl acetate and 2% osmium tetroxide in acetone for 24 h at −80°C, followed by an increase in temperature to −30°C in the same medium for 10 h. After freeze-substitution the samples were brought to room temperature, washed in 100% acetone for 15 min, rinsed in absolute ethanol for 15 min, infiltrated with LR-White (medium grade, London Resins) at room temperature for 4 h and UV-polymerized at room temperature for 24 h. The embedded cells were sectored on a Reichert Ultrotact and ultrathin sections were mounted on formvar coated gold grids or gilded copper grids.

All lectins used in this study (lectins from *Arachis hypogaea*, *Canavalia ensiformis*, *Glycine max*, *Helix pomatia*, *Lens culinaris*, and from *Triticum vulgaris*, see Table 1) were 10 nm gold-labelled, purchased from Sigma. For staining the procedure grids were preincubated in 10 mM phosphate-buffered saline containing 0.05% Tween 20, pH 7.4 (PBST) for 30 min and then transferred on to 20 µl droplets of the appropriate lectin (1 µl diluted in 40 µl PBST containing 0.5% bovine serum albumin; BSA) for 18–24 h at 5–6°C in a moist chamber. For alternative methods, preincubations were also done with 10 µM phosphate-buffered saline, pH 7.4 (PBS) or 10 mM PBST containing 0.5% BSA, and lectins were diluted with PBS or PBST. Lectin incubations were also performed with varying lectin concentrations (2 µl or 4 µl diluted in 40 µl buffer), varying duration and varying temperatures (3 h, 12 h, 24 h at room temperature; 12 h at 5–6°C). For specificity control experiments preincubations and labelling with lectins from *Helix pomatia* and from *Glycine max* were carried out in the presence of 0.45 M N-acetyl-d-galactosamine (GalNAc; Sigma). Sections labelled with lectin-gold were rinsed by a mild spray of PBS from a Pasteur pipette, transferred on to small droplets of PBS for 2 min, rinsed a second time with PBS and finally with a mild spray of double-distilled water from a plastic spray bottle. Rinsing was also done with PBST which, however, provided insufficient results. After rinsing and drying the sections were counterstained with aqueous uranyl acetate (2%) for 5 min and Reynold’s lead citrate for 2 min and examined on a Philips 400 T electron microscope at 80 kV.

Results

General polysaccharide stainings

The silver hexamine method which, of the general polysaccharide stainings yielded the best results, provided labelling of different vesicles. In *Micrasterias denticulata* numerous DVs, distributed all over the cytoplasm or still connected to the dictyosomes are stained with the same intensity when sections are treated for 30 min at 60°C during the silver step (Fig. 1A, B). The big slime-containing vesicles and the primary wall show slight staining. Under the same staining conditions a specific significant labelling is found only in vesicles that are still attached or at least situated close to the dictyosomes (Fig. 1C) in the closely-related desmid Xanthidium armatum. The dictyosomes themselves are sometimes slightly stained at the trans-side (Fig. 1B, C). In both *Micrasterias denticulata* and Xanthidium armatum distinction between different vesicle types is only possible by different staining intensities with this method. A block of pre-existing aldehyde groups (see Materials and methods) yields the same results as described above. When the duration of the silver nitrate treatment is extended to 45 or 60 min the staining becomes unspecific.

Treatment with silver proteinate at 60°C results in a slight staining of DVs, which are occasionally still connected to the dictyosomes (Fig. 1D). The silver enhancement procedure (Lewis and Knight, 1992) did not improve this staining. When silver proteinate is applied at room temperature only parts of the chloroplast (probably plastoglobuli) are stained heavily. Other methods which would allow a distinction between polysaccharides (e.g. staining of acid carbohydrates by using dialysed formvar coated gold grids or gilded copper grids.

