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The microsomal dicarboxylyl-CoA synthetase

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Dicarboxylic acids are products of the ω-oxidation of monocarboxylic acids. We demonstrate that in rat liver dicarboxylic acids (C_5–C_{16}) can be converted into their CoA esters by a dicarboxylyl-CoA synthetase. During this activation ATP, which cannot be replaced by GTP, is converted into AMP and PP_i, both acting as feedback inhibitors of the reaction. Thermolabile at 37°C, and optimally active at pH6.5, dicarboxylyl-CoA synthetase displays the highest activity on dodecanedioic acid (2 μmol/min per g of liver). Cell-fractionation studies indicate that this enzyme belongs to the hepatic microsomal fraction. Investigations about the fate of dicarboxylyl-CoA esters disclosed the existence of an oxidase, which could be measured by monitoring the production of H_2O_2. In our assay conditions this H_2O_2 production is dependent on and closely follows the CoA consumption. It appears that the chain-length specificity of the handling of dicarboxylic acids by this catabolic pathway (activation to acyl-CoA and oxidation with H_2O_2 production) parallels the pattern of the degradation of exogenous dicarboxylic acids *in vivo*.

In peroxisomes from rat liver Lazarow & de Duve (1976) demonstrated a set of reactions similar to the classical mitochondrial fatty acyl-CoA β-oxidation. The first step of the peroxisomal system, in contrast with the mitochondrial acyl-CoA dehydrogenases, is not dependent on the respiratory chain, since electrons produced are directly transferred from the peroxisomal flavoproteins to molecular O_2 with the production of H_2O_2 (see Mannaerts & Debeer, 1981).

In contrast with both these pathways, fatty acids can also be oxidized in the ω-position without formation of CoA ester intermediates (Preiss & Bloch, 1964; Robbins, 1968; Ichihara *et al.*, 1969; Björkhem & Danielsson, 1970; Björkhem, 1973, 1976). They are converted in the presence of $NADPH+H^+$ and O_2 into ω-hydroxy acids by the microsomal mixed-function oxidase system (Wakabayashi & Shimazono, 1963; Pettersen, 1972). The ω-alcohol radical is further transformed to an ω-carboxylic radical by a cytosolic ω-hydroxy acid dehydrogenase (Mitz & Heinrikson, 1961). These reactions allow the synthesis of dicarboxylic acids from monocarboxylic acids.

These dicarboxylic acids are not terminal end products of metabolism and their shortening has been already suggested by Verkade *et al.* (1935). Pettersen (1972) brought strong arguments from experiments performed *in vivo* in favour of the claim that initial ω-oxidation of fatty acids followed by β-oxidation of dicarboxylic acids can account for the urinary occurrence of medium-chain and short-chain dicarboxylic acids.

More recently, the intracellular site of the β-oxidation of dicarboxylic acids was investigated by experiments performed *in vitro* (Mortensen *et al.*, 1983). These authors showed that, when incubated with mitochondrial and peroxisomal fractions, the CoA derivative of dodecanedioic acid gives rise to small amounts of shorter dicarboxylic acids.

In the present paper, we demonstrate that dicarboxylic acids with a chain length greater than C_5 can be metabolized.

The enzyme activities we measure can account

for the utilization of dicarboxylic acids *in vivo* as studied by several authors (see Mortensen & Gregersen, 1982).

Materials and methods

Materials

Clofibric acid [2-(*p*-chlorophenoxy)-2-methylpropionic acid], uric acid, homovanillic acid and peroxidase type II were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Dicarboxylic acids were from Janssen Chemica (Beerse, Belgium). FAD, CoA, NAD^+, ATP, GTP and concanavalin A were from Boehringer Pharma (Mannheim, W. Germany). 4-Methylumbelliferyl derivatives were from Koch–Light Laboratories (Colnbrook, Bucks., U.K.) Percoll was purchased from Pharmacia (Uppsala, Sweden).

