THE ROYAL SOCIETY

Pea chloroplast carnitine acetyltransferase

C. Masterson and C. Wood*

Department of Biological and Nutritional Sciences, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

The purpose of this study was to resolve the controversy as to whether or not chloroplasts possess the enzyme carnitine acetyltransferase (CAT) and whether the activity of this enzyme is sufficient to support previously reported rates of fatty acid synthesis from acetylcarnitine. CAT catalyses the freely reversible reaction: carnitine + short-chain acylCoA ↔ short-chain acylcarnitine + CoASH. CAT activity was detected in the chloroplasts of *Pisum sativum* L. With membrane-impermeable acetyl CoA as a substrate, activity was only detected in ruptured chloroplasts and not with intact chloroplasts, indicating that the enzyme was located on the stromal side of the envelope. In crude preparations, CAT could only be detected using a sensitive radioenzymatic assay due to competing reactions from other enzymes using acetyl CoA and large amounts of ultraviolet-absorbing materials. After partial purification of the enzyme, CAT was detected in both the forward and reverse directions using spectrophotometric assays. Rates of 100 nmol of product formed per minute per milligram of protein were obtained, which is sufficient to support reported fatty acid synthesis rates from acetylcarnitine. Chloroplastic CAT showed optimal activity at pH 8.5 and had a high substrate specificity, handling C2–C4 acyl CoAs only. We believe that CAT has been satisfactorily demonstrated in pea chloroplasts.

Keywords: *Pisum sativum*; chloroplasts; carnitine acetyltransferase; acetylcarnitine

1. INTRODUCTION

Carnitine acetyltransferase or CAT (E.C.2.3.1.7) catalyses the reversible interchange of activated short-chain acyl groups between CoASH and the quaternary amine L-carnitine according to the following equation:

carnitine + short-chain acyl CoA

↔ short-chain acylcarnitine + CoASH,

$\Delta G^{0'} = 1.56\,\mathrm{kJ\,mol^{-1}}$.

As the low free energy change indicates, acylcarnitines are high-energy molecules representing activated acyl groups and the reaction is freely reversible.

CAT is just one member of a family of carnitine acyltransferase enzymes which all carry out similar reversible reactions in which an acyl group may be transferred between CoASH and carnitine. These carnitine acyltransferases have rather broad and overlapping acyl CoA specificities. CAT is the carnitine short-chain acyltransferase with a kinetic preference for short-chain acyl CoAs, typically C2 to C8 or C10 in chain length in mammals (Colucci & Gandour 1988). Carnitine and the carnitine acyltransferases are central to cellular metabolism and have numerous roles, but these generally fall into two categories (Bieber 1988). First, carnitine acts as a carrier of activated acyl groups across membranes via a three-step mechanism which ensures that acyl CoA pools in different cell compartments remain distinct and appropriate to the metabolic functions of each compartment. Acyl CoAs, which cannot permeate membranes, are converted to their corresponding carnitine ester by a carnitine acyltransferase on one side of a membrane barrier. Acylcarnitines, unlike acyl CoAs, can permeate membranes freely via a specific carrier protein (an anti-porter which permits facilitated diffusion of carnitine and acylcarnitines as a 1:1 exchange). The acyl CoA is then regenerated through the action of a second carnitine acyltransferase on the opposite side of the membrane barrier. The second role of carnitine is to buffer the acyl CoA:CoASH ratio. Because carnitine acyltransferases catalyse reactions which are close to equilibrium and use or release unesterified CoASH, they have the ability to buffer CoASH concentrations in different cell compartments.

CAT has been detected in pea (*Pisum sativum* L.) mitochondria (Thomas & McNeil 1976; Thomas & Wood 1982; Wood *et al.* 1983; Burgess & Thomas 1986; Gerbling & Gerhardt 1988; Budde *et al.* 1991). Burgess & Thomas (1986) demonstrated that this CAT was located on the matrix side of the mitochondrial inner membrane, where it was able to alleviate acetyl CoA pressure and pyruvate dehydrogenase inhibition during inhibition of the Krebs cycle. This was achieved by removal of accumulating acetyl CoA as acetylcarnitine, which was exported from the mitochondrion. This work was confirmed by Budde *et al.* (1991).

