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Bioassembly of acyl lipids in microspore-derived embryos of *Brassica campestris* L.

D. C. Taylor, A. M. R. Ferrie, W. A. Keller, E. M. Giblin, E. W. Pass, and S. L. MacKenzie

National Research Council of Canada, Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9, Canada

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Abstract

The native lipid composition and the capacity of cell-free extracts to biosynthesize acyl lipids *in vitro* were determined for the first time using the recently reported microspore-derived (MD) embryo system from the *Brassica campestris* low erucic acid line BC-2 (Baillie et al. 1992). The total lipid fraction isolated from mid-cotyledonary stage MD embryos (21 days in culture) was composed primarily of triacylglycerol (76 %) with an acyl composition quite similar to that of mature BC-2 seed. When incubated in the presence of glycerol-3-phosphate, ^{14}C 18:1-CoA, and reducing equivalents, homogenates prepared from 21-day cultured MD embryos were able to biosynthesize glycerolipids via the Kennedy pathway. The maximum *in vitro* rate of triacylglycerol biosynthesis could more than account for the known rate of lipid accumulation *in vivo*. The homogenate catalyzed the desaturation of 18:1 to 18:2 and to a lesser extent, 18:3. The newly-synthesized polyunsaturated fatty acids initially accumulated in the polar lipid fraction (primarily phosphatidic acid and phosphatidylcholine) but began to appear in the triacylglycerol fraction after longer incubation periods. As expected for a low erucic acid cultivar, homogenates of MD embryos from the BC-2 line were incapable of biosynthesizing very long chain monounsaturated fatty acyl moieties (20:1 and 22:1) from 18:1-CoA *in vitro*. Nonetheless, embryo extracts were still capable of incorporating these fatty acyl moieties into triacylglycerols when supplied with ^{14}C 20:1-CoA or ^{14}C 22:1-CoA. Collectively, the data suggest that developing BC-2 MD embryos constitute an excellent experimental system for studying pathways for glycerolipid bioassembly and the manipulation of this process in *B. campestris*.

Key Words: *Brassica campestris* - Microspore Derived Embryos - Acyl Lipid Biosynthesis

Abbreviations: CPT, *sn*-1,2-diacylglycerol cholinephosphotransferase; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; DGDG, digalactosyldiacylglycerol; G-3-P, glycerol-3-phosphate; G-3-PAT, glycerol-3-phosphate acyltransferase; LPA, *lyso*-phosphatidic acid; LPAT, *lyso*-phosphatidic acid acyltransferase; LPC, *lyso*-phosphatidylcholine; LPCAT, acyl-CoA: *lyso*-phosphatidylcholine acyltransferase; LPE, *lyso*-phosphatidylethanolamine; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PA Phosphatase, phosphatidic acid phosphatase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; TAG, triacylglycerol; 18:1-CoA, oleoyl-Coenzyme A; 18:1, oleic acid, *cis*-9-octadecenoic acid; 18:2, linoleic acid, *cis*-9,12-octadecadienoic acid; 18:3, α-linolenic acid, *cis*-9,12,15-octadecatrienoic acid; 20:1, *cis*-11-eicosenoic acid; 22:1, erucic acid, *cis*-13-docosenoic acid; all other fatty acids are designated by number of carbon atoms: number of double bonds.

Introduction

Haploid and doubled haploid plants of many species have been generated using the technique of isolated microspore culture (Wei and Harada 1986; Cho and Zapata, 1988; Coumans et al. 1989) and nowhere has this been better exploited than in the genus *Brassica* (Lichter 1982; Keller et al. 1987). Homozygous doubled haploids have been utilized in plant breeding programs to reduce cultivar development time (Pauls, 1991). Although microspore culture techniques are well established for *B. napus* L. and have been used in breeding and studies of fatty acid inheritance (Chen and Beversdorf, 1990; Pauls, 1991), mutant selection

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(Swanson et al. 1989) and biochemical/ developmental studies (Taylor et al. 1989; 1990a; 1991; 1992; Weber et al. 1989; 1992; Weselake et al. 1991; Wilberg et al. 1991), limited research has been conducted in other *Brassica* species.

The successful application of microspore culture to *B. campestris* recently reported by Baillie et al. (1992) represents a significant advancement in the development of cell technology for the genetic improvement of this relatively recalcitrant, self-incompatible diploid oilseed crop species. Here we report the first biochemical studies of native acyl lipids and *in vitro* metabolism of radiolabeled lipid substrates in this new embryogenic cell culture system.

