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Carnitine acyltransferases in chloroplasts of *Pisum sativum* L.

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Abstract. Carnitine-acetyltransferase (EC 2.3.1.7) and carnitine-palmitoyltransferase (EC 2.3.1.21) activities were shown to be present in chloroplasts of green pea leaves and possibly to occur in leaf mitochondrial and peroxisomal fractions. A role for the enzymes in the transfer of acyl groups across membranes is suggested.

Key words: Acetylcarnitine – Carnitine acetyltransferase and palmitoyltransferase – Chloroplast (carnitine metabolism) – Palmitoylcarnitine – *Pisum* (carnitine metabolism).

Introduction

Thomas et al. (1983) demonstrated the presence of carnitine-acetyltransferase activity (EC 2.3.1.7) and of carnitine-palmitoyltransferase activity (EC 2.3.1.21) in purified etiochloroplasts of barley. The enzymes catalyse, respectively, reactions (i) and (ii).

$$\text{acetyl CoA} + \text{carnitine} \rightleftharpoons \text{acetylcarnitine} + \text{CoASH} \quad \text{(i)}$$

$$\text{palmitoyl CoA} + \text{carnitine} \rightleftharpoons \text{palmitoylcarnitine} + \text{CoASH} \quad \text{(ii)}$$

It was suggested that these enzymes might be involved in the flow of acyl groups across the chloroplast envelope. The purpose of the present work was to extend the investigation by assaying for the activities of these enzymes in pea chloroplasts.

Material and methods

Growth of peas. Pea seeds (*Pisum sativum* L. cv. Alaska) were purchased from Bachelor Foods, Workshop, Notts., UK. The seeds were imbibed in running tap water overnight, sown in moist vermiculite and grown in a greenhouse for 2–3 weeks (20° C, 16-h photoperiod).

Chemicals. Acetyl CoA, acetylcarnitine, palmitoyl CoA and palmitoylcarnitine were purchased from Sigma Chemical Co., Poole, Dorset, UK or the Boehringer Corporation (London), Lewes, East Sussex, UK. Radiochemicals were purchased from Amersham International, Amersham, Bucks., UK. Bovine serum albumin, Fraction V powder, Sigma, was purified by the method of Thomas et al. (1982). (L)-carnitine was a gift from Dr. Claudio Cassava of Sigma – Taw Seda, Rome, Italy.

Preparation of chloroplasts and assays of enzymes. Chloroplasts were extracted from 2- to 3-week-old peas by the method of Miflin and Beevers (1974). Purification of the chloroplasts on sucrose density gradients was as described previously (Thomas et al. 1982). Fractions from the sucrose density gradient were assayed for the following marker enzymes: fumarase (EC 4.2.1.2) (Hill and Bradshaw 1969), cytochrome-c oxidase (EC 1.9.3.1) (Smith 1955), catalase (EC 1.11.1.6) (Aebi 1974), non-reversible $NADP^+$-linked glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.9) (Kelly and Gibbs 1973) and chloroplast $NADP^+$-linked glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.13) (Wolosiuk and Buchanan 1976). Chlorophyll was determined by the method of Arnon (1949) and protein by the method of Lowry et al. (1951). Sucrose concentrations were determined with an Abbé refractometer (Bellingham & Stanley, London, UK). Carnitine acetyltransferase was assayed and acetylcarnitine characterised by methods described by Wood et al. (1983). Carnitine palmitoyltransferase was assayed by the direct method described by Thomas et al. (1983). Characterisation of palmitoylcarnitine produced by the intact chloroplast fraction utilised the indirect assay and methods described by Thomas et al. (1982). Thin-layer chromatography was on 0.25-mm wet-thickness silicagel (Kieselgel G; Merck, Darmstadt, FRG) with an acidic solvent, methanol:acetone:HCl (90:10: 4, by vol), and a basic solvent, methanol:dioxan:NH_3 (specific gravity 0.88) (30:45:25, by vol.) (Eneroth and Lindstedt 1965).

Results and discussion

The marker enzymes and chlorophyll (Figs. 1, 2) were distributed along the gradient in a pattern essentially the same as reported for barley etiochloroplasts (Thomas et al. 1983). Intact chloroplasts were collected between 19 ml and 25 ml, as indicated by the co-sedimentation of the $NADP^+$

