Carnitine-acyltransferase activity of mitochondria from mung-bean hypocotyls

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Abstract. Carnitine-acyltransferase activity assayed with acetyl-CoA, octanoyl-CoA, or palmitoyl-CoA is associated with the mitochondria but not with the peroxisomes of mung-bean hypocotyls. Using mitochondria as an enzyme source, a half-maximal reaction rate is obtained with a palmitoyl-CoA concentration approximately twice that required with acetyl-CoA. In the presence of a saturating acetyl-CoA concentration the carnitine-acyltransferase activity is not enhanced by palmitoyl-CoA as additional substrate. However, palmitoylcarnitine is formed in addition to acetylcarnitine, and the formation of acetylcarnitine is competitively inhibited by palmitoyl-CoA. It is concluded that the mitochondria of mung-bean hypocotyls possess a carnitine acyltransferase of broad substrate specificity with respect to the chain-length of the acyl-CoA and that the demonstration of a carnitine-palmitoyltransferase activity in plant mitochondria does not indicate the presence of a specific carnitine long-chain acyltransferase.

Key words: Carnitine acyltransferase – Mitochondrion – Peroxisome – Vigna.

Introduction

Carnitine acyltransferases of animal cells are involved in transport processes related to fatty acid β-oxidation. Accordingly, carnitine acyltransferases are located in the inner mitochondrial and in the peroxisomal membranes. Concerning mitochondria and peroxisomes of higher plants, carnitine-acyltransferase activities have been reported to be present in mitochondria from pea cotyledons (Wood et al. 1983, 1984; Burgess and Thomas 1986) and possibly to occur in mitochondria and peroxisomes from pea leaves (McLaren et al. 1985). The carnitine-acyltransferase activity of the organelles has been assayed with both palmitoyl-CoA and acetyl-CoA as substrates. As carnitine-acyltransferase activity could be demonstrated with either substrate, it has been claimed that both a carnitine long-chain acyltransferase (EC 2.3.1.21) and a carnitine acetyltransferase (EC 2.3.1.7) are associated with the mitochondria of pea cotyledons (Wood et al. 1983, 1984).

The demonstrated carnitine-palmitoyltransferase activity of the mitochondria from pea cotyledons is used as one argument in favour of the occurrence of a mitochondrial β-oxidation system in this organ. The existence of a mitochondrial β-oxidation system in cells of higher plants is, however, a matter of debate (Gerhardt 1986; Wood et al. 1986). We have therefore investigated the subcellular location and some properties of the carnitine-acyltransferase activity of the mung-bean hypocotyl, an organ for which only the peroxisomal β-oxidation system could be demonstrated (Gerhardt 1984, 1986).

Material and methods

Organelle isolation. Mung-bean (Vigna radiata L.) seeds were germinated in the dark at 30 °C. Mitochondria and peroxisomes were isolated from the hypocotyls of 2- to 3-d-old seedlings on sucrose density gradients according to Gerbling and Gerhardt (1987).

Assays for carnitine-acyltransferase activity. Carnitine-acyltransferase activity was assayed in the direction of the formation of acetylcarnitine from acetyl-CoA.

i) The appearance of the free SH-group of the released CoASH was followed spectrophotometrically by the reaction of the free SH-group with 5,5'-dithio-bis-(2-nitrobenzoate) (Ellman's reagent). The reaction mixture (1 ml total volume) consisted of 200 mmol·l⁻¹ 2-amino-2-(hydroxymethyl)-1,3-pro-
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panediol(Tris)-HCl, pH 6.8, 4 mmol·l⁻¹ KH₂PO₄, 1 mmol·l⁻¹ MgSO₄, 1 mmol·l⁻¹ ethylenediaminetetraacetic acid (EDTA), 10 mmol·l⁻¹ malonate, 6 mmol·l⁻¹ 5,5-dithio-bis(2-nitrobenzoate), 10 µmol·l⁻¹ acetyl-CoA, 1 mmol·l⁻¹ l-carnitine, and 10–80 µg mitochondrial or peroxisomal fraction protein. The reaction was started by the addition of l-carnitine. Acyl-CoA or l-carnitine was omitted from the blanks. The increase of absorbance at 412 nm (ε = 13.6 cm²·µmol⁻¹) was followed.

ii) The formation of acylcarnitine from acyl-CoA was determined directly by using a radioactive assay. The reaction mixture, a modification of that described by Wood et al. (1983, 1984) contained, in a total volume of 0.5 ml, 200 mmol·l⁻¹ Tris-HCl, pH 6.8, 4 mmol·l⁻¹ KH₂PO₄, 1 mmol·l⁻¹ MgSO₄, 10 mmol·l⁻¹ malonate, 10 µmol·l⁻¹ acetyl-CoA, 2 mmol·l⁻¹ l-[methyl-¹⁴C]carnitine (1.5 MBq·mmol⁻¹), and 10–80 µg (≤ 300 µl) mitochondrial or peroxisomal fraction protein. Radioactive assays were also run with 2 mmol·l⁻¹ unlabeled instead of labeled l-carnitine and with 10 µmol·l⁻¹ [¹⁴C]palmitoyl-CoA (740 kBq·mmol⁻¹) or [¹⁴C]acetyl-CoA (740 kBq·mmol⁻¹) instead of unlabeled acyl-CoA. After incubation, the radioactive substrate and products of the reaction mixture were separated by thin-layer chromatography and their radioactivity was determined directly by scintillation counting according to Gerbling and Gerhardt (1987).

