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The activity of acetyl-CoA carboxylase is not correlated with the rate of lipid synthesis during development of oilseed rape (*Brassica napus L.*) embryos

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Abstract. Acetyl-CoA carboxylase (ACCase; EC 6.4.1.2) activity has been determined in seed tissues of oilseed rape (*Brassica napus* L.), pea (*Pisum sativum* L.) and castor bean (*Ricinus communis* L.). A new method is described which leads to significantly higher measurable activities of the enzyme in tissue homogenates than previously reported. This method does not involve either Triton X-100 or centrifugation treatments which have been used previously in the study of the enzyme. In the case of oilseed rape the activity was also increased by removal of the testa from the seed. The activity of ACCase was determined throughout the development of oilseed rape embryos. Enzyme activity increased 3.5-fold as the embryo fresh weight increased from 0.3 to 2.0 mg and then reached a plateau at 1.1 nmol malonyl-CoA·min^{-1}·embryo^{-1}. The main period of lipid accumulation commenced at an embryo fresh weight of 2.3 mg, which was after the plateau in ACCase activity had been reached. Activity of the enzyme declined after an embryo fresh weight of 3.5 mg, which was before lipid accumulation in the embryo had been completed. Comparison of the activity of ACCase and the apparent in-vivo rate of lipid synthesis on an embryo-fresh-weight basis (i.e. nmol malonyl-CoA formed or utilized·min^{-1}·mg^{-1} fresh weight) revealed that ACCase activity declines relative to the rate of lipid synthesis throughout development. The negative correlation between these two rates is discussed in relation to the role of ACCase in the regulation of accumulation of storage lipid during embryo development.

Key words: Acetyl-CoA carboxylase – *Brassica* – Embryo (development) – Fatty acid synthesis – *Pisum* – *Ricinus*

Introduction

Developing seeds of many plants accumulate storage oils although the proportion of the final weight of the seed as oil varies from species to species. The principal fatty acids present in the seed storage oil also vary considerably between species (Murphy et al. 1993). The pathway of de-novo fatty-acid biosynthesis has been studied extensively and is essentially the same in all plant tissues (Harwood 1988; Murphy et al. 1993). However, the regulation of this pathway and of the Kennedy pathway of triacylglycerol synthesis is not so thoroughly understood. Acetyl-coenzyme A carboxylase (ACCase; EC 6.4.1.2) catalyses the ATP-dependent formation of malonyl-CoA from acetyl-CoA and bicarbonate. Malonyl-CoA is utilized as the substrate for the synthesis of fatty acids and its formation represents the first committed step of de-novo fatty-acid synthesis (Harwood 1988).

Three lines of evidence suggest that ACCase may be important in regulating the rate of fatty-acid biosynthesis in plant tissues. First, the changes in the relative levels of the acyl-acyl carrier protein (acyl-ACP) pools in leaf tissues during the transition from light to darkness suggest that the in-vivo activity of ACCase is being regulated (Post-Beittenmiller et al. 1991, 1992a). Second, the measurable activity of ACCase in seeds has been reported to be only comparable to, or less than, the rate of lipid biosynthesis (Simcox et al. 1979; Turnham and Northcote 1983; Charles et al. 1986; Bettey and Smith 1990), and ACCase could therefore represent a "rate-limiting" step. Third, increases during development in the lipid content of seeds are reported to be coincident with increases in the activity of ACCase in castor bean, oilseed rape, and soybean (Simcox et al. 1979; Turnham and Northcote 1983; Charles et al. 1986).

In the course of our studies of the capacity for synthesis of acetyl-CoA by plastids isolated from mid-phase developing embryos of oilseed rape we determined the activity of ACCase (Fan and Rawsthorne 1993). Extrapolation of the ACCase activity in the plastid pellets to an embryo basis revealed activities that were considerably greater than the maximum rate reported previously for extracts from developing embryos of oilseed rape (Turnham and Northcote 1983). The aims of this study were to develop this protocol to allow sensitive measurement of ACCase activity in crude extracts of seeds of oilseed rape and to then use it to to determine the temporal changes in ACCase activity and their relationship to lipid synthesis during seed development. The suitability of the revised procedure for the determination of ACCase activity in seed tissues of other species was also assessed.