Materials and Methods

Radiolabeled substrates and biochemicals. [1-^{14}C] Oleic acid (58 mCi mmol^{-1}) and [1-^{14}C] erucic acid (52 mCi mmol^{-1}) were purchased from Amersham Canada Ltd. (Oakville, Ont.) and NEN Research Products (Mississauga, Ont.), respectively. [1-^{14}C] Eicosenoic acid was synthesized as described by Taylor et al. (1992). All 1-^{14}C-labeled fatty acids were converted to [1-^{14}C] acyl-CoAs by an enzymatic method described previously (Taylor et al. 1990b). ATP, coenzyme A (CoASH), NADH, NADPH, PVPP, Hepes buffer, unlabeled acyl-CoAs and most other biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Neutral lipid standards were obtained from Nu-Chek Prep Inc. (Elysian, MN), while polar lipid standards were obtained from Sigma. Silica gel 60 H was purchased from E. Merck (Darmstadt, Germany) and HPLC-grade solvents (Omni-Solv, BDH Chemicals) were used throughout these studies.

Plant Growth Conditions. The *Brassica campestris* L. breeding line BC-2, a low erucic acid strain, was originally developed by Dr. D. Hutcheson at the Agriculture Canada Research Station, Saskatoon. Plants were grown in a growth cabinet under a 16 h photoperiod with light intensity of 1500 µmol m^{-2} s^{-1} and a day/night temperature of 20/15°C. Prior to bolting, the temperature was lowered to 10/5°C (day/night). Throughout the growth period, plants were fertilized with 14-14-14 Nutricote 100 (slow release fertilizer) and watered bi-weekly with 0.35 g L^{-1} of 15-15-18 (N-P-K) nutrient solution.

Microspore culture. Microspore-derived embryos were produced from genotype BC-2 using the protocol described by Baillie et al. (1992). Optimal embryo production occurred when microspores were cultured first for 48 hours in modified Lichter (1982) medium (NLN-17; 17% sucrose, pH 6.2) and then transferred to NLN-10 (10% sucrose) medium. At 21 days, the culture was sieved through 250 µm nylon mesh to isolate the mid-cotyledonary stage MD embryos. All experiments were performed with embryos at this developmental stage.

Preparation of homogenates. MD embryos were harvested, rinsed thoroughly with distilled water and suction filtered to remove excess moisture. After weighing, embryos were homogenized at 4°C by mortar and pestle in the presence of a small amount of acid-washed silica, grinding medium (80 mM Hepes-NaOH, pH 7.2, containing 0.32 M sucrose, 1 mM DTT, 1 mM EDTA) at a proportion of 5 mL/g fresh wt. of embryos and 150 mg PVPP/ mL grinding medium. After homogenization, an equal volume of grinding medium was added to the paste, and the slurry filtered through two layers of Miracloth. The filtered homogenate (10 mL/g fresh wt.) was used directly for the isolation and analysis of total acyl lipids, protein, or for *in vitro* acyl lipid bioassembly studies, as described below.

Analysis of acyl composition in lipids from MD embryos and mature seeds. Total acyl lipids were extracted immediately from fresh homogenates of MD embryos. Aliquots (300 µL; 30 mg fresh wt. equivalent) of homogenate were mixed with 1 mL iso-propanol, tubes capped (teflon liners in caps) and the mixture boiled for 5 min. The solution was cooled briefly and 0.5 mL CH_2Cl_2 added. After sitting at room temperature for 30 min with occasional vortexing, the organic and aqueous phases were separated by the sequential addition of 2 mL CH_2Cl_2 and of 2 mL 1 M KCl in 0.2 M H_3PO_4. Following centrifugation (500 x g for 5 min) the lower organic phase was saved and the aqueous phase washed twice with 2 mL CH_2Cl_2. The organic phases were combined and the solvents removed using a gentle stream of nitrogen to yield the total acyl lipid extract (TLE). In the analysis of mature seed of line BC-2, dry seeds (300 mg) were initially ground in 300 µL water mixed with 1 mL isopropanol using a Polytron at high speed for 1-2 min. The extract was then boiled for 5 min. before adding 0.5 mL CH_2Cl_2. The subsequent phase separation and extraction procedures were identical to those described above. Lipid species were resolved by TLC on Silica H plates and recovered and analyzed for acyl composition as described by Taylor et al. (1991). Prior to transmethylation and GC analysis, 2-10 µg of 17:0 fatty acid was added to each sample as an internal standard for the quantitative determination of fatty acid content.

Dry weight and protein determinations. Several samples (30-100 mg fresh wt. each) were taken from each batch of MD embryos for the determination of dry

weight following dessication at 100°C for 48 h. Protein present in the homogenates was assayed by the method of Bradford (1976) using bovine serum albumin as a standard.