CAT activity was detected in pea chloroplasts by McLaren *et al.* (1985). Masterson *et al.* (1990*a*) showed that acetylcarnitine could act as a substrate for fatty acid biosynthesis in these chloroplasts, being incorporated into fatty acids at a rate of $172\,\mathrm{nmol\,min^{-1}\,mg^{-1}}$ chlorophyll. It was proposed that chloroplasts could be supplied with acetylcarnitine by mitochondria under certain metabolic conditions. However, Roughan *et al.* (1993) were unable to detect CAT activity in whole leaf homogenates of spinach, amaranthus and pea or in sucrose gradient- and Percoll-purified chloroplasts isolated from spinach leaves and pea shoots. They concluded that there was insufficient CAT activity present in the leaves to account for *in vivo* rates of fatty acid synthesis. This paper addresses the controversy by reinvestigating the existence of chloroplastic CAT in pea leaves.

*Author for correspondence (clifford.wood@ncl.ac.uk).

Received 23 August 1999 *Accepted* 12 October 1999

2. MATERIAL AND METHODS

(a) *Material*

General acids, bases and inorganic salts (AnalaR grade where possible) were supplied by BDH Chemicals, Poole, UK. All organic buffers, osmotica, bovine serum albumin (BSA), L-carnitine, L-acetylcarnitine, D,L-acetylcarnitine and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma (London) Chemical Co., Poole, UK. Anti-rabbit IgG-AP secondary antibody was obtained from Boehringer Mannheim, Lewes, UK. Bio-Rad protein assay dye reagent was obtained from Bio-Rad Laboratories Ltd, Hemel Hempstead, UK. Protein standards and nitrocellulose were supplied by Pharmacia Biotech, St Albans, UK. Dyematrex gel blue B affinity chromatography medium was supplied by Amicon Ltd, Upper Mill, Stonehouse, UK. [1-^{14}C]Acetyl CoA was from Amersham, UK. Acrylamide was supplied by NBL, Cramlington, UK. Pea seeds were supplied by Batchelor Foods Ltd, Worksop, UK.

(b) *Growth of plants*

Pea seeds (*P. sativum* L. cultivar Conquest) were imbibed overnight in running tap water, sown in moist vermiculite and grown for two to three weeks under glass in natural sunlight.

(c) *Isolation and purification of chloroplasts*

Chloroplasts were extracted from two- to three-week-old pea leaves by the method of Miflin & Beevers (1974) and purified on sucrose density gradients as described previously (Thomas *et al.* 1982). Chloroplasts, when not required intact, were ruptured osmotically or by freeze–thawing and released or soluble stromal proteins were separated from membrane-bound proteins by centrifuging at 144 000 *g* for 30 min. The soluble and released proteins in the supernatant fraction were precipitated with $(NH_4)_2SO_4$ at 75% saturation. Proteins obtained in this way could be stored at −80 °C for at least two months without loss of CAT activity.

(d) *Assays*

The reactions catalysed by CAT are defined as the forward reaction and the reverse reaction according to the following convention (Bieber & Farrell 1983):

$$\text{forward reaction} \rightarrow$$
$$\text{acetyl CoA} + \text{carnitine} \leftrightarrow \text{acetylcarnitine} + \text{CoASH}$$
$$\leftarrow \text{reverse reaction.}$$

In the forward direction, acetyl CoA and carnitine are used as substrates and CAT was assayed either using the radioactive product formation assay of Cederblad & Lindstedt (1972) as used by Wood *et al.* (1983) or by monitoring CoASH release spectrophotometrically with the SH reagent DTNB (Pearson *et al.* 1974). This latter assay was also used to determine the substrate specificity of CAT by using acyl CoA substrates of varying chain lengths. In the reverse direction, acetylcarnitine and CoASH are used as substrates and CAT activity was measured by following thioester formation spectrophotometrically at 232 nm (Pearson *et al.* 1974).

In the radioactive forward assay, the reaction mixture consisted of 50 mM *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethane sulphonic acid] (HEPES), pH 7.5, 0.33 M sorbitol, 1 mM $MgSO_4$, 0.5% (w/v) BSA, 1 mM L-carnitine and 10 µM acetyl CoA containing 9.25 kBq (0.25 µCi) [1-^{14}C]acetyl CoA (SA 59 mCi mmol^{-1}). The reaction was started by the addition of 0.5 ml of sample giving a total reaction volume of 1 ml. The reaction was allowed to proceed for 30 min at 27 °C in the dark. At termination, 0.5 ml of the reaction mixture, together with 1 mg D,L-acetylcarnitine carrier, was applied to a 2 ml column of Dowex 2 × 8 (200–400 mesh) resin in the Cl^- form. Radioactive acetylcarnitine was eluted from the column with water whilst the acetyl CoA substrate was retained by the column. The eluate was subjected to thin layer chromatography (TLC) analysis and the chromatogram developed using the acidic solvent of Eneroth & Linstedt (1965) which consisted of methanol:acetone:HCl (90:10:4 by volume). Radioactive spots from the TLC plates were visualized using a Birchover spark chamber (Birchover Instruments Ltd, Letchworth, UK) using Polaroid 667 film. Spots scraped from the TLC plate were counted in 10 ml Optiphase scintillant for 10 min. The identity of the acetylcarnitine spot was confirmed by TLC with an authentic standard and by mass spectrometry as described by McLaren *et al.* (1985).

