Volume 229, number 1, 215–218 FEB 05638 February 1988

Occurrence and possible roles of acetoacetyl-CoA thiolase and 3-ketoacyl-CoA thiolase in peroxisomes of an *n*-alkane-grown yeast, *Candida tropicalis*

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Received 23 December 1987

Two kinds of 3-ketoacyl-CoA thiolases were found in the peroxisomes of *Candida tropicalis* cells grown on *n*-alkanes (C_{10}–C_{13}). One was a typical acetoacetyl-CoA thiolase specific only to acetoacetyl-CoA, while another was 3-ketoacyl-CoA thiolase showing high activities on the longer chain substrates. A high level of the latter thiolase activity in alkane-grown cells was similar to that of other enzymes constituting the fatty acid β-oxidation system in yeast peroxisomes. These facts suggest that the complete degradation of fatty acids to acetyl-CoA is carried out in yeast peroxisomes by the cooperative contribution of acetoacetyl-CoA thiolase and 3-ketoacyl-CoA thiolase.

Acetoacetyl-CoA thiolase; 3-Ketoacyl-CoA thiolase; β-Oxidation system; Peroxisome; (Alkane-grown yeast, *Candida tropicalis*)

1. INTRODUCTION

Peroxisomes are one of the subcellular organelles common to eukaryotic cells and have an essential role in the fatty acid degradation. The peroxisomal fatty acid β-oxidation system is composed of acyl-CoA oxidase coupled to catalase, a marker enzyme of peroxisomes, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase [1]. This system is different from the mitochondrial one, in which the initial step is catalyzed by acyl-CoA dehydrogenase coupled to the respiratory chain.

In mammalian cells, fatty acids of long chains are degraded to medium chain acyl-CoAs in peroxisomes, which are transported to mitochondria with a carnitine shuttle [2]. Mitochondria serve to degrade the transported medium chain acyl-CoAs to acetyl-CoA, the substrate of the tricarboxylic acid cycle. At the final step of β-oxidation, peroxisomal 3-ketoacyl-CoA thiolase is responsible for the degradation of long chain acyl-CoAs, while the mitochondrial enzyme has a broad substrate specificity for the chain length [3,4].

In the case of the *n*-alkane-assimilating yeast, *Candida tropicalis*, all the enzymes constituting the fatty acid β-oxidation system are exclusively detected in peroxisomes, the results suggesting that fatty acids are completely degraded to acetyl-CoA in peroxisomes [5,6]. Although acyl-CoA oxidase [7] and the bifunctional enzyme, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase [8], have been purified from this yeast and characterized, no information is available on 3-ketoacyl-CoA thiolase participating in the final step of the fatty acid β-oxidation.

This paper deals with the presence of two kinds of 3-ketoacyl-CoA thiolases in yeast peroxisomes (one is specific to acetoacetyl-CoA and the other to long chain 3-ketoacyl-CoAs), and their possible roles in the peroxisomal β-oxidation cycle.

2. MATERIALS AND METHODS

2.1. *Cultivation of yeast*

C. tropicalis pK 233 (ATCC 20336) was cultivated to the exponential growth phase in a medium containing glucose or an

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

Table 1

Activity levels of 3-ketoacyl-CoA thiolase in glucose-grown and alkane-grown *C. tropicalis* on different substrates

Substrate	Thiolase activity (nmol · min^{-1} · mg protein^{-1})		*B/A*
	Glucose-grown cells (*A*)	Alkane-grown cells (*B*)	
Acetoacetyl-CoA	43	110	2.6
3-Ketohexanoyl-CoA	0.92	190	210
3-Ketooctanoyl-CoA	3.1	540	170
3-Ketodecanoyl-CoA	5.5	1000	180
3-Ketododecanoyl-CoA	6.5	740	110

Thiolase activity was measured with cell-free extracts

n-alkane mixture (C_{10}–C_{13}) as the sole source of carbon and energy [9].

2.2. *Preparation of cell-free extracts and subcellular fractionation*

Cell-free extracts were prepared by disintegrating the cells with a Braun cell homogenizer [9]. Protoplasts prepared from *n*-alkane-grown cells were homogenized with a teflon homogenizer and then fractionated by differential centrifugations [6]. The fractions obtained were as follows; S_2 fraction (20 000 × *g* supernatant) composed of cytosol; P_2 fraction (20 000 × *g* pellets) consisted of mitochondria and peroxisomes. To separate peroxisomes and mitochondria, P_2 fraction was subjected to a discontinuous sucrose density gradient centrifugation [6].

