Glycerol 3-phosphate acylation by microsomal fractions from avocado mesocarp.

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Triacylglycerol synthesis in plants occurs via the classical Kennedy pathway [1,2]. The quality and physical properties of the triacylglycerol products are determined by the substrate specificities of the acyltransferases involved [2]. The initial step is catalysed by glycerol 3-phosphate acyltransferase and involves the acylation of the sn-1 position of glycerol 3-phosphate to form lysophosphatidic acid. This acylation occurs at much slower rates than the second acylation and thus limits formation of phosphatidate, a key intermediate in lipid formation. Little is known about the mechanism of action of the acyltransferases of the Kennedy pathway, as they are membrane bound in the endoplasmic reticulum, and there has been little success in their purification. In this work, a microsome fraction from avocado mesocarp has been used as a source of glycerol 3-phosphate acyltransferase in order to study its mode of action, especially with respect to substrate specificities.

Triacylglycerol synthesis by the microsome fraction of avocado mesocarp was studied using radiolabelled substrates. In viro incubation with [1-14C]glycerol 3-phosphate showed carbon flux through the intermediates of the Kennedy pathway, including lysophosphatidic acid, phosphatidic acid and diacylglycerol. The amounts of labelled intermediates produced could be varied by altering the incubation conditions. For example, inclusion of 10 mM EDTA in the incubations increased labelling in lysophosphatidate and phosphatidate by 189% and 25%, respectively. Incubations with mixed acyl-CoA substrates showed that glycerol 3-phosphate acyltransferase had higher rates with saturated acyl-CoA species whilst 1-acyl glycerol 3-phosphate acyltransferase utilised monounsaturated acyl-CoA species preferentially. Both the acyltransferases would utilise other substrates thus forming uncharacteristic triacylglycerol species. Incubations using radiolabelled acyl-CoA substrates were found to be of limited use because of the high rates of transacylation catalysed by the avocado microsomes.

In order to determine the site of the glycerol 3-phosphate acyltransferase, we carried out limited proteolysis of the microsomal vesicles [3]. In these experiments the total amount of protein digestion was kept to a minimum (see also [3]).

Solubilisation of the membrane bound glycerol 3-phosphate acyltransferase activity has been achieved using detergents and protein denaturants. Treatment with urea results in solubilisation of 30% of the particulate activity. Of a variety of detergents tested CHAPS (0.05% w/v) treatment was most effective and solubilised 15% of particulate activity. This activity was stable at 4°C for 24 hours, but was unstable to freezing, even in the presence of glycerol. In contrast, particulate activity fell to 15% of the original after a similar period at 4°C.

Application of dye column chromatography methods used in purification of the E. coli glycerol 3-phosphate acyltransferase [5] to the solubilised avocado preparations was of limited use because of the poor yields of activity. However, size exclusion chromatography and different affinity columns have yielded preparations with considerably enhanced specific activity.

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