Animals

Adult male Wistar rats (200–300g) were fed on a standard laboratory animal chow, containing less than 3% of fat. Treated animals were fed on a solid diet made by mixing the powdered animal chow with 0.5% (w/w) neutralized clofibric acid. The treatment of animals with 3-methylcholanthrene and with phenobarbital was performed in accordance with Roberfroid *et al.* (1983).

Assay of enzyme activities and metabolites

Dicarboxylyl-CoA synthetase activity is measured by the rate of disappearance of CoA in the presence of various dicarboxylic acids. The assay mixture (0.5 or 1ml) contained 50mM-potassium phosphate buffer, pH6.5, 0.5mM-CoA, 10mM-ATP + 10mM-$MgCl_2$ and either 1mM-dicarboxylic acid for the test or water for the blank. At different incubation times, 0.15ml samples were withdrawn and mixed with trichloroacetic acid [5% (w/v) final concentration]. After centrifugation, 0.1ml of supernatant was added to 0.9ml of reaction medium containing 100mM-Tris/HCl buffer, pH8.0, and 2mM-5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent). The absorbance was read at 412nm.

Dicarboxylyl-CoA oxidase activity was assayed by measuring the dicarboxylyl-CoA-dependent production of H_2O_2. The CoA derivative was formed in the reaction mixture from the corresponding dicarboxylic acid and CoA in the presence of ATP + $MgCl_2$. The assay mixture (0.5ml) contained 1mM-dicarboxylic acid, 0.2mM-CoA, 8mM-ATP and 16mM-$MgCl_2$, 0.02mM-FAD, 80mM-glycylglycine buffer, pH8.3, 10mM-homovanillate and 0.1mg of horseradish peroxidase. H_2O_2 was determined by the fluorimetric method of Guilbault *et al.* (1967), in which homovanillic acid reacts with H_2O_2 in the presence of peroxidase to form a fluorescent dimer (Vamecq & Van Hoof, 1984).

The lysosomal hydrolase *N*-acetyl-β-glucosaminidase was assayed by the method of Van Hoof & Hers (1968), with 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside as substrate. Lactate dehydrogenase activity was measured as described by Hohorst (1963).

Cytochrome *c* oxidase was assayed by the method of de Duve *et al.* (1955), and arylsulphatase C was measured with 4-methylumbelliferyl sulphate as substrate (Vamecq & Van Hoof, 1984).

D-Amino acid oxidase was assayed as described by Gaunt & de Duve (1976) except that the substrate was 100mM-D-proline instead of 25mM-D-alanine. Urate oxidase activity was measured by monitoring the decrease of uric acid concentration at 292.5nm, as described by Baudhuin *et al.* (1964). Glycollate oxidase was assayed as previously described (Vamecq & Van Hoof, 1984). Tyramine oxidase (monoamine oxidase) was assayed by its H_2O_2 production, similarly to glycollate oxidase, except that 20mM-glycollate was replaced by 20mM-tyramine as substrate.

One unit of enzyme activity is the amount of enzyme that catalyses the conversion of 1μmol of substrate/min.

AMP was measured by the method of Adam (1963).

Proteins were measured by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Extraction of dodecanedioyl-CoA

Dodecanedioyl-CoA, the product of the reaction catalysed by dicarboxylyl-CoA synthetase, was extracted by using the procedure described by Krisans *et al.* (1980) for palmitoyl-CoA. With the use of this technique, dodecanedioyl-CoA was present in the aqueous phase and separated from dodecanedioic acid, which remains in the organic phase. The aqueous phase was freeze-dried, and the dry residue was dissolved in distilled water and adjusted to pH7.0–7.5 with 0.1M-NaOH.

