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EFFECT OF CARNITINE ON GREENING BARLEY LEAVES

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Abstract—Carnitine increases chlorophyll production in greening barley leaves. [Methyl-^{14}C]carnitine fed to greening leaves was not utilized as a carbon source; most label was recovered in unchanged carnitine. Choline and betaine did not substitute for carnitine in promoting chlorophyll production, indicating that carnitine is unlikely to act as a nitrogen source. It is suggested that carnitine may play a role in facilitating acyl group transfer in the barley leaves.

INTRODUCTION

The presence of carnitine ($Me_3N^+CH_2CH(OH)CH_2COO^-$) in plants has been known since 1951 [1]. Panter and Mudd [2] detected carnitine in wheat seeds, oat seedlings, cauliflower and avocado. Carnitine was also detected in germinating pea cotyledons [3] and is present in barley leaves [4]. It has been shown that carnitine enhances fatty acid oxidation by avocado slices [5]. McNeil and Thomas [6] found that palmitate oxidation by mitochondria isolated from pea cotyledons was stimulated by 25–30% with carnitine. This stimulation of palmitate oxidation was similar to that reported earlier for liver and heart mitochondria [7]. In the present paper, further effects of carnitine have been investigated, and its utilization in plant tissue studied.

RESULTS

Figure 1 demonstrates the effect of carnitine on chlorophyll production in etiolated barley leaves exposed to light for 24 hr. Carnitine at concentrations below 20 mM stimulated chlorophyll production, the maximum stimulation occurring at 10 mM. At and above 25 mM concentration, chlorophyll production was below that of the control. Betaine and choline were tested also for their effect on the production of chlorophyll in greening barley (Fig. 1). Choline was found to stimulate at 5 mM but not to the same extent as 5 mM carnitine, and at higher concentrations inhibition was observed. Betaine was inhibitory at all concentrations tested. Although these two compounds, like carnitine, possess a quaternary ammonium group, they did not have the same effect as carnitine on the greening of etoliated barley leaves.

Greening barley leaves, pretreated with a range of concentrations of carnitine, were supplied $NaH^{14}CO_3$. The incorporation of radioactivity into ethanol-extractable material was greatest in leaves pretreated with 10 mM carnitine (Fig. 2). Dark fixation was only slightly affected by carnitine. It seems that carnitine, as it affects chlorophyll synthesis and hence CO_2 fixation, accelerates chloroplast development in greening barley leaves.

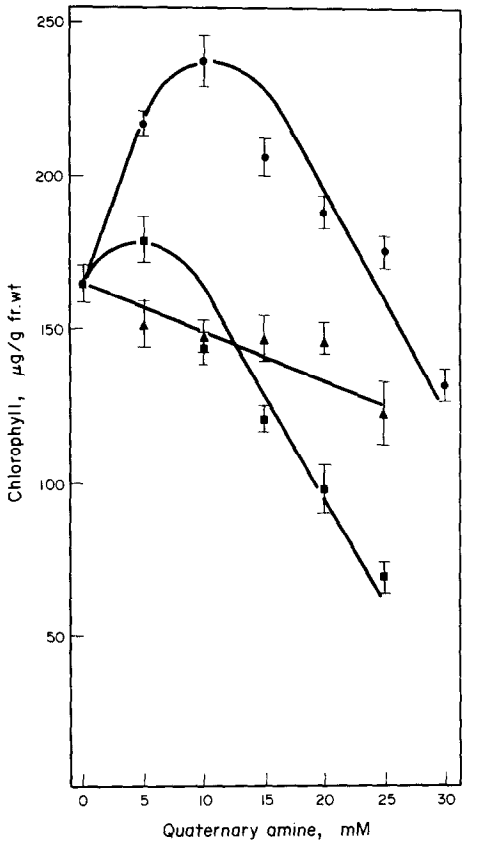

Fig. 1. The effect of quaternary amines on chlorophyll production in greening barley leaves. (●——●) carnitine, (▲——▲) betaine, (■——■) choline.

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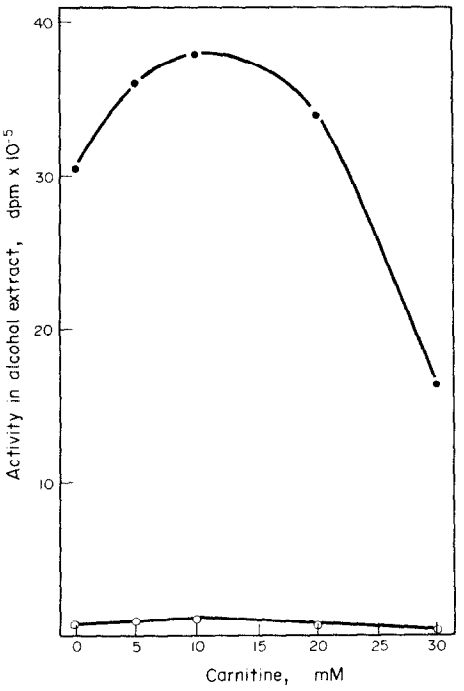

Fig. 2. The effect of (±)-carnitine on ^{14}C incorporation into alcohol-soluble products of greening barley leaves. (●——●) ^{14}C incorporation during 1 hr exposure to $NaH^{14}CO_3$ in light, (○——○) ^{14}C incorporation during 1 hr exposure to $NaH^{14}CO_3$ in darkness.

