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The Role of Phospholiphase D in Phospholiphid Transaction During Cotton Seeds Maturation

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Abstract: The role of phospholipase D (EC 3.1.4.4) was studied in the changing of phospholipid content during cotton seed (Gossipium hirsutum 108-G) maturation.

To define phospholipase D activity cotton seed homogenates were incubated in a water-methanol environment at 37 °C for various time intervals (15, 30, 60, 120, and 300 min). Enzyme activity was judged from the formation of hydrolysis products - phosphatidic acid and phosphatidylmethanol. The phospholipid content was defined by using thin-layer chromatography with a follow up analysis of phosphorus quantity in phospholipid fractions.

It was shown that phospholipase D displayed two functions during cotton seed maturation: transferase and hydrolase. Hydrolase function could be defined from the increase of the product (phosphatidic acid) concentration during the complete maturation of the seed. We noticed that the concentration increased. Transferase activity could be defined through the increase of the phosphatidylmetanol concentration during the incubation of phospholipids in a water-methanol environment. The experimental data showed that phospholipase D plays an important role in phospholipid metabolism.

Key Words: Phospholipase D, Phospholipids, Cotton seeds.

Introduction

Much research has been conducted to investigate the phospholipid metabolism process and its enzymatic aspects (1-12). This process is very important in cell membrane regeneration and formation.

As a result of a great deal of research, it was identified that quality and quantity changes took place in the structure of phospholipids during plant seed development because they undergo hydrolysis by phospholipase D, which is capable of acting as hydrolase and transferase (1,4-6, 12). Investigating the phospholipid metabolism in cabbage leaves, Dawson found that phospholipase D could act as transferase, i.e. it catalyses the reaction of transalkylation - the transaction of alkyl residues between phospholipids (2). These aspects of phospholipid metabolism have been studied in many plants, though little work has been done on seed maturation in cotton.

During cotton seed maturation, phospholipase D acts as hydrolase (5), but the transferase function of this enzyme has not been studied.

After investigating the phospholipid metabolism, we identified that the main cotton seed phospholipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and the product of their hydrolysis - phosphatidic acid (6-8).

It was also shown that phospholipase D catalyses the reaction of phosphatidylinositol formation from the product of phytin degradation - inositol and the substitution reaction of the alkyl residues of the phospholipids (choline and ethanolamine) by inositol (6). In this reaction the phospholipase D transferase effect plays a very important role. It was interesting to study the dynamic of phospholipid changes and the role of phospholipase D in the process of cotton seed maturation.

In this work, we show hydrolase and transferase functions of phospholipase D and its role in the phospholipid metabolism during cotton seed maturation.

Materials and Methods

To define the role of phospholipase D (which belong to class 3 hydrolases, to subclass etherase (EC 3.1.4) and catalyses the reaction of the hydrolytic splitting of phosphoglycerides (EC 3.1.4.4)) cotton (*Gossypium hirsutum*, *108-F*) seeds grown by a common-adopted method were used in this research (9). The seed samples were selected at ten-day maturation intervals. Maturing

seeds were taken from cotton buds and then homogenised.

For the identification of phospholipids, seeds homogenates were incubated in a water-methanol (50:1) environment at 37 °C for various time intervals (15, 30, 60, 120, 300 min). Then homogenates were mixed with a solution of 2 ml methanol and 4 ml chloroform and were left for 130 min at room temperature under constant blending. The homogenates were then filtrated and the residue was washed three times by a 2:1 chloroform-methanol mixture. Then 5 ml of 0.2% $CaCl_2$ was added to the extracts and blended. After stratification, the supernatants were extracted again by 2 ml of chloroform. The chloroform extracts were unified and their volumes were increased to 20 ml. Then they were fully evaporated to dryness. The residues were dissolved in 16 ml of cooled acetone and then centrifuged. Enzyme activity was identified by the formation of the hydrolysis product - phosphatidic acid or phosphatidylmethanol. The residue was dissolved in 0.2 ml chloroform and phospholipid content was determined by double-dimension thin-layer chromatography with a following analysis of phosphorus quantity in every phospholipid fraction (10). For this thin-layer plates with silicagel KSK-2 were used and as solution system the following solutions were used: chloroform-methanol-25% ammonium hydroxide (65:25:5 in volume) and chloroform-methanol-acetone-acetic acid-water (relation 100:20:40:20:10).

