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EFFECT OF METHANOL ON PHOSPHOLIPID COMPOSITION OF GERMINATING COTTON SEEDS

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The effect of methanol on the change of phospholipid composition of germinating cotton seeds was studied. It was shown that adding methanol to the germination medium caused the formation of the new phospholipid phosphatidylmethanol.

Key words: phospholipids, germination, methanol, phosphatidylmethanol.

Inositol is one of the metabolic products of cotton seed germination and is formed by hydrolytic cleavage of phytin by an activated form of phytase [1]. Inositol formed from phytin should participate directly in exchange reactions with cotton phospholipids and replace alcohols in the polar part of phospholipids to form phosphatidylinositol (PI) and the corresponding alcohols. We established previously that methanol was the best alcoholic substrate of phospholipase D in model experiments and that it can compete with inositol in transalkylation reactions. Phosphatidylmethanol, a phospholipid atypical of the natural specimens including cotton, was formed [2]. The formation of phosphotidylmethanol was accompanied by suppression of PI synthesis under various conditions [3, 4].

It is known that methanol is not involved in phospholipid synthesis through the reaction of alcohols with cytidine diphosphoacylglycerol [5]. Its capability to compete with inositol enables it to be used as a unique "tool" to detect the possible synthesis of PI in transalkylation reactions of phospholipids catalyzed by phospholipase D if this in fact occurs during the natural development of seeds, for example, during their germination when a significant quantity of inositol is formed from phytin [1]. Therefore, our goal was to investigate the possible synthesis of phosphatidylmethanol during germination of cotton seeds in media containing methanol.

Our experiments established that methanol under *in vivo* conditions is the best alcoholic substrate and is capable of competing in transalkylation reactions with inositol. Phosphatidylmethanol, which is not encountered in the plant itself, is also formed in germinating seeds.

Table 1 lists the experimental changes of content for the principal phospholipid components of cotton seeds during germination in water and in a medium of aqueous methanol (1%).

Germination in water was accompanied by extensive biosynthesis of all studied phospholipids. Thus, the content of phosphotidylcholine (PC) increased by 22%; of phosphatidylethanolamine (PEA), by 5 times; PI, by 7 times; and phosphatidic acid (PA), by 50 times. Adding methanol to the germination medium caused formation of the new phospholipid phosphatidylmethanol (PM), the content of which reached a maximum on the third day of growth and then decreased. The contents of the other phospholipids did not increase under these conditions. The contents of PC and PEA in 5-day sprouts decreased by 3 times compared with the norm; of PI, by 6 times; of PA, by 50 times.

Figure 1 shows that germination in medium containing methanol also changed the total amount of phospholipids. Thus, this index increased 4 times in control specimens on the eighth day of growth. In the presence of methanol, it not only did not increase but also decreased by 30%. The decrease was accompanied by a redistribution of the relative content of the studied phospholipids. Thus, the relative content of PEA on the third day of growth was 8.1% as compared with 11% in the control; PI, 18.2 and 34.4; PA, 5.8 and 16.7%. The PM content increased and reached 30.6% of the total phospholipids.

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TABLE 1. Change of Phospholipid Content During Germination of Cotton Seeds in water and in Medium with Methanol (1%) (μg P Per Single Plant)

Specimen	PC	PEA	PI	PA	PM
Dormant seeds	1.47 ± 0.039	0.13 ± 0.003	0.47 ± 0.011	0.05 ± 0.001	-
1-Day sprouts	1.80 ± 0.045	0.31 ± 0.018	0.81 ± 0.029	0.17 ± 0.005	-
	1.73 ± 0.041	0.25 ±0.007	0.49 ± 0.012	0.09 ± 0.005	0.18 ± 0.004
3-Day sprouts	1.80 ± 0.046	0.52 ± 0.016	1.63 ± 0.040	0.79 ± 0.021	-
	1.48 ± 0.039	0.32 ± 0.009	0.72 ± 0.019	0.23 ± 0.006	1.21 ± 0.031
5-Day sprouts	1.82 ± 0.043	0.65 ± 0.017	3.32 ± 0.092	2.49 ± 0.069	-
	0.74 ± 0.020	0.29 ± 0.008	0.57 ± 0.014	0.05 ± 0.001	0.58 ± 0.017

*Numerators are values obtained for germination of cotton seeds in water; denominators, in methanol (1%) (averages of three experiments with 100 seeds each).

