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FUNCTION OF GOLGI VESICLES IN RELATION TO CELL WALL SYNTHESIS IN GERMINATING PETUNIA POLLEN. IV. IDENTIFICATION OF CELLULOSE IN POLLEN TUBE WALLS AND GOLGI VESICLES BY X-RAY DIFFRACTION

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SUMMARY

The residues of Golgi vesicles and tube walls from germinating *Petunia* pollen were studied by application of the X-ray diffraction technique after different chemical treatments. In the Golgi vesicles as well as in the tube walls the presence of a cellulose component was demonstrated. This result led to the assumption that the machinery for cellulose synthesis is already present in the Golgi vesicles.

1. INTRODUCTION

Two alternative hypotheses have been put forward with respect to the site of cellulose synthesis. The first hypothesis claims the cellulose synthesis to occur at the outside of the plasmalemma or in the cell wall itself (PRESTON 1963, ROELOFSEN 1965, STAEHELIN 1966, 1968, MÜHLETHALER 1967, BARNETT 1969, FREY-WYSSLING 1969, BARNETT & PRESTON 1970, ROBINSON & PRESTON 1971, 1972). The other hypothesis stresses the likelihood of the cellulose synthesis to be located within the cytoplasm in specific organelles (LEDBETTER & PORTER 1963, MARX-FIGINI & SCHULTZ 1966, BROWN et al. 1970, HERTH et al. 1972, GAMALEI 1973).

Until 1970 the first hypothesis was generally accepted (COLVIN 1972), although the evidence up to then was only obtained by morphological studies. However, the evidence obtained by chemical and morphological studies performed with the alga *Pleurochrysis scherffelii* strongly supports the second hypothesis (BROWN et al. 1970, HERTH et al. 1972). In this organism a cellulosic compound was found to be present in Golgi vesicles as scales. These are transported via the Golgi system to the outside of the plasma membrane.

Chemical data obtained by analysing the Golgi vesicles from germinating *Petunia* pollen has led to the assumption that the alkali-resistant material found in the Golgi vesicles as well as in the tube wall material might be of cellulosic nature (ENGELS 1974a). Till now no evidence has been obtained which indicated that the fibrillar material observed in pollen tube walls consists of cellulose. Only assumptions have been made in this direction (SASSEN 1964, KROH 1964, FLYNN 1968). The present paper deals with a study of X-ray dif-

fraction patterns of material from tube walls and Golgi vesicle contents obtained from germinated pollen after different extraction procedures. This study is intended to obtain evidence about the presence of cellulose in these materials.

2. MATERIAL AND METHODS

Golgi vesicles and pollen tube walls from germinated *Petunia* pollen were isolated as described previously (ENGELS 1973, 1974a). The isolated material was treated with N-HCl at 100 °C for 1 hr. The residue of the Golgi vesicles was subsequently treated with 2N KOH for 15 hrs at room temperature. In addition a chloroform extraction was carried out on the KOH residue of the Golgi vesicles during 60 hrs at room temperature. The residues from the tube wall after N-HCl and the Golgi vesicles after chloroform extraction were treated with 20% NaOH at 100 °C for 1 hr. All solutions used for extraction were centrifuged at 27,000 rpm in a SW 27.1 for 1 hr before they were applied, to avoid contamination with cell wall material. Even after artificial addition of cellulose fibrils from tube walls to the isolation medium of Golgi vesicles no fibrils were found in the supernatant in which the Golgi vesicles accumulated.

Cotton hairs treated with N-HCl and 20% NaOH under conditions as described previously served as a reference for cellulose.