Lipid bioassembly studies. Studies comparing the incorporation of ^{14}C-labeled oleoyl (18:1), eicosenoyl (20:1) and erucoyl (22:1) moieties into acyl lipids were conducted in reaction mixtures containing 90 mM Hepes-KOH, pH 7.4, 1 mM ATP, 1 mM CoASH, 2 mM $MgCl_2$, 200 µM glycerol-3-phosphate, 0.5 mM NADH, 0.5 mM NADPH, 0.4 to 0.6 mg homogenate protein and either 180 µM [1-^{14}C] 18:1-CoA (10-50 nCi $nmol^{-1}$), 180 µM [1-^{14}C] 20:1-CoA (47 nCi $nmol^{-1}$) or 180 µM [1-^{14}C] 22:1-CoA (34 nCi $nmol^{-1}$) in a final reaction volume of 0.5 mL. All incubations were carried out at 30°C, in open tubes rotated at 100 r.p.m. for 30 min to 4 h . Reactions were stopped by adding 2 mL CH_2Cl_2 : MeOH (1:2 v/v) and phases separated as described above. The radiolabeled neutral and polar lipid products were then fractionated by TLC on Silica H as described previously (Taylor et al. 1991). Individual lipid species were transmethylated and the ^{14}C fatty acid methyl ester composition determined by radio-HPLC as described by Taylor et al. (1992). The incorporation of ^{14}C acyl moieties into each glycerolipid species was expressed either by % distribution of ^{14}C, or as a specific activity (pmol min^{-1} mg $protein^{-1}$), based on the known radio-specific activity (nCi $nmol^{-1}$) of each [1-^{14}C] acyl-CoA substrate.

Results and Discussion

Lipid, protein and dry weight content in developing MD embryos of B. campestris line BC-2.
Mid-cotyledonary stage MD embryos of *B. napus* cv. Reston have been shown to be highly active in the biosynthesis and accumulation of acyl lipids (Taylor et al. 1991; 1992). Thus, this was the stage chosen for similar measurements in the present study. The dry weight of *B. campestris* MD embryos was about 18 % of their fresh weight. Protein content among batches of MD embryos was 30-35 mg/g fresh wt. or 17-20 % protein on a dry weight basis (166-195 mg protein/g dry wt.). The total acyl lipid content of MD embryo preparations (measured as total fatty acid methyl esters) was 80-110 µg/mg dry weight to or 480-600 µg/mg protein (Table 1). The values for all of these parameters are well within the ranges previously reported for mid-cotyledonary stage MD embryos from *B. napus* cv Reston (Holbrook et al. 1992).

Lipid composition of developing MD embryos vs mature seed of B. campestris line BC-2.
The total lipids of mid-cotyledonary MD embryos of the low erucic acid line BC-2 (Table 1) consisted primarily of oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) in amounts of about, respectively, 50, 23 and 12 wt %. The proportions of the very long chain monounsaturated fatty acids erucic (22:1) and eicosenoic (20:1) were characteristically low at less than 0.1 and 1 wt %, respectively. This acyl distribution is

Table 1. Acyl composition of total lipid extract of mid-cotyledonary MD embryos (MDE) and mature seed of *B. campestris* line BC-2.*

Acyl Composition		16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0	24:1	Total
Weight %	MDE	7.0	4.2	49.4	22.9	12.4	1.5	0.8	0.8	0.04	0.5	0.5	100
	Seed	4.4	1.7	56.9	24.4	10.5	0.5	0.8	0.3	0.12	0.1	0.2	100
µg/mg dry wt.	MDE	7.7	4.7	54.6	25.3	13.7	1.6	0.8	0.9	0.05	0.6	0.6	110.6
µg/mg protein	MDE	42.8	25.8	303.2	140.5	76.2	8.9	4.6	5.0	0.3	3.5	3.4	614.2

*** n = 2; the SD of all determinations was less than 5 %.**

similar to that reported previously for both MD and zygotic embryos of the low erucic acid *B. napus* cultivar Topas, although it more closely reflects the composition of late- to very late-, rather than mid-cotyledonary stages (Taylor et al. 1990a; Pomeroy et al. 1991). More importantly, the acyl distribution was qualitatively very similar to that found in mature seed of *B. campestris* line BC-2 (Table 1). Thus, mid-cotyledonary MD embryos possessed the ability to synthesize and accumulate lipids containing the same types of fatty acids found in fully mature zygotic embryos (seed) of the parent breeding line.

The acyl compositions of the various glycerolipid species are shown in Table 2. It is reasonable to assume that a quantitative measurement of the fatty acids present in the individual glycerolipid classes approximates the relative sizes of these pools, given that there are 3 fatty acids/TAG, 2 fatty acids/PC etc. The neutral lipids (TAG + DAG + MAG) contained more than 80 % of the fatty acids in the total lipid extract (TLE). The majority of these were found in TAGs, which comprised about 77 % of the TLE. The major fatty acids in TAGs, were 18:1, 18:2 and 18:3, comprising about 34 %, 16 % and 6 % respectively, of the total fatty acids present. About 15 % of the total fatty acids accumulated in the polar lipids, with LPC/PC and LPE/PE/DGDG making up about 8 and 6 %, respectively. As expected, these membrane lipid components were predominantly comprised of 18:1 and 18:2 , with lesser quantities of 18:3 and 16:0. The pools of LPA, PA , MAG and FFA were quite low, and indicate that the extraction method did not lead to significant hydrolysis of polar lipids by endogenous phospholipases. This was probably prevented by the use of boiling iso-propanol as the initial solvent (Christie, 1982). These overall trends in the accumulation of various glycerolipid classes parallel those reported previously for developing MD embryos of *B. napus* cultivars (Taylor et al. 1990a; 1991; Holbrook et al. 1992) and, in particular, the acyl composition of the accumulating TAGs is similar to that reported in the low erucic acid *B. napus* cultivar Topas (Pomeroy et al. 1991).