2.3. *Enzyme and protein assay*

The activity of 3-ketoacyl-CoA thiolase was measured at 30°C, as described by Staak et al. [10], except that the acetoacetyl-CoA concentration was 40 μM. Catalase, cytochrome oxidase and protein were assayed by the methods described in [11].

2.4. *Chemicals*

Acetoacetyl-CoA was purchased from Sigma (St. Louis, MO). All other 3-ketoacyl-CoA compounds were prepared enzymatically from corresponding enoyl-CoAs [12], synthesized by the mixed anhydride method [13]. Other chemicals were obtained from commercial sources.

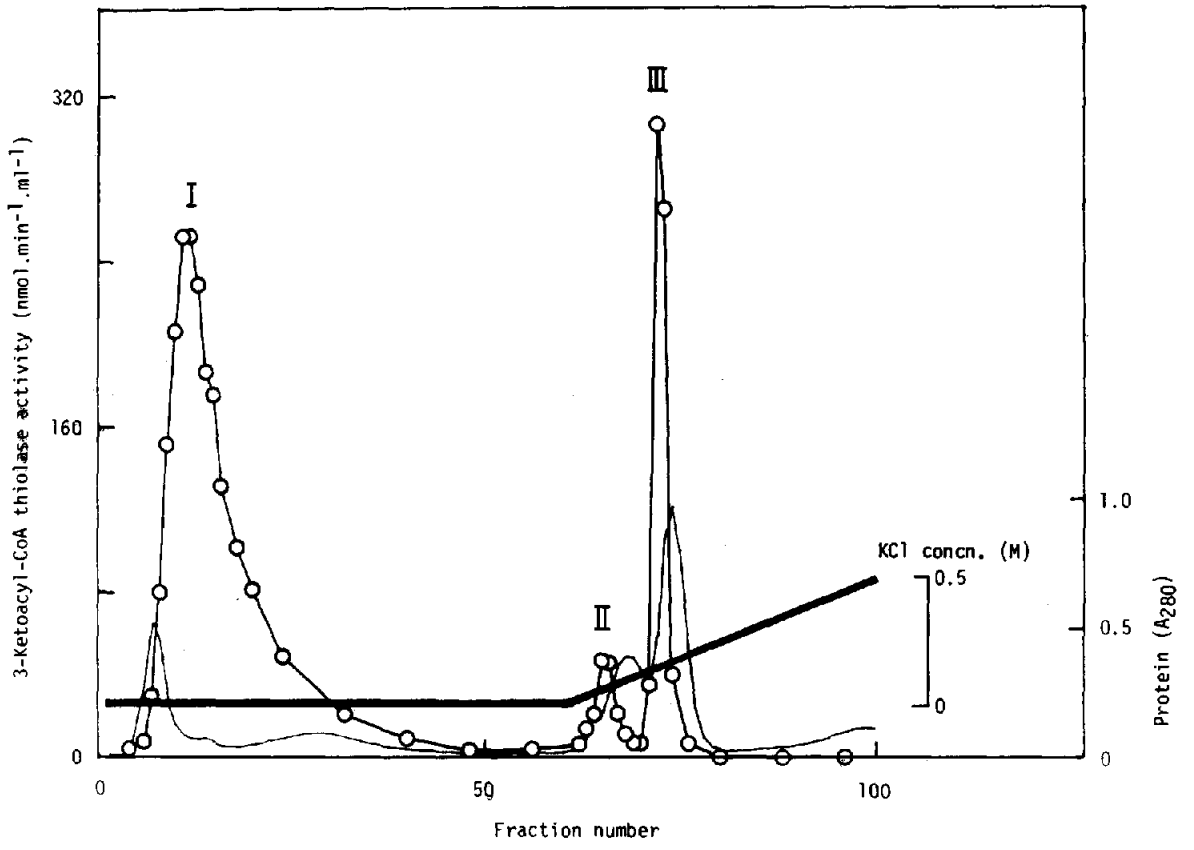

Fig.1. DEAE-Sepharose CL-6B column chromatography of the P_2-S fraction. Volume of each fraction, 5 ml. (○) 3-Ketoacyl-CoA thiolase activity on acetoacetyl-CoA; (——), protein; (▬), KCl concentration.

