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THE EFFECT OF LECTINS ON GERMINATING POLLEN OF LILIUM LONGIFLORUM II. EFFECT OF CONCANAVALIN A ON PHOSPHOLIPID TURNOVER AND ON BIOSYNTHESIS OF PECTIC POLYSACCHARIDES

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SUMMARY

The present study describes the effect of Concanavalin A (Con A, 200 μg/ml) on phospholipid turnover and on the biosynthesis of pectic polysaccharides in germinating pollen of *Lilium longiflorum*. Both phenomena are directly involved in pollen tube growth. The turnover rate of phospholipids was reduced with about 50% by the presence of Con A during the first 4 hours of germination. When Con A was added after 4 hours of incubation in the absence of lectin, a sudden shift of tritium label was observed from phospholipids to 1,2-diacylglycerol. This shift is probably due to inhibition of phospholipid synthesis during the turnover process, while degradation continues. The biosynthesis of pectic polysacharides was also inhibited with about 50% upon addition of Con A either at the onset of germination or to actively growing pollen tubes which were preincubated for 4 hours in the absence of lectin.

1. INTRODUCTION

In the last decade the effect of lectins on pollen tube growth has been frequently described in relation to the incompatibility response (HESLOP-HARRISON & HESLOP-HARRISON 1982, for other references see PIERSON et al. 1986, this issue). From these lectins Con A** had the most significant effect on pollen germination and pollen tube growth (SOUTHWORTH 1975, SHARMA et al., 1985). In an accompanying study (PIERSON et al. 1986, this issue) we showed that Con A treatment results in inhibition of 'in vitro' germination and tube growth in pollen of *Lilium longiflorum* and in disorganization of microfilaments in the pollen tube.

Several other aspects of the growth mechanism in pollen tubes, characterized as tip growth, have been described in the past. One of these is the intensive membrane flow from the endoplasmic reticulum via the dictyosomes and dictyo-

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** For abbreviations see p. 249

some-derived vesicles to the plasma membrane (for reviews see MOLLENHAUER & MORRÉ 1980, PICTON & STEER 1982). Thus the extending plasma membrane is provided with phospholipids, which are synthesized in the endoplasmic reticulum (MUDD 1980). Membrane flow requires a rapid turnover of the phospholipid pool, which can be quantified by the incorporation of ^{32}Pi (only for phospholipids) or [2-^{3}H]glycerol (HELSPER et al. 1986). Another aspect of tip growth, the synthesis of cell wall polysaccharides, can be assayed by the incorporation of *myo*-[2-^{3}H]inositol in pectic polysaccharides (KROH & LOEWUS 1968).

Little research had been done on the biochemical aspects of the influence of Con A or other lectin-like components on pollen germination and pollen tube growth. The present report describes the effect of Con A on phospholipid turnover and on the biosynthesis of pectic polysaccharides in germinating pollen of *Lilium longiflorum*.

2. MATERIALS AND METHODS

2.1. Pollen culture

Pollen of *Lilium longiflorum* Thun. cv. Arai no. 5 was cultured in liquid pentaerythritol-borate medium (DICKINSON 1968) as described by PIERSON et al. (1986, this issue). The incubation period was 4 to 5 hours. Con A (final concentration 200 μg/ml) was given either for the first four hours of incubation or for one hour after 4 hours of preincubation in the absence of lectin. *myo*-[2-^{3}H]inositol (5 μCi, 2 Ci/mmol) or [2-^{3}H]glycerol (5 μCi, 1 Ci/mmol) were present throughout the incubation periods.

After incubation pollen tubes were washed twice with 2 ml fresh medium and subsequently held for 10 minutes at 100 °C (boiling water bath) to destroy phospholipase activity (HELSPER et al. 1986).

2.2. Fractionation of radioactive pollen tubes

After the heating step washed pollen tubes were homogenized in 1 ml methanol. Fractionation into pectic polysaccharides, lipid, and alcohol-soluble, non-lipid cell components was carried out according to HELSPER et al. (1986). The lipid fraction was further analysed by TLC with chloroform-methanol-acetic acid (60:25:8 by vol.) as a solvent for phospholipid separation and with petroleum ether (40/60) – diethylether-formic acid (60:40:1 by vol.) for the separation of triacylglycerol and 1,2-diacylglycerol. Radiotracer experiments were repeated at least twice.