In vitro lipid bioassembly studies

The ability of homogenates from the BC-2 MD embryos to incorporate ^{14}C 18:1-CoA into glycerolipids was studied in time-course experiments. The overall distribution of ^{14}C during the experiment showed that label moved through the Kennedy pathway intermediates LPA/PA, PC, DAG and accumulated in TAG (Fig. 1). Initially, the greatest proportion of ^{14}C was found in the intermediates PA and PC (0.5 h), but by 2 h, the proportion in these intermediates decreased, while that in DAG and especially TAG, increased.

This pattern is similar to that displayed *in vitro* by the MD embryo system from *B. napus* (Taylor et al. 1991; 1992) and confirms that the present *B. campestris* MD embryo cell-free system functionally displays all of the enzyme activities necessary for TAG biosynthesis via the Kennedy pathway (Fig. 2; Barron and Stumpf, 1962; Stymne and Stobart, 1987). The rapid increases in ^{14}C content of the TAG pool concomitant with minimal increases in ^{14}C in the DAG pool during the time course experiment, are entirely consistent with the role of DAG as an intermediate in the conversion of

Table 2. Acyl composition of lipid species isolated from a total lipid extract (TLE) of mid-cotyledonary MD embryos of *B. campestris* line BC-2.

	Fatty Acyl Composition (µg fatty acid/lipid species/mg dry wt)											Total	% of TLE[++]
Lipid Species	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0	24:1		
LPA + PA	0.20	0.17	0.17	0.04	nd	0.01	0.08	0.04	nd[+]	nd	nd	0.71	1.5
LPC + PC	0.59	0.25	2.20	1.12	0.30	0.01	0.02	nd	0.02	nd	nd	4.51	8.0
LPE + PE + DGDG	0.52	0.20	0.79	0.72	0.38	0.04	0.01	0.02	0.04	0.01	nd	2.73	5.6
MAG	0.10	0.05	0.06	0.02	0.03	0.01	nd	0.01	nd	0.01	nd	0.29	1.0
DAG	0.31	0.12	1.05	0.55	0.19	0.02	0.02	0.02	0.01	0.01	nd	2.30	4.0
TAG	4.79	2.11	33.45	16.00	6.41	0.80	0.69	0.52	0.08	0.34	0.22	65.41	76.4
FFA	0.35	0.34	0.10	0.02	0.01	0.02	nd	0.02	0.01	nd	nd	0.87	3.0
MGDG	0.27	0.14	0.07	0.06	0.04	nd	nd	nd	0.02	nd	nd	0.60	1.0

[+] nd = not detected; < 0.01 µg fatty acid/lipid species/mg dry wt.

[++] % of total lipid extract calculated assuming 3 fatty acids/TAG, 2 fatty acids/DAG or phospholipid (PA, PC, PE, MGDG, DGDG), 1 fatty acid/MAG, FFA or *lyso*- phospholipid.