Cell-fractionation studies

Self-generating Percoll gradients were prepared as described by Mannaerts *et al.* (1982) except that, before centrifugation, rat liver postnuclear supernatants instead of λ fractions were layered on isoosmotic 30% (w/v) Percoll. The fractions obtained were further submitted to isopycnic centrifugation on a sucrose gradient as previously described (Vamecq & Van Hoof, 1984).

These techniques of cell fractionation were used to achieve, in a first step, the separation of both peroxisomes and microsomes from lysosomes and

mitochondria and, in the next step, the separation of microsomes and peroxisomes.

High-speed centrifugation corresponds to a 100000*g* centrifugation during 30min in a vertical rotor (Ti 50; Beckman Instruments, Spinco Division, Palo Alto, CA, U.S.A.).

Differential centrifugation techniques were used to prepare the ML, the P and the S fractions as described by de Duve (1975).

In tissue homogenates prepared for all fractionation investigations, 1mM-EDTA was added in order to prevent aggregation between subcellular components.

Results

Characteristics of dicarboxylyl-CoA synthetase

The progress of the overall reaction catalysed by dicarboxylyl-CoA synthetase can be measured with the Ellman's reagent. A CoA consumption is recorded when rat liver homogenates are incubated with dodecanedioic acid, ATP + $MgCl_2$ and CoA. When assayed on whole liver homogenates or on their postnuclear supernatants (2mg or less of protein/ml) CoA consumption proceeds linearly with time for a period of 10–15min before reaching a plateau (Fig. 1).

In rats dicarboxylyl-CoA synthetase is found in liver only, and we have been unable to detect its activity in kidney, jejunum, heart, lung, skeletal and smooth muscles, cerebrum, cerebellum and brain stem.

With dodecanedioic acid as substrate, the optimum pH for the synthetase activity is 6.5 in phosphate buffer. The specificity of the enzyme for dicarboxylic acids of different chain lengths is optimal for dodecanedioic acid (Fig. 2) and is not significantly modified when the assay is performed at pH7.5 (phosphate buffer) or at pH8.5 (glycylglycine buffer), but the rate of the reaction was decreased by 40% and 70% respectively. At pH6.5 the activity of dodecanedioyl-CoA synthetase in rat liver is about 2units/g wet wt.

In Fig. 3(*a*) the activity of dicarboxylyl-CoA synthetase expressed is plotted as a function of dodecanedioic acid concentration. Plotting the results in accordance with the Lineweaver–Burk method (Fig. 3*b*) allows calculation of an apparent $V_{max.}$ of about 33munits/mg of protein and an apparent K_m of about 2.5mM.

Dicarboxylyl-CoA synthetase is quite labile. Preincubation at 37°C of a fresh liver homogenate in the absence of substrate and cofactors leads to a loss of 75% of its activity within 30min (Fig. 4). Freezing and thawing of liver homogenates also strongly diminishes its activity within several hours (results not shown). The effect of deoxycholate is reported below; 0.1% and 1% (w/v) Brij and 0.1% Triton X-100 are without effect on the enzyme reaction. Fig. 5(*a*) shows that ATP concentrations between 2 and 10mM are optimal. The apparent K_m of the enzyme for ATP is about 0.55mM. The apparent $V_{max.}$ is about 10munits/mg of protein. GTP is not a substrate. The reaction is inhibited by its products and AMP is more inhibitory than pyrophosphate (Figs 5*b* and 5*c*).

No thiol consumption is recorded when the enzyme is assayed with reduced glutathione, L-cysteine or thioglycollate instead of CoA.

When assayed with dodec-2-enedioic acid (traumatic acid) the activity of dicarboxylyl-CoA synthetase is about 40% of its activity on dodecanedioic acid.