Lectin stainings

Among the lectins used for the present investigations both the lectins from *Helix pomatia* (HPA) and from *Glycine max* (SBA) resulted in a significant labelling of the primary wall-forming DVs in untreated young developing *Micrasterias* cells (Table 1). After numerous variations in method (see Materials and methods), it was found that preincubation of the grids in PBST, followed by lectin incubation (1 µl diluted in 40 µl PBST containing...
0.5% BSA) for 18–24 h at 5–6 °C and rinsing with PBS, provided the best staining results.

Specific lectin staining was detectable in DVs either already released from the dictyosomes or still attached to distal Golgi cisternae, but not in regions of the dictyosomes themselves (Fig. 2A). Staining of the same intensity was found in DVs of the central cytoplasm where the dictyosomes are mostly located in a growing half cell of *Micrasterias denticulata* as well as in the DVs of the cortical cytoplasm and in those already incorporated into the cell wall (Figs 2B; 3A–D). Moreover, random gold staining from HPA and SBA was detectable in all regions of the primary wall without any visible correlation to the cell pattern (Fig. 3B-D). The staining intensity in the primary wall varied slightly from cell to cell.

Except for the primary cell wall and the Golgi-derived DVs no other cellular structures revealed gold labelling from HPA or SBA treatment. Not even the A-vesicles, also involved in primary wall formation (Fig 3A), nor the large mucilage vesicles (Figs 2A, B; 3B, D), which have been shown to contain polysaccharides, were stained by application of this method.

Experiments for specificity control showed that no staining occurred in DVs nor in the primary cell wall nor in any other cytoplasmic structures after incubation with HPA or SBA when the sections were preincubated with GalNAc (Figs 2C; 3E, F). Incubations with lectins from *Tricornucrum vulgaris* (WGA), *Lens culinaris* (LCA), *Canavalin ensiformis* (Con A), and *Arachis hypogaea* (PNA) did not result in any specific gold labelling (Fig. 2D, E). Secretory pathway of *Micrasterias denticulata* as well as in the DVs cans and glycoproteins (Lewis and Knight, 1992), theof the cortical cytoplasm and in those already incorporated into the cell wall (Figs 2B; 3A–D). Moreover, random gold staining from HPA and SBA was detectable in all regions of the primary wall without any visible correlation to the cell pattern (Fig. 3B-D). The staining intensity in the primary wall varied slightly from cell to cell.

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Source</th>
<th>Abbreviation</th>
<th>Specificity</th>
<th>Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut agglutinin</td>
<td><em>Arachis hypogaea</em></td>
<td>PNA</td>
<td>β-D-Gal(1-3)-D-GalNAc</td>
<td>Negative</td>
</tr>
<tr>
<td>Canavanin A</td>
<td><em>Canavalin ensiformis</em></td>
<td>Con A</td>
<td>α-D-Man, α-D-Glc</td>
<td>Negative</td>
</tr>
<tr>
<td>Soybean agglutinin</td>
<td><em>Glycine max</em></td>
<td>SBA</td>
<td>D-GalNAc</td>
<td>Dark vesicles</td>
</tr>
<tr>
<td><em>Helix pomatia</em> agglutinin</td>
<td><em>Helix pomatia</em></td>
<td>HPA</td>
<td>D-GalNAc</td>
<td>Primary wall</td>
</tr>
<tr>
<td><em>Lens culinaris</em> agglutinin</td>
<td><em>Lens culinaris</em></td>
<td>LCA</td>
<td>α-D-Man</td>
<td>Primary wall</td>
</tr>
<tr>
<td>Wheat germ agglutinin</td>
<td><em>Triticum vulgaris</em></td>
<td>WGA</td>
<td>(D-GlcNAc)αβ, NeuNAc</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Table 1. Specifications and results of gold-labelled lectins used on ultrathin sections of *Micrasterias denticulata***

**Discussion**

Two different methodological approaches have been used in this study to distinguish vesicle populations involved in primary wall growth and pattern formation of *Micrasterias denticulata* and/or *Xanthidium armatum*, the general polysaccharide stainings and the lectin–gold labelling experiments.