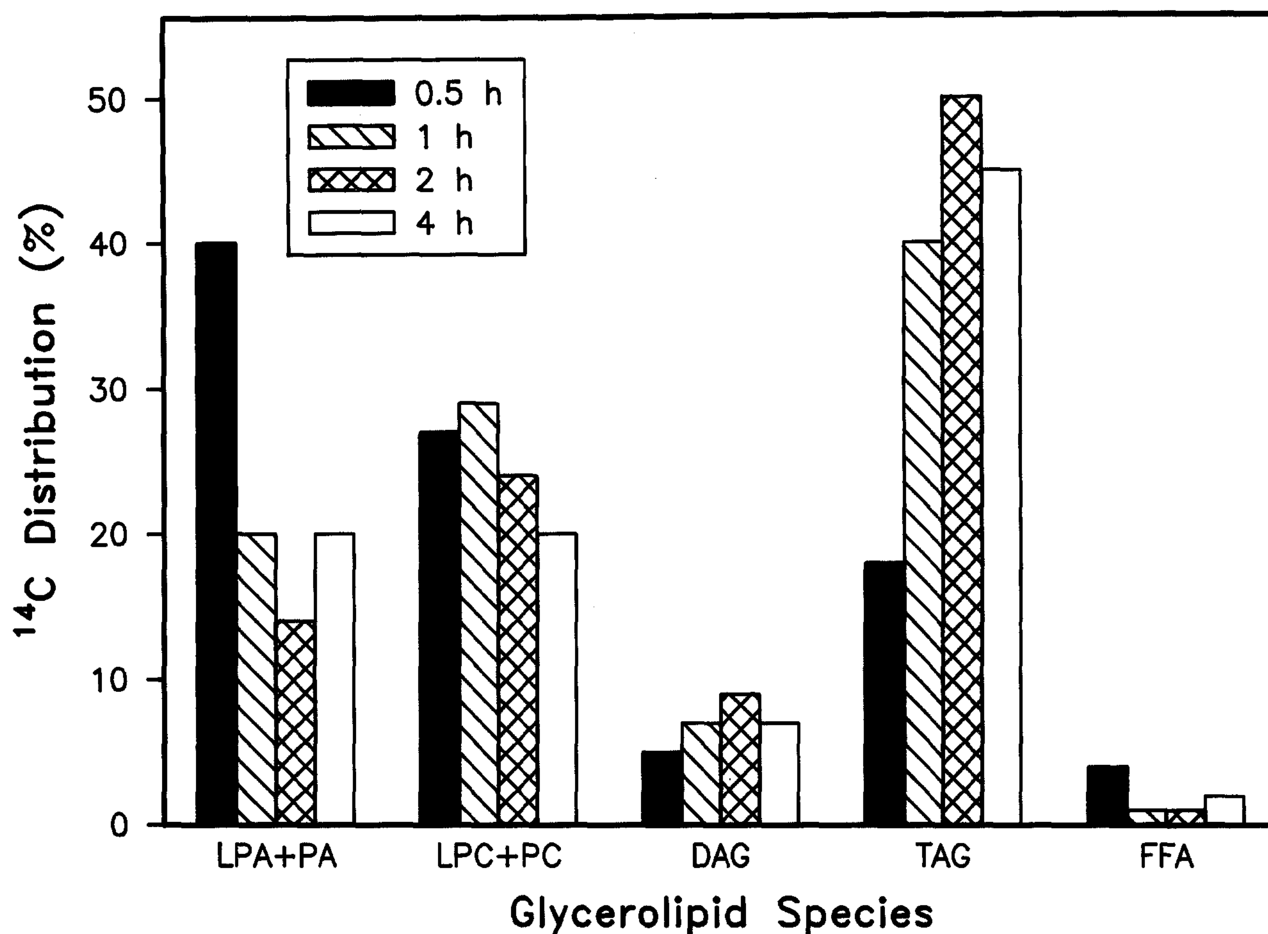

Fig. 1. Distribution of ^{14}C in major glycerolipid species during time course experiment. Homogenates from *B. campestris* line BC-2 MD embryos were incubated with ^{14}C 18:1-CoA in the presence of G-3-P as described in "Materials and Methods".

PA or PC to TAG. As in previous studies (Taylor et al. 1991; 1992), other glycerolipids such as MGDG, DGDG, PE, PG and MAG collectively accumulated less than 5 % of the total label from ^{14}C 18:1-CoA and this pattern remained virtually unchanged over the time course (data not shown). Thus these lipid species appear to be relatively unimportant in the overall mechanism for TAG biosynthesis.

The rate of TAG biosynthesis *in vitro,* (measured as incorporation of ^{14}C 18:1-CoA into the TAG fraction) was maximal at 50-70 pmol/min/mg protein over the first 30-60 min of incubation (Fig. 3). Thereafter, the rate of synthesis decreased although radiolabeled TAGs continued to accumulate. The maximum rates documented here agree well with those reported previously for 18:1 incorporation into TAGs *in vitro* by preparations from the *B. napus* MD Reston embryo system (Taylor et al. 1991; 1992). Furthermore, given that significant lipid accumulation in MD embryos begins at the early cotyledonary stage (*ca* 14 days in culture; data not shown), it can be estimated that by the mid-cotyledonary stage (21 days in culture) the native lipid (600 µg/mg protein) accumulated primarily over a 7-day period (day 14-21). Assuming the major lipid component to be TAG (*cf* Table 2) as triolein (MW 884), the *in vivo* rate of TAG accumulation over the early-mid cotyledonary transition becomes 67.3 pmol/min/mg protein. Thus, the maximum *in vitro* rates of TAG bioassembly (*cf* Fig. 3) can more than account for the known *in vivo* rate of TAG accumulation. The ability of the MD embryo system to accumulate TAGs *in vitro* at rates approaching those observed *in vivo* (Taylor et al. 1991) is, to our knowledge, an advantage afforded by only one other experimental oilseed system, the safflower microsome system (Stymne and Stobart, 1987).

After 4 hours, when the rate of TAG biosynthesis had decreased (Fig. 3), there was some evidence of phospholipase D activity contributing to an increase in

phosphatidic acid (PA) pools via the degradation of PC (see Fig. 1). Such activity was documented previously in studies of ^{3}H PC metabolism using homogenates from the *B. napus* MD embryo system (Taylor et al. 1991). Phospholipase D is known to be generally present in oilseeds (Roughan et al. 1978; Christie, 1982) and, more recently, has been detected in somatic embryos of *Catharanthus roseus* (Wissing et al. 1992).