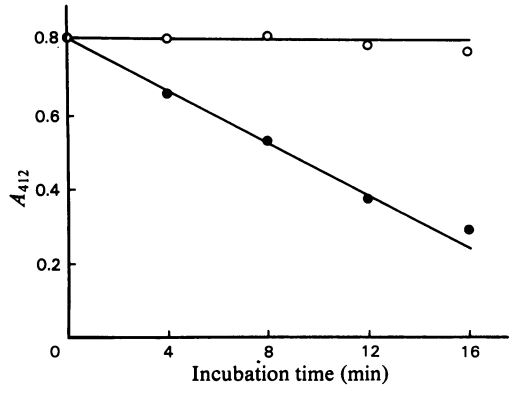


Fig. 1. *Assay of dicarboxylyl-CoA synthetase with a rat liver homogenate*
For experimental details see the text. A linear decrease in the absorption at 412nm is recorded when dodecanedioic acid is present (●). It corresponds to a CoA consumption and accordingly to dodecanedioyl-CoA production. The blank (○) is run in the absence of added dicarboxylic acids.

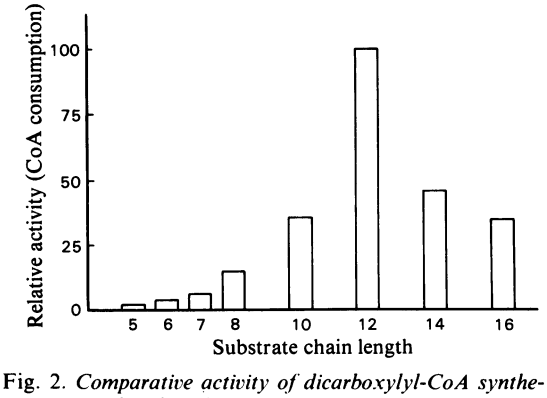


Fig. 2. *Comparative activity of dicarboxylyl-CoA synthetase on dicarboxylic acids of various chain lengths*
For experimental details see the text.

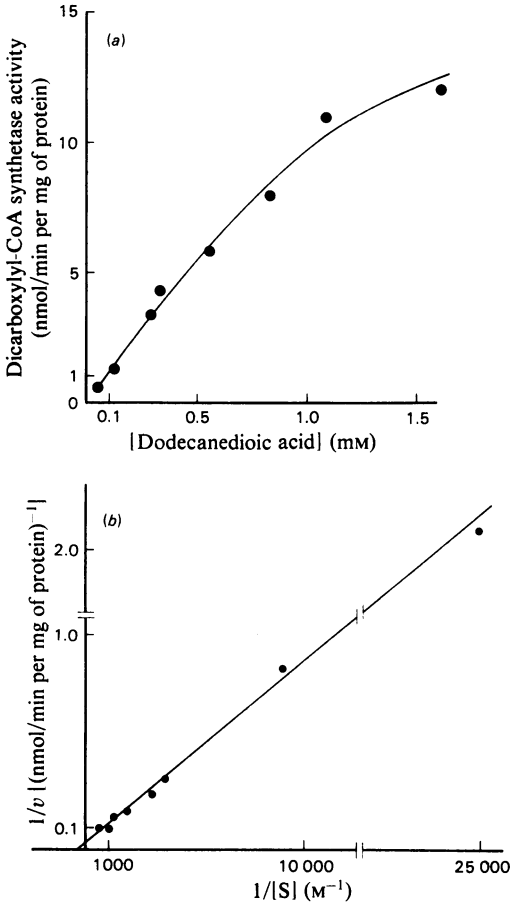


Fig. 3. (*a*) *Effect of increasing concentrations of dodecanedioic acid on the activity of rat liver dicarboxylyl-CoA synthetase and* (*b*) *apparent* $V_{max.}$ *and apparent* K_m *of dicarboxylyl-CoA synthetase*
The assay conditions were as described in the Materials and methods section. The results in (*a*) are presented in the form of a Lineweaver–Burk plot in (*b*).