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Abbreviations: ACCase = acetyl-CoA carboxylase; BSA = bovine serum albumin; Bicine = N,N-bis[2-hydroxyethyl]glycine; DAA = days after anthesis; DTT = dithiothreitol; PMSF = phenylmethanesulfonyl flouride

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Materials and methods

Chemicals. All chemicals were of Analar grade or equivalent and were purchased from BDH (Poole, Dorset, UK) or Sigma Chemical Co. Ltd. (Poole, Dorset, UK). All radiochemicals were purchased from Amersham International plc (Amersham, UK).

Plant material. Plants of oilseed rape (*Brassica napus* L. cv. Topas), pea [*Pisum sativum* L., BC1/10RR, derived from JI430 (John Innes Germplasm Collection) by Hedley et al. 1986], and castor bean (*Ricinus communis* L.) were grown in unlit glasshouses. Day and night temperatures were 18°C and 12°C respectively for oilseed rape and pea, and 25°C and 18°C for castor bean. All plants were grown in John Innes No.1 compost. Seeds of oilseed rape were harvested from siliques at several stages of development. Siliques were removed from three independent batches of oilseed rape plants between July and September 1992. All the embryos were removed from a single silique, their testas removed and the average fresh weight determined. The embryos were then kept on ice for up to 1 h before being used for enzyme assays or were frozen in liquid nitrogen and stored at −80°C before extraction for lipid analysis. For one of the batches of plants the flowers were tagged and the fresh weights of embryos were recorded at regular time intervals throughout development. These data allowed calculation of the rate of lipid accumulation.

Seeds of pea and castor bean were harvested at stages of development when lipid accumulation and/or ACCase activity had previously been shown to be maximal (Simcox et al. 1979; Bettey and Smith 1990).

Preparation of seed extracts. For the rapeseed extracts, seeds or embryos were homogenized in an all-glass homogenizer with 1 ml of ice-cold extraction buffer per five embryos. Older embryos (fresh weight > 2.5 mg·embryo^{-1}) were first homogenized using an ice-cold mortar and pestle. Two homogenization buffers were used. Medium A was that which we had used for preparation of intact plastids from homogenates of developing embryos: 0.5 M sorbitol, Hepes-NaOH (pH 7.4), 10 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA, 10% (v/v) ethanediol, 5 mM dithiothreitol (DTT), 1% (w/v) bovine serum albumin (BSA). Medium B was that used previously by Turnham and Northcote (1983) and contained 100 mM Tris-HCl (pH 7.5), 20% (v/v) glycerol, 0.2 mM phenylmethanesulfonyl fluoride (PMSF), and 15 mM 2-mercaptoethanol. Triton X-100 was added to some extracts to give a final concentration of 0.5% (v/v). The extracts were then incubated for 20 min with end-over-end mixing at 4°C. Some extracts, with or without added Triton, were spun at 12 000·*g* for 10 min at 4°C and the supernatant was used for the enzyme assays.

The embryos of pea and the embryo plus endosperm of *Ricinus* were removed from the testa and homogenized in medium A as described above. Centrifugation and Triton treatments were also as described above.

Assay of ACCase activity. The assay procedure was based upon that described by Turnham and Northcote (1982, 1983). Activity was determined by measuring the incorporation of ^{14}C from $NaH^{14}CO_3$ into malonyl-CoA. Incubations were at 25°C for 10 min and contained in a total volume of 200 µl, 60 mM N,N-bis[2-hydroxyethyl]glycine (Bicine)-NaOH (pH 8.5), 4 mM ATP, 7 mM $MgCl_2$, 8 mM $NaH^{14}CO_3$ (23 MBq·mmol^{-1}), 0.8 mM acetyl-CoA. The reaction was started by adding 40 µl of extract and stopped by the addition of 50 µl 6 M HCl. Two 100-µl samples of each stopped assay mixture were spotted onto Whatmann (Maidstone, Kent, UK) 3MM paper (1.5 cm^2), then dried and subjected to scintillation counting. Each extract was assayed in duplicate and control incubations contained all components except acetyl-CoA. Activity is expressed as the acetyl-CoA-dependent incorporation of $H^{14}CO_3^-$ into acid-stable compounds.

