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Research paper

Evidence for a different metabolism of PC and PE in shoots and roots

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Abstract

We investigated phosphatidylcholine (PC) and phosphatidylethanolamine (PE) labelling in shoots and roots from leek plantlets, maize seedlings and *Arabidopsis thaliana* through the incorporation of radiolabelled acetate. Regardless of the pathway followed in shoots, PC labelling was always higher than PE labelling. However, we obtained an opposite situation in leek and *A. thaliana* roots since PC labelling was much lower than PE labelling. Several hypotheses to explain the origin(s) of these discrepancies between roots and shoots were tested. Among them, neither the level of the respective *AAPT* activities, nor specific regulations of PC biosynthesis through the mRNA levels of several enzymes (choline citidylyltransferase (*CCT*), ethanolamine citidylyltransferase (*ECT*), phosphoethanolamine methyltransferase (*PEAMT*)), nor the fatty acyl chain composition of PC, PE, and diacylglycerol, were responsible for the differences observed between PC and PE metabolism in roots and shoots. Finally, we investigated the acylation of PC and PE in vitro in both shoots and roots of *A. thaliana* seedlings, and demonstrated that some specific remodelling of PC and PE by acylation was responsible for the differences in labelling observed in vivo.

Keywords: Roots; Shoots; Lipid acylation; Phosphatidylcholine; Phosphatidylethanolamine

1. Introduction

The phosphatidylcholine (PC) biosynthetic pathways appear to vary among different plant species (see Fig. 1 for the various biosynthetic routes [1] and references therein). For example cytidine diphosphate (CDP)-methylethanolamine is the primary substrate used by *AAPT* to produce PC in soybean leaves whereas the major substrate is CDP-choline in *Lemna* and castor bean endosperm. In contrast, CDP-methylethanolamine, CDP-dimethylethanolamine and CDP-choline can be involved in PC synthesis in carrot. Therefore several different PC biosynthetic pathways can be described in plants (Fig. 1).

The Kennedy pathway is constituted by choline kinase (*CK*) + choline citidylyltransferase (*CCT*) + *AAPT* and depends on the availability of choline (CHO) for *CK* [2]. Moreover, the reaction catalysed by *CCT* is considered as the limiting step [12,14,15,24].

The second pathway is made of ethanolamine kinase (*EK*) + phosphoethanolamine methyltransferase (*PEAMT*) + *CCT* + *AAPT*. This route begins by the formation of phosphoethanolamine (P-EA), which is then methylated to phospho methyl ethanolamine (P-MEA); additional methylations lead to the synthesis of phosphocholine (P-CHO), which is substrate for the *CCT*. N-methylation by *PEAMT* has been found to be a committing step in PC synthesis in leaves and other vegetative tissues [2,6,25].

A third metabolic route can be described which bypasses the use of P-CHO by *CCT*. In this case, either *PEAMT* will form P-MEA and phospho dimethyl ethanolamine (P-DMEA), or mono-methyl ethanolamine (MEA) and di-methyl ethanola-

Abbreviations: AAPT, amino-alcohol phosphotransferase; CCT, choline citidylyltransferase; CDP, cytidine diphosphate; CHO, choline; CK, choline kinase; CoA, coenzyme A; DAG, diacylglycerol; DMEA, di-methyl ethanolamine; DMPE, di-methyl PE; EA, ethanolamine; ECT, ethanolamine citidylyltransferase; EK, ethanolamine kinase; G3P, glycerol 3 phosphate; GroP, glycerolphosphate; LPA, lyso phosphatidic acid; MEA, mono-methyl ethanolamine; MPE, mono-methyl PE; PA, phosphatidic acid; PAP, PA phosphatase; PC, phosphatidylcholine; P-CHO, phosphocholine; P-DMEA, phospho dimethyl ethanolamine; PE, phosphatidylethanolamine; P-EA, phosphoethanolamine; PEAMT, phosphoethanolamine methyltransferase; P-MEA, phospho methyl ethanolamine.

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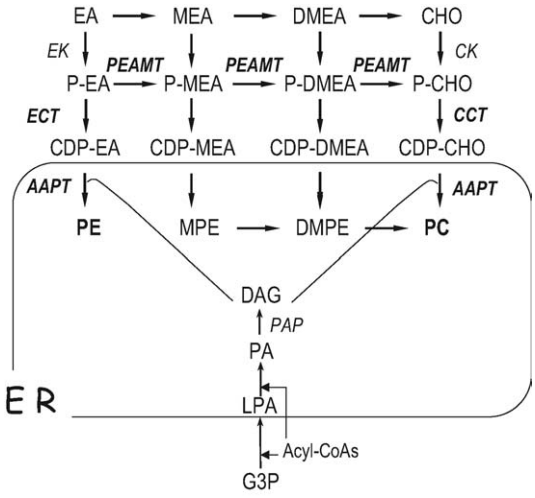

