THE SEPARATION AND PROPERTIES OF TWO FORMS OF CARNITINE PALMITOYLTRANSFERASE FROM OX LIVER MITOCHONDRIA

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Summary Ox liver mitochondria contain two types of carnitine palmitoyltransferase, which have been separated by ion exchange chromatography and isoelectric focusing. One is readily obtained in soluble form, and is inactivated by 2-bromopalmitoyl derivatives of CoA or carnitine. The other is tightly bound to membrane material, from which it may be released by butanol, and uses the bromopalmitoyl compounds as substrates. Other differences in substrate specificity are reported. It is proposed that the former enzyme is the "outer" transferase of intact mitochondria, which is accessible to added acyl-CoA, while the latter represents the "inner" transferase available only to CoA in the mitochondrial matrix.

Undamaged mitochondria will oxidise the Coenzyme A derivatives of fatty acids only in the presence of carnitine; furthermore, isolated mitochondria require carnitine for the oxidation of long-chain fatty acids when these are added in low concentrations in the presence of serum albumin. Fritz and Yue (1963) proposed a scheme in which acyl-CoA, formed from long-chain fatty acids in an outer compartment of the mitochondria, was separated from the enzymes of β-oxidation by a barrier impermeable to CoA but not to carnitine or its esters. The existence of two pools of carnitine palmitoyltransferase (EC 2.3.1.23), one on each side of the barrier, would permit the transfer of acyl groups to the site of β-oxidation, carnitine acting as a fatty acyl carrier:

$$\text{Palmitoyl-CoA} + \text{carnitine} \xrightarrow[\text{Outer transferase}]{} \text{palmitoylcarnitine} + \text{CoASH}$$

$$\text{CoASH} + \text{palmitoylcarnitine} \xrightarrow[\text{Inner transferase}]{} \text{palmitoyl-CoA} + \text{carnitine}$$

Further work from a number of laboratories has provided support for a scheme of this general type (for a discussion, see Greville and Tubbs, 1968). Tubbs and Chase (1967) reported that soluble carnitine palmitoyltransferase from ox liver was powerfully inhibited by 2-bromoacyl-CoA in the presence of carnitine (for the probable mechanism of this see Chase and Tubbs (1969)), and that these acyl-CoA analogues abolish the oxidation of palmitoyl-CoA, but not of palmitoylcarnitine, by rat liver mitochondria. These results, and those of Garland and Yates (1967), show that some of the carnitine palmitoyltransferase of intact mitochondria is inaccessible to added acyl-CoA, in accord with the proposal of Fritz and Yue.

We now wish to report the separation and partial purification of two carnitine palmitoyltransferases from ox liver. One, which is readily solubilised and is inhibited by 2-bromopalmitoyl-CoA, probably corresponds to the outer enzyme of Fritz and Yue; the other is tightly bound to membrane material, is not inhibited by bromoacyl-CoA and is likely to represent the inner transferase. It appears that the two carnitine palmitoyltransferases differ in their enzymic properties as well as in their intramitochondrial location.

EXPERIMENTAL

Carnitine palmitoyltransferase activity was routinely assayed at 30° by following the increase in extinction at 412 nm of a system containing 38 µM palmitoyl-CoA, 1.25 mM L-carnitine, 125 µM 5,5'-dithio-bis (2-nitrobenzoic acid), 100 mM tris-HCl (pH 8.0) and enzyme. 1 unit of enzyme catalysed the release of 1 µmole of CoASH per minute in this system.

Separation of carnitine palmitoyltransferase isoenzymes

Frozen ox liver (58 g.) was minced and then homogenised for 5 min. with 3 vol. of water at 0° using a Polytron overhead blender (Kinematica GmbH, Lucerne, Switzerland). After centrifugation at 20,000 g for 25 min., the supernatant was equilibrated with 5 mM potassium malonate, pH 5.7, by

gel filtration. The extract (190 ml) was applied to a column (4 x 20 cm.) of carboxymethylcellulose equilibrated with the same malonate buffer. The column was washed with 300 ml of 5 mM malonate and further protein eluted with a gradient of 5-150 mM malonate, pH 5.7; 50 ml fractions were collected. Three peaks of carnitine palmitoyltransferase activity (labelled A, B and C in Fig. 1) were found and the fractions corresponding to each peak combined.