An analysis of the radiolabeled glycerolipid products by transmethylation followed by radio-HPLC (Table 3) indicated that, in the presence of reducing equivalents, further desaturation of radiolabeled 18:1 to 18:2 was able to occur *in vitro*. After 30 min, radiolabeled 18:2 was found primarily in the LPA/PA and LPC/PC pools,and began to appear more prominently in DAGs and TAGs at 1 to 4 hours. The mechanism for desaturation of 18:1 and the subsequent distribution of 18:2 to other glycerolipids in MD embryos of *B. campestris* is probably similar to that known to occur in other oilseeds (*cf* Fig. 2): That is, 18:1 is desaturated while esterified to PC. Polyunsaturated C_{18} fatty acids can then (1) re-enter the acyl-CoA pool via the action of acyl-CoA: *lyso*-phosphatidylcholine acyltransferase (LPCAT) to acylate G-3-P, LPA or DAG, or (2) the PC can be interconverted to DAG via *sn*-1,2-diacylglycerol

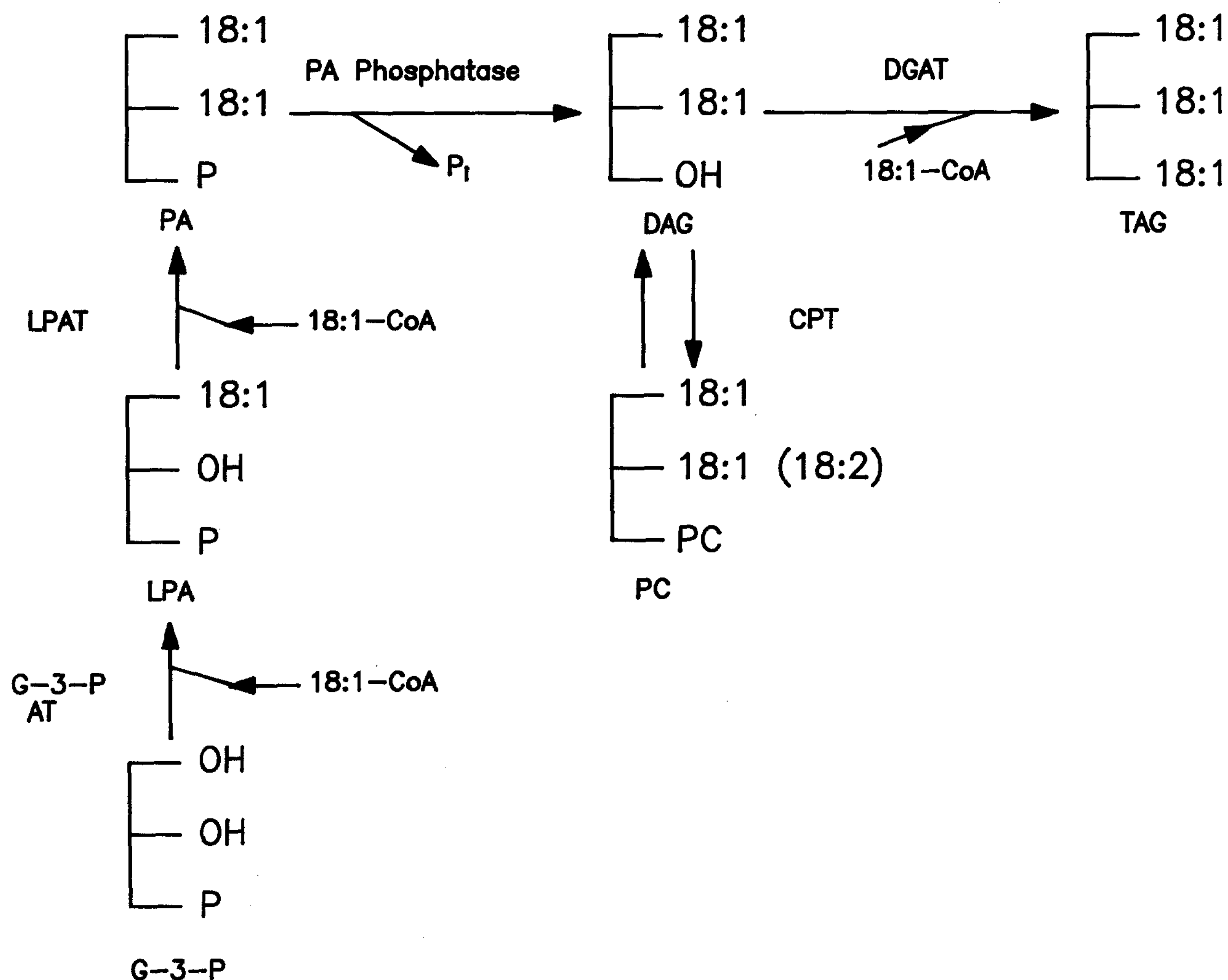

Fig. 2. Scheme for triacylglycerol bioassembly (Kennedy) pathway in developing oilseeds. After desaturation on the PC molecule, polyunsaturated C_{18} fatty acids can enter the acyl-CoA pool via the enzyme acyl-CoA:*lyso*-phosphatidylcholine acyltransferase (LPCAT; not shown). Adapted from Stymne and Stobart (1987). 18:1-CoA, oleoyl-Coenzyme A; 18:2, linoleic acid; G-3-P, glycerol-3-phosphate; G-3-PAT, glycerol-3-phosphate acyltransferase; LPA, *lyso*-phosphatidic acid; LPAT, *lyso*-phosphatidic acid acyltransferase; PA, phosphatidic acid; PA Phosphatase, phosphatidic acid phosphatase; PC, phosphatidylcholine; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; TAG, triacylglycerol; CPT, *sn*-1,2-diacylglycerol choline-phosphotransferase.