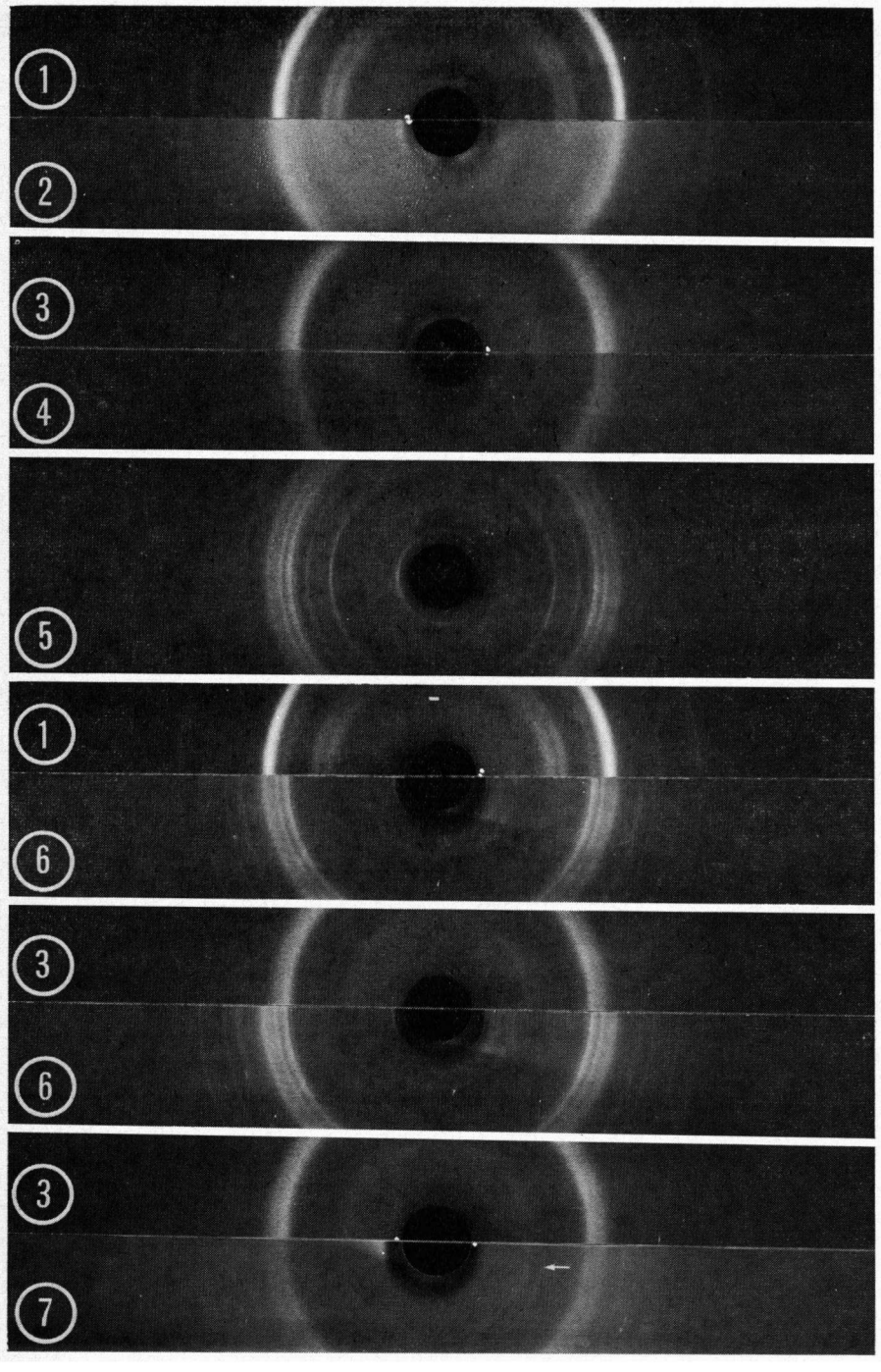
X-ray patterns obtained from the different probes were made in a Debije and Scherrer camera with 0.5 mm ⌀ collectors. The exposure time was 17 hrs.

3. RESULTS

The X-ray pattern of cotton hairs treated with N-HCl consists mainly of 4 main diffraction lines (*fig. 1*). The two inner lines are of equal intensity and correspond to angles of diffraction of 15° and 16.5°. The two outer lines are of unequal intensity and correspond to angles of diffraction of 20.5° and 22.8°, respectively. Such an X-ray pattern is characteristic for cellulose I. After the same treatment pollen tube walls give an X-ray diffraction pattern as represented in *fig. 2*, which corresponds in its essential features to that of the cellulose I pattern of cotton hairs. The two inner lines are very low in intensity and hardly visible. The two outer lines show the same relative difference in intensity as observed in the cotton sample.

Treatment of the cotton hairs with 20% NaOH results in an X-ray pattern consisting mainly of 3 lines (*fig. 3*). The inner line of the paired ones has the

Figs. 1–7. X-ray diffraction patterns obtained from purified residues of Golgi vesicles, pollen tube walls and cotton hairs.
Fig. 1. Cotton hairs after N-HCl extraction.
Fig. 2. N-HCl extracted tube walls.
Fig. 3. Cotton hairs after NaOH treatment.
Fig. 4. Tube walls after NaOH treatment.
Fig. 5. Golgi vesicles after N-HCl and 2N KOH extraction.
Fig. 6. Golgi vesicles after additional chloroform purification.
Fig. 7. Golgi vesicles after NaOH treatment.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highest intensity. The lines correspond to angles of diffraction of 11.6°, 20.0 and 21.0° for inner, middle, and outer line, respectively. The pattern obtained from the pollen tube walls treated with NaOH produces essentially the same diagram. Both are characteristic for cellulose II (*fig. 4*).

The residue of N-HCl purified Golgi vesicles produces a very diffuse pattern from which no further information could be obtained. After treatment with 2N KOH a pattern composed of fine lines became visible (*fig. 5*). This pattern is assumed to originate partly from the presence of fatty material. Indeed some lines disappear when the material is treated with chloroform, which results in a pattern suggesting the presence of both cellulose I and II (*fig. 6*). The pattern obtained from the residue of the Golgi vesicles treated with NaOH corresponds unambiguously with that of cotton hairs after the same treatment (*fig. 7*).

