LOCALIZATION OF CARNITINE ACETYLTRANSFERASE IN PEROXISOMES AND IN MITOCHONDRIA OF *n*-ALKANE-GROWN *CANDIDA TROPICALIS*

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1. Introduction

The profuse appearance of peroxisomes (microbodies) in the *n*-alkane-grown cells of various yeasts has been demonstrated [1,2]. Subsequent isolation of the peroxisomes from *n*-alkane-grown *Candida tropicalis* was accomplished and the localization of the following enzymes was confirmed in the isolated peroxisomes: Catalase, D-amino acid oxidase, isocitrate lyase, malate synthase, NADP-linked isocitrate dehydrogenase, uricase and fatty acid β-oxidation system [3–5].

In spite of the fact that isocitrate lyase and malate synthase, the key enzymes of glyoxylate cycle essential for gluconeogenesis in alkane-utilizing yeasts [6], were located in the peroxisomes, the other glyoxylate cycle enzymes common to the TCA cycle, malate dehydrogenase, citrate synthase and aconitase, were detected exclusively in the mitochondria [3]. Furthermore, the fatty acid β-oxidation system, which participates in acetyl-CoA production from alkane substrates, was essentially peroxisome-associated under the experimental conditions employed [5]. These results strongly indicate that cooperation of peroxisomes with mitochondria is necessary for the complete operation of the glyoxylate cycle and that the acetyl-CoA utilized by citrate synthase in the mitochondria must be supplied from the peroxisomes.

The purpose of this report is to provide evidence for the localization of carnitine acetyltransferase (EC 2.3.1.7, acetyl-CoA:carnitine *O*-acetyltransferase) (CAT) both in the peroxisomes and in the mitochondria of alkane-grown *C. tropicalis*. The possible existence of a shuttle system for the transport of acetyl units from peroxisomes to mitochondria is discussed.

2. Materials and methods

2.1. *Cultivation of yeast*

Candida tropicalis (Castellani) Berkhout strain pK 233 was cultivated for 16 h at 30°C in a medium containing an alkane mixture (C_{10}–C_{13}) as sole carbon source, as in [4].

2.2. *Subcellular fractionation*

Peroxisomes and mitochondria were isolated from the alkane-grown cells of *C. tropicalis* by means of differential centrifugation and sucrose density gradient centrifugation. The procedure was the same as in [3], except that homogenization of the protoplasts was carried out for 10 min and sucrose density gradient centrifugation was done at 20 000 rev./min (g_{av} = 49 600) for 5 h. Microsomal fraction (P_3) was obtained by centrifugation of S_2 (20 000 × *g* supernatant) at 44 000 rev./min (g_{av} = 139 000) for 2 h.

2.3. *Enzyme assay*

Catalase, cytochrome oxidase and protein were assayed by the methods in [7]. Carnitine acetyltransferase (CAT) was assayed spectrophotometrically at 30°C by following the release of CoA-SH from acetyl-CoA using the general thiol reagent 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) [8]. The reaction mixture contained 40 mM potassium phosphate

buffer (pH 7.8), 0.05 mM acetyl-CoA (lithium salt, Sigma Chemical Co., St Louis), 0.12 mM DTNB, 2.2 mM DL-carnitine (β-hydroxy-γ-trimethyl-ammonium butyrate) chloride (Wako Pure Chemical Industries, Osaka) and enzyme in final vol. 1.5 ml. The reaction was initiated by adding the enzyme and the increase in absorbance was followed at 412 nm. Carnitine-independent release of CoA-SH from acetyl-CoA was not observed under the conditions employed. When 1.1 mM L-carnitine chloride (P-L Biochemicals, Milwaukee), which is known to be the active isomer for CAT activity [8], was used in place of 2.2 mM DL-carnitine chloride in the reaction mixture, the activity showed almost the same value.

3. Results

As shown in table 1 and fig.1, in *n*-alkane-grown cells of *C. tropicalis*, CAT was found mainly in the particulate fraction P_2 (20 000 × *g* pellets) composed of peroxisomes and mitochondria [3]. Further fractionation of P_2 by means of sucrose density gradient centrifugation showed that CAT in P_2 was detected both in fraction 2 (30–40% sucrose fraction) and in fraction 5 (42.5–50% sucrose fraction) (fig.2). Cytochrome oxidase, a marker enzyme of mitochondria, was detected mainly in fraction 2, and catalase,