Protein was determined by a modification (Gerhardt 1983) of the Lowry method (Lowry et al. 1951).

Thin-layer chromatography. Aliquots (3, 5 or 10 µl) of the reaction mixture of the radioactive carnitine-acyltransferase assay were directly applied to thin-layer chromatoplates (20 × 20 cm²) coated with a 0.1-mm layer of cellulose (Merck, Darmstadt, FRG). Chromatograms were developed twice with butanol:glacial acetic acid:water (5:2:3, by vol.) at room temperature. Radioactive spots were detected using a radio-thin-layer chromatography scanner (model II; Berthold, Wildbad, FRG). The substrates l-carnitine, palmitoyl-CoA and acetyl-CoA were located on the chromatogram by co-chromatography of the corresponding labeled compounds. The products palmitoyl-, octanoyl-, and acetylcarnitine were located by co-chromatography of an aliquot of a reaction mixture for preparing the corresponding labeled reference substances. The composition of this reaction mixture was similar to that of the radioactive assay for measuring the carnitine-acyltransferase activity except that organelle protein was replaced by 2 nkat carnitine acetyltransferase. In addition, radioactivity which had been added to the reaction mixture as L-[¹⁴C]carnitine in the radioactive assay (see Material and methods) of carnitine-acyltransferase activity. The activity of the mitochondria from mung-bean hypocotyls was determined with acetyl-CoA (a) or palmitoyl-CoA (b) as substrate. At 0 min and 10 min reaction time, five µl of the assay mixture were subjected to thin-layer chromatography. The radioactivity of the spots of carnitine and acetylcarnitine was determined by scintillation counting.

Table 1. Carnitine-acyltransferase activity of the mitochondria from mung-bean hypocotyls as determined by the release of CoASH from acyl-CoA (spectrophotometric assay) or by the formation of acylcarnitine (radioactive assay). Spectrophotometric and radioactive assays were performed in parallel samples with 10 µM acyl-CoA as substrate.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Substrate</th>
<th>Activity (pkat·mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometric</td>
<td>Palmitoyl-CoA</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Octanoyl-CoA</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Acetyl-CoA</td>
<td>91</td>
</tr>
<tr>
<td>Radioactive</td>
<td>Palmitoyl-CoA</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Octanoyl-CoA</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Acetyl-CoA</td>
<td>89</td>
</tr>
</tbody>
</table>

Table 2. Recovery of the radioactivity employed as l-[methyl-¹⁴C]carnitine in the radioactive assay (see Material and methods) of carnitine-acyltransferase activity. The activity of the mitochondria from mung-bean hypocotyls was determined with acetyl-CoA (a) or palmitoyl-CoA (b) as substrate. At 0 min and 10 min reaction time, five µl of the assay mixture were subjected to thin-layer chromatography. The radioactivity of the spots of carnitine and acetylcarnitine was determined by scintillation counting.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reaction time (min)</th>
<th>Radioactivity (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>a Carnitine</td>
<td>782</td>
<td>511</td>
</tr>
<tr>
<td>Acetylcarnitine</td>
<td>0</td>
<td>254</td>
</tr>
<tr>
<td>b Carnitine</td>
<td>788</td>
<td>660</td>
</tr>
<tr>
<td>Palmitoylcarnitine</td>
<td>0</td>
<td>125</td>
</tr>
</tbody>
</table>

protein and proceeded linearly for 20 min. Optimal activity occurred at pH 6.8–7.0.

The mitochondrial reaction exhibited a 1:1 stoichiometry of CoASH release and acylcarnitine formation (Table 1), indicating that the measured acyl-CoA- and carnitine-dependent reaction (data not shown) is due to the activity of a carnitine acyltransferase. In addition, radioactivity which had been added to the reaction mixture as L-[¹⁴C]carnitine but could not be recovered as [¹⁴C]carnitine after the incubation, was confined to the formed acylcarnitine (Table 2).

At a substrate concentration of 10 µM the carnitine-acyltransferase activity decreased with increasing chain-length of the acyl-CoA (Table 1). Using mitochondria as an enzyme source, half-maximal reaction rates estimated by Lineweaver-Burk plots, occurred with acetyl-CoA, octanoyl-CoA, and palmitoyl-CoA at substrate concentrations of 1.3, 1.5, and 2.9 µM, respectively. The corresponding Vmax values were estimated to be 0.09, 0.07, and 0.04 nkat·mg⁻¹ mitochondrial fraction protein.