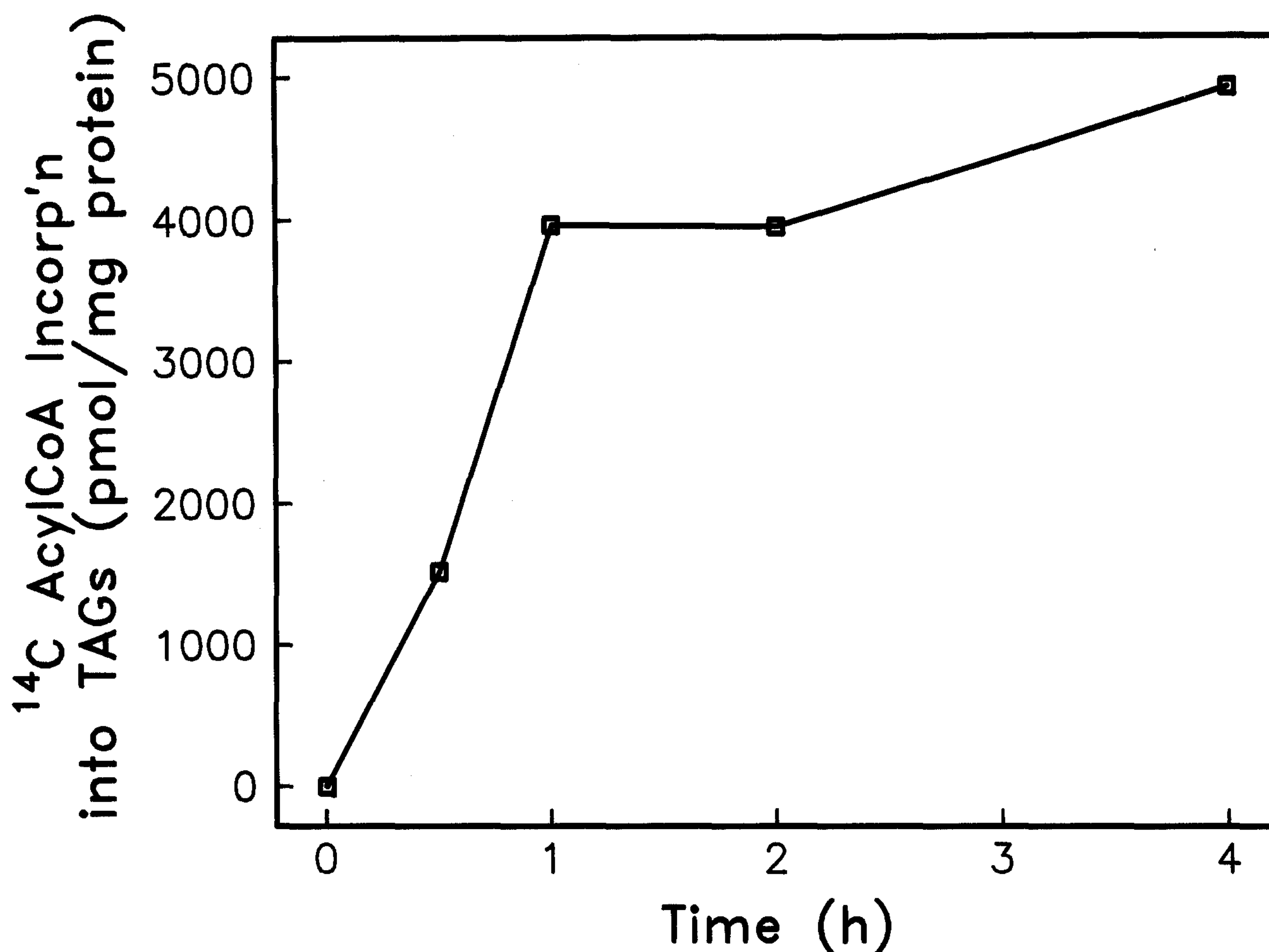

Fig. 3. Time course for incorporation of ^{14}C 18:1-CoA into the triacylglycerol fraction by an homogenate of 21-day cultured MD embryos of *B. campestris* line BC-2. Values are the means of three replicates; SD for each time point < ± 5%.

cholinephosphotransferase (CPT). Either route will lead to DAGs and subsequently TAGs enriched in polyunsaturated fatty acids (Stymne and Stobart, 1987). Given the distribution of the radiolabeled 18:2 moieties incorporated early in the time course experiment, (greatest proportion found in LPA/PA and LPC/PC after 30 min), it would seem that the LPCAT route is responsible for remobilizing most of the newly-formed ^{14}C 18:2 from PC into other acyl lipids. In addition to ^{14}C 18:2, small amounts of ^{14}C 18:3 (only 1-5% of total ^{14}C acyl moieties incorporated) were found in LPA/PA, LPC/PC and DAG over the first hour; after 4 hours, all of the 18:3 was sequestered in the TAG fraction (data not shown).