The phospholipids were identified by using as control phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol from cotton seeds (11). The phosphatidic acid was obtained as pure phosphatidylcholine by using phospholipids from the rootlets of Middle Asian radish (12).

Results and Discussion

Statistical analyses

The investigations were carried out in two biological replicates to three determinations in each replicate. The average arithmetical data and the deviations from the average of the three determination are given in diagrams.

Figures 1-4 present the changes in phospholipid content as a result of endogenie phospholipase D action. These dates show that during the incubation of the plant seed homogenates in a water environment over exact periods there take place various changes in the quality content of phospholipids. For example, it was identified that the accumulation of phosphatidylinositol increased during the incubation of the homogenates of seeds and by 30 min it reached a maximum in all variations, from day 10 to 70. Then the constant volume of phosphatidylinositol decreased and after 5 h of incubation it was only 10%. Concerning the other phospholipids, it was identified that the content volume of phosphatidylcholine decreased 60%, and phosphatidylethanolamine 64% in the 10 days of

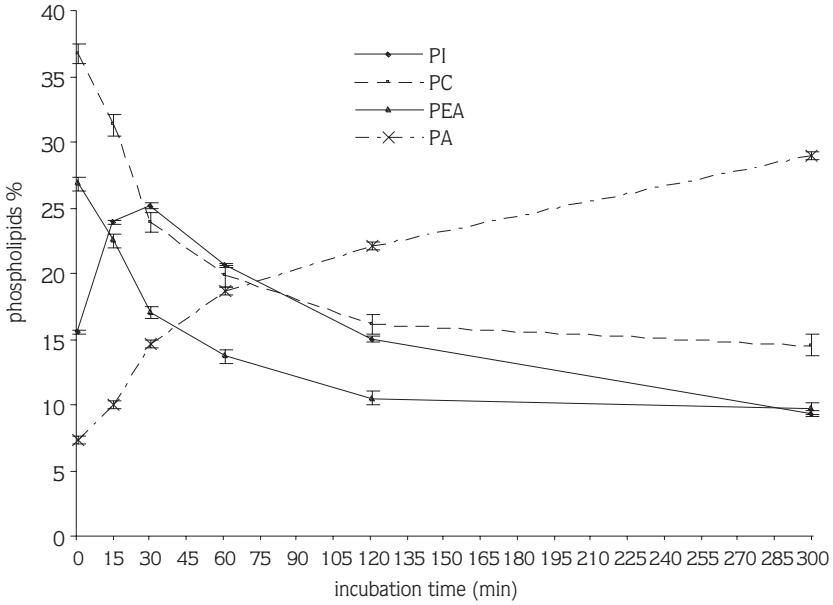

Figure 1. The changes of main phospholipids in cotton seeds under the influence of endogenic phospholipase D on day 10 of maturation

PI-phosphatidylinositol
PC-phosphatidylcholine
PEA-phophatidyletanolamine
PA-phosphatidic acid
PM-phophatidylmethanol