The effect of carnitine may be caused by its capacity to act as an acyl carrier [7] and so increasing the availability of precursors to synthesizing sites of essential components for chloroplast development and function. Alternatively, carnitine may act as a carbon or nitrogen source in the greening leaf. To test the latter possibility, L-[^{14}C-Me]-carnitine, D-[U-^{14}C]glucose and $NaH^{14}CO_3$ were fed to greening barley leaves for 1 hr in the light. The results demonstrated that carnitine was ineffective as a carbon source (Table 1). Only 40 pmol supplied carnitine contributed to the CO_2 evolved as compared to 5430 pmol from supplied glucose. Much lower levels of ^{14}C from carnitine were recovered in any fraction than when either glucose or $NaHCO_3$ was supplied to the leaves. Most of the ^{14}C recovered in fractions of carnitine-fed leaves was in unchanged carnitine as revealed by TLC (Table 2). A second radioactive spot on TLC plates of both the basic fraction of the ethanol extract and the protein hydrolysate was observed. TLC in acidic solvent was carried out and this second radioactive spot cochromatographed in both acidic and basic solvents with authentic palmitoylcarnitine.

DISCUSSION

Figure 2 and Table 1 indicate that the effect of carnitine in promoting chlorophyll synthesis in barley leaves would not be explained by suggesting that it acts as a carbon source. Carnitine is unlikely to act as a methyl donor group not only because little ^{14}C is incorporated into other components but also because the other 'onium' compounds, choline and betaine, do not substitute for carnitine in promoting chlorophyll synthesis. In animals, carnitine was unable to substitute for dietary choline in the prevention of fatty liver formation in rats [8] or as a growth factor in several choline-requiring organisms [9]. Choline added *in vitro* was without effect on respiration or fatty acid oxidation by liver preparations [7] and pea mitochondrial preparations [6] whereas carnitine added *in vitro* stimulated long chain fatty acid oxidation by both animal and plant preparations [6, 7]. Thus, it would seem that carnitine and choline independently influence separate aspects of lipid metabolism. Betaine did not substitute for carnitine and its failure to replace carnitine in exerting a stimulatory effect on greening also suggests that these compounds are involved in different aspects of plant metabolism. That choline and betaine, both quaternary amines, did not stimulate chlorophyll production in greening barley demonstrates that they did not act as nitrogen sources hence the similar quaternary nitrogen in carnitine is unlikely to act as a nitrogen source.

When [^{14}C] carnitine was supplied to greening barley leaves, 65% of the label recovered was in unchanged carnitine, i.e. 177 pmol remained as carnitine (Tables 1 and 2). In the protein hydrolysate and in the basic fraction of the ethanol extract (amino acids), 82% and 93.6% of the activity respectively was recovered in unchanged

Table 1. Carbon recovery following feeding of ^{14}C-labelled carnitine, CO_2 and glucose to green barley leaves

Fraction	Substrate		
	CO_2 (pmol/g fr. wt)	Glucose (pmol/g fr. wt)	Carnitine (pmol/g fr. wt)
CO_2 evolved	—	5430	40
Ethanol extract	13 600	761	233
Acidic fraction	8010	167	8
Basic fraction	3270	41	189
Neutral fraction	2260	536	21
Solid residue	299	8	4

Each solution contained 40 μatoms carbon (5 μCi) in 4 ml 25 mM KH_2PO_4 buffer, pH 6.5. Green barley leaves were supplied isotope for 1 hr in white light (6.4 W/m^2). The ethanol extracts were fractioned on ion exchange columns into acid, basic and neutral fractions.

Table 2. ^{14}C recovery in fractions of barley leaves fed with carnitine

Carnitine fraction	Total carbon recovered (pmol/g fr. wt)	Total carbon recovered as carnitine (pmol/g fr. wt (%))	Total carbon recovered as recovered as carnitine (pmol/g fr. wt (%))
Basic fraction of ethanol extract	189	177 (93.6)	11.6 (6.1)
Basic fraction of acid hydrolysed residue	3.9	3.2 (82.0)	0.32 (8.2)

Palmitoylcarnitine co-chromatographed with authentic palmitoylcarnitine with an R_f in basic solvent of 0.61 and in acidic solvent of 0.62. R_fs of carnitine in the solvents were 0.10 and 0.47 respectively.

carnitine. This evidence is not compatible with the suggestion that carnitine was acting as a carbon or nitrogen source in the greening barley leaves.