4. DISCUSSION

A number of data has been presented illustrating the presence of cellulose in pollen tube walls of *Petunia*. The X-ray patterns from the HCl resistent residue of tube walls and cotton hairs correspond with one another in their essential features. Both are characteristic for cellulose I. From the weak rings present in the tube wall pattern one may conclude that the cristallinity of the tube wall cellulose is poor and/or that impurities are still present. Chemical analysis of the HCl residue with 2N KOH reveal the presence of a polysaccharide composed of several monosaccharides (ENGELS 1974a). Tube wall residue and cotton hairs treated with NaOH produce again similar X-ray patterns, which, however, are now characteristic for cellulose II. A conversion of a cellulose I- into a cellulose II-lattice by NaOH treatment is characteristic for native cellulose. A chemical analysis of the 2N KOH residue of tube wall material has revealed the presence of glucose as the only detectable monosaccharide (ENGELS 1974a). Examination of this material by EM showed a network of microfibrils with diameters between 15 and 20 nm (ENGELS 1974b). The data obtained from the present study lead to the conclusion that the microfibrils are composed of native cellulose, thus substantiating earlier assumptions made by SASSEN (1964).

After treatment with NaOH the material of Golgi vesicles and cotton hairs show a similar X-ray pattern characteristic for cellulose II. An additional line (arrow *fig. 7*) is an indication for the presence of another substance besides cellulose. In *Pleurochrysis* it has been found that a protein is present which is strongly bound to cellulose and which could not be removed by the methods used (HERTH et al. 1972). The control experiments rule out the possibility of impurities by other compounds. Hence it must be concluded that Golgi vesicles contain a polymer chain of the cellulose type.

An extensive study of sections and freeze-etch replicas of Golgi vesicles did not reveal any indication for the presence of fibrils in Golgi vesicles (ENGELS 1973). The cellulose component found in the Golgi vesicles is, therefore, not present in the form of a crystalline structure. Therefore one has to assume that the cellulose is masked by protein and/or lipid which prevents its cristallization.

A cellulose intermediate bound to a glycolipid has been found in *Pisum* (WINTER et al. 1970). In *Phaseolus aureus* an acid labile cellulose intermediate has been reported which contains a glycolipid enzyme complex (VILLEMEZ & CLARK 1969). RAY et al. (1969) found a particulate membrane complex in Golgi membranes in *Pisum sativum* which apparently showed β, 1–4 glucan-synthetase activity.

After HCl extraction the contents of the Golgi vesicles of *Petunia* did not produce an X-ray diagram which could be interpreted. This is possibly caused by the high protein content in comparison with the amount of polysaccharides present in Golgi vesicles (ENGELS 1974a). By KOH extraction a part of the protein is removed and a pattern of fine lines is obtained which suggests the presence of cellulose. After additional extraction with chloroform an X-ray pattern is produced which is indicative for the presence of a mixture of cellulose I and II (*fig. 6*) The assumed intermediate, protected by protein and lipid, may be able to cristallize in the configuration of cellulose I after extraction of the protective substances. During prolonged treatment with KOH, cellulose I may partly transform into cellulose II. A solution of mercerated cellulose has been reported to produce a mixture of cellulose I and II when recristallization is carried out with caution (MACCHI et al. 1968, MACCHI & PALMA 1969).

Synthesis of cell wall polysaccharides with the exception of cellulose is generally accepted to take place in Golgi vesicles in higher plants (NORTHCOTE 1971). Cellulose synthesis is thought to take place exclusively at the outside of the plasmalemma or in the cell wall (PRESTON 1963, ROELOFSEN 1965, STAEHELIN 1966, 1968, MÜHLETHALER 1967, BARNETT 1969, FREY-WYSSLING 1969, BARNETT & PRESTON 1970, ROBINSON & PRESTON 1971, 1972, NORTHCOTE 1969a, b, 1971).

BROWN et al. (1970) and HERTH et al. (1972) indicated a cellulosic component in the Golgi vesicles of the alga *Pleurochrysis scherffelii*. Recently GAMALEI (1973) described structures in thin sections of Golgi vesicles of *Picea abies* interpreted as cellulose microfibrils.

Our results indicate that the Golgi vesicles in *Petunia* pollen tubes contain polysaccharides which are very similar in their composition to those of the tube wall (ENGELS 1974a). The results obtained from the X-ray studies lead us to the assumption that the Golgi vesicles also contain cellulose in addition to other polysaccharides. It is generally assumed that in connection with cell wall synthesis Golgi vesicles migrate to and fuse with the plasmalemma. This fusion implicates that the inside of the unit membrane of the Golgi vesicles resembles morphologically as well as physiologically the outside of the plasmalemma (FINERAN 1973 review). From this point of view cellulose synthesis within the Golgi vesicles needs not to be considered contrary to synthesis of cellulose at the outside of the plasmalemma.

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