Radioactive analysis was performed as described before (HELSPER et al., 1986).

2.3. Chemicals

All chemicals were reagent grade. Radiochemicals were obtained from Amersham International Inc. (U.K.). Con A was purchased from Sigma (St. Louis, USA).

3. RESULTS

3.1. Effect of Concanavalin A on the incorporation of [2-^{3}H]glycerol in lipids

When pollen was incubated in the presence of [2-^{3}H]glycerol, tritium was incorporated in lipids at a high rate from 1 to 4 hours (*table 1*; *fig. 1*). Then about 50% of the total ratioactivity was recovered in this fraction (*table 1*). After 4 hours the tritium level remained almost constant. Less than 1% of the label was observed in other cell fractions, such as pectic polysaccharides and alcohol-soluble, non-lipid cell components. Further analysis of the lipid fraction revealed that phosphatidylcholine, phosphatidylethanolamine, 1,2-diacylglycerol and triacylglycerol each contained about 20% of the lipid-bound radioactivity (*table 1*). Phosphatidylglycerol, phosphatidylinositol and phosphatidic acid contained 12, 7 and 1%, respectively. The polyphosphoinositides, forming part of the other lipid components, contained less than 0.5% of the total label in lipids. The time course of incorporation in the polyphosphoinositides was very irregular.

Addition of Con A (200 μg/ml) during the first four hours of incubation with [2-^{3}H]glycerol resulted in a 50% lower tritium level in the lipid fraction (*table 1*). The distribution of label over the individual phospholipids and neutral lipids was hardly affected, except for an increase of about 5% in 1,2-diacylglycerol.

When Con A was added after 4 hours of preincubation with [2-^{3}H]glycerol in the absence of lectin, the total radioactivity in the lipid fraction only slightly changed during the next hour of incubation (*table 1*). A fast and significant

Table 1. Effect of Concanavalin A (Con A, 200 μg/ml) on the incorporation of tritium in the lipid fraction (in dpm) and on the distribution of label in radioactive phospholipids and neutral lipids (in % of the total lipid-bound radioactivity). Con A was present either during the first four hours of incubation with 11 × 10^6 dpm [2-^{3}H]glycerol or for one hour following four hours of incubation in the absence of this lectin. In the 'control' Con A was omitted.

Lipid component	Incubation period			
	4 hours	5 hours		
		Presence of Con A		
	control	0–4 h	control	4–5 h
	dpm	dpm	dpm	dpm
Total lipid	5,477,500	2,929,500	5,474,800	5,140,200
	%	%	%	%
Phosphatidylcholine	21	20	22	16
Phosphatidylethanolamine	17	15	17	11
Phosphatidylglycerol	12	12	11	9
Phosphatidylinositol	7	7	8	6
Phosphatidic acid	1	1	1	2
1,2-Diacylglycerol	17	22	16	32
Triacylglycerol	24	22	24	23
Others	1	1	1	1