The DTNB forward assay is based on measuring the initial rates of CoASH released from acyl CoA substrates using the SH reagent DTNB which forms a yellow coloured 5′-thio-2-nitrobenzoate anion with CoASH which absorbs strongly at 412nm. The assay medium contained 0.1 M Tris–HCl, pH 7.8, 0.125 mM DTNB, 1.25 mM ethylenediaminetetra-acetic acid (disodium salt) (EDTA), 0.15 mM acyl CoA and 1 mM L-carnitine. Enzyme was added to the assay medium (final volume 3 ml) to begin the reaction which was monitored at 412 nm at room temperature. Control assays carried out in the absence of L-carnitine were used to measure any CoASH released by competing reactions. These activities were subtracted from all measurements taken in the presence of L-carnitine to give the CAT activity.

In the 232 reverse assay, the initial rate of acetyl CoA formation from acetylcarnitine substrate was followed by measuring the increase in absorbance at 232 nm. This assay was only possible with partially purified CAT preparations because of the high 232 backgrounds with crude preparations. The assay medium contained 50 mM HEPES, pH 7.8, 0.125 mM EDTA (disodium salt), 0.25 mM CoASH and 2.5 mM L-acetylcarnitine. The reaction was started by the addition of 50 µl enzyme (final volume 3 ml). Control assays were carried out in the absence of CoASH.

Protein was measured by the method of Bradford (1976) using Biorad protein assay dye reagent and BSA as a standard protein. Chlorophyll was measured by the method of Arnon (1949).

(e) *SDS–PAGE and Western blotting*

Slab SDS–PAGE was performed as described by Laemmli (1970) using a 10% separating and 4% stacking gel. The same standards were used for all gels: phosphorylase *b* (94 000 Da), BSA (67 000 Da), ovalbumin (43 000 Da), carbonic anhydrase (30 000 Da), trypsin inhibitor (20 100 Da) and α-lactalbumin (14 400 Da).

After transfer to nitrocellulose membranes and washing in phosphate-buffered saline (PBS), the blots were blocked with 3% (w/v) fat-free milk protein in PBS and washed. They were incubated with antibody solution (1:1000 dilution) for 12 h at 4 °C, then washed thoroughly with PBS. The blots were then decorated with alkaline phosphatase-conjugated secondary antibody at 1:1000 dilution for 4 h at room temperature. Finally, they were washed, stained with fast blue RR, destained and dried.

