SYNTHESIS OF STORAGE OIL IN DEVELOPING SEEDS OF *BRASSICA NAPUS* L.

M. J. HILLS, D.H. HOBBS AND D.J. LACEY

Brassica and Oilseeds Research Department, John Innes Centre, Colney, Norwich, Norfolk, NR4 7UH, U.K.

ABSTRACT

Storage triacylglycerols are synthesized by the endoplasmic reticulum from acyl chains generated by the plastid in developing seeds of rape. It is likely that acyl-CoAs are transported from the plastid to the endoplasmic reticulum by acyl-CoA binding proteins. Separation of membranes and organelles in homogenates of developing rape seeds by sucrose density centrifugation revealed two fractions of membranes which synthesize storage oil (triacylglycerols). One of these contained endoplasmic reticulum including enzymes involved in structural phospholipid synthesis. The other fraction which synthesized triacylglycerols showed relatively low activities of the enzymes of phospholipid synthesis. The data suggest that the endomembrane system has regions which are dedicated to triacylglycerol synthesis which either become detached from the reticulum during homogenization or which are already separated from the ER *in vivo*.

INTRODUCTION

It is thought that storage triacylglycerols are synthesized during seed development by the endoplasmic reticulum and deposited as oil bodies which, in the mature seed are found to be surrounded by a coat of oleosins and phospholipid. The acyl groups of the TAGs are synthesized in plastids by fatty acid synthetase and, by an as yet unknown mechanism, exported from the plastid. The fatty acids are activated to acyl-CoAs and transported to the ER where glycerol-3-phosphate is sequentially acylated to form triacylglycerol. The membrane structural phospholipid, phosphatidylcholine, is also formed by this pathway by the transfer of a phoshocholine group to diacylglycerol (Murphy et al 1993). The oil is deposited as droplets by a mechanism that is not well understood and becomes coated with oleosins, a very unusual protein which is thought to prevent coalescence of the oil during desiccation of the seed (Cummins *et al* 1993). Upon rehydration the oil bodies remain as discrete entities and can therefore be efficiently hydrolysed by lipases (Hills and Mukherjee 1994).

EXPERIMENTAL

Synthesis of triacylglycerols by sub-cellular fractions of rape embryos

Homogenates of developing rape seeds were fractionated by sucrose density gradient centrifugation. Two fractions were found to incorporate either radiolabeled glycerol-3-phosphate (G3P) or oleoyl moieties into triacylglycerols at high rates indicating that the full Kennedy pathway was operating in both fractions. One fraction had a density of 1.10 and was found to contain the endoplasmic reticulum marker enzymes CDP-choline:DAG cholinephosphotransferase, *lyso*phosphatidylcholine:acyl-CoA acyltransferase and antimycin A and cyanide insensitive NADH:cytochrome c oxidoreductase. The lighter fraction, however, contained only very low levels (1-2%) of activity of the enzymes involved in the synthesis of phosphatidylcholine and it could not be reliably demonstrated that the cytochrome c reductase activity present in the light fraction was associated with the enzymes of the Kennedy pathway.

TABLE 1. Relative rates of lipid synthesis by light membrane and ER fractions

Density of membrane fraction	1.05	1.10
Experiment		
[^{14}C] G3P into TAG	120	100
[^{14}C] oleate into PC	1.4	100
[^{14}C] choline into PC	2.5	100

Rates of incorporation of the radiolabeled precursors into lipid given relative to that by the ER = 100. TAG (triacylglycerol), PC (phosphatidylcholine).

Since the fraction which contained the enzymes of TAG synthesis but lacked those of phospholipid synthesis had a low density, it would be expected that they contain much more neutral lipid (triacylglycerols and diacylglycerols) than the endoplasmic reticulum

fraction. Detailed analysis of the lipid composition of these fractions revealed that the light fraction contained mainly triacylglycerols although the proportion of diacylglycerols in each fraction was similar. Analysis of the oleosin content of the light fraction showed that it was unlikely that the triacylglycerol rich vesicles were derived from oil bodies which had become broken during the homogenisation of the developing seeds. It would therefore appear that the triacylglycerol rich vesicles are derived from the endoplasmic reticulum, though whether or not they were attached to the endomembrane system in the intact embryo or were vesicle which were already separate remains to be established.

ACKNOWLEDGEMENTS

The authors wish to thank the Biotechnology and Biological Sciences Research Council, UK and the John Innes Foundation (DJL) for financial support.

REFERENCES

Murphy, D.J., Rawsthorne, S. and Hills, M.J.(1993). Storage lipid formation in seeds *Seed Science Research*, 3 79-95

Cummins, I., Hills, M.J., Ross, J.H.E., Hobbs, D.H., Watson, M.D. and Murphy, D.J. (1993). Differential, temporal and spatial expression of genes involved in storage oil and oleosin accumulation in developing rapeseed embryos: implications for the role of oleosins and the mechanisms of oil-body formation. *Plant Molecular Biology*, **23**, 1015-1027

Hills, M.J. and Mukherjee, K.D. (1994) Lipases from plants. In *Lipases: their structure, biochemistry and application*. Eds P. Woolley and S.B. Petersen. pp.49-75 Cambridge: Cambridge University Press.