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TOTAL AND POLAR LIPID BIOSYNTHESIS DURING GROWTH OF *CROTALARIA JUNCEA* POLLEN TUBES

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Key Word Index—*Crotalaria juncea*; Leguminosae; pollen tube growth; polar lipids; acetate incorporation; boric acid; cyclic-AMP.

Abstract—[1-^{14}C]-Acetate incorporation into total and polar lipids was studied in the growing pollen tubes of *Crotalaria juncea*. Ungerminated pollen had phosphatidyl inositol, phosphatidyl serine, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, monogalactosyl diglyceride, digalactosyl diglyceride, sulpholipid and steryl glycosides. In the growing pollen tubes considerable [1-^{14}C]-acetate incorporation was observed into the individual polar lipids. The exogenous carbon source significantly influenced lipid biosynthesis. Boric acid (20 mg/l.) promoted both pollen tube growth and acetate incorporation into phospholipids. In comparison to 5′-adenosine monophosphate, cyclic-3′,5′-adenosine monophosphate (cAMP) promoted tube growth and also enhanced phospho- and glycolipid biosynthesis. The regulation of membrane component biosynthesis by cAMP is suggested.

INTRODUCTION

Literature dealing with the lipid metabolism of pollen is scanty. Most of the studies have been directed towards pollen sterols [1] or identification of various polar lipid classes from the ungerminated pollen [2, 3]. A single report is available which demonstrated the incorporation of [1-^{14}C]-acetate into membrane lipids during pollen tube growth [4]. Using inhibitors of nucleic acid and protein synthesis these authors proposed that enzymes for the synthesis of these lipids were preformed in the ungerminated pollen. Since the growth of pollen tubes is an active phenomenon and involves active membrane biosynthesis, it may be accompanied by simultaneous synthesis of membrane components. Furthermore, the tube growth process is influenced by various growth hormones and other metabolites and hence it is a suitable system to study membrane lipid biosynthesis in relation to such treatments.

RESULTS

Data on pollen tube growth under various culture conditions are given in Table 1. *Crotalaria* pollen germinated and produced long pollen tubes even in distilled water, although tube length was greater in a basal medium. Addition of boric acid to water enhanced the pollen tube length considerably. Cyclic-3′,5′-adenosine monophosphate (cAMP) in a basal medium markedly enhanced tube length while 5′-adenosine monophosphate (5′ AMP) did not produce any noticeable effect.

The polar lipids from the ungerminated pollen separated by TLC comprised both phospho- as well as glycolipids. The phospholipids were phosphatidyl inositol (PI), phosphatidyl serine (PS), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) and phosphatidyl glycerol (PG). The glycolipids consisted of monogalactosyl diglyceride (MGDG), digalactosyl diglyceride (DGDG), sulpholipids (SL) and steryl glycosides (SG). There was no change in the various polar lipid components with pollen tube growth. Since there was overlapping of spots of PE with PG, and PS with MGDG, the radioactivity counts from these spots were pooled respectively.

Table 1. Effect of boric acid, cAMP and 5′ AMP on pollen tube length. Pollen were cultured in water or basal medium for 3 hr

Treatment	Tube length (μm)
Water (control)	120 ± 10
Water + boric acid (20 mg/l.)	270 ± 9
Water + cAMP (5 mg/l.)	193 ± 17
Water + 5′AMP (5 mg/l.)	120 ± 9
Basal medium (control)	345 ± 9
Basal medium + cAMP (5 mg/l.)	465 ± 13
Basal medium + 5′AMP (5 mg/l.)	375 ± 14

For determining the pollen tube length 100 observations were recorded and the standard error was computed from the means.

Data given in Table 2 indicate that a considerable amount of lipids were synthesized during pollen tube growth, both in water and in the basal medium. The incorporation of label into the total lipids was *ca* 3 times higher in the latter case. Addition of boric acid to water caused higher incorporation, in the total lipids. No significant change occurred with cAMP treatment in water, whereas in basal medium, cAMP enhanced lipid biosynthesis. 5′ AMP treatment produced an opposite effect on acetate incorporation compared to cAMP.

Table 2. Effect of boric acid, cAMP and 5′ AMP on [1-^{14}C]-acetate incorporation in total lipids. Data are given as cpm × 10^3 [1-^{14}C]-acetate incorporated/30 mg pollen/3 hr incubation

	Treatment	cpm × 10^3
	Water (control)	205
a	Boric acid (20 mg/l.)	230
a	cAMP (5 mg/l.)	201
a	5′AMP (5 mg/l.)	232
b	Basal medium (control)	641
b	cAMP (5 mg/l.)	835
b	5′AMP (5 mg/l.)	626

Two sets of experiments, a and b were conducted; in a water was used as culture medium and in b basal medium was used for pollen culturing.