It would appear that the effect of carnitine on greening is a special physiological or biochemical effect and is unrelated to any function as a carbon or nitrogen source. It was of interest that a radioactive spot of the same R_f as authentic palmitoyl carnitine was obtained on TLC plates of the basic fraction of the ethanolic extract and on TLC plates of the protein hydrolysate (Table 2). It is known that carnitine exerts an effect on fatty acid metabolism in avocado slices [5] and pea mitochondria [6] and it has been suggested that carnitine acyltransferase enzymes occur in plant tissues [6, 10]. It may be that such enzymes play a role in the morphogenesis of plastids. Possibly carnitine acts as a carrier of long chain acyl groups from cytosol donor sites across young plastid membranes to plastid-synthesizing sites. Alternatively carnitine acts as a 'saver' of CoA in a manner previously outlined [6]. The effect of carnitine on chlorophyll synthesis is suggested to be indirect, the increase in chlorophyll content and CO_2 fixation produced by carnitine seeming to be merely a consequence of the increased availability of cytosol acyl groups to plastid-synthesizing sites, this increased availability being mediated by the supplied carnitine.

EXPERIMENTAL

Barley seed, *Hordeum vulgare* var. Zephyr 2 M, was purchased from West Cumberland Farmers Ltd., Hexham, Northumberland. Chemicals were obtained from BDH Ltd; D,L- and L-carnitine from Koch-Light or Sigma; and radiochemicals from the Radiochemical Centre, Amersham, U.K.

Barley was grown for 7 days at 25° in the dark. The etiolated leaves were cut in darkness and the cut ends immersed in the test solns. These solns were buffered with KH_2PO_4 to a final concn of 25 mM, pH 6.5. The leaves were left in continuous white light (6.4 W/m^2) at 22° for 24 hr. The light source was four 40 W 'Universal White' fluorescent tubes.

Chlorophyll contents of the leaves were determined by the method of ref. [11].

Isotope feeding. (a) $NaH^{14}CO_3$. Etiolated barley leaves grown as above were excised and their cut ends immersed in L-carnitine solns. The leaves were illuminated in continuous white light as above for 18 hr. They were then cut into 1 cm lengths and floated on 10 ml 0.1 M HEPES buffer, pH 7, contained in sealed flasks. $NaH^{14}CO_3$ (10 μCi) was mixed with the buffered soln at the start of the expt and remained in contact with the leaf tissue for 1 hr. The leaves were killed and successively extracted with boiling 70% EtOH containing in the first extraction 1 ml HCO_2H. Bulked filtrates were assayed (0.1 ml aliquots) for radioactivity in 10 ml NE 260 scintillant on a Packard Liquid Spectrometer Model 3385. The efficiency of the counting procedure was 75% after allowing for the efficiency of the counting apparatus and correcting for quenching.

(b) D-[U-^{14}C]Glucose; D,L-[Me-^{14}C]carnitine and $NaH^{14}CO_3$. The radioisotopes were diluted with carrier to provide the final concns in 4 ml as follows: $NaHCO_3$, 40 μmol; D-glucose, 6.68 μmol; and to the D,L-[Me-^{14}C] carnitine was added sufficient carrier L-carnitine to give 5.68 μmol. Each soln so prepared contained 40 μatoms of carbon in 4 ml. Seven-day-old etiolated barley plants were placed in white light at 22° for 24 hr. The green barley leaves were cut 5 cm below the apex and the bottom 10 mm immersed in 4 ml buffered soln contained in glass vials. The buffer was 25 mM KH_2PO_4, pH 6.5. Each vial contained 2 g leaf tissue. A small vessel containing 0.5 ml 10% (w/v) KOH and a paper wick was suspended from a sealing cap above the leaves supplied carnitine or glucose to absorb evolved CO_2. Isotope (5 μCi) was added to the 4 ml soln with a syringe and the leaves were illuminated in white light (6.4 W/m^2) for 1 hr. Absorbed $^{14}CO_2$ in KOH was counted in 10 ml NE260 scintillant. Leaf samples were rinsed thoroughly in a Buchner funnel with H_2O, blotted dry and extracted in boiling 70% EtOH; the residue was retained. Aliquots of the ethanolic extract were counted in NE260 scintillant and the remainder fractionated into acid, basic and neutral fractions by ion exchange in Zeokarb 225 (H^+ form) and Deacidite FF(CO_3^{2-} form). Aliquots were removed for counting and where appropriate for TLC. The residue remaining after EtOH extraction was heated at 110° for 12 hr in sealed ampoules with 6 M HCl. The mixture was diluted with H_2O, filtered and the filtrate passed through Zeokarb 225 (H^+ form) and the retained basic fraction later recovered from the column. This basic fraction, the protein hydrolysate, was counted and subjected to TLC.

TLC was carried out in Si gel in either of the following solvents: (i) MeOH–Me_2CO–11.6 M HCl (45:5:2), (ii) MeOH–dioxan–18 M NH_4OH (6:9:5). Radioactive spots were removed, counted and subjected to further TLC for authentication.

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