CONTROL OF CARBON PARTITIONING TO STORAGE PRODUCTS IN DEVELOPING OILSEED EMBRYOS

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ABSTRACT

In oilseed rape (*Brassica napus* L.) there is a transient accumulation of starch during the early stages of cotyledon filling although the mature seed contains predominantly oil and protein as storage products. In order to understand how the carbon which enters the developing embryo is partitioned to these storage products, and how this is regulated, we have studied the pathways of carbon metabolism involved in their accumulation. The approach has been based upon the measurements of the activities of enzymes involved and on experiments with plastids isolated from the embryos. These data suggest that during starch and oil accumulation, partitioning of carbon from imported sucrose could occur within the plastid, the cytosol or both. They also reveal that cytosolic hexose and hexose phosphates may well be an important source of carbon for fatty acid synthesis in rapeseed.

INTRODUCTION

Seeds contain oil, starch, and protein in proportions which depend upon the plant species, cultivar and the stage of development. In the mature seed of oilseed rape, the main storage products are oil and protein (Murphy and Cummins, 1989) but starch accumulates transiently during the early phase of oil deposition (da Silva and Smith, personal communication). The synthesis of starch and fatty acids occurs in the plastids and in non-photosynthetic tissues this requires the supply of carbon precursors from the cytosol. In photosynthetic tissues the carbon is derived directly from photosynthetic metabolism. The supply of precursors from the cytosol to the plastids has been studied in a few seed tissues but nothing is known about which cytosolic precursors are utilized by the plastids from oilseed rape.

To date, most studies suggest that in non-photosynthetic tissues either glucose-1- or glucose-6-phosphate are imported from the cytosol and used to synthesize the ADP-glucose for starch synthesis, depending upon the plant species (Smith and Martin, 1993). For fatty acid synthesis a wide range of carbon precursors have been proposed (see Murphy *et al.*, 1993) with pyruvate, malate and acetate receiving the most attention. Whichever precursor is utilized it is ultimately metabolized to acetyl-Coenzyme A and then utilized by acetyl-CoA carboxylase in the first committed step of fatty acid synthesis. It has been thought that this enzyme plays an important role in determining the rate of fatty acid synthesis in both leaves and seeds (Murphy *et al.*, 1993). However, in developing rapeseed embryos there is no correlation between its activity and the rate of oil accumulation (Kang *et al.*, 1993).

In order to understand how partitioning of carbon is controlled we have investigated

the plastidial pathways involved in the provision of acetyl-CoA and ADP-glucose, focusing upon which cytosolic precursors are utilized and how these pathways interact.

EXPERIMENTAL

A prerequisite for these studies was the development of a method for the isolation of intact, functional plastids from developing embryos of oilseed rape. This was achieved using embryos which were at a stage of development when both starch and oil were accumulating (Kang and Rawsthorne, 1994). The isolated plastids were essentially free from cytosolic contamination and the plastid fractions were on average 65% intact. These plastids contain all the enzymes of hexose/hexose phosphate interconversion, a complete glycolytic pathway, the pyruvate dehydrogenase complex, and enzymes of the oxidative pentose phosphate pathway. The presence of chlorophyll, ribulose-1,5-bisphosphate carboxylase and fructose-1,6-bisphosphatase suggest that these plastids are somewhat chloroplast like in their development. This enzymatic machinery would allow the plastids to utilize a diverse range of cytosolic metabolites as precursors for their major biosynthetic pathways. In fact a variety of exogenously-supplied ^{14}C-labelled substrates where taken up and metabolised by the plastids to starch and/or fatty acids in an intactness-and ATP-dependent manner (Table 1). Plastids were incubated for 1h and the reactions stopped. Incorporation into starch was determined as that in the methanol/KCl insoluble material and into fatty acids as that in the chloroform soluble material extracted after saponification of the incubation mixtures.

Table 1. Incorporation of ^{14}C from labelled metabolites into starch and fatty acids by isolated plastids. The rates of incorporation have been normalized by expressing them per unit of activity of a plastidial marker enzyme (NADP-glyceraldehyde-3-phosphate dehydrogenase). The data represent the mean ± SE for at least three separate plastid preparations

^{14}C-Substrate	Rate of incorporation	
	Fatty acids (nmol acetate h^{-1})	Starch (nmol hexose h^{-1})
Glucose-6-phosphate	178.1 ± 34.7	195.1 ± 14.9
Glucose	-	53.3 ± 13.4
Fructose	-	182.5 ± 36.5
Dihydroxyacetone phosphate	140.1 ± 18.0	49.5 ± 10.5
Malate	105.4 ± 35.5	-
Pyruvate	256.9 ± 10.4	-
Acetate	47.5 ± 8.7	-

The greatest rates of starch and fatty acid synthesis were from glucose-6-phosphate (G-6-P) and pyruvate, respectively although G-6-P was also an effective precursor for fatty acid synthesis. These data demonstrate that the glycolytic pathway is functional in the rapeseed plastids and that pyruvate, produced by either plastidial or cytosolic glycolysis could be an effective precursor for fatty acid synthesis. The latter observation confirms several other observations with isolated plastids (e.g. Liedvogel and Bauerle, 1986;

Smith *et al.*, 1992). However, this is the first report that hexose phosphate can act as a precursor for plastidial fatty acid synthesis.

It is notable that G-6-P was metabolized to starch and to fatty acids by the same plastid preparation. Given that the first two enzymes of the oxidative pentose phosphate pathway (OPP) are present in these plastids the possibility that G-6-P could be metabolized through three separate plastidial pathways was raised. To test this incubation conditions were established which allowed the simultaneous determination of G-6-P metabolism through the above three routes. The partitioning of G-6-P in these experiments was highly consistent with 51.5%, 22.7% and 25.8% of the supplied substrate being utilized in starch or fatty acid synthesis, and by the OPP, respectively.

These experiments have yielded data which suggest that the partitioning of carbon towards plastidial pathways could occur in the plastids, in the cytosol, or in both. The ability of plastids to utilize multiple substrates will be discussed in the context of enabling a balance between the metabolic requirements of these pathways for carbon precursors and reducing power. Furthermore, factors which may contribute to the regulation of provision of acetyl-CoA *in vivo*, and so to the rate of fatty acid synthesis will also be discussed.

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