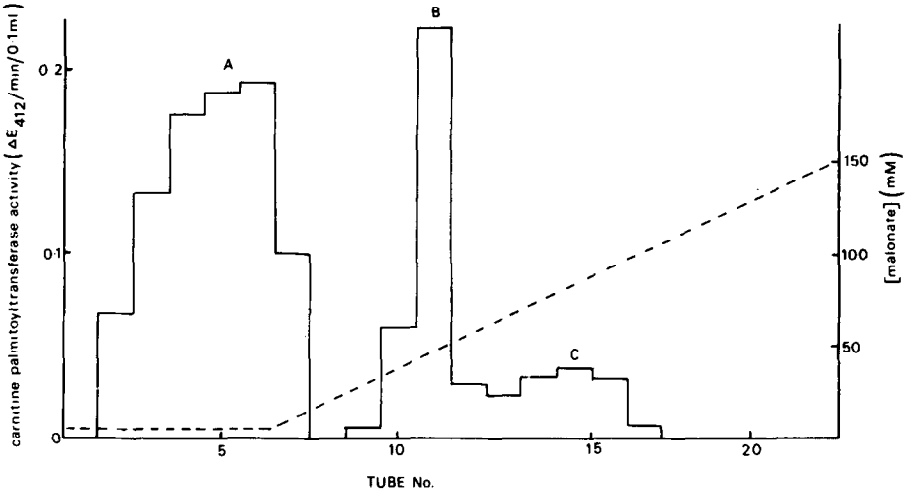

Figure 1. Separation of ox liver carnitine palmitoyltransferases on carboxymethylcellulose; for details see text.

Samples of enzyme were preincubated with 2-bromopalmitoyl-CoA in the presence of L-carnitine before the addition of palmitoyl-CoA to initiate the transferase reaction (for conditions see Fig. 2). The enzyme in peak B was almost completely inhibited, but the material from peak A and C remained active. Peak A was a cloudy suspension from which all carnitine palmitoyltransferase activity could be sedimented by centrifugation at 100,000 g for 30 min. at pH 5.7 or 7.1. Peaks B and C contained only soluble protein.

A similar pattern of carnitine palmitoyltransferase activity was obtained when a 0.5% Triton X-100 extract of frozen ox liver mitochondria

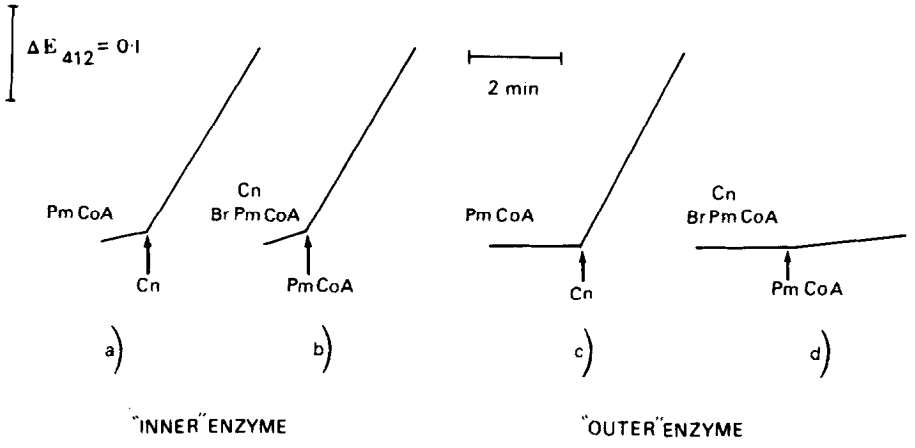

Figure 2. Effect of bromopalmitoyl-CoA on carnitine palmitoyltransferases. The routine assay was used; when present, the concentration of DL-2-bromopalmitoyl-CoA was 3 μM. (a) Purified "inner" enzyme (see text) incubated with palmitoyl-CoA for 2 min. before starting the transferase reaction with carnitine. (b) "Inner" enzyme incubated with bromopalmitoyl-CoA plus carnitine for 2 min.; reaction started with palmitoyl-CoA. (c) As (a), but using purified "outer" enzyme. (d) As (b), but using "outer" enzyme.

was subjected to electrofocusing for 3 days at 15° in an LKB-8101 electrofocusing column using Ampholine pH 3-10 in a 20-60% (v/v) gradient of glycerol. Enzyme activity was focused into three regions, corresponding to material with iso-electric points of pH 4.8 (66% of the activity recovered), pH 5.7 (29%) and pH 7.5 (5%). Under the conditions described in Fig. 2, the enzyme preparations with iso-electric points at pH 4.8 and 7.5 were insensitive to inhibition by 2-bromopalmitoyl-CoA, whereas the pH 5.7 material was inactivated.