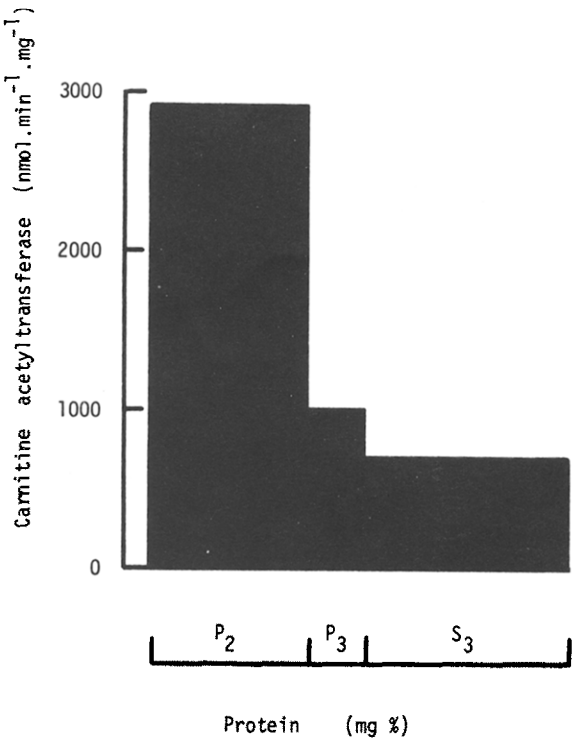

Fig.1. Subcellular localization of CAT in *Candida tropicalis*: P_2, 20 000 × *g* pellets (peroxisomes and mitochondria); P_3, 139 000 × *g* pellets (microsomes); S_3, 139 000 × *g* supernatant (cytoplasm).

Table 1
Subcellular localization of enzymes in alkane-grown *C tropicalis* cells

	Fraction		
	S_1	P_2	S_2
Protein (mg)	77	27	37
Cytochrome oxidase			
rel. act. (%)	100	96	0.5
spec. act. (nmol/min × mg protein)	86	240	0.9
Catalase			
rel. act. (%)	100	79	41
spec. act. (μmol/min × mg protein)	364	838	315
Carnitine acetyltransferase			
rel. act. (%)	100	53	22
spec. act. (nmol/min × mg protein)	1910	2910	877

The experimental procedures were the same as in [3]. S_1, 3000 × *g* supernatant of the protoplast homogenate; P_2, 20 000 × *g* pellets of S_1; S_2, 20 000 × *g* supernatant of S_1. Activities in S_1 were expressed as 100%

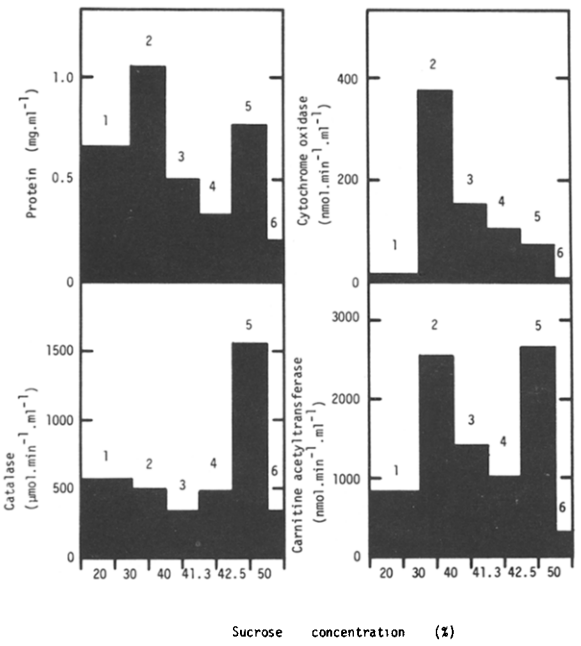

Fig.2. Particulate localization of enzymes in P_2. The experimental procedures were as in [3]. The volume of each fraction was as follows: 1, 3.75 ml; 2–5, 2.5 ml each; 6, 1.25 ml.

a marker enzyme of peroxisomes, predominantly in fraction 5. The results indicate that CAT is located both in the peroxisomes and in the mitochondria of the yeast.

S_2 (20 000 × *g* supernatant) was further centrifuged at a higher speed (139 000 × *g*) to obtain the microsomal fraction (P_3) and the cytoplasmic fraction (S_3). Most of the CAT activity in S_2 was recovered in S_3 (fig.1), suggesting the localization of the enzyme in the cytoplasm. However, the possibility cannot be excluded that this may be due, at least in part, to disruption of the organelles during the preparation steps; CAT does not appear to occur in the cytoplasm of animal cells. The localization of CAT in the microsomal material is not conclusive.