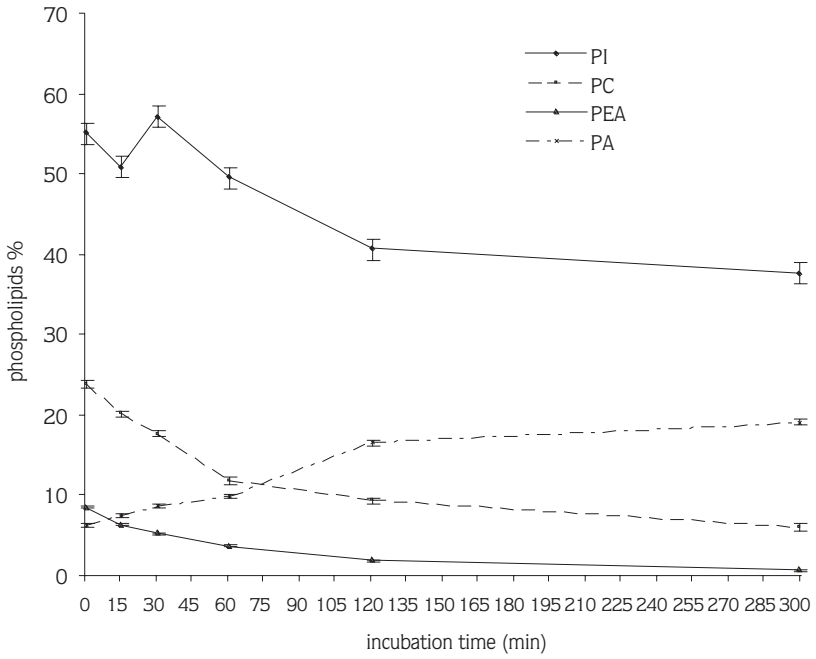

Figure 2. The changes of main phospholipids in cotton seeds under the influence of endogenic phospholipase D on day 30 of maturation

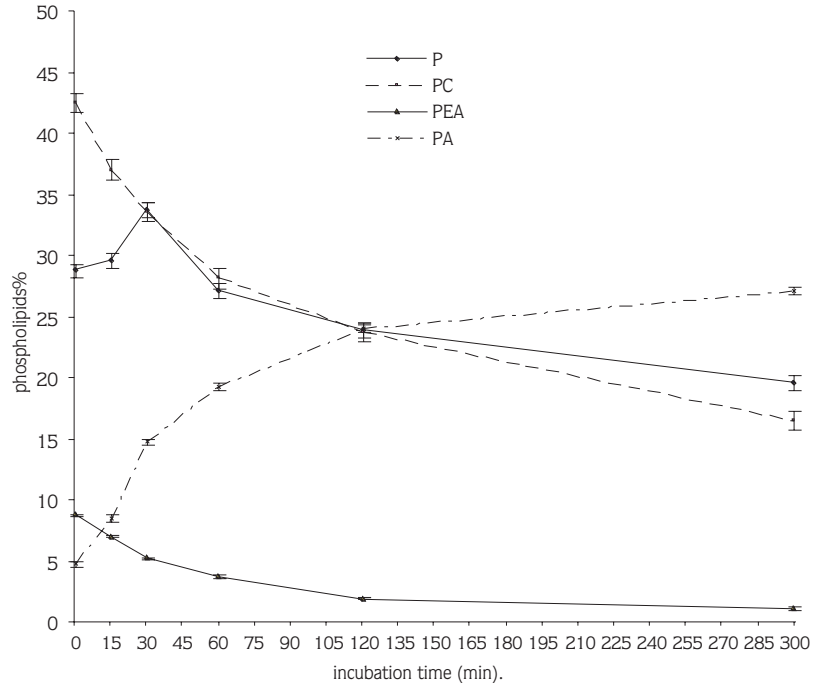

Figure 3. The changes of main phospholipids in cotton seeds under the influence of endogenic phospholipase D on day 50 of maturation

maturation. On day 30 of maturation the content of phosphatidylcholine decreased 75%, and phosphatidylethanolamine 92%.

In the following day of seed maturation the content of phosphatidylcholine and phosphatidylathenolamine decreased during the incubation, but the quality of these phospholipids decreased unsteadily and independent of seed maturation terms. For example, on day 50 the content of phosphatidylcholine decreased 61% and phosphatidylethanolamine 87%; on day 70, the content of phosphatidylcholine decreased 43%, and phosphatidylethanolamine 65%. Contrary to given data,