In water-cultured pollen the incorporation of acetate was in the order: PS + MGDG > PI > SL > PC > DGDG > PE + PG > SG (Table 3). Basal medium cultures showed a higher incorporation in PS + MGDG, PE + PG, PC, SL and SG fractions (Table 4). A decrease in incorporation was observed in the case of PI and no marked change occurred in DGDG fraction. Addition of boric acid to water cultures enhanced the acetate incorporation into PC, PE + PG and PS + MGDG although the incorporation into PI, DGDG, SL and SG was essentially unaffected (Table 3).

Compared to a water control, the cAMP treatment increased incorporation into PC, PE + PG, PS + MGDG and SG fractions but it was lower in the PI, SL and DGDG fractions (Table 3). In contrast to this, cAMP when added to the basal medium increased the amount of labelling in the individual phospho- and glycolipids (Table 4).

Table 3 clearly shows that treatment with 5′AMP in water cultures resulted in higher synthesis of the PI and PE + PG fractions, PS + MGDG, DGDG and SL fractions showed a decrease, and there was no change in the case of PC and SG (Table 3). 5′AMP supplemented to the basal medium resulted in a generally decreased synthesis of polar lipids with the exception of PI, SG and SL fractions (Table 4).

DISCUSSION

The major polar lipid components in the ungerminated *Crotalaria* pollen were similar to those reported previously for *Petunia*, *Zea mays*, *Cucurbita maxima*, *Lilium lancifolium* and *Elacis guineensis* pollen [2–4]. The present data indicate no change in the various lipid classes in the ungerminated pollen and pollen tubes. Water-cultured pollen had limiting endogenous carbon reserves for lipid biosynthesis, as is evident from the much higher incorporation of label into basal medium-cultured pollen. Exogenous addition of sucrose could provide the carbon skeleton for lipid biosynthesis and this can be one of the reasons for the observed increase in the tube length. The higher acetate incorporation in the PI fraction in water cultures suggests that this lipid, beside being a membrane component, possibly also served as an inositol storage component, as has already been proposed by Stanley [7]. This is further supported by the decreased incorporation into the PI fraction in the basal medium. Incorporation studies into various polar lipid types also indicated membrane biosynthesis during pollen tube growth. MGDG and DGDG are major polar lipid components of the plastid membrane. The significance of their synthesis in pollen tube growth is not known since pollen/pollen tubes do not have active plastids.

Table 3. Effect of boric acid, cAMP and 5′ AMP added to water cultures on [1-^{14}C]-acetate incorporation in various polar lipid components. Data expressed as cpm × 10^3 [1-^{14}C]-acetate incorporated/30 mg pollen/3 hr incubation

	Treatment			
Polar lipid	Water (control)	Boric acid (20 mg/l.)	cAMP (5 mg/l.)	5′AMP (5 mg/l.)
PI	7.63	7.38	6.19	14.58
PC	3.20	6.14	4.53	3.33
PE*	1.23	3.14	2.38	3.83
+	1.48	3.61	2.87	4.22
PG	0.25	0.47	0.49	0.39
PS*	4.68	8.59	6.51	2.64
+	8.38	12.02	10.32	5.65
MGDG	3.70	3.43	3.81	3.01
DGDG	1.72	1.79	1.07	0.88
SL	5.02	4.46	3.52	2.61
SG	0.25	0.31	0.98	0.24

* Since there was some overlapping of spots in the case of PE + PG and PS + MGDG, counts from these spots were pooled respectively.

Table 4. Effect of cAMP and 5′ AMP added to basal medium cultures on [1-^{14}C]-acetate incorporation into various polar lipid components. Data expressed as cpm × 10^3 [1-^{14}C]-acetate incorporated/30 mg pollen/3 hr incubation

Polar lipid	Treatment		
	Basal medium (control)	cAMP (5 mg/l.)	5′ AMP (5 mg/l.)
PI	4.96	18.45	15.86
PC	8.14	17.66	6.09
PE*	8.30	14.53	5.85
+	10.81	17.75	7.42
PG	2.51	3.22	1.57
PS*	2.56	6.89	2.42
+	15.62	25.09	13.40
MGDG	13.06	18.20	10.98
DGDG	1.97	2.89	0.95
SL	6.37	7.97	8.77
SG	1.24	2.09	2.43

* As in Table 1.