4. Discussion

As reported in [3], yeast peroxisomes contain the key enzymes of glyoxylate cycle, isocitrate lyase and malate synthase, while the rest of the enzymes in the cycle, common to the TCA cycle also, are located in the mitochondria. Furthermore, the activity of fatty acid β-oxidation has been detected only in the peroxisomes but not in the mitochondria [5]. Although the possibility of the presence of a different β-oxidation system in the mitochondria would not be excluded as the case of rat liver [9], these results strongly suggested that a certain transport system of acetyl units from the peroxisomes to the mitochondria should operate in the alkane-utilizing yeast cells.

Carnitine acyltransferases, CAT and carnitine palmitoyltransferase (EC 2.3.1.23, acyl-CoA:carnitine acyltransferase) (CPT), especially the latter enzyme, have been shown to be essential in the transport of fatty acyl residues from cytoplasmic acyl-CoA into the mitochondrial matrix [10]. Recently, carnitine acyltransferases have been detected not only in mitochondria but also in the peroxisomes of mammalian cells [11–13]. The results presented in this paper suggest that an 'acetylcarnitine shuttle' may have an important role in the transfer of acetyl units formed in the peroxisomes by β-oxidation to the mitochondria for the complete operation of the glyoxylate cycle in *Candida*.

In addition, when *C. tropicalis* is grown on relatively short chain alkanes, such as undecane (C_{11}), the de novo synthesis pathway for fatty acids is operative [14]. Fatty acids derived from alkanes are activated to acyl-CoA in the peroxisomes [15] and are degraded to acetyl-CoA in the peroxisomes [5]. CAT in the peroxisomes may also participate in the transfer of acetyl units from the peroxisomes to the cytoplasm, and the enzyme in the cytoplasm may convert acetyl-carnitine to acetyl-CoA, which can be utilized for the fatty acid synthesis. A possible role of the peroxisomes and mitochondria, and also that of CAT in alkane-assimilating yeasts are illustrated in fig.3.

In *Candida*, it has been established that several enzymes concerning lipid metabolism are located in the peroxisomes: acyl-CoA synthetase II [15], the fatty acid β-oxidation system [5,15], CAT (this study) and NAD-linked glycerophosphate dehydrogenase (unpublished results). The peroxisomes seem to be one of the major site of lipid metabolism in *Candida* utilizing *n*-alkanes and higher fatty acids.

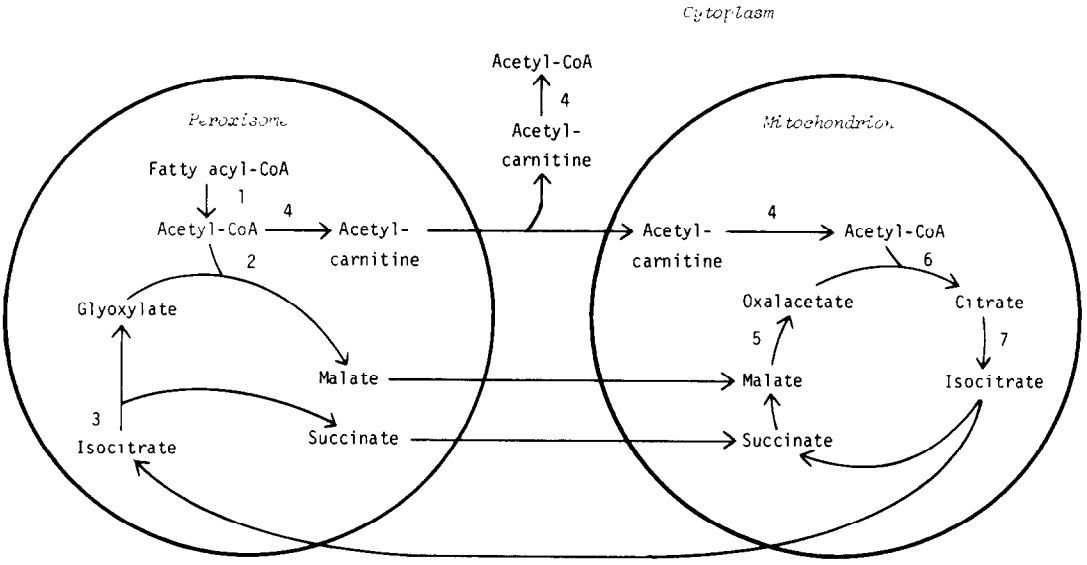

Fig.3. Possible role of peroxisome and mitochondrion in alkane assimilation by yeasts: 1, β-oxidation system; 2, malate synthase; 3, isocitrate lyase; 4, carnitine acetyltransferase (CAT); 5, malate dehydrogenase; 6, citrate synthase; 7, aconitase.

Acknowledgement

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