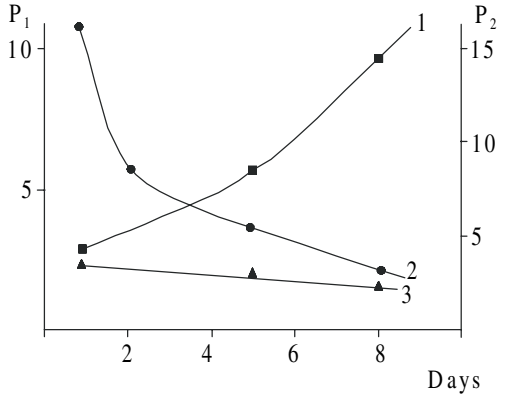

Fig. 1

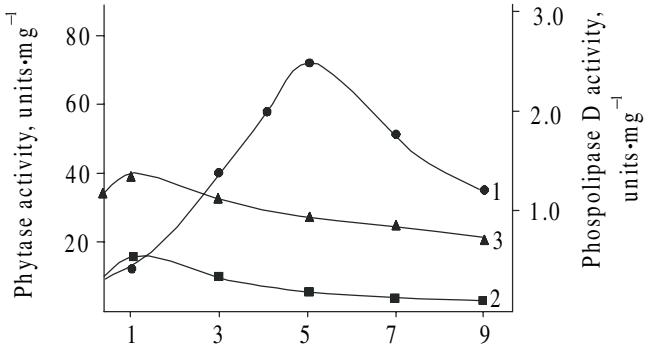

Fig. 2

Fig. 1. Phospholipid content P of cotton sprouts. Total phospholipids P of sprouts germinated in water (μg per g dry wt., P_1) (1); the same calculated per single plant (P_2) (2); phospholipids P of sprouts germinated in medium with methanol (1%) calculated per single plant (P_2) (3).

Fig. 2. Phytase and phospholipase (3) activities of germinating seeds in water (1, 3) and methanol (1%) (2).

Significant changes in the sprouts were also observed during germination in the medium with methanol. The length of 5-day rootlets was 40% of the control. The biomass accumulation decreased by 50%. Development was completely inhibited during the subsequent days.

The activities of phytase and phospholipase D were also investigated during germination in the medium with methanol. Methanol prevented the activation of phytase (Fig. 2, 2) and had no significant effect on the hydrolytic activity of phospholipase D (Fig. 2, 3). Under these conditions, the activity of phytase was almost completely inhibited. This affected the overall condition of the plants, due to which the phospholipid exchange decreased, the transferase function of phospholipase D was suppressed, and the exchange was disrupted.

Hence it can be said that methanol also inhibited PI synthesis during germination of seeds through a transalkylation reaction catalyzed by phospholipase D.

Previous results [2] and those of this work suggest that extensive biosynthesis of PI was observed during germination of cotton seeds (Table 1). It is possible that endogenous phospholipase D is involved in this process.

Adding methanol to germination medium of cotton seeds inhibited substantially the biosynthesis of the studied phospholipids. The PI content in 5-day sprouts was less than in the control (Table 1). Also, extensive accumulation in seeds of PM was noted.

Thus, methanol is capable of competing with inositol in a transferase reaction to form unnatural phosphatidylmethanol. In our opinion, the most important result is the fact that methanol in plants can act as substrate for forming a new phospholipid and that the reaction is catalyzed by phospholipase D.

EXPERIMENTAL

We investigated seeds and sprouts of cotton (*Gossypium hirsutum* L.) variety 108-F. Experiments were carried out under laboratory conditions. Seeds were germinated by the previous method [1]. The phospholipid composition was determined by micro-TLC [6]. Phospholipids were separated using two-dimensional micro-TLC and the following solvent systems: $CHCl_3$:CH_3OH:NH_4OH (25%) (65:25:5, 1), $CHCl_3$:$(CH_3)_2CO$:CH_3OH:CH_3CO_2H:H_2O (100:40:20:20:10, 2). Chromatographically pure PC, PI, and PM and PA synthesized using radish PL-D were used as standards [7, 8]. Phospholipid components on chromatograms were detected using known reactions [6]. Isolated phospholipids were analyzed quantitatively on the basis of P content [9].

Phytase activity of cotton seeds and sprouts was determined as before [1]; phospholipase D activity, from the amount of choline released by hydrolysis of phospholipids by the literature method [10]; transferase activity of PL-D, from the rate of transalkylation of PC in the presence of methanol, inositol, or other alcoholic substrates.

The standard reaction mixture (total volume 2.5 mL) contained PC (5 mM), absolute methanol (4-16%), $CaCl_2$ (30 mM), silica gel (40 mg), sodium acetate buffer (200 μM), pH 5.6, and enzyme preparation (50 mg) and was incubated at 30°C for 20 min. The course of reactions was monitored by two-dimensional TLC [1].

REFERENCES

1. M. M. Rakhimov, M. M. Abdullaeva, M. N. Valikhanov, and M. U. Babaev, *Physiol. Rast.*, **33**, No. 6, 1121 (1986).
2. M. M. Abdullaeva, *Khim. Prir. Soedin.*, 139 (2004).
3. M. M. Rakhimov, Sh. R. Mad′yarov, and M. U. Babaev, *Uzb. Biol. Zh.*, No. 3, 7 (1979).
4. W. Frank, T. Munnik, K. Kerkmann, F. Salamini, and D. Bartels, *Plant Cell*, **12**, 111 (2000).
5. A. L. Lehninger, *Biochemistry*, 2nd Ed., Worth, New York (1975).
6. M. Kates, *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*, Elsevier, New York (1973).
7. R. Haftendorn, G. Schwarze, and R. Ulbrich-Hofmann, *Chem. Phys. Lipids*, **104**, 57 (2000).
8. M. M. Rakhimov, R. Akhmedzhanov, M. U. Babaev, V. Kkhole, Sh. R. Mad′yarov, and B. A. Tashmukhamedov, *Biokhimiya*, **46**, No. 2, 240 (1981).
9. I. Kahovcova and R. Odavic, *J. Chromatogr.*, **40**, 90 (1969).
10. M. M. Adbullaeva, M. M. Mulladjanova, and M. M. Umarova, *Turk. J. Biol.*, **26**, No. 4, 241 (2002).