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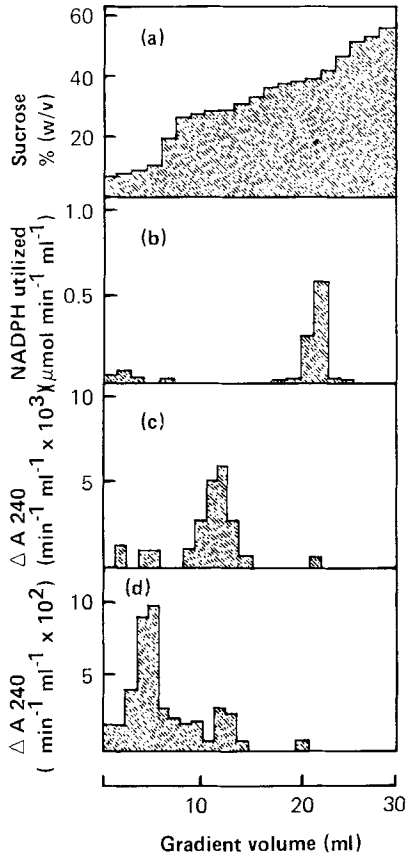

Fig. 1 a–d. Sucrose-density-gradient profiles of washed chloroplasts of pea leaf. Distribution of (**a**) sucrose, (**b**) NADP$^+$-glyceraldehyde-3-phosphate dehydrogenase, (**c**) fumarase and (**d**) catalase

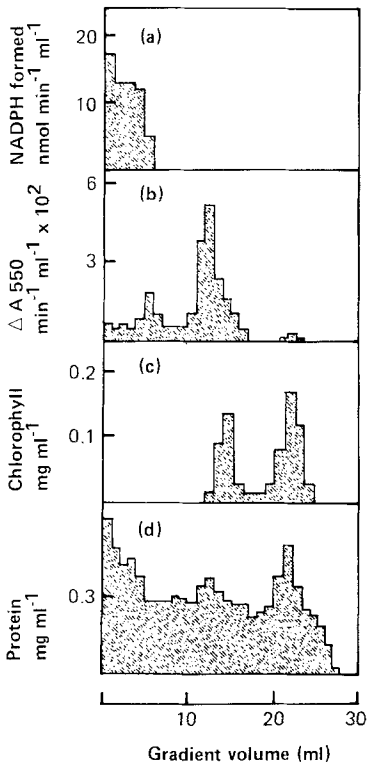

Fig. 2 a–d. Sucrose-density-gradient profiles of washed chloroplasts of pea leaf. Distribution of (**a**) non-reversible glyceraldehyde-3-phosphate dehydrogenase, (**b**) cytochrome-c oxidase, (**c**) chlorophyll and (**d**) protein

-glyceraldehyde-3-phosphate-dehydrogenase activity and chlorophyll. This region of the gradient showed a very minor contamination with the peroxisomal (catalase) and mitochondrial (fumarase and cytochrome c) markers but was entirely devoid of activity of the cytosolic marker non-reversible NADP$^+$-glyceraldehyde-3-phosphate dehydrogenase.

Carnitine acetyltransferase (Fig. 3 a) was found distributed between four fractions, coinciding with the markers for, respectively, the cytosolic, peroxisomal, mitochondrial and intact-chloroplast fractions. The presence of this enzyme activity in mitochondria from pea cotyledons has been reported previously (Wood et al. 1983) and was also found in the mitochondrial and peroxisomal fractions off the sucrose density gradients of barley etio-chloroplasts (Thomas et al. 1983). As Thomas et al. (1983) pointed out for barley etiochloroplasts, the procedures used in the present study were designed primarily for the isolation of purified intact chloroplasts and hence the putative presence of carnitine acetyltransferase in pea leaf peroxisomes requires further investigation. Currently, the presence in the cytosolic fraction of the short-chain acyltransferase is interpreted as attributable to contamination with enzyme released from organelles during the isolation procedure. Three peaks of activity of carnitine palmitoyltransferase were detected (Fig. 3 b) in the peroxisomal, mitochondrial and intact chloroplast fractions. The high activity of both acyltransferases in the chloroplast fraction cannot be attributed to contamination by other organelles because of the low recovery of peroxisomal and mitochondrial markers in the chloroplast fraction.

Following the acyltransferase assays, the tubes comprising the peroxisomal, mitochondrial and chloroplast fractions were bulked and subjected to thin-layer chromatography (TLC). The acetylcarnitine extracts yielded one radioactive spot in both the acidic (R_f 0.49) and basic (R_f 0.28) solvents, coinciding with authentic acetylcarnitine. Thin-layer chromatography of alkaline hydrolysates produced a non-radioactive spot of carnitine (R_f 0.46 in acidic solvent, R_f 0.1 in basic solvent). The