3. RESULTS AND DISCUSSION

3.1. *Induction of 3-ketoacyl-CoA thiolase in* Candida tropicalis

The activities of 3-ketoacyl-CoA thiolase in *C. tropicalis* cells grown on *n*-alkanes and glucose were measured with 3-ketoacyl-CoAs of various chain lengths (table 1). The level of the 3-ketoacyl-CoA thiolase activity was several hundred-times higher in alkane-grown cells than in glucose-grown cells with 3-ketoacyl-CoAs of longer chains as substrates, while the level to degrade acetoacetyl-CoA was only about three-times higher in alkane-grown cells. These facts suggested the presence of more than one 3-ketoacyl-CoA thiolase exhibiting the different substrate specificity and inducible nature.

3.2. *Presence of three 3-ketoacyl-CoA thiolases*

P_2 fraction containing peroxisomes and mitochondria was treated with a vortex-mixer under hypotonic conditions (50 mM potassium phosphate buffer, pH 7.2) in the presence of 10% (w/v) glycerol, 1 mM dithiothreitol and 0.2 mM phenylmethylsulfonyl fluoride to solubilize the enzymes, and centrifuged at 127 000 × *g* for 1 h. The supernatant obtained (P_2-S) was applied to a DEAE-Sepharose CL-6B column (2.2 × 17 cm) equilibrated with 50 mM potassium phosphate buffer (pH 7.2) containing 10% glycerol and 1 mM dithiothreitol, and proteins were eluted with a linear concentration gradient of KCl (fig.1). Three peaks of the 3-ketoacyl-CoA thiolase activity (I, II and III) were obtained with acetoacetyl-CoA as the substrate. A similar result was also obtained by the chromatography of the peroxisomal fraction after the subcellular fractionation (not shown). Respective thiolase activities in the P_2-S fraction were as follows: I, 14; II, 0.86; III, 3.3 ($\mu mol \cdot min^{-1}$) using acetoacetyl-CoA as the substrate; and I, 0.14; II, 9.5; III, 110 ($\mu mol \cdot min^{-1}$) using 3-ketooctanoyl-CoA as the substrate. The ratio of respective thiolase activities to acetoacetyl-CoA or 3-ketooctanoyl-CoA revealed that the contribution of thiolase II activity must be very low. Table 2 shows that thiolase I was specific only to acetoacetyl-CoA, while thiolases II and III had high activities for 3-ketodecanoyl-CoA and 3-ketododecanoyl-CoA. These results indicate that thiolase I and thiolases II and III are classified to different types of the enzymes.

Table 2

Substrate specificity of 3-ketoacyl-CoA thiolases I, II and III

Substrate	Thiolase		
	I	II	III
Acetoacetyl-CoA	1	1	1
3-Ketohexanoyl-CoA	0.01	6.7	8.3
3-Ketooctanoyl-CoA	0.01	11	33
3-Ketodecanoyl-CoA	0.02	14	46
3-Ketododecanoyl-CoA	0.02	13	47

The respective enzyme activities on acetoacetyl-CoA are expressed as 1 (unit)

3.3. *Peroxisomal thiolases I, II and III*

The subcellular localization of thiolases I, II and III was examined by using acetoacetyl-CoA and 3-ketooctanoyl-CoA as the substrates. Fig.2 shows that the distribution of the respective activities of 3-ketoacyl-CoA thiolase to acetoacetyl-CoA and 3-ketooctanoyl-CoA corresponded to that of catalase, a marker enzyme of peroxisomes. These results, together with those obtained from the DEAE-Sepharose CL-6B column chromatography of the peroxisomal fraction, have demonstrated that thiolases I, II and III were peroxisomal enzymes. In the mitochondrial fraction, containing cytochrome oxidase as a marker, no significant peak of the thiolases was found (not shown). 3-Ketoacyl-CoA thiolase recovered in the cytosolic fraction (S_2) was only specific to acetoacetyl-CoA, although we have no information on its function.

