CARNITINE LEVELS IN SOME HIGHER PLANTS

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1. Introduction

In animals, L-carnitine is important in mitochondrial β-oxidation [1, 2], but the distribution and role of carnitine in higher plants is not known, although peanut seedlings, for example, β-oxidize fatty acids [3]. Using a biological assay [4] which has several disadvantages [5], Fraenkel found that wheat seeds and alfalfa seedlings contain carnitine, but the extracts from six other plants did not have measurable amounts. Here we present the results of enzymic assays for carnitine in plants, using the highly specific acetyl-CoA:carnitine O-acetyltransferase (EC 2.3.1.7) [6], and discuss the relationship of distribution of carnitine and its possible function in these tissues.

2. Materials and methods

Acetyl-CoA was synthesized by the method of Simon and Shemin [7]. L-carnitine was obtained from Mann Research Laboratories, New York, and acetyl-CoA:carnitine O-acetyltransferase from C.F. Boehringer und Soehne GmbH, Mannheim, Germany. The enzymic assay of L-carnitine using 5,5-dithio*bis*-2-nitrobenzoic acid (DTNB) was that of Marquis and Fritz [5]. The reaction was started by adding enzyme, the total change in absorbance at 412 nm being measured on a Cary Model 15 recording spectrophotometer. Any change in absorbance on adding the enzyme itself without plant extract was subtracted from the total change with extract.

For the preparation of extracts from avocado mesocarp and peanut, 10 g material was ground with 50 ml water in a mortar and pestle, and the lipids extracted using the procedure of Bligh and Dyer [8]. The methanol-water fraction was taken to dryness under vacuum at 50°, the residue dispersed in about 30 ml water, and the clear supernatant assayed for carnitine. In the case of the other plants listed in table 1, 10 g material and 200 ml dilute $HClO_4$ were blended at full speed for 30 sec, then this was made 50% with respect to ethanol and neutralized with KOH. After standing the suspension in ice for 1 hr, the precipitate was removed, the supernatant evaporated, and the residue dispersed in water and assayed as described above.

Table 1
Carnitine levels in some higher plants.

Plant Material	L-carnitine (μg/g dry wt)
Wheat seed	4
Wheat germ	12
Barley seed	0
Oat seed	1
Oat seedling (80–90 hr)	14
Spinach leaf (freeze-dried)	0
Cabbage head leaf	0
Castor bean (minus seed coat)	0
Peanut seed (minus seed coat)	1
Peanut seed embryo	8
Avocado mesocarp	48
Cauliflower inflorescence	14

Conditions for assay are the same as described in the legend to fig. 1.

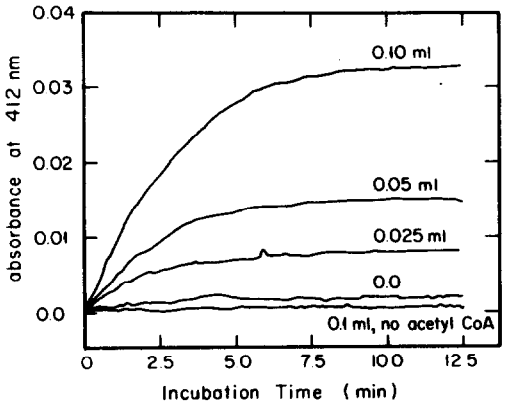

Fig. 1. Assay for carnitine in extract of avocado mesocarp. The reference cell contained Tris buffer pH 7.5 (100 μmoles), DTNB (100 mμmoles), acetyl-CoA (50 mμmoles), extract (0–0.1 ml, as indicated) and water to 1.0 ml. The sample cell was similar but was made up to 0.95 ml with water. The reaction was started by adding to the sample cell 0.05 ml carnitine acetyltransferase diluted to a suitable concentration in phosphate buffer pH 7.5, 0.1 M. Incubation was at 30°.

3. Results

The levels of carnitine in various plants are shown in table 1. For those extracts in which carnitine is listed present, the change in absorbance was proportional to the volume of extract added to the cuvette over the range of concentration used for the assay (fig. 1). The reaction was strongly inhibited when no acetyl-CoA was added. With standard L-carnitine, which was three times more concentrated than any plant extract, the reaction went to completion in less than 15 min. Furthermore, on adding standard carnitine together with extracts, the change in absorbance was additive, and no inhibition of the enzyme was observed.

4. Discussion

The enzymic assays indicate that carnitine is present in avocado, cauliflower, oat seedlings and some seeds. In avocado as well as in cauliflower, carnitine could be important for the mitochondrial β-oxidation of fatty acids, and the difference in carnitine concentration in these two tissues a rough measure of the β-oxidative capacitiy of each. The role, if any, of carnitine in germination is of interest, particularly in the case of fatty seeds. Acylcarnitines could be involved in β-oxidation in the seedling, or serving as a store of energy in the seed. In the seeds without measurable carnitine, biosynthesis of the carnitine may take place after germination. For wheat, oats and peanuts, the concentration of carnitine was significantly higher in embryonic or seedling tissue than in the seed itself. Mature leaves of spinach and cabbage do not appear to contain carnitine, and the rate of β-oxidation in these tissues is expected to be correspondingly small. Further work on the distribution and metabolism of carnitine in plants is now in progress.

Acknowledgements

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