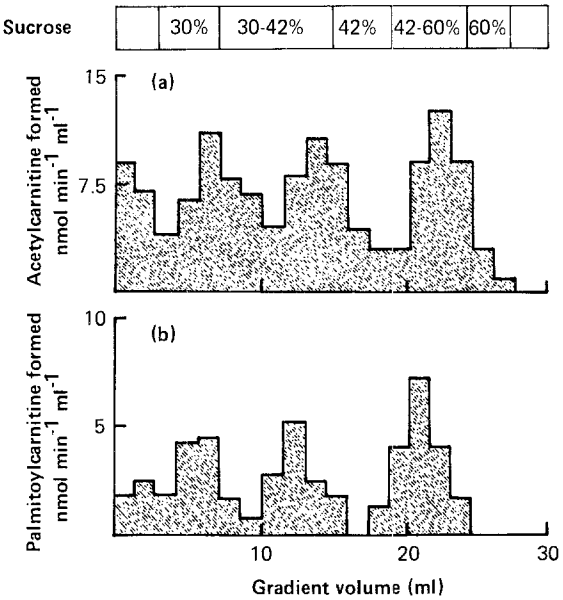

Fig. 3 a, b. Sucrose-density-gradient profiles of washed chloroplasts of pea leaf. Distribution of (**a**) carnitine acetyltransferase (**b**) carnitine palmitoyltransferase

Table 1. Recrystallization to constant specific activity of acetylcarnitine produced by pea chloroplasts. The solvent at each step was butan-l-ol

Crystallization step	Acetylcarnitine (Bq/2 mg)
1	4.10
2	3.83
3	3.25
4	3.13
5	3.16

palmitoylcarnitine extracts gave radioactive spots co-chromatographing with authentic palmitoylcarnitine (R_f 0.64 in acidic, R_f 0.61 in basic solvent) and alkaline hydrolysis of the extracts followed by TLC of the hydrolysates yielded a spot of radioactive carnitine (R_f 0.46 in acid solvent, R_f 0.1 in basic solvent).

Radioactive acetylcarnitine and palmitoylcarnitine produced by the chloroplasts were extracted and re-crystallized to constant radioactivity (Tables 1, 2). The identity of the purified crystal compound was confirmed by TLC as described above and by mass spectrometry. Mass spectra identical to those published (Hvistendahl et al. 1970) and to those of authentic compounds were obtained.

Although it is well established that fatty-acid synthesis in leaves is located in the chloroplast, the source of the precursor, acetyl CoA, is not clear. Pyruvate dehydrogenase has been detected in pea chloroplasts (Elias and Givan 1979; Williams and Randall 1979) and pyruvate has been shown to enter pea chloroplasts mainly by diffusion although at low concentrations its passage through the envelope may be carrier-mediated (Proudlove and Thurman 1980). Thus pyruvate could act as one source of acetyl CoA in pea chloroplasts. Murphy and Stumpf (1981) failed to find the pyruvate-dehydrogenase complex in mature spinach chloroplasts although there was some activity of the enzyme detected in butter-lettuce chloroplasts. This enzyme, however, was not CoASH-dependent which gives rise to doubt whether they assayed the pyruvate-dehydrogenase complex as Williams and Randall (1979) have shown this enzyme in pea chloroplasts to have a strict CoASH-dependence.

Table 2. Recrystallization to constant specific activity of palmitoylcarnitine produced by pea chloroplasts

Crystallization step	Solvent	Palmitoylcarnitine (Bq/2 mg)
1	Chloroform	9.53
2	Chloroform	5.10
3	Chloroform	4.98
4	Water	5.18
5	Water	5.12

Murphy and Stumpf (1982) Liedvogel and Stumpf (1982) and Murphy and Walker (1982) all suggested that a source of acetyl CoA in the spinach chloroplast originates from acetyl CoA produced in the mitochondria. They proposed that the acetyl CoA produced from pyruvate in mitochondria is hydrolysed by the enzyme acetyl CoA hydrolase to form free acetate and CoASH with release of energy. The free acetate then moves to the chloroplast where it reforms acetyl CoA.

The data presented here confirm that mature-pea chloroplasts, like the etio-chloroplasts of barley and the mitochondria of pea cotyledons, contain carnitine-short-chain-acyltransferase and carnitine-long-chain-acyltransferase activities. It is possible that another source of acetyl CoA is acetylcarnitine derived from pyruvate oxidation in mitochondria and exported from there in the manner proposed by Thomas and Wood (1982) and Wood et al. (1983). Thus the presence of carnitine short-chain acyltransferase in the chloroplast would allow acetyl CoA originating in the mitochondria to be imported into the chloroplast as acetylcarnitine. The presence of carnitine long-chain acyltransferase in the chloroplast would allow the export of acyl CoAs synthesised within the chloroplast, with concomitant conservation of thioester-bond energy (Thomas et al. 1983) to cystosol sites.

The presence of these enzymes would allow carnitine/acylcarnitine couples to buffer CoASH/acylCoA couples in the chloroplast in the manner suggested previously for barley chloroplasts (Thomas et al. 1982) and for pea mitochondria (McNeil and Thomas 1976; Thomas and Wood 1982).

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