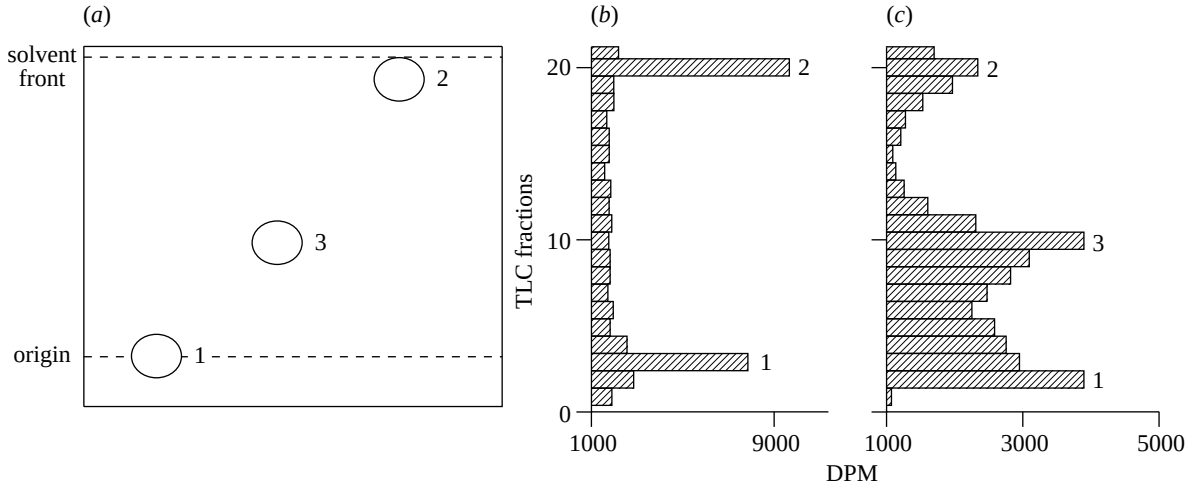

Figure 1. TLC analysis of acetate, acetyl CoA, acetylcarnitine and chloroplast incubations. CAT was assayed in the forward direction by the radioenzymatic assay of Cederblad & Lindstedt (1972) as described in § 2. Samples of the radioactive product eluted from the Dowex columns were separated by TLC with methanol:acetone:HCl (90:10:4 by volume) as the solvent. One centimetre fractions were scraped from the developed plate and counted in 10 ml Optiphase scintillant. (*a*) Running position of the authentic standards. (*b*) Radioactivity recovered from intact chloroplasts fed ^{14}C-acetyl CoA and carnitine. (*c*) Radioactivity recovered from osmotically ruptured chloroplasts fed ^{14}C-acetyl CoA and carnitine. Peak 1, acetyl CoA; peak 2, acetate; peak 3, acetylcarnitine.

(f) ***Dyematrex blue B column chromatography***

Dyematrex gel blue B in 20 mM Tris–HCl, pH 7.5 (buffer A) was poured into a column (1.6 cm internal diameter × 25 cm) to give a 50 ml volume column and equilibrated with buffer A. The stromal protein fraction precipitated by $(NH_4)_2SO_4$ at 75% saturation was resuspended in buffer A and dialysed against two changes of 1l buffer A to remove the $(NH_4)_2SO_4$. The sample volume was reduced to *ca.* 5 ml in dialysis sacs using dry polyethylene glycol (PEG) 20 000 and loaded on the dyematrex column. The column was washed with three column volumes of buffer A. CAT was then eluted with buffer A containing 1.5 M KCl. The eluate was dialysed against buffer A to remove the KCl and then reduced in volume to *ca.* 5 ml in dialysis sacs using dry PEG 20 000.

3. RESULTS

It has been reported earlier that pea mitochondria possess CAT activity (Wood *et al.* 1983; Burgess & Thomas 1986). It was therefore important to ensure that our chloroplast fraction was free of mitochondrial contamination. Confirmation of purity came from marker enzyme assays (Burgess *et al.* 1985; McLaren *et al.* 1985) and the probing of Western blots with antibodies for organelle-specific proteins. Proteins from purified chloroplasts or mitochondria were run on SDS–PAGE gels and, after transfer to nitrocellulose, probed with either rabbit anti-fescue rubisco antibody or rabbit anti-pea mitochondrial stress 70 protein antibody. The Western blots were developed following decoration with an anti-rabbit IgG-AP secondary antibody. Only two proteins in the chloroplast track cross-reacted with the rubisco antibody (with M_rs of 55 000 and 16 000 corresponding to the large and small subunits of rubisco, respectively) and only one protein from the mitochondrial protein track (M_r 70 000) reacted with the stress 70 antibody. No signal was seen with the rubisco antibody in the mitochondrial track and no signal was seen with the stress 70 protein antibody in the chloroplast track (results not shown). Therefore, any CAT activity detected in the chloroplasts could not be due to mitochondrial contamination.

Intact and broken chloroplasts were assayed for CAT activity in the forward direction using [1-^{14}C]acetyl CoA as a substrate according to the method of Cederblad & Lindstedt (1972). Eluates from the Dowex columns were subjected to TLC analysis. Acetylcarnitine formation could only be detected in ruptured chloroplasts (figure 1). Figure 1*a* shows the running position of the acetyl CoA, acetylcarnitine and acetate standards. Figure 1*b*,*c* shows the distribution of ^{14}C-acetylcarnitine obtained with incubations of intact and ruptured chloroplasts, respectively. No ^{14}C-acetylcarnitine was formed with intact chloroplasts. A peak of acetylcarnitine was only found with the ruptured chloroplasts. Thus, CAT is located on the inside (stromal side) of the chloroplast membrane barrier and the [1-^{14}C]acetyl CoA substrate is unable to penetrate the intact chloroplast membrane. Activity was only obtained when the chloroplasts were osmotically ruptured and the substrate had access to the internal enzyme.

Intact chloroplasts were ruptured by freeze–thawing and soluble or released stromal proteins were separated from membrane-bound proteins by centrifugation at 144 000 *g* for 30 min. Table 1 shows that CAT activity was only detected in the soluble protein fraction and no activity was associated with the membrane pellet.