Thin-layer chromatography of reaction products. The ACCase assay was as described above except that it was stopped by the addition of 50 µl of 25% (w/v) trichloroacetic acid (TCA). The TCA was removed prior to chromatography by extraction with diethyl ether. Diethyl ether (750 µl) was added to each tube of stopped reaction mixture and the tube was vortex-mixed and left to stand for 1 min. The top, diethyl-ether layer which contained the TCA but none of the ^{14}C was removed and discarded. The extraction was repeated three more times then residual diethyl ether was removed with a stream of nitrogen gas. Aliquots of 30 µl of the aqueous fraction and 15 µl of 2-[^{14}C]-malonyl-CoA (7.4 kBq·ml^{-1}) were chromatographed on a cellulose TLC plate (Eastman Kodak Company, Rochester, N.Y., USA) in *n*-butanol:acetic acid:water (12:3:5, by vol.) and the plate was dried and subjected to autoradiography. The relative positions of radioactive compounds were determined and these areas on the plates were scraped and counted in a scintillation counter. Recovery of label in these fractions was calculated as a percentage of that which had been applied to the origin.

Determination of lipid contents of seeds and embryos. Total lipid content of the embryos was determined by quantitative fatty-acid methyl-ester (FAME) analysis. Total lipids were extracted as described by Murphy and Cummins (1989) except that a known amount of triheptadecanoin was added to the embryos prior to extraction. Extracts were methylated with sodium methoxide (Christie 1989) and methyl esters were separated using a gas chromatograph as described by Murphy and Cummins (1989). Individual fatty acids were quantified with respect to the heptadecanoic-acid internal standard.

The lipid accumulation per embryo was converted to a rate by differentiating the curve fitted to the data of total lipid content per embryo plotted against embryo fresh weight (Fig. 2) and by using the relationship between embryo fresh weight and days after anthesis (DAA). The daily rate of lipid accumulation was then converted to nmol malonyl-CoA utilized·min^{-1} by calculating the total number of malonyl-CoA equivalents required to synthesize the number of moles of each individual fatty acid present at the respective developmental stage. This calculation assumes no turnover of lipids and therefore derives the net or apparent rate of lipid synthesis. During embryo development of oilseed rape, lipid accumulation is predominantly as triacylglycerol (Perry and Harwood 1993a) which is unlikely to turn over to any great extent.

Results

Determination of ACCase activity. An essential prerequisite to thorough characterization of ACCase activity during embryo development of oilseed rape was the optimization of extraction and assay procedures for the enzyme. The ACCase activity in extracts from embryos made using medium A (the plastid-isolation medium which had previously given high activities; Fan and Rawsthorne 1993), was compared first with that in extracts from whole seeds prepared using the method described by Turnham and Northcote (1983), i.e. using medium B. The effects of addition of Triton X-100 and centrifugation on ACCase activity were also assessed. The maximum ACCase activity (1.02 nmol malonyl-CoA·min^{-1}·embryo^{-1}) was obtained in crude untreated homogenates of embryos in medium A and was decreased by addition of Triton X-100 or centrifugation, either separately or in combination (Fig. 1a). The ACCase activity in extracts from whole seeds using medium B was between two- and tenfold less than that in the untreated embryo extract, depending upon the additional treatment.