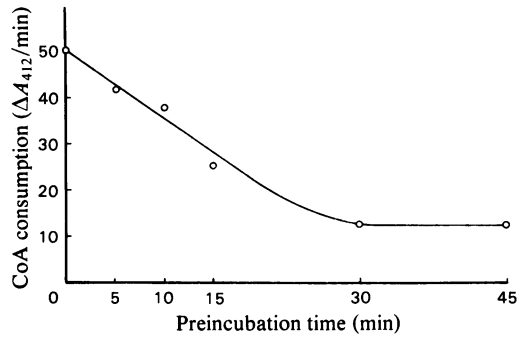


Fig. 4. *Thermostability of dicarboxylyl-CoA synthetase from rat liver*
The whole homogenate (approx. 20mg of protein/ml) was incubated in 10mM-phosphate buffer, pH6.5, at 37°C in the absence of added dicarboxylic acids. The activity of the enzyme is not modified by the incubation at 0°C for a few hours.

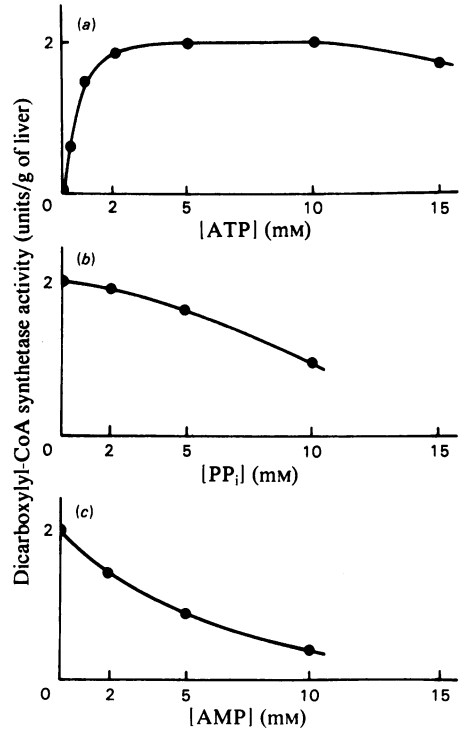


Fig. 5. *Effect of various concentrations of ATP,* PP_i *and AMP on the activity of dicarboxylyl-CoA synthetase with dodecanedioate as substrate*
For experimental details see the text.

For the demonstration of AMP production, a microsomal fraction rich in dicarboxylyl-CoA synthetase activity (48 munits/mg of protein) was used. Such a preparation is, however, contaminated by the 5′-nucleotidase that is associated with hepatic cell membranes. The latter enzyme can be inhibited by 0.1mM-concanavalin A (Riemer & Widnell, 1975; Williamson *et al.*, 1976), in contrast with its cytosolic isoenzyme, which is by far less active (Van den Berghe *et al.*, 1977). An initial investigation with the microsomal material without inhibition of the 5′-nucleotidase failed to demonstrate that AMP was a product of the synthetase reaction. In the presence of 0.1mM-concanavalin A, which had previously been shown to be without inhibitory effect on dicarboxylyl-CoA synthetase activity, AMP was found to be produced in 1:1 molar ratio with the CoA consumed. However, in some experiments we have

observed that AMP production can proceed even after the consumption of most of the CoA. One possible explanation might be the contamination of the enzyme preparation by a dicarboxylyl-CoA hydrolase activity, which would create a futile cycle by regenerating the substrates of the synthetase reaction. This hypothesis could also account for the fact that in some assays of dicarboxylyl-CoA synthetase the plateau of CoA consumption has been followed by an apparent CoA release (results not shown).

Fate of dicarboxylyl-CoA

Dodecanedioyl-CoA ester produced by the synthetase reaction can serve as substrate for an oxidase that forms H_2O_2. The resulting 2-enoyl-CoA ester would probably (see Mortensen *et al.*, 1983) be converted into decanedioyl-CoA through the other three steps of β-oxidation, as indicated in Scheme 1.