Enhanced pollen tube growth in the presence of boric acid is generally attributed to increased sugar uptake and its utilization [8]. In our experiments boric acid did not produce any effect on glycolipids and PI, although it enhanced the synthesis of other phospholipid components. Shkol'nik and Kopmane [9] and Shkol'nik and Alekseeva [10] reported a decrease in phospholipids of sunflower, and flax and maize leaves respectively, under boron-deficiency conditions. Since boric acid is known to influence inositol metabolism, through the myoinositol pathway, this alternate channelization could account for the observed effect of boric acid on the PI fraction.

Effect of exogenous application of cAMP on pollen tube growth and even its control of some oxidoreductases during pollen tube growth have been reported [11]. Recently, cAMP has also been reported from pollen [12, 13]. The present experiments were designed to find out its role, if any, on lipid biosynthesis during pollen tube growth. To ascertain the specificity of this nucleotide its non-cyclic counterpart 5′ AMP was also used. The poor response of cAMP in water cultures may be due to the low availability of endogenous carbohydrates since cAMP is known to promote various oxidoreductases during *Crotalaria* pollen tube growth [14], thus utilizing the endogenous carbon reserves through other metabolic pathways. The effect of 5′ AMP on acetate incorporation into the total lipids is the reverse of cAMP and this effect of 5′ AMP may be indirect since 5′ AMP inhibited and/or had no effect on oxidoreductases [14]. Consequently more sugars were available to effect lipid biosynthesis. This also explains the increased incorporation in the PI fraction. 5′ AMP produced a variable response in the two media, hence it may not have any direct effect on membrane biosynthesis. cAMP, on the other hand, evoked a uniform response in the case of phospho- and glycolipids, at least in the basal medium cultures. Apparently, the effect of cAMP appears to be due to the specificity of its cyclic ring. The role of cAMP in membrane lipid biosynthesis in higher plant tissues has not been previously reported and the present study is the first information.

EXPERIMENTAL

Materials. [1-^{14}C]-Acetate was from the Radioisotope Division of BARC, Trombay (India); cAMP and 5′ AMP were from Sigma. Some lipid standards were purchased from Sigma and the Biochemical Unit of VP Chest Institute, New Delhi. *Crotalaria juncea* L. (sun hemp) was raised in the Botanical Gardens of Punjab Agricultural University, Ludhiana.

Culture conditions. Pollen (30 mg) collected from freshly opened flowers were cultured in sterilized Petri dishes containing H_2O alone or basal medium (3% sucrose + 20 mg/l. boric acid) at 28 ± 2°. Since pollen tube growth enters into the exponential phase of growth after 90 min of culturing, a 3 hr period was selected for recording the effect of different treatments on tube length and incorporation studies. Boric acid (20 mg/l.) was added to H_2O cultures only, whereas cAMP and 5′ AMP (5 mg/l. each) were supplemented both in H_2O and basal medium.

Incorporation studies. For the incorporation studies 1 μCi of [1-^{14}C]-acetate (sp. act. 46.15 mCi/mmol) was added per ml of the culture medium. Cultures were harvested after 3 hr and centrifuged at 3000 *g* for 15 min at 10° in a refrigerated centrifuge to remove the culture solns. The pellet was washed with H_2O until no residual radioactivity was left in the supernatants.

Lipid extraction and analysis. Pollen tubes were homogenized in $CHCl_3$–MeOH (2:1) for lipid extraction [5]. H_2O-soluble impurities were removed by using 0.9% NaCl soln. The $CHCl_3$–MeOH phase was subsequently evapd at low temp., and pure lipids were again dissolved in 3 ml of $CHCl_3$–MeOH (2:1). 1 ml of this prepn was taken for total lipid estimation and the remaining 2 ml were used for the detection of various polar lipid classes by Si gel G TLC using $CHCl_3$–MeOH–7 N NH_3 (65:25:4). Spots were visualized with I_2 vapours and marked. Identification of individual polar lipid components was achieved using different spray reagents and by comparison of R_f values with those of standards [6]. For measuring radioactivity, spots were eluted into the non-aq. toluene-based scintillation fluid

consisting of PPO(2,5-diphenyl oxazole) 3 g; POPOP(1,4-bis-2,5(phenyl oxazolyl benzene) 100 g and toluene, 1 l. The incorporation studies were repeated twice and the data represent observations from one such reproducible set of expts.

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