The BC-2 MD embryo preparations had no detectable capacity for elongating ^{14}C 18:1-CoA to ^{14}C-labeled 20:1 or 22:1 (VLCMFA biosynthesis) even when reactions were conducted in the presence of 10 mM malonyl-CoA (data not shown) as described previously (Taylor et al. 1992). This is as expected, since low erucic acid *B. campestris* and *B. napus* cultivars are deficient in the elongation system normally present in high erucic acid strains (Stumpf and Pollard, 1983; Downey and Craig, 1964; Röbbelen and Thies, 1980). However, while the capacity for VLCMFA biosynthesis is undetectable *in vitro* in the BC-2 embryos, the ability to *incorporate* VLCMFAs into glycerolipids was not impaired. As shown in Table 4, both ^{14}C-20:1-CoA and ^{14}C 22:1-CoA were readily incorporated into TAGs by cell-free homogenates from the BC-2 MD embryos. Similar findings have been reported in studies of MD embryos from the low erucic acid cultivar Topas (Weber et al. 1992). Interestingly, 20:1-CoA was incorporated to some degree into all Kennedy pathway intermediates; in contrast, ^{14}C 22:1-CoA, when supplied exogenously,

Table 3. Incorporation of ^{14}C 18:1-CoA and its desaturation product (^{14}C 18:2) into glycerolipids by an homogenate fraction from *B. campestris* MD embryos*.

Time	^{14}C Fatty Acid	^{14}C Fatty Acyl Distribution in Glycerolipid Species (%)				
		LPA + PA	LPC + PC	DAG	TAG	FFA
30 min	18:1	16.6	35.3	9.7	35.4	3.0
	18:2	72.3	25.8	0**	1.8	0
1 hour	18:1	17.8	39.2	4.1	37.4	1.6
	18:2	65.8	25.1	1.6	3.0	4.5
4 hour	18:1	12.0	18.6	14.9	50.6	3.9
	18:2	58.1	22.5	7.4	12.0	0

* Total ^{14}C fatty acyl moieties incorporated into glycerolipids at each time point were: 7.7 nmol/mg protein (30 min), 9.9 nmol/mg protein (1 hour) and 12.9 nmol/mg protein (4 hour). These include small amounts of ^{14}C 18:3, typically, 1-5% of the total.

** not detected.

Table 4. Incorporation of ^{14}C 20:1-CoA and ^{14}C 22:1-CoA into glycerolipids by an homogenate fraction from *B. campestris* MD embryos

^{14}C Acyl-CoA	Time (min)	^{14}C Fatty Acyl Incorporation into Glycerolipid Species (pmol/mg protein)						
		LPA/PA	LPC/PC	DAG	TAG	FFA	PE	Total
20:1-CoA	30	430	76	210	2050	400	0	3170
	60	980	260	200	2730	610	0	4780
22:1-CoA	30	0	0	0	2730	640	0	3370
	60	0	0	0	3460	960	0	4420

was found only in the TAG and FFA fractions (Table 4). The MD embryo system from high erucic acid *B. napus* cv Reston has exhibited similar trends during *in vitro* metabolism experiments (Taylor et al. 1992). In that study it was shown that the incorporation of ^{14}C-22:1 moieties into TAGs indeed occurs via the Kennedy pathway, but is intimately linked to *de novo* 22:1 biosynthesis by successive C_2 chain extensions of ^{14}C 18:1-CoA. Work is currently in progress in this laboratory to produce MD embryos from a high erucic acid cultivar of *B. campestris*. Only then will we be able to test whether a linked elongation/incorporation mechanism, similar to that observed in the Reston system, indeed operates in the assembly of TAGs containing 22:1 in *B. campestris* MD embryos.

In conclusion, the MD embryo system from the BC-2 line of *B. campestris* is ideally suited for biochemical studies related to lipid accumulation and triacylglycerol bioassembly. The system behaves in a manner identical to *B. napus* MD embryo systems currently in use for studies of embryo development, lipid accumulation and the regulation of these and related processes (Taylor et al. 1990a; 1991; 1992; Weber et al. 1992; Holbrook et al., 1992; Weselake et al. 1991; Wilberg et al, 1991; van Rooijen et al. 1992). The advantages afforded to *B. campestris* breeders by microspore culture technology (Baillie et al. 1992) are encouraging. Now, biochemists, developmental and molecular biologists, working alongside breeders, can take advantage of the fact that the *B. campestris* MD embryo system mimics the developing oilseed in accumulating acyl lipids. The added features of culture manipulability and the relative ease of accessing large numbers of stage-specific developing embryos will no doubt facilitate research to manipulate oil quality in this crop.

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