From the PAS methods which mainly label homoglu-can and glycoproteins (Lewis and Knight, 1992), the silver hexamine method yielded the most satisfying results by staining all types of secretory vesicles (including mucilage vesicles) during primary wall formation to different degrees as well as the primary cell wall of *Micrasterias* cells itself. Staining has also been found in dictyosome-associated vesicles of *Micrasterias* and *Xanthidium*, starting at the middle Golgi compartments, where DV formation begins (Meindl et al., 1992) and continuing towards the trans-Golgi cisternae. The distinction between the different vesicle types with this method is only possible by the staining intensity which was much higher in DVs than in mucilage vesicles. The heavy staining of special areas of the chloroplast (probably plastoglobuli) which has been shown to contain polysaccharides, were stained by application of this method.

Experiments for specificity control showed that no staining occurred in DVs nor in the primary cell wall nor in any other cytoplasmic structures after incubation with HPA or SBA when the sections were preincubated with GalNAc (Figs 2C; 3E, F). Incubations with lectins from *Tricornucrum vulgaris* (WGA), *Lens culinaris* (LCA), *Canavalin ensiformis* (Con A), and *Arachis hypogaea* (PNA) did not result in any specific gold labelling of any kind of vesicles nor of the cell wall, even after repeated experiments and after using different procedures (Fig. 2D, E).

**Fig. 1.** (A–C) Ultrastructure of growing semi-cells of *Micrasterias denticulata* and *Xanthidium armatum*. Staining: periodic acid-silver hexamine (silver step 30 min at 60 °C), in (B) pre-existing aldehyde groups blocked with sodium bisulphate. (A) Cortical cytoplasm and primary wall of *Micrasterias denticulata*. Dark vesicles (arrows) stained strongly, whereas large vesicles (LV) and primary wall (PW) show slight staining. (B) Dictyosomes of *Micrasterias denticulata*. Vesicles attached to the dictyosomes or released into the cytoplasm stain strongly, dictyosomal cisternae only slightly stained in trans-areas. Arrows point at dark vesicles. LV large vesicles. (C) Dictyosome of *Xanthidium armatum*. Vesicles (arrows) are stained. Golgi cisternae only slightly stained. (D) Dictyosomes and vesicles of *Micrasterias denticulata* stained with periodic acid silver proteinate (30 min at 60 °C at the silver step). No staining of dictyosomal cisternae, fine staining of vesicles (arrows; bars = 0.5 μm).
Localization of N-acetyl-D-galactosamine in desmids

Fig. 3. (A–D) Cytoplasm of growing semi-cells of *Micrasterias denticulata* after high pressure freeze fixation and staining with lectin from *Helix pomatia*. Specific gold staining in dark vesicles (thick arrows) and the primary cell wall (PW). A-vesicles (AV) not stained. (D) arrow points at dark vesicle which has just been incorporated into the cell wall (E, F) Control, pre-treated with N-acetyl-galactosamine. No specific gold staining after incubation with lectin from *Helix pomatia* in the central cytoplasm (E), in the cortical cytoplasm and in the cell wall (F). Thick arrows point at dark vesicles, thin arrows indicate A-vesicles. LV large vesicles (bars = 0.25 μm).