In order to determine whether the differences in ACCase activity described above were due to the differences

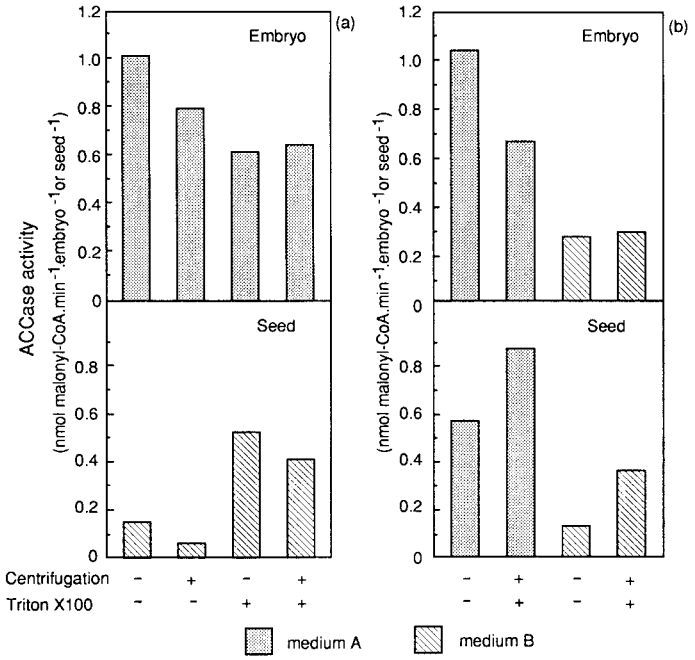

Fig. 1a,b. Effect of post-extraction treatment and removal of the testa on the activity of ACCase in crude homogenates of seeds of oilseed rape. Seeds were used which contained embryos with a fresh weight of approximately 2.5 mg·embryo^{-1}. Two extraction media were used. Medium A contained Hepes, sorbitol, KCl, $MgCl_2$, EDTA, ethanediol, DTT and BSA. Medium B contained Tris, glycerol, PMSF and 2-mercaptoethanol (Turnham and Northcote 1983). **a** Effect of addition of Triton X-100 and/or centrifugation on the ACCase activity of embryos extracted in medium A and of whole seeds extracted in medium B. **b** Effect of extraction medium and Triton/centrifugation treatment on ACCase activity in embryo or whole-seed homogenates. Each column represents the mean of two determinations of ACCase activity made on separate batches of seeds. Replicate values differed from the means by an average of 6%

between the extraction media or to the use of whole seeds versus embryos the enzyme activity was compared in extracts from either embryos or whole seeds in either medium A or B, with or without the Triton/centrifugation treatment (Fig. 1b). The effect of the medium was greater than that of the type of tissue extracted and the activities in extracts in medium B were on average 33% of those in comparable extracts in medium A. The presence of the testa caused an average decrease in activity of 47% which was alleviated partially by the Triton/centrifugation treatment, but only in the case of whole-seed extracts. We have not characterized which component(s) of medium A were responsible for the increases in measurable ACCase activity.

The assay for ACCase was optimized with respect to the concentration of assay components, buffer and pH using embryos extracted in medium A with no further treatment. The use of Bicine instead of Tris (used by Turnham and Northcote 1983) in the ACCase assay led to a twofold increase in activity (data not shown). Optimum conditions are given in the *Materials and methods*. We believe that the greater incorporation of ^{14}C from bicarbonate into acid-stable compounds obtained using embryos extracted in medium A, rather than seeds extracted in medium B with Triton/centrifugation treatments, represents increased ACCase activity for the following reasons. First, the assay was linear for at least 10 min and was dependent upon both acetyl-CoA and ATP. Second, thin-layer chromatography of the reaction products revealed that 96% of the applied ^{14}C-label cochromatographed with authentic [^{14}C]malonyl-CoA (data not shown). The activity also showed saturation kinetics for [^{14}C]bicarbonate with a maximum rate at 8 mM which is comparable to other values published previously (pea: 12 mM, Bettey and Smith 1990; oil palm: 7 mM, Turnham and Northcote 1982). However, the acetyl-CoA concentration required to give a maximum rate in these crude extracts was 0.8 mM, which is considerably greater than the saturating concentration or K_m values for acetyl-CoA published previously for oilseed rape and other plant ACCases (activity saturates typically at between 0.05 and 0.1 mM: Finlayson and Dennis 1983; Nikolau and Hawke 1984; Slabas and Hellyer 1985; Bettey et al 1992). The activity required a Mg^{2+} concentration which was 3 mM greater than that for ATP, in agreement with data for the purified enzyme of pea embryos (Bettey et al. 1992). The ACCase activity reached near-saturation at 4 mM ATP but further increases occurred up to 8 mM ATP and continued to increase beyond this concentration. The basis of this increase was not investigated but the ^{14}C incorporation was exclusively into malonyl-CoA at 8 mM ATP (data not shown).

