Dimethyl Sulfoxide: Reversible Inhibitor of Pollen Tube Growth

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Abstract. Five percent dimethyl sulfoxide (DMSO) completely inhibited tube initiation, stopped tube growth and suppressed the high respiration associated with tube growth of lily pollen. The effect of DMSO on respiration was indirect because uncoupling concentrations of 2,4-dinitrophenol abolished the inhibition of respiration. Five percent DMSO did not inhibit rapid starch synthesis during the first 30 minutes of incubation, nor did DMSO inhibit the period of high respiration associated with rapid starch synthesis. DMSO did not cause permanent damage to the cells since normal pollen tube growth occurred after its removal. Dimethyl sulfoxide is not a general inhibitor of pollen metabolism, but it may be a specific inhibitor of a process required for tube growth.

While determining whether dimethyl sulfoxide (DMSO) protected lily pollen from freezing damage, we observed that pollen tube initiation did not occur when DMSO was present in the culture medium but that tube growth occurred after the DMSO was removed. It seemed likely that one or more metabolic steps leading to pollen tube initiation were reversibly inhibited by DMSO. The sequence of reactions required for pollen tube initiation and subsequent tube growth is not known. Identifying the reactions blocked by DMSO might provide information concerning the regulation of tube growth in germinating pollen. Furthermore, DMSO might become generally useful in studies of growth if it inhibits a particular reaction associated with plant cell elongation.

The effect of DMSO on cell growth and development has received relatively little attention. Sciuchetti et al. (10) reported that Datura seedlings which had received several applications of 2% aqueous DMSO were slightly taller than control plants. Proliferation in vitro of fibroblasts is not affected by 1% DMSO, but 3% and 5% reduce proliferation (1). Similarly, 1% DMSO has relatively little effect on HeLa cells, but 3% and 4% inhibit nucleic acid synthesis and cause 95% loss of viability after 70 hours of exposure (8).

The experiments reported here were conducted to obtain quantitative data on pollen tube initiation and tube growth as affected by DMSO. Inhibition of pollen tube initiation by DMSO did not seem to be accompanied by a general inhibition of cellular metabolism.

Materials and Methods

Pollen from Lilium longiflorum, variety Ace, was used in all experiments. Anthers were taken each morning from freshly opened flowers, allowed to dehisce and dry under a microbiological hood for about 10 hours, and then stored at 2° until use.

The standard culture medium (pH 5.2 in the presence or absence of DMSO) contained 0.29 mM sucrose, 1.27 mM Ca(NO₃)₂, 0.16 mM H₃BO₃, 0.99 mM KNO₃, 3.0 mM KH₂PO₄, and 10 µg/ml tetracycline. All pollen germination experiments were conducted at 30°. In experiments where DMSO (Baker Chemical Company) was added to the standard culture medium, the final concentration of DMSO in the medium is expressed as percent (v/v). A freshly opened bottle of DMSO was used for experiments reported here. The bottle was stored at 2° during the 3 month period in which the experiments were done.

Pollen (10 mg) and culture medium (1.7 ml) were placed in 25 ml Erlenmeyer flasks during studies of the effect of DMSO on tube growth. Average tube lengths and percent germination were determined photographically (3). The flasks were incubated on a water bath shaker. Aliquots were taken from the flasks and photographed as germination proceeded.

Respiration was measured manometrically, and each 15 ml Warburg flask contained 10.0 mg pollen and 1.4 ml culture medium in the main compartment. Materials to be tipped in during the experiment were placed in the side arm with 0.3 ml culture medium. Side arms of control flasks received culture medium only. 2,4-Dinitrophenol (DNP) was added in 0.01 ml of stock solution. Adding the same volume of water to controls did not affect

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PLANT PHYSIOLOGY

TUBE LENGTH (mm)

0 0.2 0.4 0.6 0.8 1.0 1.2 1.4

TIME (HOURS)

0 1 2 3 4 5

CONTROL

0.5% DMSO

5% DMSO ADDED AT 2 hr

1% DMSO

5% DMSO INITIALLY, DILUTED TO 1% DMSO AT 2 hr

% GERMINATION

0 10 20 30 40 50 60 70

TIME (HOURS)

0 1 2 3 4 5

CONTROL

0.5% DMSO

1% DMSO

5% DMSO ADDED AT 2 hr

5% DMSO INITIALLY, DILUTED TO 1% DMSO AT 2 hr

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pollen respiration or tube growth in preliminary experiments. The Warburg flasks were shaken continuously at 30° and readings were taken at 5 or 10 minute intervals. Flasks were placed in ice at the end of respiration experiments. At that time aliquots were removed and photographed to obtain growth data.

The study of starch accumulation was similar to the growth study described above except that each Erlenmeyer flask contained 1.3 ml of culture medium. Hot ethanol was added to each flask after a predetermined incubation time to give a final concentration of 80% (v/v) ethanol (5). Groups of 5 replicate flasks were pooled, and a starch fraction was determined according to Hassid and Neufeld (7). Starch was measured with anthrone reagent using glucose standards, and the data were calculated as µg glucose/mg pollen.

Absorption spectra of the starch-iodine complex were obtained by Krismann's procedure (9). Spectra were obtained with a Beckman DB-G spectrophotometer which scanned at 50 mµ/min and at a constant band width of 0.2 mµ. Cells having 1 cm light paths were used. α-Amylase (type II-A, 1100 units/mg, from Sigma) was used to digest a sample of starch prior to adding iodine. The reaction mixture initially contained 226 µg starch, 366 amylase units/ml and 13 mm phosphate buffer (pH 6.9) in a total volume of 0.6 ml. The mixture was incubated 15 minutes at 20°, and the reaction was stopped by immersion in boiling water.