The soluble stromal protein fraction was concentrated in dialysis sacs using dry PEG (20 000 Da) and the CAT assays were repeated with incubations containing [1-^{14}C]acetyl CoA in the presence or absence of carnitine. Figure 2 shows that, in the absence of carnitine, a second radioactive compound was obtained from the radioactive acetyl CoA. The identity of this compound is unknown but was not acetate produced by acetyl CoA hydrolysis. The incubation containing carnitine produced radioactive acetylcarnitine with a great reduction of the unknown compound, showing that the stromal CAT competes

Table 1. *Location of chloroplastic CAT*

(CAT was assayed as in figure 1. One unit of activity is 1 pmol of acetylcarnitine formed per minute.)

sample	total units	total protein (mg)	specific activity (units mg^{-1} protein)
thawed chloroplasts	15 587	137	113.8
supernatant after centrifugation at 144 000 *g* for 30 min (soluble and released proteins)	15 587	58	268.7
pellet after centrifugation at 144 000 *g* for 30 min (membrane-bound proteins)	0	79	—

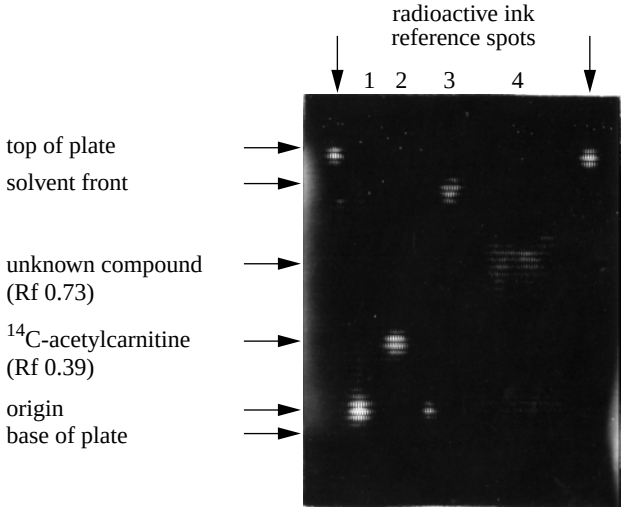

Figure 2. Spark chamber print showing the radioactive products resulting from incubation of stromal protein with ^{14}C-acetyl CoA in the presence and absence of carnitine. The assay used was the radioenzymatic assay of Cederblad & Lindstedt (1972). The incubations and TLC separations were carried out as described in § 2. Track 1, [1-^{14}C]acetyl CoA standard; track 2, incubation of stromal protein in the presence of 1 mM carnitine; track 3, [1-^{14}C]acetate standard; track 4, incubation of stromal protein in the absence of 1 mM carnitine.

favourably for acetyl CoA even in the presence of other acetyl CoA-handling enzymes. These results confirm that chloroplasts possess a CAT enzyme requiring carnitine as a substrate together with acetyl CoA.

CAT was concentrated and stabilized by precipitating the stromal protein with $(NH_4)_2SO_4$ at 75% saturation. When this precipitated protein was resuspended and assayed spectrophotometrically at 232 nm in the reverse direction using acetylcarnitine as a substrate, high activity rates in the range of 100–300 nmol min^{-1} mg^{-1} protein were obtained, with no activity exhibited by the various controls (figure 3*a*). This is a much higher activity than that obtained in the forward direction with acetyl CoA as a substrate (0.1–0.3 nmol min^{-1} mg^{-1} protein). In fact activity can only be detected in the forward direction using the sensitive radioactive assay. If the spectrophotometric DTNB assay is used to measure activity in the forward direction, no activity is detectable because there is such a high background rate of CoASH release in the absence of carnitine. Clearly in this impure preparation there is competition from other enzymes for the acetyl CoA substrate, making CAT activity difficult to measure. When the CAT is purified further by passage through a Dyematrex blue B column, an equally high activity of 150 nmol min^{-1} mg^{-1} protein is obtained in the forward direction using the DTNB assay (figure 3*b*).

The acyl CoA specificity of chloroplastic CAT was tested using acetyl, butyryl, hexanoyl, octanoyl and palmitoyl CoA as substrates. Chloroplastic CAT was found to be highly specific. The activity measured with butyryl CoA was only 3.4% of the activity measured with acetyl CoA. No activity was detected with C6, C8 and Cl6 acyl CoAs.

Chloroplastic CAT exhibited optimal activity at pH 8.5, which is the physiological pH of the stroma in the light. This contrasted with CAT activity from pea cotyledon mitochondria, which showed optimal activity at pH 7.0.

