Phytochemistry, 1978, Vol 17, pp. 1185–1186. © Pergamon Press Ltd. Printed in England

0031-9422/78/0701-1185$02.00/0

SHORT REPORTS

MYO-INOSITOL CONTENT OF LILY POLLEN*

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(*Received* 25 *November* 1977)

Key Word Index—*Lilium longiflorum*; Liliaceae; Easter lily; pollen; germination; *myo*-inositol 1-phosphate synthase; *myo*-inositol.

myo-Inositol (MI) metabolism in germinated pollen of the Easter lily (*Lilium longiflorum* Thunb.) has been the subject of several recent studies ([1] and refs cited therein) but information on MI content and MI biosynthesis in this tissue is lacking. This paper examines the free MI content of resting and germinating lily pollen and the activity of MI-1-P synthase (EC 5.5.1.4) in cell-free extracts of lily pollen.

The free MI content was measured in 70% EtOH extracts of pollen that had been ground in a glass homogenizer using the same solvent as suspending medium. Extracts were fractionated on columns of powdered cellulose using Me_2CO–water to recover MI which was assayed by GLC as its *n*-butyl boronic acid ester. MI was measurable at levels as low as 0.05 mg per g of pollen. Data representative of lily pollen samples used in our metabolic studies are given in Table 1. Some data obtained on pollen from other sources are also included in this table. All had free MI contents ranging from 1 to 6 mg per g of pollen, values similar to that found in *Alnus* [2]. Grass pollens, including corn (*Zea mays*), are reported to have substantially higher MI contents [2, 3]. With regard to samples taken from stored pollen, Nielsen [4] showed that the MI content was unaffected by prolonged storage.

Table 1. Free *myo*-inositol content of selected pollens

Source	Collection date	*myo*-Inositol content (mg/g of pollen)
Pinus lambertiana	1966	5.3
Fraxinus americana	1966	6.3
Pyrus communis	1966	6.4
Malus	1967	4.5
Typha latifolia	1976	2.3
Lilium longiflorum		
cv Ace	1974	6.0
cv Ace	1976	1.3
cv Nellie White	1976	3.6 ± 1.0*

* Average value of 7 samples.

During pollen germination, endogenous sources of free MI are rapidly depleted. A sample of Nellie White lily pollen showed a decline in free MI from 3.3 mg/g of pollen in the resting state to 0.3 mg/g after 12 hr of tube growth in pentaerythritol medium that was devoid of metabolizable carbohydrate. In a separate experiment, it was determined that most of this loss took place during the first 6 hr of germination. Dickinson [5] has noted depletion of carbohydrate reserves and cessation of starch formation when lily pollen is germinated in pentaerythritol beyond 6 hr.

Lily pollen contains MI-1-P synthase, the enzyme that converts D-glucose-6-P to MI-1-P [6, 7]. The 30–90% $(NH_4)_2SO_4$-precipitated fraction from resting pollen contains about 50 pkat per g of pollen. As seen in Fig. 1a, little change occurs in synthase activity during germination. This observation was confirmed with Ace lily pollen gathered in 1977. In the latter study, synthase activity remained unchanged in the 30–60% $(NH_4)_2SO_4$ fraction over a 6 hr germination period.

An acid phosphatase (EC 3.1.3) is also present in resting pollen and exhibits increased activity during germination (Fig. 1b). Whether this enzyme resembles the Mg^{2+}-dependent phosphatase that has been found with MI-1-P synthase in *Acer* [6] is not known.

Calculations based on total MI-1-P synthase activity in lily pollen indicate a synthetic capacity of less than 1 μmol of MI-1-P per g of pollen during the first 6 hr of germination. Newly synthesized MI can only account for a few percent of the free MI utilized during this period. Biosynthesis of MI from D-glucose-6-P seems to have an insignificant role as a source of MI for oxidative conversion to uronsyl and pentosyl constituents of pollen tube wall polysaccharides during early stages of pollen tube development but may contribute to such processes in later stages when pollen tube elongation leads to dependence on nutritional sources in the pistil [8].

EXPERIMENTAL

Pollen sources. L. longiflorum, cv Ace, 1972 and 1974, was

* This work was supported by Grants No. GM-12422 and GM-22427 from the National Institute of General Medical Sciences, National Institutes of Health, US Public Health Service. Scientific paper no. 4708, project 0266, College of Agriculture Research Center, Washington State University, Pullman.