Purification of two forms of carnitine palmitoyltransferase

a) Membrane bound enzyme. The material sedimented on centrifugation of peak A (Fig. 1) at 100,000 g for 30 min. was suspended in 20 mM triethanolamine-HCl, pH 7.5, homogenised with 0.3 vol. of cold n-butanol and recentrifuged. A clear aqueous solution containing nearly all of the carnitine palmitoyltransferase activity was obtained, which, after equilibration with 5 mM malonate containing 20% glycerol, passed un-

retarded through a carboxymethylcellulose column. Further purification was achieved by chromatography on DEAE-cellulose at pH 7.5, followed by adsorption onto and elution from calcium phosphate gel. Carnitine palmitoyltransferase with a specific activity of 0.4 units/mg. protein was obtained in this way; it was not inhibited by 2-bromopalmitoyl-CoA (Fig. 2).

b) Soluble enzyme. The supernatant obtained from a homogenate of frozen liver by centrifuging at 20,000 g for 20 min. was treated with 5 mM lead acetate and the resultant precipitate discarded. This gave a soluble form of carnitine palmitoyltransferase, which was purified by successive chromatography on DEAE- and carboxymethyl-cellulose, and cellulose phosphate. Further purification and concentration using calcium phosphate and alumina gels yielded enzyme with a specific activity in excess of 10 units/mg. protein, and which was totally inactivated by treatment with bromopalmitoyl-CoA (Fig. 2); this, and the enzyme's chromatographic behaviour, indicated that it corresponded to peak B of Fig. 1.

Full details of the purification of the two forms of carnitine palmitoyltransferase will be published elsewhere.

DISCUSSION

The above results demonstrate that extracts of whole liver or of mitochondria contain at least two forms of carnitine palmitoyltransferase. One of these (peak A of Fig. 1) is tightly bound to membrane material. This enzyme, even when solubilised by butanol treatment, is not inhibited by incubation with bromopalmitoyl-CoA in the presence of carnitine. The other major enzyme form (peak B, Fig. 1) is in a soluble form in crude extracts and is inactivated by bromopalmitoyl-CoA. A minor peak of activity (peak C, Fig. 1; also observed after electrofocusing) was not inhibited by bromopalmitoyl-CoA; this may perhaps represent material of peak A type which has become separated from acidic membrane components.

It seems likely that the enzyme inhibited by bromopalmitoyl-CoA corresponds to the "outer" pool of transferase in intact mitochondria,

i.e. the enzyme accessible to added acyl-CoA. Thus both Tubbs and Chase (1967) and Garland and Yates (1967) found carnitine-dependent oxidation of acyl-CoA by rat liver mitochondria to be inhibited by bromoacyl-CoA, while palmitoylcarnitine oxidation, which requires only the "inner" carnitine palmitoyltransferase pool to be active, was unimpaired.

It is known that nearly all of the carnitine palmitoyltransferase of liver mitochondria is associated with the inner-membrane fraction (Haddock, Yates and Garland, 1970), and that the "inner" transferase pool represents the major part of this enzyme in such mitochondria (Garland and Yates, 1967). Furthermore, Tubbs and Chase (1970) found that the acylcarnitine analogue 2-bromomyristoyl-thiocarnitine, although powerfully inhibiting the mitochondrial oxidation of palmitoylcarnitine, did not inhibit the "inner" pool of carnitine palmitoyltransferase in intact mitochondria. Similar results have been obtained with 2-bromopalmitoyl-L-carnitine (Biochem. J. in preparation) and we have found that, whereas the

TABLE 1

Acylcarnitine Specificity of Carnitine Palmitoyltransferase Isoenzymes

L-Carnitine Ester	"Outer" Enzyme		"Inner" Enzyme	
	V_{max}	K_m (μM)	V_{max}	K_m (μM)
Acetyl	10	500	0	-
Propionyl	95	338	0	-
Butyryl	96	320	0	-
Hexanoyl	101	200	19	353
Octanoyl	68	58	93	460
Dodecanoyl	68	28	152	426
Palmitoyl	100*	19	100*	60
DL-2-Bromopalmitoyl	Inhibited		22	73

* V_{max} with palmitoylcarnitine taken as 100 (arbitrary units). Rates were measured at 30° in a 2.0 ml system containing 100 mM tris-HCl, pH 8.0, 120 μM CoASH, varied amounts of acylcarnitine and enough of the appropriate enzyme (purified as in the text) to give an extinction change of 0.005-0.050 per minute at 232 nm in a 10 mm light path.

peak B (Fig. 1) enzyme is inhibited by bromopalmitoylcarnitine in the presence of CoASH, the peak A enzyme is not. All these considerations support the view that the non-inhibited enzyme of peak A represents the "inner" mitochondrial transferase pool.

Substrate specificity. Table 1 shows the results of an investigation of the activity of the two carnitine palmitoyltransferases using carnitine esters of fatty acids of different chain-length. The partially purified "inner" enzyme uses as substrates only carnitine derivatives of fatty acids with 6 or more carbon atoms; bromopalmitoylcarnitine is also a substrate. The "outer" enzyme, however, is active with fatty acids containing 2-12 carbon atoms and, as mentioned above, is inactivated by bromopalmitoyl-carnitine.

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