In conclusion, thiolase I was a typical acetoacetyl-CoA thiolase and thiolases II and III were 3-ketoacyl-CoA thiolases having high activities to longer chain 3-ketoacyl-CoAs. Thiolase III of the two 3-ketoacyl-CoA thiolases seems to be mainly responsible for the degradation of fatty acids derived from *n*-alkanes of long chains, while the degradation of acetoacetyl-CoA is carried out preferentially by thiolase I, thus the fatty acid β-oxidation cycle completely operating in the yeast peroxisomes. The presence of acetoacetyl-CoA thiolase and 3-ketoacyl-CoA thiolase in the yeast peroxisomes will be noticeable in studying the evolution of fatty acid metabolism.

Acknowledgment: This work was supported in part by a Grant-in-Aid for Research from the Ministry of Education, Science and Culture, Japan.

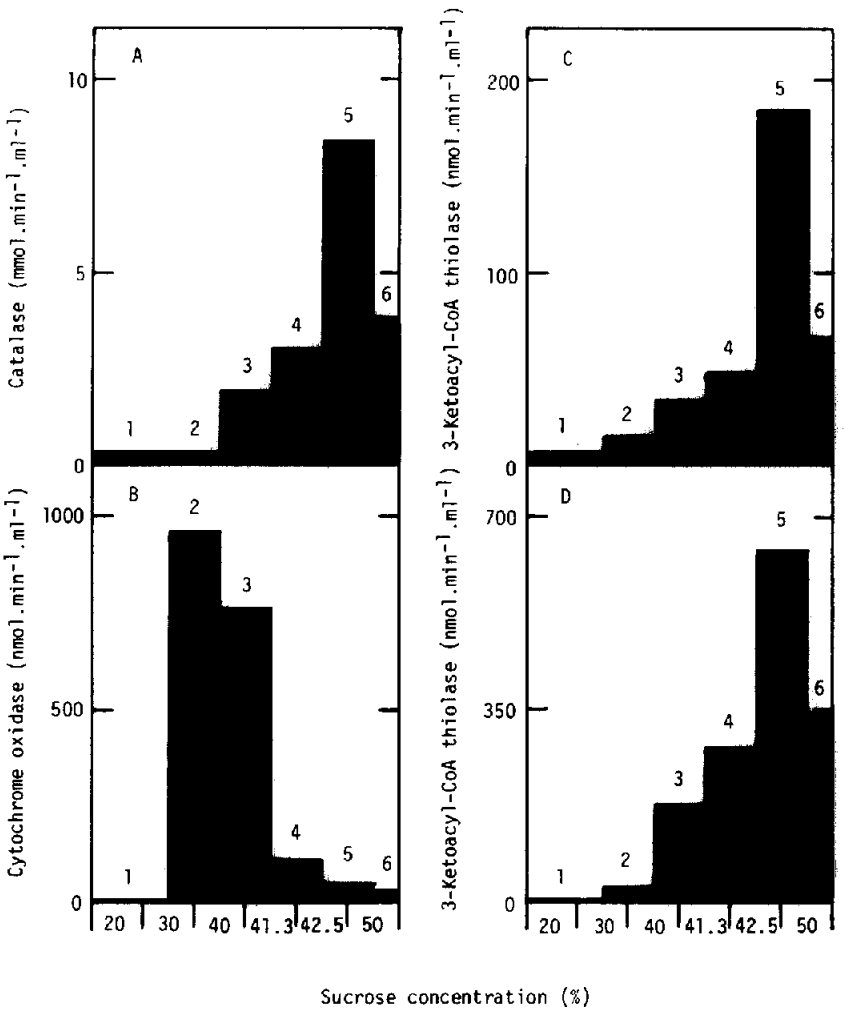

Fig.2. Particulate localization of enzymes in the P_2 fraction. The experimental procedures were described in the previous paper [6]. The volume of each fraction was as follows: 1, 3.75 ml; 2–5, 2.5 ml each; 6, 1.25 ml. (A) Catalase; (B) cytochrome oxidase; (C) 3-ketoacyl-CoA thiolase measured with acetoacetyl-CoA as the substrate; (D) 3-ketoacyl-CoA thiolase measured with 3-ketooctanoyl-CoA as the substrate.

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