Activity of ACCase and accumulation of lipid during rapeseed development. The revised extraction and assay procedure enabled us to detect much higher ACCase activity in rapeseed embryos at a single stage of development and this led us to re-examine the relationship between ACCase activity and storage-oil accumulation throughout embryo development. The pattern of ACCase activity during embryo development (Fig. 2) represents the combined data from embryos harvested between July 1992 and September 1992 from three independent batches of oilseed rape plants. For each of the different batches of plants the pattern of ACCase activity was highly reproducible (data not shown).

Acetyl-CoA carboxylase activity was readily detected in the smallest embryo size measured (average fresh weight = 0.3 mg). It increased from this point by 3.5-fold to reach an average value of 1.07 nmol malonyl-CoA produced·min^{-1}·embryo^{-1} (Fig. 2). This level of activity was maintained between an embryo size of 2.0 and 3.5 mg, corresponding to 27 to 40 DAA. Activity then declined as embryo fresh weight increased and the decline continued during embryo drying and maturation (Fig. 2). The decrease in ACCase activity during the later stages of development is not an artefact produced by a developmental increase of endogenous factors which reduce the extractable activity. When embryos of early, middle and late stages were co-extracted there was no loss of activity compared to that which would be predicted from the sum of the activities of each embryo class when extracted separately (Table 1).

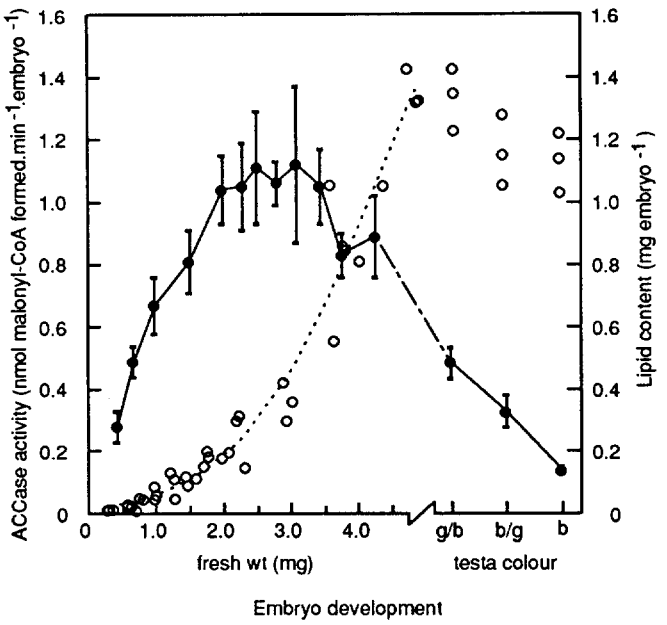

Fig. 2. Developmental changes in the activity of ACCase (●) and the lipid content (○) of embryos of oilseed rape. Development is expressed on the basis of increasing fresh weight of the embryo up to the end of lipid accumulation, after which the embryos dehydrated and testa colour has been used to provide a phenotypic scale (*g/b*, testa green with some browning, embryo green; *b/g*, testa predominantly brown, embryo yellowing; *b*, testa dark brown, embryo pale yellow). Each ACCase value represents the mean ±SE of between four and nine individual extractions made using embryos harvested from three separate batches of plants. Individual values of total lipids (determined as fatty-acid methyl esters) are given to which a curve (*dotted line*) has been fitted up to the end of lipid accumulation