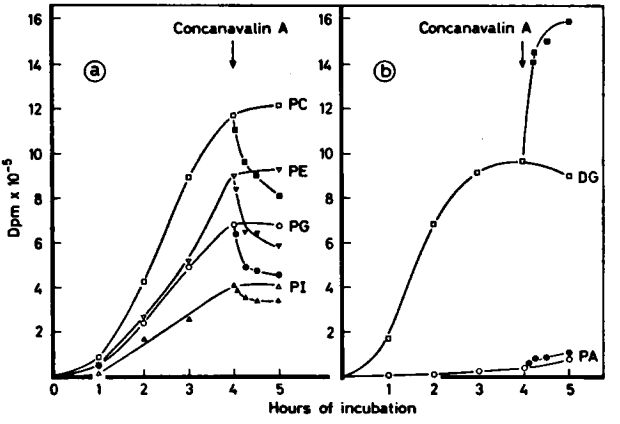

Fig. 1. Effect of Concanavalin A (200 μg/ml, closed symbols), added after 4 hours of preincubation with [2-^{3}H]glycerol (11 × 10^6dpm), on the redistribution of tritium in lipids in pollen of *Lilium longiflorum*. Open symbols represent the incorporation in the absence of Concanavalin A.
Fig. 1a) □ = phosphatidylcholine (PC); ▽ = phosphatidylethanolamine (PE); ○ = phosphatidylglycerol (PG); △ = phosphatidylinositol (PI).
Fig. 1b) ○ = phosphatidic acid (PA); □ = 1,2-diacylglycerol (DG).

change was observed in the distribution of label in phospholipid and 1,2-diacylglycerol (*fig. 1, table 1*), while the amount of tritium in triacylglycerol was hardly affected (*table 1*). The tritium level in all phospholipids, except in phosphatidic acid, decreased within 5 minutes after addition of Con A (*fig. 1a*). There was a slight increase in radioactivity in phosphatidic acid, but the majority of the label accumulated in 1,2-diacylglycerol (*table 1, fig. 1b*).

3.2. Effect of Concanavalin A on the incorporation of *myo*-[2-^{3}H]inositol in pectic polysaccharides and phosphatidylinositol

When Con A was present during the first four hours of incubation the incorporation of tritium from *myo*-[2-^{3}H]inositol in pectic polysaccharides and phosphatidylinositol was about half the value observed in the control experiment in which Con A was absent (*table 2*). The incorporation rates in the control experiment were consistent with earlier observations (HELSPER et al. 1986).

Table 2. Effect of Concanavalin A (200 μg/ml) on the incorporation of tritium from *myo*-[2-^{3}H]inositol in pectic polysaccharides and phosphatidylinositol by germinating pollen of *Lilium longiflorum*. Results are expressed as dpm incorporated during 4 hours of incubation with 11 × 10^6 dpm *myo*-[2-^{3}H]inositol.

Labeled fraction	Concanavalin A absent	present
	dpm	dpm
Pectic polysaccharides	1,177,500	450,000
Phosphatidylinositol	568,500	272,000

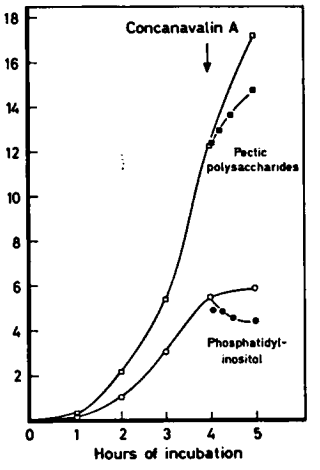

Fig. 2. Effect of Concanavalin A (200 μg/ml, closed symbols), added after 4 hours of preincubation with *myo*-[2^3H]inositol (11 × 10^6dpm) on the incorporation of tritium in pectic polysaccharides (□) and phosphatidylinositol (○) by pollen of *Lilium longiflorum*. Open symbols represent the incorporation in the absence of Concanavalin A.

Addition of Con A to pollen tubes, which were labeled with *myo*-[2-^{3}H]inositol for four hours in the absence of Con A, also resulted in a reduction with about 50% of the incorporation rate in pectic polysaccharides during the next hour of incubation (*fig. 2*). The tritium level in phosphatidylinositol decreased as response to Con A in a similar way as illustrated in *fig. 1a* for pollen tubes labeled with [2-^{3}H]glycerol.

4. DISCUSSION

In germinating pollen of *Lilium longiflorum* Con A (200 μg/ml) inhibits phospholipid turnover and biosynthesis of pectic polysaccharides. The inhibitory response to Con A is detectable within 5 minutes when the lectin is added to actively growing pollen tubes which were cultured for 4 hours without this lectin.

