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ETHANOLAMINE INCORPORATION INTO MEMBRANES OF PEA EMBRYONIC AXES DURING GERMINATION

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Key Word Index—*Pisum sativum*; Leguminosae; imbibition; germination; endoplasmic reticulum; ethanolamine; plasma membrane; temperature; phospholipids.

Abstract—Changes in the ethanolamine pool of the embryonic axes of pea seeds exposed to different temperatures during imbibition and germination were followed. The ethanolamine pool decreased except during imbibition at 25°. Label from ethanolamine was incorporated almost entirely into phosphatidylethanolamine with incorporation into phosphatidylcholine being observed only after imbibition and germination at 25°. The incorporation of ethanolamine was apparently less sensitive to temperature than that of choline and glycerol, previously reported. Preliminary results also show an effect of the imbibition temperature on some of the membrane proteins, but most did not seem to be affected.

INTRODUCTION

We have previously reported on the effect of temperature of imbibition on a number of metabolic changes occurring in the membranes of the embryonic axes of germinating peas [1–3] and on the ultrastructural changes associated with them [4]. One of the major constituents of the phospholipids of the membranes is phosphatidylethanolamine (PE) [5]. It was therefore of interest to determine how ethanolamine is incorporated into the phospholipids of membranes, as compared to the incorporation of glycerol and choline, previously studied. This aspect of phospholipid metabolism has hardly been studied in plants, although it has been reported that *N*-acyl-phosphatidylethanolamine is rapidly metabolized during early germination [6] and that ethanolamine may be liberated. In the following we will report on the incorporation of ethanolamine into membrane phospholipids in response to the temperature of imbibition.

RESULTS AND DISCUSSION

The amount of ethanolamine in the embryonic axes changes during germination (Fig. 1). Exposure of the seeds to 25° during imbibition resulted in a very sharp increase in their ethanolamine content so that high values were observed after 5 hr. After 20 hr the ethanolamine content was lower than that in the dry seeds, whether the seeds were kept at 25° or transferred to 5°. Exposure to 5° during inbibition completely prevented this rise, irrespective of whether the seeds were subsequently kept at 5° or transferred to 25°. The amount of ethanolamine in the seed embryonic axes is about twice that of choline, at least after imbibition, and similar to the glycerol content, after imbibition at 25°. Subsequent levels are low, as were the levels of the other precursors of phospholipids.

Since PE makes up 15–20% of the phospholipids in the endoplasmic reticulum (ER) [3] we determined how it is incorporated into the seed membranes during germination following different temperature treatments. The results for the incorporation of ethanolamine into PE in the total membrane fraction are shown in Table 1, expressed either on a fr. wt or dry wt basis.

Initially ethanolamine was quickly incorporated into PE, during the 5 hr imbibition period at 25°, but label was lost during subsequent germination at this temperature if calculated on a fr. wt basis; on a dry wt basis the loss was much smaller (Table 1). This loss of label was markedly retarded if the seeds were transferred to 5° after im-

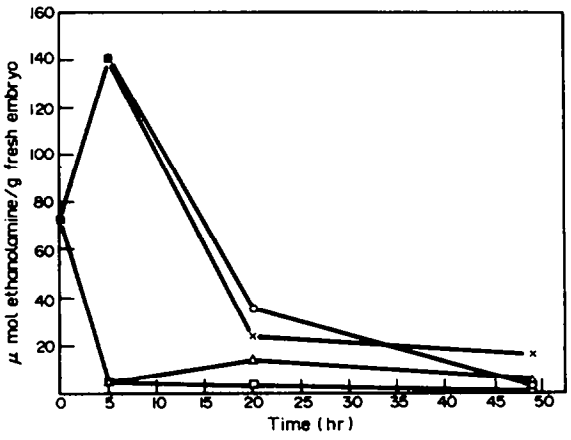

Fig. 1. Effect of temperature of imbibition and germination on the free ethanolamine content of embryonic axes of peas. × --- × Imbibition and germination at 25°; △---△ imbibition and germination at 5°; ○---○ imbibition at 25° and germination at 5°; □---□ imbibition at 5° and germination at 25°.

Table 1. Effect of temperature of imbibition and germination on incorporation (dpm) of [2-^{14}C]ethanolamine into phosphatidyl ethanolamine in membranes of pea embryonic axes

Temperature		mg dry wt			mg fr. wt		
Time (hr)		5(6)*	20	48	5(6)*	20	48
Imbibition	Germination						
25°	25°	1310 ± 220	1200 ± 22	1010 ± 110	570	330	44
25°	5°	1310 ± 220	950 ± 5	890 ± 33	570	420	350
5°	5°	310 ± 44	875 ± 160	780 ± 110	130	380	310
5°	25°	310 ± 44	850 ± 55	680 ± 5	130	270	50

*In case of imbibition at 5°, 6 hr.

bibition. Imbibition at 5° reduced initial incorporation, but incorporation continued for at least 20 hr and also almost stopped the loss of label, if the seeds were kept at 5°. The treatment of 5° imbibition and germination at 25° resulted in low incorporation followed by subsequent loss on a fr. wt basis, quite similar to the behaviour at 25° throughout, but if expressed on a dry wt basis it resembled the 5–5° treatment. This contrasts quite considerably with the behaviour of choline incorporation [1].