The total lipid content of the same oilseed rape embryos increased slowly at first and then much more rapidly between embryo fresh weights of 2.3 and 4.7 mg·embryo^{-1} (corresponding to 33–50 DAA) (Fig. 2). The onset of this rapid increase occurred approximately 15 d later than the increase in ACCase activity. The maximum lipid content of the embryos was 1.33 μg·embryo^{-1} at 50 DAA (embryo fresh weight = 4.9 mg). During subsequent stages of embryo maturation and dehydration (which we defined on the basis of embryo and testa colouration) there was an apparent net decrease in the total lipid content. It is not known whether this represents a real decrease in lipid content or a decrease in the efficiency of lipid extraction with increasing embryo maturity.

In order to make a direct comparison between ACCase activity and lipid accumulation we have expressed these parameters as nmol malonyl-CoA synthesized or utilized·min^{-1}·mg^{-1} embryo fresh weight, respectively (see *Materials and methods*). Acetyl-CoA carboxylase activity declined steadily throughout development to reach a value of 0.21 nmol malonyl-CoA·min^{-1}·mg^{-1} embryo fresh weight at the end of the phase of lipid accumulation (Fig. 3). In contrast, the apparent rate of lipid synthesis dropped slightly followed by a twofold increase to a maximum of 0.36 nmol malonyl-CoA·min^{-1}·mg^{-1} embryo fresh weight during the same period.

Table 1. Acetyl-CoA carboxylase activity in crude extracts of developing oilseed rape embryos. Extracts were prepared from embryos at defined stages of development or from mixtures of embryos at different developmental stages (early, 0.7–1.1 mg·embryo^{-1}; mid, 2.3–3.0 mg·embryo^{-1}; late, testa colour brown, embryo yellow). The activities given represent the mean ± SE of extracts prepared from three separate groups of embryos at each developmental stage. The figures in parentheses are the actual activity determined for each mixed extract expressed as a percentage of the predicted activity (predicted activity is the sum of the activities of each embryo class when extracted separately)

Embryo stage	No. of embryos extracted	ACCase activity (nmol malonyl-CoA·min^{-1})
Early	10	7.63 ± 0.34
Mid	10	22.70 ± 0.88
Late	6	2.51 ± 0.35
Early + Mid	6 + 5	20.74 ± 1.87 (130)
Early + Late	6 + 3	6.54 ± 0.55 (112)
Mid + Late	6 + 3	13.41 ± 0.39 (90)

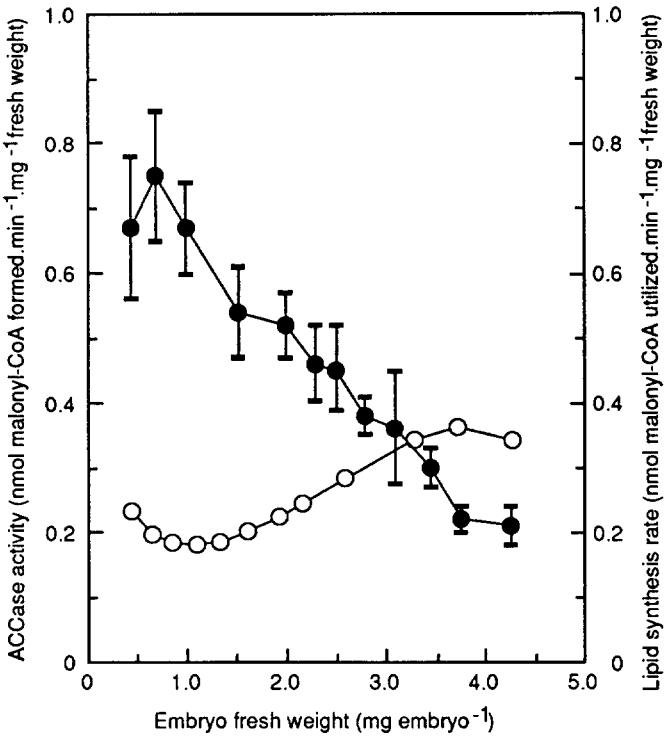

Fig. 3. Developmental changes in the activity of ACCase (●) and the calculated rate of lipid synthesis in vivo (○) expressed on an embryo-fresh-weight basis. Values are given for the period up to the end of lipid accumulation and for ACCase represent the mean ±SE of between four and nine individual extractions made using embryos of oilseed rape harvested from three separate batches of plants. The rate of lipid synthesis was derived from the curve fitted to the data for lipid content as shown in Fig. 2