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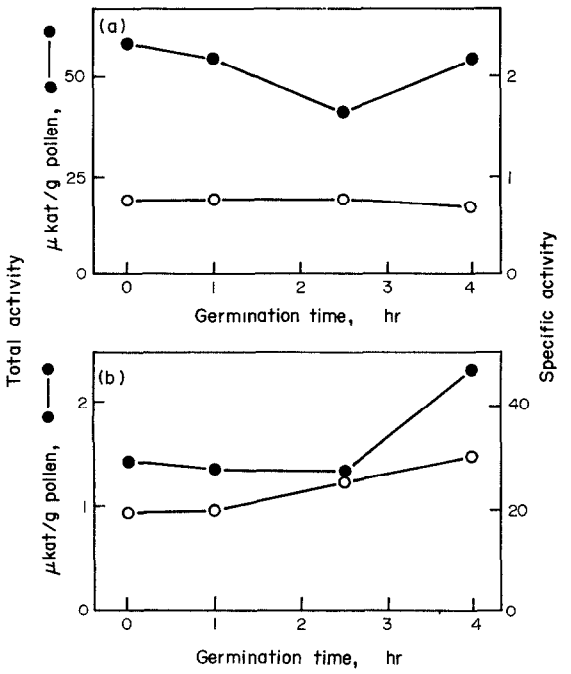

Fig. 1. *myo*-Inositol-1-P synthase (a) and acid phosphatase (b) activities in the 30–90% sat. $(NH_4)_2SO_4$ fraction from extracts of resting and germinated lily pollen (1 g).

harvested from commercial greenhouses. *L. longiflorum*, cv Ace, 1976 and Nellie White, 1976 and 1977, were harvested from field-grown flowers in Brookings, Oregon. Anthers were removed from buds 1 day prior to anthesis and air-dried for 3 days at 20°. Pollen was recovered on a No. 10 stainless-steel sieve, loosely packed in perforated 20 ml polyethylene vials and stored at 4°. Pollen from *Typha latifolia* L. (common cattail), 1976, was gathered locally. Other pollens listed in Table 1 were stored samples.

Recovery of MI. Pollen (0.5 g) was ground in 70% EtOH (5 ml) in a motor-driven Kontes Duall glass homogenizer. Residues were resuspended in fresh solvent and the process repeated twice. To combined extracts was added a trace amount of MI-[U-^{14}C] (50000 cpm, 324 Ci/mol) to monitor experimental losses during subsequent steps. After removal of EtOH in a rotary evaporator, the aq. soln was deionized by passage through columns of Dowex 50 (H^+) and Dowex 1 (formate) resins. Effluent and wash were combined, evapd to 1 ml and loaded on a column of powdered cellulose (Whatman CF-11, 30 × 1 cm) that had been prewashed with Me_2CO-H_2O (9:1). The column was eluted with the same solvent mixture (50 ml) followed by Me_2CO-H_2O (4:1) which removed MI. Fractions containing MI were dried, dissolved in Py containing *n*-butyl boronic acid (5 mg/ml), heated at 100° for 20 min, cooled and assayed by GLC for the MI-*n*-butyl boronate [9]. On electronic integration the MI content of each sample was determined from a standard curve.

Enzymic activity. Pollen (1 g) was germinated in pentaerythritol-based medium [5]. Germinated pollen was recovered by filtration on Miracloth and then ground at 0° in 20 mM Tris–HCl buffer (30 ml), pH 8, containing 0.5 mM GSH. Samples were centrifuged at 20000 *g* for 1 hr at 5°. Fractionation of the supernatant fluid with $(NH_4)_2SO_4$ (0.16 g/ml) pptd 15% of the MI-1-P synthase activity. The remaining activity was pptd by additional $(NH_4)_2SO_4$ (0.4 g/ml), the 30–90% fraction. Dialyzed prepns were assayed for soluble protein [10], MI-1-P synthase [7] and acid phosphatase [11].

Acknowledgements—We thank Ms. Mary Ann Mitchell for technical assistance, Dr Mary W. Loewus for confirmatory studies on synthase activity in germinated pollen, and Mr Walt Schroeder, Gold Beach, Oregon and Dr Al Roberts, Oregon State University, Corvallis, for helpful advice. Permission to harvest lily flowers in commercial fields was given by Mr A. G. Tony Rose, Mr Pete Cowan, and Mr Albert Nelson, all of Brookings, Oregon.

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