The efficient incorporation of [2-^{3}H]glycerol in lipids shows that pollen tubes of *Lilium longiflorum* are an appropriate system for the preparation of radioactive lipids, which are predominantly labeled in the glycerol moiety. The absence of tritium in the lipoidal products, formed by methanolysis of these lipids (J. P. F. G. HELSPER c.s. to be published), indicates the absence of label in the acyl chains. The decrease of tritium incorporation from [2-^{3}H]glycerol between 3 and 4 hours is probably due to saturation of the lipid-bound glycerol pool. Since the molar amounts of the different phospholipids hardly change throughout the incubation (HELSPER et al. 1986), their specific activity will remain almost constant beyond 4 hours.

The 50% inhibition at 200 μg Con A/ml, observed for the incorporation of *myo*-[2-^{3}H]inositol in pectic polysaccharides and of [2-^{3}H]glycerol in lipids is

in good agreement with the decrease in growth rate observed in *Lilium* pollen tubes as response to Con A (PIERSON et al. 1986, this issue). A similar inhibition of [^{3}H]inositol incorporation in pectic polysaccharides is observed when Con A is added after 4 hours of incubation in the absence of lectin. The latter situation more closely resembles that in which putative lectin-like components in the pistil may start to regulate pollen tube growth as part of the incompatibility response.

The shift in tritium distribution from phospholipids to 1,2-diacylglycerol, when Con A is added after 4 hours preincubation with [2-^{3}H]glycerol, cannot be explained by considering only incorporation rates. In fact the tritium level in lipids remains almost constant beyond 4 hours of incubation, both in the absence and presence of Con A. The accumulation of tritium in 1,2-diacylglycerol at the expense of phospholipids as response to Con A treament may be due to a general stimulation of phospholipase C activities. This seems not very likely because it would imply simultaneous stimulation of different phospholipases. A more acceptable explanation is that Con A inhibits phospholipid synthesis during the turnover cycle while degradation continues. As 1,2-diacylglycerol is a general phospholipid precursor (MUDD 1980) it will accumulate as result of such a process. Of course more experimental evidence is required to support this hypothesis.

Involvement of a 'phosphatidylinositol response' as described by BERRIDGE (1984) is also not probable. This type of response, which has not yet been described in plant tissues, involves the stimulus-induced increase in turnover of specifically phosphatidylinositol and its phosphorylated derivatives. Con A induces a change in the tritium level of all phospholipids after interaction with pollen tubes. Therefore the response of pollen tubes to Con A closely resembles that observed in homogenates of soy bean hypocotyls after treatment with the synthetic auxin 2,4-dichlorophenoxyacetic acid (MORRÉ et al., 1984). The ultimate result is in both cases a significant change in phospholipid composition of the membranes, which will probably influence the growth capacity.

A close relationship exists between pectin biosynthesis, organization of microfilaments, phospholipid turnover and pollen tube growth. The biosynthesis of precursors for pectic polysaccharides occurs inside dictyosome-derived secretory vesicles (RAY et al. 1976, VAN DER WOUDE et al. 1971). The transport of these secretory vesicles to the pollen tube tip is probably dependent on a structured organization of microfilaments (PICTON & STEER 1982). The intensive membrane flow in pollen tubes, which also involves production and transport of secretory vesicles and their fusion with the plasma membrane, requires a high turnover rate of membrane phospholipids (HELSPER et al. 1986). Interference of Con A with one of these processes will influence the others and also pollen tube growth. This may explain their collective reaction to Con A, which was described in this report and in an accompanying paper (PIERSON et al., 1986, this issue). Moreover, Con A may affect the incorporation of polysaccharide precursors in the cell wall, after their release from secretory vesicles, by interference with the appropriate glycosyltransferases. A similar mechanism has been suggested by HESLOP-HARRISON & HESLOP-HARRISON (1982) for the regulation

of pollen tube growth by lectin-like incompatibility factors.

In summary, the interaction of Con A with pollen tubes of *Lilium longiflorum* leads to reactions that strongly affect the growth rate (see also PIERSON et al. 1986, this issue) and thus the fertilization potency of sensitive pollen. Similar regulatory processes may occur in the incompatibilty response as result of pollen-pistil interactions.

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