ATTN: Continuation request for Grant No. DE-FG02-87ER13729

Understanding Acyl Chain and Glycerolipid Metabolism in Plants
John Ohlrogge
Michigan State University

Final Report:

Acyl-editing in initial eukaryotic lipid assembly in soybean seeds.

The growing value of plant oils as food and fuel has placed great interest in engineering plants to accumulate more oil per plant. To accurately understand the synthesis of plant oils we must identify not only the enzymes involved in triacylglycerol (TAG) synthesis but also the flow of substrates to each enzymatic step. We recently demonstrated through *in vivo* labeling in pea leaves that the flow of nascent fatty acids (FA) from synthesis in the plastid to incorporation into extraplastidial glycerolipids involves acyl editing with phosphatidylcholine (PC) (Bates et al., 2007). Specifically, we concluded that newly synthesized 16:0 and 18:1 FA are exported from the plastid and directly incorporated into the *sn*-1 or *sn*-2 position of PC through acyl editing. If the membrane lipid pool plays an intermediate role in TAG synthesis similar to its role in *de novo* lipid synthesis in pea leaves then the flux of acyl chains and DAG in and out of membrane lipids need to be considered for logical engineering of increased TAG synthesis.

To determine the flow of acyl chains and glycerol into TAG of developing soybeans we utilized an embryo culture system in which soybean development closely mimics *in planta* growth. Rapid *in vivo* labeling with [^{14}C]acetate and [^{14}C]glycerol was used to analyze the early kinetics of acyl chain and glycerol backbone incorporation into PC, DAG and TAG. Additionally stereochemical and molecular species analysis of labeled lipids was used to determine the initial species of each lipid produced from *de novo* synthesis and acyl editing. The major flux of nascent [^{14}C]18:1 FA out of the plastid was into the *sn*-2 position of PC alongside a previously synthesized FA, similar to the acyl editing we observed in pea leaves. Thus, the kinetics of glycerol backbone labeling and the stereochemistry of newly synthesized acyl chain labeling indicate that the major flux of acyl chains from the plastid in soybean seeds occurs first into PC, rather than into phosphatidic acid.

Identification and characterization of two *Arabidopsis thaliana* lysophosphatidyl acyltransferases with preference for lysophosphatidylethanolamine

Two Arabidopsis genes, At1g80950 and At2g45670, both annotated to encode proteins with acyltransferase regions and with sequence similarity to a recently identified lung lysophosphatidylcholine acyltransferase (LPCAT) were characterized. To identify their substrate specificity and biochemical properties, the two Arabidopsis acyltransferases, designated AtLPEAT1 and AtLPEAT2 were expressed in yeast knockout lines *ale1* and *slc1*, that are deficient in microsomal acyltransferases. Lysophosphatidyl acyltransferase activity is almost null in the yeast knockout *ale1* and expression of AtLPEAT1 in this background exhibited strong acylation activity of lysophosphatidylethanolamine (LPE).and lysophosphatidate (LPA) with lower activity on LPC and LPS. AtLPEAT2 was more selective for LPE > LPC > LPS > LPA.

Both acyltransferases preferred 18:1-LPE over 16:0-LPE as acceptor and preferred palmitoyl-CoA as acyl donor in combination with 18:1-LPE. Both acyltransferases showed no or minor response to Ca^{2+}, despite the presence of a calcium binding EF-hand region in AtLPEAT2. AtLPEAT1 was more active at basic pH while AtLPEAT2 was equally active between pH 6.0 - 9.0. These results represent the first description of plant acyltransferases with a preference for LPE.

Characterization and subcellular distribution of lysolipid acyl transferase activity of pea leaves.

Our *in vivo* labelling studies (Bates et al, 2007) indicate that the initial acylation step that occurs after fatty acids leave the plastid occurs on PC. In order to address the subcellular location of this acylation, protoplasts were prepared from pea leaves, gently lysed and the subcellular compartments separated on sucrose gradients. At least 30% of the total lysophosphatidyl choline acyltransferase (LPCAT) activity of the cells could be attributed to chloroplasts. These results support the hypothesis that LPCAT is an enzyme activity involved in export of FA from chloroplasts. This result also suggests that PC rather than acyl-CoA may be the form in which acyl chains are exported from chloroplast to ER. If true, current models of plant lipid metabolism which show acyl-CoA export of acyl groups will need revision.

Publications

1.Bates PD, Ohlrogge JB, Pollard M. (2007) Incorporation of newly synthesized fatty acids into cytosolic glycerolipids in pea leaves occurs via acyl editing. *J Biol Chem*. 282:31206-16.

2.Durrett T, Benning C, Ohlrogge J, (2008) Plant Triacylglycerols as Feedstocks for the Production of Biofuels. ***Plant J*** (in press)

3.Allen DK, Shachar-Hill Y, Ohlrogge JB. (2007) Compartmental-Specific Labeling Information in ^{13}C Metabolic Flux Analysis of Plants *Phytochemistry* 68:2197-2210.

4.Alonso A, Goffman F, Ohlrogge J, Shachar-Hill Y. (2007) Carbon conversion efficiency and central metabolic fluxes in developing sunflower (*Helianthus annuus* L.) embryos *Plant Journal* 52:296-308.