4. DISCUSSION

This report confirms that pea leaf chloroplasts possess CAT activity. This activity cannot be attributed to mitochondrial contamination of the chloroplast preparations and was located exclusively on the stromal side of the chloroplast inner envelope. When assayed in the forward direction with acetyl CoA as a substrate, chloroplasts had to be ruptured to permit access of the acetyl CoA substrate to the enzyme in order to detect any activity. In the normal situation with intact chloroplasts acetyl CoA is excluded by the membrane barrier, thus preventing the detection of CAT activity.

Chloroplastic CAT is either a soluble stromal protein or a peripheral membrane protein which is weakly attached to the membrane and easily released without the use of detergents. We have consistently found no activity associated with pelleted chloroplast membranes. This is consistent with the findings that mammalian mitochondrial and peroxisomal CAT is found in the soluble matrix and not associated with a membrane fraction (Markwell *et al.* 1976; Colucci & Gandour 1988; Ramsay & Arduini 1993).

The CAT activity was dependent on acetyl CoA and carnitine as substrates in the forward direction and on acetylcarnitine and CoASH as substrates in the reverse direction. No activity was obtained with the various controls in the absence of substrate or when using boiled enzyme. In crude chloroplast preparations only low activities were detectable in the forward direction due to other competing reactions using the acetyl CoA substrate and masking the CAT activity. As the CAT preparation was purified further, higher activities were detected, in the region of 100 nmol min^{-1} mg^{-1} protein. Knowing the total CAT activity for the partially purified preparation and knowing

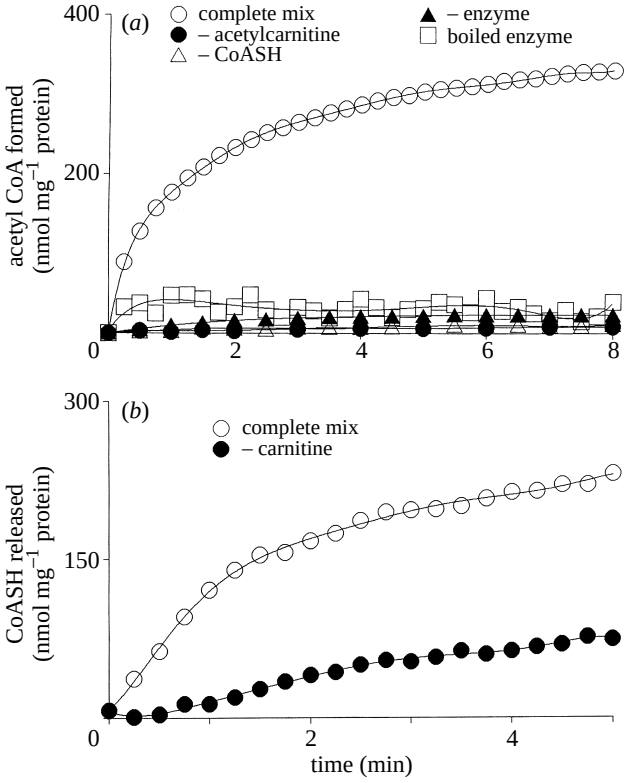

Figure 3. (*a*) The 232 reverse assay for CAT activity. The rate of acetyl CoA formation from acetylcarnitine substrate was followed by measuring the increase in absorbance at 232 nm. The incubations were carried out as described in § 2 using stromal protein precipitated by $(NH_4)_2SO_4$ at 75% saturation. The various control assays are also shown. (*b*) The DTNB forward assay for CAT activity. The rate of CoASH released from acetyl CoA substrate was followed using the SH reagent DTNB. The incubations were carried out as described in § 2 using stromal CAT which had been partially purified by passage through a Dyematrex blue B column. The control assay in the absence of carnitine is also shown.

the total chlorophyll content of the chloroplasts from which the CAT was isolated, it is possible to estimate a CAT activity of 200 nmol min^{-1} mg^{-1} chlorophyll, which is sufficient activity to account for the fatty acid synthesis rates of 172 nmol min^{-1} mg^{-1} chlorophyll reported by Masterson *et al.* (1990*a*) using acetylcarnitine as a substrate.

Chloroplastic CAT was highly substrate specific, using only C2–C4 acyl CoA substrates. In this respect chloroplastic CAT resembles the CAT found in the yeast *Torulopsis bovina* which also shows a very narrow chain length specificity, rather than mammalian CAT which has a broader acyl specificity showing activities with substrates of up to C8 or C10 acyl chain length (Emaus & Bieber 1983). Chloroplastic CAT also showed optimal activity at pH 8.5 which is the pH of the chloroplast stroma in the light. This is consistent for an enzyme proposed to be active in the chloroplast stroma during conditions of fatty acid synthesis in the light.