Results and Discussion

Preliminary experiments of several hours duration showed that DMSO at 1% or less had little or no effect on lily pollen germination, while 5% and 10% prevented pollen tube initiation. No tube initiation occurred when pollen was incubated as long as 24 hours in 5% DMSO. Percent germination and rate of tube elongation were progressively reduced by concentrations between 1% and 5%. Pollen produced normal tubes after incubation in 5% DMSO if the grains were rinsed on a suction filter and then resuspended in culture medium which lacked DMSO. However, the transfer of pollen from filter paper to fresh culture medium was not quantitative, and some pollen grains were ruptured.

The effects of various concentrations of DMSO on average tube length and percent germination are shown in figure 1. One percent DMSO inhibited tube elongation slightly while 0.5% DMSO had no effect. These concentrations did not affect percent germination adversely. There was no pollen tube initiation in 5% DMSO. Furthermore, tube elongation was markedly inhibited when 5% DMSO was added to rapidly growing pollen tubes. Reversibility of the DMSO effect on tube initiation is also shown in figure 1. Samples of pollen were incubated in 5% DMSO for 2 hours; then the concentration was reduced to 1% by dilution. After dilution, the time course of pollen tube initiation and the rate of tube elongation resembled that of the controls except that the abscissa was displaced 2 hours.

The effect of DMSO on respiration is shown in figure 2. Average tube lengths and percent germination were measured at 180 minutes when the respiration experiment terminated. Control flasks exhibit the typical respiratory pattern which was reported earlier (2). The initial period of high respiration is associated with starch synthesis while the second period of high respiration is associated with pollen tube growth (5). The pattern of growth and respiration of pollen germinating in 1% DMSO was similar to that of the control. An initial period of high respiration was also exhibited by pollen incubated in 5% DMSO, although the rate of O2 uptake was somewhat less than in the control. Transition to a lower rate of O2 uptake occurred at 30 minutes in all cases, and in 5% DMSO the rate remained low.

The low rate of O2 uptake imposed by 5% DMSO was relieved by DNP. The DNP-stimulated rate in control flasks was only slightly greater than the rate observed in the presence of 5% DMSO (fig 2). Hence, respiratory pathways were not directly inhibited by DMSO, and the low rate of respiration was probably caused by limited availability of ADP. Respiratory quotients (R.Q.) were measured in another experiment. Inhibition of O2 uptake by 5% DMSO was accompanied by a comparable inhibition of CO2 production, since the R.Q. remained about 1 the same as in control flasks which lacked DMSO. The effect of DMSO contrasts with that of oligomycin which inhibits O2 uptake but stimulates CO2 output by lily pollen to give R.Q. values as great as 3 (4).

Fig. 1. Effect of DMSO on pollen germination. Tube lengths (above), and percent germination (below) are data from a single experiment. Each point is an average from a 10 mg sample of pollen incubated in 1.7 ml of culture medium. The averages for percent germination represent at least 100 pollen grains. The averages for tube lengths represent at least 8 tubes at the 1 hour interval and at least 65 tubes at 2 and 3 hours. One control (—□—) contained standard culture medium, and the other control (—○—) contained culture medium diluted 10% with water. Four volumes of culture medium lacking DMSO were added at 2 hours to the samples labelled (—▲—).
Since the initial period of high respiration is associated with rapid starch synthesis (5), the initial high respiration in 5% DMSO indicated that considerable starch synthesis occurred even though tube growth was prevented. Accordingly, pollen was incubated in the presence or absence of 5% DMSO, and starch content was measured (Fig. 3). Growth data were obtained from replicate samples (Fig. 3). During the first 30 minutes, starch accumulated at similar rates whether or not pollen was incubated in 5% DMSO. After 30 minutes there was a progressive decrease in rate of starch accumulation in both treatments, but the decrease in the DMSO medium was more pronounced.

I₂-KI was added to a portion of the starch isolated at 3 hours (no DMSO in culture medium), and an absorption spectrum was obtained. A broad absorption maximum occurred at about 540 mµ (Fig. 4). Incubation with α-amylase completely destroyed the ability to form a colored complex with iodine. Calcium increased the optical density 3.6-fold and shifted the absorption maximum to about 520 mµ. The properties of the starch were not altered by germination in DMSO, since similar spectra were obtained with starch isolated from pollen incubated 3 hours in 5% DMSO. The absorption spectra and the Ca effect are quite similar to results obtained with amylpectin (9) and a branched polysaccharide from potato tubers (6). A similar starch sample (3 hr, no DMSO) was incubated with α-amylase (915 units/ml, 5 hrs), and ethanol was added. Almost all (98%) of the starch fraction became soluble in 80% ethanol,
indicating the predominance of α-1,4-glucosidic linkages.

DMSO is not a general inhibitor of pollen metabolism, but it is capable of inhibiting pollen tube initiation and pollen tube growth. DMSO concentrations adequate to prevent tube initiation do not permanently damage the cells as indicated by tube initiation and rapid tube growth after removal or dilution of the DMSO. The respiration data are consistent with the idea that 5% DMSO inhibited reactions which utilize high energy phosphate and that there was no direct effect on respiratory control or oxidative phosphorylation of pollen mitochondria. DMSO may reversibly inhibit reactions necessary for tube initiation and tube growth. However, if DMSO penetrates slowly, the observed inhibition of growth may be an osmotic effect. It is of interest that pollen accumulated considerable starch in the presence of a concentration of DMSO that prevented tube initiation.

DMSO may become a useful tool in studies of plant cell elongation. Experiments with intact cells and cell fractions are needed to learn how rapidly DMSO penetrates the cells and whether DMSO specifically inhibits any of the synthetic events that occur during lily pollen germination.

Literature Cited