Fig. 2. (A–E) Cytoplasmic areas of high pressure frozen growing semi-cells of *Micrasterias denticulata*. (A, B) Incubation with lectin from *Glycine max*: specific gold staining in dark vesicles (arrows) released from the dictyosome, still attached to distal Golgi cisternae (A) or located in the cortical cytoplasm (B). (C) Control staining with lectin from *Glycine max* after preincubation with N-acetyl-galactosamine. No staining. Arrows point at dark vesicles. (D, E) Incubation with lectin from *Triticum vulgaris* (D) and from *Arachis hypogea* (E). No staining. Arrows point to dark vesicles. LV large vesicles (bars = 0.25 μm).
and discriminate Golgi products. During these experiments it turned out that gold-labelled lectins with affinity to GalNAc residues like HPA or SBA stained only primary wall forming DVs whereas other vesicle populations like A-vesicles also involved in primary wall formation, or mucilage vesicles remained unstained. As GalNAc is generally not found in N-linked glycoproteins (Roth, 1984) this result probably indicates that DVs contain O-linked glycoproteins such as hydroxyproline-rich glycoproteins or arabinogalactans which have also been identified in other algae cell walls (Voigt et al., 1994). In this respect, the finding of this experiment strongly corroborates earlier studies on various higher plants pointing towards the presence of galactosamines in hydroxyproline-rich cell wall glycoproteins (Wold and Hillestad, 1976; Hori, 1978). On the contrary, O-glycosylation in algae and higher plant cells is currently thought to occur mainly by the addition of galactose and arabinose (Showalter and Varner, 1989; Staehelin and Moore, 1995).

Gold staining with HPA or SBA in *Micrasterias* has been found in DVs still associated with the dictyosomes, but not in cis- or medial Golgi compartments indicating that either addition of GalNAc takes place in trans-Golgi cisternae at almost mature DVs or, that GalNAc is not present at terminal positions in more cis-oriented Golgi compartments. These considerations could be correlated to results from earlier investigations on *Micrasterias* cells treated with BFA (Salomon and Meindl, 1996), which among others is known to prevent the synthesis of O-linked saccharide chains of membrane glycoproteins (Kimura et al., 1993). On an ultrastructural level BFA treatment causes a gradual decrease in the number of Golgi cisternae from the cis-side leading to a marked reduction and subsequently to an almost complete loss of Golgi bodies in the young semi-cell of *Micrasterias*. As soon as dictyosomes have disappeared from the young semi-cell, DVs are no longer visible near the plasma membrane, indicating that prevention of the synthesis of O-linked saccharide chains of membrane glycoproteins inhibits the formation of primary wall material containing vesicles by disrupting Golgi structure and function (Salomon and Meindl, 1996).

GalNAc residues were also detected in the primary wall cell of *Micrasterias* which confirms the observation of Kiermayer (for summary see Kiermayer, 1981) that DVs are involved in primary wall formation. The fact that A-vesicles are not stained by lectins with affinity to GalNAc residues may indicate that their function in primary wall formation is different from that of DVs as presumed by Meindl et al. (1992). Based on the assumption that a locally different extensibility of the cell wall is due to varying chemical and physical properties, the latter authors assumed that A-vesicles may contain material not for extension growth, but for thickening and/or hardening of the cell wall at the indentations of a *Micrasterias* cell.

Except those of HPA and SBA, no other lectins used in this study caused specific staining in *Micrasterias*. This fact may not necessarily mean that carbohydrates or carbohydrate residues other than GalNAc are absent, as an influence of osmium on the reactivity of carbohydrate sites to lectins may not be excluded (Lewis and Knight, 1992). However, in *Scenedesmus* labelling with golddcoupled Con A, WGA and PNA was found in the cell wall, at the plasma membrane and in distal Golgi compartments after freeze-substitution with osmium tetroxide and uranyl acetate (Hayashi and Ueda, 1987).

Both the general polysaccharide stainings and lectin labelling indicate that polysaccharides, in secretory vesicles of desmids which are detected by the methods used, are present from the onset of vesicle production in distal Golgi-compartments up to vesicle fusion with the plasma membrane. This is in contrast to findings in higher plant cells (Pickett-Heaps, 1968; Van der Woude et al., 1971) and to observations of Menge (1976) on *Micrasterias* revealing an increase in staining intensity of secretory vesicles with increasing distance from the dictyosomes which is interpreted as a continuous modification of polysaccharides during their transport. Further information on the secretory pathway in desmids might result from the application of antibodies of the JIM family recognizing epitopes of glycoproteins or cell wall polysaccharides (Knox, 1992).

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References