Roughan *et al.* (1993) failed to find any CAT activity in isolated chloroplasts from pea or spinach. The fact that they felt it necessary to add CoASH to the medium for fatty acid synthesis would suggest that their chloroplasts were damaged in some way. Intact chloroplasts should be self-sufficient in CoASH for metabolism as CoASH cannot cross the chloroplast membrane barrier (Brooks & Stumpf 1965). The addition of an external, non-penetrating CoASH should have no discernible effect upon fatty acid synthesis. If the chloroplasts were damaged then the soluble stromal CAT would have been lost during isolation.

It is suprising that Roughan *et al.* (1993) found no CAT activity in homogenates of pea shoots and spinach and amaranthus leaves as it is fairly well established that mitochondria at least possess CAT activity (Thomas & Wood 1982; Wood *et al.* 1983; Burgess & Thomas 1986; Gerbling & Gerhardt 1988; Budde *et al.* 1991) and this mitochondrial CAT should have been detected.

So what is the function of this chloroplastic CAT? Masterson *et al.* (1990*a*,*b*) showed that acetylcarnitine is the predominant precursor for fatty acid synthesis in pea and that CAT presumably converts this substrate to acetyl CoA inside the chloroplast. The efficiency of acetylcarnitine as a substrate for fatty acid synthesis has been questioned (Preiss *et al.* 1993; Roughan *et al.* 1993). Preiss *et al.* (1993) reported that acetylcarnitine was not efficiently incorporated into fatty acids in barley chloroplasts but they did report that an equimolar concentration of acetylcarnitine inhibited acetate incorporation by 70% which is consistent with our findings (Masterson *et al.* 1990*b*). Roughan *et al.* (1993) reported that when they used our paper chromatographic system to purify their acetylcarnitine, it 'inhibited fatty acid synthesis from acetate' but 'later preparations purified by TLC did not' (p. 1159). Whilst claiming to have failed to repeat our experiments using our methods, their assay for fatty acid synthesis included not only acetylcarnitine but also free CoASH and carnitine, both of which would interfere with the balance of the Thomas–Wood carnitine mechanism proposal (Thomas & Wood 1986; Masterson *et al.* 1990*b*) as summarized in Wood *et al.* (1992). Indeed, Wood *et al.* (1992) showed that free CoASH is a competitive inhibitor with respect to carnitine and acylcarnitine, both in mitochondrial and chloroplastic CAT function.

The present study demonstates that chloroplasts do indeed possess a CAT enzyme, which has been questioned in the past. The activity measured is in excess of that required for the fatty acid biosynthesis rates from acetylcarnitine reported by Masterson *et al.* (1990*a*). The chain length specificity and pH optimum of the enzyme are consistent with the enzyme functioning in the supply of acetyl CoA for chloroplastic fatty acid synthesis as proposed by Masterson *et al.* (1990*a*,*b*) and this enzyme clearly warrants further investigation.

The pea seeds were a kind gift from Batchelor Foods Ltd, Worksop, UK. We thank Dr J. A. Miernyk of the United States Department of Agriculture, Peoria, Illinois, USA for both the gift of the rubisco and Stress 70 antibodies and also for helpful discussions during the preparation of this manuscript. C. Masterson thanks The Royal Society for her university research fellowship. This work was part funded by Biotechnology and Biological Sciences Research Council grant BOM00602.

REFERENCES

Arnon, D. I. 1949 Copper enzymes in isolated chloroplasts. Polyphenol-oxidase in *Beta vulgaris*. *Plant Physiol.* **24**, 1–15.

Bieber, L. L. 1988 Carnitine. *A. Rev. Biochem.* **57**, 261–283.

Bieber, L. L. & Farrell, S. 1983 Carnitine acyltransferases. In *The enzymes*, vol. 16 (ed. P. D. Boyer), pp. 627–644. New York: Academic Press.

Bradford, M. M. 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

Brooks, J. L. & Stumpf, P. K. 1965 A soluble fatty acid synthesizing system from lettuce chloroplasts. *Biochim. Biophys. Acta* **98**, 213–216.

Budde, R. J. L., Fang, T. K., Randall, D. D. & Miernyk, J. A. 1991 Acetyl coenzyme A can regulate activity of the mitochondrial pyruvate dehydrogenase complex *in situ*. *Plant Physiol.* **95**, 131–136.