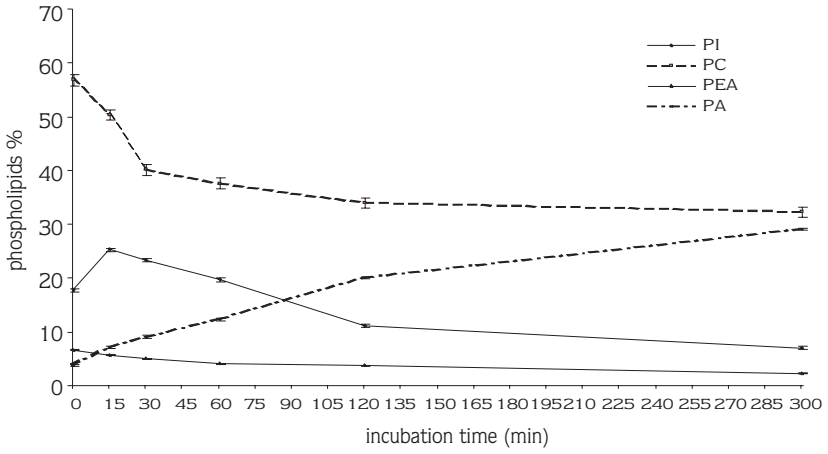

Figure 4. The changes of main phospholipids in cotton seeds under the influence of endogenic phospholipase D on day 70 of maturation

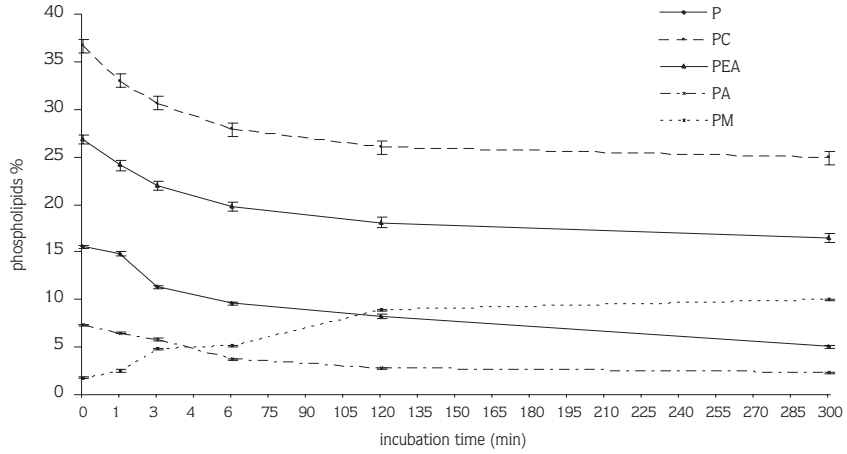

Figure 5. The transalkylation of main phospholipids in cotton seeds under the unfluence of phospholipase D on day 10 of maturation

PI-phosphatidylinositol
PC-phosphatidylcholine
PEA-phosphatidylethanolamine
PA-phosphatidic acid
PM-phosphatidylmethano

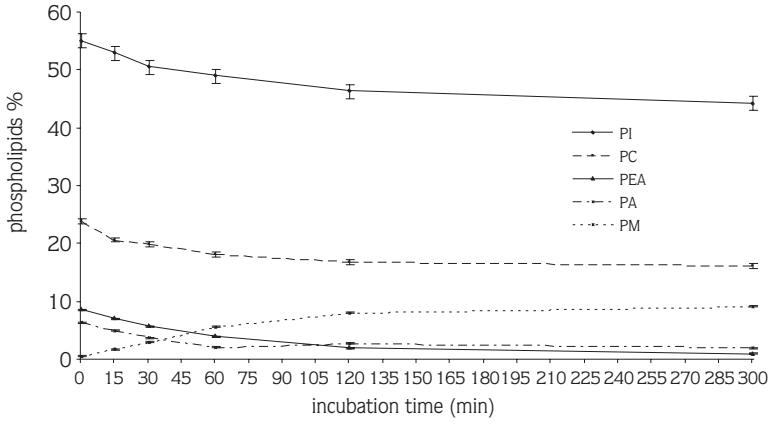

Figure 6. The transalkylation of main phospholipids in cotton seeds under the unfluence of phospholipase D on day 30 of maturation

the content of phosphatidic acid from days 10 to 70 of maturation increased during the incubation 75-85%. These results show that during cotton seed maturation phospholipase D acts as hydrolase and the quantity of this reaction product, phosphatidic acid, is increased in all variations.