Fig. 1. PC and PE biosynthetic pathways in plant cells.
The sizes of the arrows in the pathways for PC and PE synthesis indicate the importance of each pathway as determined for several plant species.
Abbreviations: *AAPT*, amino-alcohol phosphotransferase; *CCT*, choline citidylyltransferase; CDP, cytidine diphosphate; CHO, choline; *CK*, choline kinase; CoA, coenzyme A; DAG, diacylglycerol; DMEA, di-methyl ethanolamine; DMPE, di-methyl PE; EA, ethanolamine; *ECT*, ethanolamine citidylyltransferase; *EK*, ethanolamine kinase; ER, endoplasmic reticulum; G3P, glycerol 3 phosphate; GroP, glycerolphosphate; LPA, lyso phosphatidic acid; MEA, mono-methyl ethanolamine; MPE, mono-methyl PE; PA, phosphatidic acid; *PAP*, PA phosphatase; PC, phosphatidylcholine; P-CHO, phosphocholine; P-DMEA, phospho dimethyl ethanolamine; PE, phosphatidylethanolamine; P-EA, phosphoethanolamine; *PEAMT*, phosphoethanolamine methyltransferase; P-MEA, phospho methyl ethanolamine.

mine (DMEA), which will be phosphorylated, and then used by *CCT* to form CDP-MEA and CDP-DMEA. These substrates could be taken up by *AAPTs* to produce mono-methyl PE (MPE) and di-methyl PE (DMPE), which will be methylated to PC [6,31]. It must be underlined that a direct methylation of phosphatidylethanolamine (PE) to synthesise PC, although not totally ruled out in spinach [21] and castor bean [22], has never been demonstrated in other plant systems [2,6,25]. To our knowledge, the first putative methylation of PE leading to MPE has never been demonstrated in plants [2,6,25]. Moreover, there is only a weak contribution of the methylation pathway to PC synthesis in *Arabidopsis thaliana* leaves, as demonstrated by in vivo incorporation of labelled CHO and ethanolamine (EA) [29]. Therefore, the Kennedy pathway can be considered as the main pathway in this case.

As PC synthesis shows variations among different plant species, we asked the question: is PC synthesised similarly in different tissues of a given plant?

We investigated PC and PE labelling in shoots and roots from leek plantlets, maize seedlings and *A. thaliana* through the incorporation of radiolabelled acetate. Regardless of the pathway followed in shoots, PC labelling was always found to be higher than PE labelling. However, using the same acetate precursor, PE labelling was much higher than that of PC in roots, indicating a possible differential metabolism of PC and PE in shoots and roots. In order to determine why acetate incorporation in roots led to a lower labelling of PC and to a higher labelling of PE, when compared to that of shoots, we tested several hypotheses: 1. We measured *AAPT* activities in leek shoots and roots to determine whether the enzymatic activity in the nucleotide pathway for PC synthesis in roots was comparable to the normal shoot capacity for PC synthesis. 2. We analysed the putative regulation of PC biosynthesis by measuring the mRNA levels encoding several enzymes (*CCT*, ethanolamine citidylyltransferase (*ECT*), *PEAMT*) in the shoots and roots from *A. thaliana* by quantitative PCR. 3. We considered if the fatty acyl chains in both phospholipids were sufficiently different to explain the variations in the labelling of PC and PE from labelled acetate between the shoots and the roots. To investigate this possibility, we analysed the fatty acyl chain composition of PC and PE from shoots and roots of *A. thaliana* plantlets, by measuring the radioactivity associated to the different fatty acids after incorporation of radiolabelled acetate in vivo. We also determined the fatty acyl composition of the diacylglycerol (DAG) species from shoots and roots. 4. We measured the acylation of PC and PE in vitro by incorporation of radiolabelled oleoyl-coenzyme A (CoA) in both the shoots and roots of *A. thaliana* plantlets.

2. Results

2.1. PC labelling in shoots and roots of leek, maize and A. thaliana seedlings

We first incorporated radiolabelled acetate in 7-day-old leek seedlings in vivo and analysed the radioactivity associated with PC and PE after various times of incubation up to 120 min. As shown in Fig. 2, the labelling of PC in shoots was systematically higher than that of PE regardless of the time of incubation (the ratio of PC to PE labelling in shoots was 1.57 ± 0.12). On the contrary, the labelling of PC in roots was always lower than that of PE regardless of the time of incubation (the ratio of PC to PE labelling in roots was 0.57 ± 0.06). Although the total amounts of PC and PE were different in the shoots and the roots of leek seedlings, we observed a similar lipid composition and identical PC to PE ratios in both tissues: 1.95 ± 0.22 in shoots and 1.91 ± 0.24 in roots. Identical results were also obtained with shoots and roots from 15-day-old maize seedlings (the ratios of PC to PE labelling were 1.66 ± 0.18 in shoots and 0.49 ± 0.08 in roots). The ratios of total PC to PE were also similar for both tissues: 1.27 ± 0.08 in shoots and 1.55 ± 0.28 in roots.

We incorporated radiolabelled acetate in the shoots and roots of 3-week-old *A. thaliana* plantlets in vivo and analysed the radioactivity associated with PC and PE after various times of incubation (Fig. 3). As observed with leek seedlings, the labelling of PC in shoots was higher than that of PE as a function of the time of incubation (the ratio of PC to PE labelling in shoots was 1.20 ± 0.10), and the labelling of PC in roots was also always lower than that of PE regardless of the time of incubation (the ratio of PC to PE labelling in roots was 0.36 ± 0.05). As observed in leek and maize, PC and PE contents of *A. thaliana* shoots and roots showed similar PC to PE ratios in both tissues (1.70 ± 0.21 in shoots and 1.51 ± 0.07 in