Burgess, N. & Thomas, D. R. 1986 Carnitine acetyltransferase in pea cotyledon mitochondria. *Planta* **167**, 58–65.

Burgess, N., Beakes, G. W. & Thomas, D. R. 1985 Separation of mitochondria from microbodies of *Pisum sativum* (L. cv. Alaska) cotyledons. *Planta* **166**, 151–155.

Cederblad, G. & Lindstedt, S. 1972 A method for the determination of carnitine in the picomole range. *Clin. Chim. Acta* **37**, 235–243.

Colucci, W. J. & Gandour, R. D. 1988 Carnitine acetyltransferase: a review of its biology, enzymology and bioorganic chemistry. *Biol. Chem.* **16**, 307–334.

Emaus, R. K. & Bieber, L. L. 1983 A biosynthetic role for carnitine in the yeast *Torulopsis bovina*. *J. Biol. Chem.* **258**, 13 160–13 165.

Eneroth, P. & Linstedt, G. 1965 Thin-layer chromatography of betaines and other compounds related to carnitine. *Anal. Biochem.* **30**, 275–278.

Gerbling, H. & Gerhardt, B. 1988 Carnitine acyltransferase activity of mitochondria from mung bean hypocotyls. *Planta* **174**, 90–93.

Laemmli, U. K. 1970 Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

McLaren, I., Wood, C., Jalil, M. N. H., Yong, B. C. S. & Thomas, D. R. 1985 Carnitine acyltransferases in pea chloroplasts. *Planta* **163**, 197–200.

Markwell, M. A. K., Tolbert, N. E. & Bieber, L. L. 1976 Comparison of the carnitine acyltransferase activities from rat liver peroxisomes and microsomes. *Arch. Biochem. Biophys.* **176**, 479–488.

Masterson, C., Wood, C. & Thomas, D. R. 1990*a* L-acetylcarnitine, a substrate for chloroplast fatty acid synthesis. *Plant Cell Environ.* **13**, 755–765.

Masterson, C., Wood, C. & Thomas, D. R. 1990*b* Inhibition studies on acetyl group incorporation into chloroplast fatty acids. *Plant Cell Environ.* **13**, 767–771.

Miflin, B. J. & Beevers, H. 1974 Isolation of intact plastids from a range of plant tissues. *Plant Physiol.* **53**, 870–874.

Pearson, D. J., Tubbs, P. K. & Chase, J. F. A. 1974 Carnitine and acylcarnitines. In *Methods in enzymological analysis* (ed. H. E. Bergmeyer), pp. 1758–1771. Weinheim, Germany: Chemie.

Preiss, M., Rosidi, B., Hoppe, P. & Schultz, G. 1993 Competition of CO_2 and acetate as substrates for fatty acid synthesis in immature chloroplasts of barley seedlings. *Plant Physiol.* **142**, 525–530.

Ramsay, R. R. & Arduini, A. 1993 The carnitine acyltransferases and their role in modulating acyl-CoA pools. *Arch. Biochem. Biophys.* **302**, 307–314.

Roughan, G., Post-Beittenmiller, D., Ohlrogge, J. & Browse, J. 1993 Is acetylcarnitine a substrate for fatty acid synthesis in plants? *Plant Physiol.* **101**, 1157–1162.

Thomas, D. R. & McNeil, P. H. 1976 The effect of carnitine on the oxidation of saturated fatty acids by pea cotyledon mitochondria. *Planta* **132**, 61–63.

Thomas, D. R. & Wood, C. 1982 Oxidation of acetate, acetyl CoA and acetylcarnitine by pea mitochondria. *Planta* **154**, 145–149.

Thomas, D. R. & Wood, C. 1986 The two β-oxidation sites in pea cotyledons. Carnitine palmitoyltransferase: location and function in pea mitochondria. *Planta* **168**, 261–266.

Thomas, D. R., Noh Hj Jalil, M., Cooke, R. J., Yong, B. C. S., Ariffin, A., McNeil, P. H. & Wood, C. 1982 The synthesis of palmitoylcarnitine by etio-chloroplasts of greening barley leaves. *Planta* **154**, 60–65.

Wood, C., Noh Hj Jalil, M., Ariffin, A., Yong, B. C. S. & Thomas, D. R. 1983 Carnitine short-chain acyltransferase in pea mitochondria. *Planta* **158**, 175–178.

Wood, C., Masterson, C. & Thomas, D. R. 1992 The role of carnitine in plant cell metabolism. In *Plant organelles: compartmentation of metabolism in photosynthetic cells* (ed. A. K. Tobin), pp. 229–263. Cambridge University Press.