Ethanolamine can also act as precursor of phosphatidylcholine (PC), by one of a number of reactions. PE may be methylated to form PC and ethanolamine may be converted to choline [5]. In fact only treatment at 25° during imbibition resulted in any appreciable incorporation of label from ethanolamine into PC and incorporation was less than 10% of incorporation into PE (Table 2). In the treatments of 5° imbibition and transfer to 5 and 25° imbibition and transfer to 5°, incorporation was negligible. In the 5–25° treatment, again there was incorporation, after 48 hr, but its level was very low, less than 5% of that into PE. Clearly these reactions are of secondary importance during the stages of germination studied in this work. A low degree of incorporation of ethanolamine into PC has also been reported in other tissues [7, 8].

If we compare the results for incorporation and metabolism of ethanolamine with those for glycerol and choline in the membranes of the embryonic axes of peas, a number of differences are at once apparent. The size of the ethanolamine and the choline pools drops between 5 and 20 hr while that of glycerol changes only by *ca* 30% during this time [1, 2]. In contrast choline incorporation into phospholipid continues for at least 20 hr in the 25–25° treatment while ethanolamine incorporation virtually stops after 5 hr, and label is then lost if related to the fr. wt. This loss cannot be fully accounted for by the increase in fr. wt of the axes, which increased 4-fold, while the apparent decrease in labelling was by a factor of *ca* 7–8. Such a loss could be due to an exchange reaction between PC and ethanolamine [5, 9], if there were a large pool of ethanolamine. However, our results show that this is not the case. The loss of label must therefore be interpreted as showing a very rapid metabolism of PE.

An additional difference between ethanolamine incorporation and that of both glycerol and choline is the fact that the 'memory' effect observed for the last two compounds is less evident in the case of ethanolamine. Imbibition at 5° followed by germination at 25° follows the pattern of imbibition and germination at 25° and not that of both at 5°, being more or less intermediate between 25–25° and 5–5°.

These results suggest that not all components of the membrane respond to the temperature of imbibition in the same way. It has recently been shown that the regulation of PC is independent of that of other phospholipids [10, 11]. This strengthens the view that an effect of temperature of imbibition on metabolism of a definite phospholipid could be significant in the temperature sensing process. This conclusion is supported by preliminary results of experiments in which we followed the level of total proteins and of proteins of different molecular weight in the plasma membrane and the ER. The amount of protein present in the plasma membrane and the ER increased during germination in all the temperature treatments, although the increases were smaller in the 5–5° treatment and the 25–5° treatments than in the

Table 2. Effect of temperature of imbibition and germination on incorporation of [2-^{14}C]ethanolamine into phosphatidyl choline into membranes of pea embryonic axes (Results as pmol [^{14}C]ethanolamine/g dr axes)

Temperature				
Time		5(6)*	20	48
Imbibition	Germination			
25°	25°	5 ± 1	90 ± 10	100 ± 40
25°	5°	5 ± 1	6 ± 2	15 ± 4
5°	5°	4 ± 0.5	4 ± 1	5 ± 0.5
5°	25°	4 ± 0.5	35 ± 2	50 ± 0

*In case of imbibition at 5°, 6 hr.

others. Thus, incorporation of protein into the membrane fractions was common to all the temperature treatments. Most of the protein components showed no clear response to temperature of imbibition. However, the imbibition temperature did effect the amount of an M_r 37000 protein formed in the plasma membrane. This protein was absent in the dry seeds, but appeared following imbibition. Under conditions leading to germination its amount did not increase much more following imbibition. However, in seeds which had been exposed to low temperatures during imbibition its amount continued to increase. Germination or imbibition at 5° also induced increases in at least two other proteins of M_r 80000 and 94000 in the ER. The higher germination temperature induced increases in a 31000 M_r protein and a 45000 M_r protein in the ER. These proteins have not yet been identified.

It is clear that further studies should concentrate on the enzymes involved in phospholipid synthesis and on the protein components of the membranes. This will lead to a better understanding of the mechanism by which the effect of temperature on the membranes is brought about.

EXPERIMENTAL

Seeds of *Pisum sativum* cv. Alaska were imbibed at 5 and 25° and germinated as previously described in ref. [1]. Seeds were fed with labelled [2-^{14}C] ethanolamine (49.3 mCi/mmol) during imbibition and then transferred to germination conditions at 5 or 25°. Membranes were extracted from the embryonic axes after imbibition or germination as described in ref. [1] and used for extraction of phospholipids without separation into plasma membrane and ER. Phospholipids were separated by TLC [12], identified and removed from the plates for counting as described in ref. [1]. Ethanolamine was determined as follows. Axes were ground with CaO and then extracted in a Sohxlet with Et_2O containing picronolic acid for 4 hr [13]. The picronalate of ethanolamine was decomposed with conc HCl and the free ethanolamine extracted. This was oxidized with periodate and the HCHO formed determined colourimetrically at 570 nm using chromotropic acid [14]. Protein in the membranes was determined using the Folin–Ciocalteu reagent [15]. Proteins in the membranes were extracted and separated electrophoretically according to ref. [16].

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