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Biochemical characterisation and potential physiological function of acyl-CoA:glycero-3-phosphocholine acyltransferases.

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ABSTRACT

Phosphatidylcholine (PC) is the main lipid of eukaryotic membranes and has a central function in the metabolism of plant lipids. PC is a substrate for various types of desaturases - membrane enzymes that produce unsaturated, polyunsaturated and atypical fatty acids (PUFAs). These acids are the object of interest of scientists due to the impact on human health, as well as possible use in industry. Glycero-3-phosphocholine (GPC) is a product of complete deacylation of phosphatidylcholine (PC). For a long time, scientific opinion was that GPC is not a substrate for direct PC resynthesis, until recently discovered activity of acyl-CoA:glycerophosphocholine acyltransferase (GPCAT) in bakery yeast (*Saccharomyces cerevisiae*) (Stålberg et al., 2008), which leads to resynthesis PC, bypassing the stage of GPC degradation to free choline, choline activation to CDP-choline and transfer of choline to diacylglycerol molecule (DAG). Despite attempts to identify the enzyme responsible for this reaction, the Stålberg team (Stålberg et al. 2008) had failed to identify the appropriate protein. The gene responsible for the synthesis of GPCAT in yeast was identified in 2014 by a team of prof. Sten Stymne from the Swedish University of Agriculture in Alnarp, of which I was a member at the time. On the basis of homology, we were able to identify the plant genes (*Arabidopsis thaliana, Ricinus comunis, Brassica napus*) that were cloned into the pYES 2.1 vector and complimented the deletion of GPCAT activity in the yeast line lacking corresponding gene (Głąb et al. 2016).

The most important and a breakthrough result of my research was the discovery of completely new, previously undescribed activity of GPCAT. I described the existence of a transacylation reaction transferring acyl residues from other lysophospholipids, i.e. lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylserine (LPS) to a GPC molecule (Głąb et al., 2016). Discovered activity explained earlier data on acylation of the GPC molecule without the addition of acyl-CoA in tests with microsomes prepared from developing oilseed plants (Lager, Głąb et al., 2015).

In the course of the research, apart from experiments explaining the reacylation mechanism of the GPC molecule, biochemical characterization was performed and the substrate specificity of yeast GPCAT was determined as well as its homologous form of *A. thaliana,* castor (*Ricinus comunis*) and rape (*Brassica napus*). Additionally, GPCAT (GPCAT dependent acylation and acyl-CoA independent acylation) activity was investigated in microsomal fractions of developing seeds of *Carthamus tinctorius*, *Ulmus glabra*, *Crambe abyssinica* and *Camelina sativa* (in microsomal fractions prepared from the seeds of these last two species GPCAT activity was not detected). As part of the work, a number of studies were carried out in attempts to determine whether GPCAT possesses the properties of LPC:LPC acyltransferase, both in yeast microsomal fractions and microsomal fractions prepared from developing seed with active GPCAT. A number of indications have been obtained that GPCAT-type enzymes may be responsible for this activity, but no definitive evidence confirming or denying this hypothesis. The last stage of the research was to perform experiments aimed to determine the impact of a knock-out and overexpression of the GPCAT gene in baker's yeast and in Arabidopsis. In the case of yeast, differences in the rate of growth of liquid cultures were discovered and fatty acid composition (increase in unsaturated fatty acids in yeast with *gpc1* deletion) were observed, but only under osmotic stress. In the case of plants, a decrease in the amount of chloroplast galactolipids in plants containing T-DNA insertions within the gene encoding GPCAT (At5g35460) was observed, as well as a drastic decrease in the amount of produced seeds in plants with overexpression of this gene, in comparison to control plants. Analysis of plant morphology with reduced expression of the enzyme and overexpression did not show significant differences.

The research presented in this work was carried out partially at the Institute of Plant Breeding of the Swedish Agricultural University in Alnarp and at the Laboratory of Plant Biochemistry, MWB UG and GUMed.