Activity of ACCase in seeds of other species. In view of the decreases caused by Triton/centrifugation on the extractable activity of ACCase from oilseed rape embryos we studied two other species, one oleogenic (castor bean) and the other non-oleogenic (pea), to determine whether the same was true for extracts from their seed tissues. All extractions were made in medium A using either the em-

Table 2. Effect of extraction conditions on the activity of ACCase in crude extracts of developing pea embryos and castor-bean seeds. The extracts were subjected to centrifugation and/or the addition of Triton X-100. Extracts were treated with Triton X-100 and then centrifuged when both treatments were applied. Each value represents the mean ± SE of extracts prepared from three separate batches of seeds

Treatment	Enzyme activity (nmol malonyl-CoA $min^{-1} \cdot seed^{-1}$)	
	Pea embryo	Castor-bean seed
None	7.41 ± 0.62	18.4 ± 1.4
Triton X-100	5.30 ± 0.68	17.8 ± 0.2
Centrifugation	5.09 ± 0.60	10.7 ± 1.4
Triton plus centrifugation	4.18 ± 0.61	13.4 ± 1.7

bryo for pea or the embryo and endosperm for castor bean. Untreated extracts gave the highest activities, and the addition of Triton X-100 or use of centrifugation caused decreases in the measurable ACCase activity for both species that were comparable to those observed for oilseed rape (Table 2).

Discussion

We have developed an extraction and assay procedure which considerably increases the extractable activity of ACCase in total extracts of seed tissues of oilseed rape compared to the method published by Turnham and Northcote (1983). The maximum activity that we determined during development of glasshouse-grown rapeseed in 1992 was 1.13 nmol malonyl-CoA formed·min^{-1}·embryo $^{-1}$. This is fivefold greater than the maximum activity published previously (Turnham and Northcote 1983). Furthermore, replicated measurements made using our new protocol and embryos from comparable glasshouse-grown plants in 1993 gave ACCase activities per embryo which were ninefold greater than the value published previously (Table 1). The increase in extractable activity that we have reported is due to several factors. Changing the extraction medium from a Tris-based buffer to a more complex, Hepes-based one (plastid-isolation medium), removal of the testa before homogenization, not adding Triton X-100 or centrifuging the extract, and assaying in Bicine buffer with relatively high concentrations of acetyl-CoA and ATP all contributed to the increase in activity. The increase in ACCase activity when a Bicine-based rather than a Tris-based buffer is used in the assay was noted previously by Slabas and Hellyer (1985). We have also found that our protocol is equally suitable for use with seed tissues of other species. The activities that we determined for castor bean and pea (Table 2) were substantially greater than those published previously for each species: 1.1 and 1.3 nmol·min^{-1} per embryo or seed, respectively (Simcox et al. 1979; Bettey and Smith 1990).

Using our revised protocol, we have determined that ACCase activity per embryo increases by only 3.5-fold as embryos increase in size from less than 0.3 mg to the mid-stage of development, after which it is essentially constant for a considerable period during the main phase of lipid accumulation by the embryo. The increase in ACCase activity preceded considerably the main period of lipid accumulation by approximately 15 d. These observations contrast with the pattern described by Turnham and Northcote (1983) in which ACCase activity increased rapidly, peaked and then declined sharply. Furthermore, in this earlier study the ACCase activity and oil content increased simultaneously after 15 DAA. Notwithstanding these differences, both of these ACCase-activity profiles reveal a decline in enzyme activity before lipid accumulation has been completed.