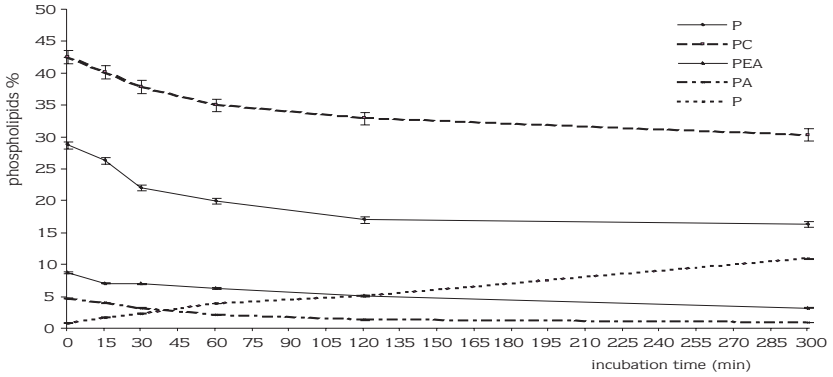

Figure 7. The transalkylation of main phospholipids in cotton seeds under the unfluence of phospholipase D on day 50 of maturation

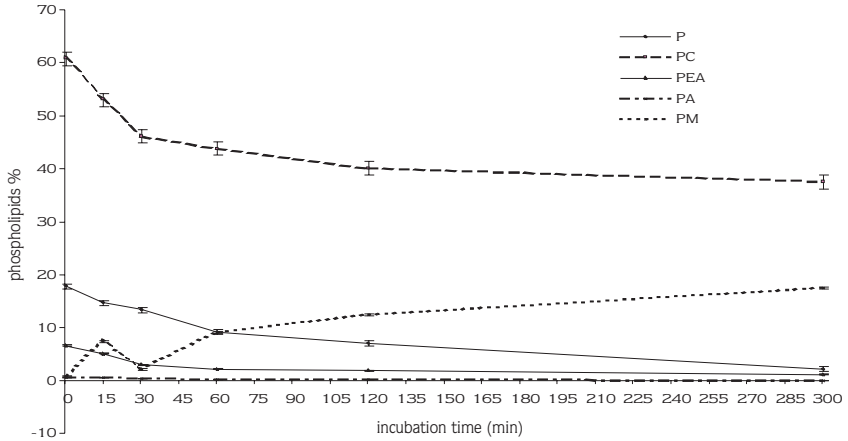

Figure 8. The transalkylation of main phospholipids in cotton seeds under the unfluence of phospholipase D on day 70 of maturation

Statistic analises

The investigations were carried out in two replicates to three determinations in each replicate. The average arithmetical data and the deviations from an average of three replicate are given in idagrams.

As seen in Figures 5-8, the incubation of homogenate in a water-methanol environment led to formation not only in phosphatidic acid, but phosphatidylmethanol too. In these experiments the content of phosphatidylcholine decreased during incubation 29-39%. It was found that the content of phosphatidylinositol has a tendenay to decrease during incubation from 20% on day 30 to 88% (on day 70). The quantity of phosphatidic acid decreased during the incubation 68-83%, and the quantity of phosphatidylmethanol increased 82-96%. The quantity of phosphatidic acid did not increase when methanol was added.

The formation of phosphotidylmethanol shows that in the matured seeds of cotton the reaction of phospholipids transalkylation under the influence of phospholipase D takes place. On the basis of these results we conclude that enzymatic changes of phospholipids have similar biochemical ways as during the growth of seeds and during their maturation. These dates show that in cotton seeds phospholipase D could act as hydrolase and transferase too.

Conclusion

These experimental results show that phospholipase D plays an important role in phospholipid exchange during cotton seed maturation.

It was found that during cotton seed maturation, phospholipase D acts as hydrolase and transferase. Phytase plays an important role in the manifestation of transferase activity. Phytase catalyses the reaction of inositol formation. Inositol is the substrate that takes part in the exchanging of reactions with phospholipids. The product of this reaction is phosphatidylinositol. It shows that enzymatic change in phospholipids which are catalysed by phospholipase D, make an important contribution to the phospholipid metabolism.

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