Determination of the rate of lipid accumulation in this present study has allowed a direct comparison of the maximum extractable activity of ACCase with the apparent in-vivo rate of lipid synthesis. This direct comparison shows that during lipid accumulation by the embryo the ACCase activity declined continuously relative to the apparent rate of lipid synthesis. The ratio of the in-vitro rate of malonyl-CoA synthesis to that of in-vivo lipid synthesis (expressed as malonyl-CoA utilization) was almost 4:1 at the beginning of the rapid phase of lipid accumulation but was reduced to less than 1:1 at the end of it. Although we have been able to increase substantially the measurable activity of ACCase in rapeseed embryos, our in-vitro assay is still unable to account for the in-vivo rate of lipid accumulation during the later stages of embryo development. Given the complexity of the extraction medium that we used, we have not attempted to determine whether further increases in ACCase activity are possible. There is clearly scope for improvements to the extraction and assay procedures.

Whilst our data reveal that ACCase activity in seed tissues is much greater than was previously thought, this does not preclude earlier suggestions that ACCase activity could represent a regulatory point in lipid accumulation. Indeed the steady decline in specific activity of the enzyme during rapeseed development suggests that ACCase activity might become more limiting during the later stages of lipid accumulation. In contrast to the measurements of levels of acyl intermediates that suggest that ACCase may be regulating fatty-acid synthesis in vivo in leaves (Post-Beittenmiller et al. 1991, 1992a), a similar study using castor seeds does not implicate ACCase in the regulation of fatty-acid synthesis in non-photosynthetic tissues (Post-Beittenmiller et al. 1992b). Nevertheless it is interesting to note that the changes during development in the rate of [^{14}C]acetate incorporation into lipids by whole embryos of rapeseed (Perry and Harwood 1993b) are very similar to those reported in the present study for the activity of ACCase on a per embryo basis. The rate of [^{14}C]acetate incorporation per embryo increases early in development, reaches a plateau, and then declines before the completion of lipid accumulation. This suggests that the pattern of in-vitro ACCase activity might correlate with changes in the in-vivo flux to lipids during development. However, it is not clear whether the rate of [^{14}C]acetate incorporation by whole isolated embryos represents the actual flux to lipids in vivo. An analysis of the relationship between the developmental changes in the extractable ACCase activity and the flux

of carbon to fatty acids will require a much more rigorous approach in which the maximum catalytic activity of ACCase is altered and the effects on the in-vivo rate of lipid synthesis are determined.

We have shown that the determined rates of ACCase and lipid accumulation are not correlated in any way during the development of rapeseed embryos. This clear conclusion was only possible because we determined the rate of lipid accumulation rather than making a comparison between the enzyme activity and the amount of lipid per embryo, as employed in many previous studies of oilseed development. A similar approach should be applied to such studies in the future in order that developmental profiles of activities of enzymes involved in lipid accumulation can be interpreted meaningfully. For example, using the data presented by Weselake et al. (1993) for developing oilseed rape it is possible to determine the relative rates of diacylglycerol acyltransferase (DAG-AT) and lipid accumulation. The activity of DAG-AT increases relative to the rate of lipid accumulation throughout the period when lipid accumulation is rapid and then shows a relative decrease during the final stages of embryo development. The activities of component constituents of the fatty-acid synthetase complex remain high throughout lipid accumulation in rapeseed and will therefore show a different pattern again (Slabas et al. 1986; 1987; MacKintosh et al. 1989). Acetyl-CoA carboxylase, fatty-acid synthetase and DAG-AT represent initial, middle and final steps in the pathway of storage-oil synthesis. Differences in the relative activities of different steps of this pathway during embryo development suggest that the amount of control that they exert over flux to storage oil during the development of oilseed rape embryos may well vary during development. What is now clear is that there is no justification for considering ACCase to be a "rate-limiting enzyme" in rapeseed embryos on the basis that its activity is low relative to that of lipid synthesis or that its temporal pattern of activity is related to that of lipid synthesis.

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