We measured fluorimetrically the amount of H_2O_2 formed by rat liver homogenates from dodecanedioate in the presence of CoA and ATP+Mg^{2+}, a blank being run without dicarboxylic acid. After a brief lag period, a linear production of H_2O_2 could be recorded that was roughly equivalent to the CoA consumption assayed in the same conditions (Fig. 6). Effects of ATP+Mg^{2+}, PP_i and AMP were similar to that reported for dicarboxylyl-CoA synthetase, as was the chain-length specificity. When its substrate is produced by the action of the corresponding synthetase, dicarboxylyl-CoA oxidase displays an optimal activity between pH8.0 and pH8.3 in glycylglycine buffer. No H_2O_2 production can be recorded when rat liver homogenates are incubated with dicarboxylic acids in the absence of ATP+Mg^{2+} and/or of CoA, indicating that both cofactors are actually required for the previous synthesis of dicarboxylyl-CoA.

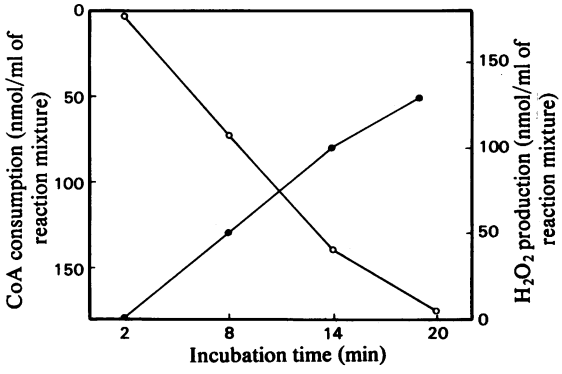
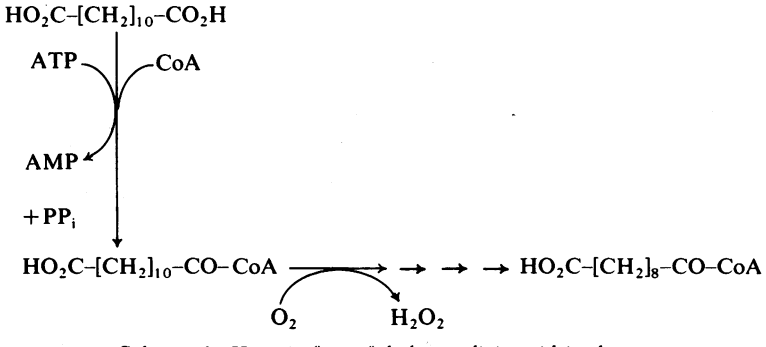


Fig. 6. *Correlation between the consumption of CoA for the synthesis of dodecanedioyl-CoA* (○) *and the production of H_2O_2 during the oxidation of this CoA ester at pH8.3* (●) *by the same rat liver homogenate*

After 20min of incubation at 37°C, about one-third of the total CoA has been consumed.

Cell-fractionation studies

Fig. 7 illustrates the distribution of proteins and enzyme activities in rat liver fractions obtained after centrifugation of postnuclear supernatants layered on a 30% Percoll/0.25M-sucrose solution. Marker enzymes are cytochrome *c* oxidase for mitochondria, β-hexosaminidase for lysosomes, arylsulphatase C for endoplasmic reticulum, D-proline oxidase for peroxisomal matrix and urate oxidase for peroxisomal nucleoid. The activity distribution of D-proline oxidase includes the enzyme activity present in the intact peroxisomes, that released from these organelles during homogenization and that of the cytosolic enzyme. The activity distribution of urate oxidase corresponds to that of the enzyme activity linked to the nucleoids present in the intact peroxisomes or that has been released from these organelles during homogenization. Usually in this kind of Percoll gradient mitochondria and lysosomes are expected to migrate to the bottom of the tube, whereas peroxisomes and endoplasmic reticulum remain in the upper half of the gradient.



Scheme 1. *Hepatic fate of dodecanedioic acid in the rat*

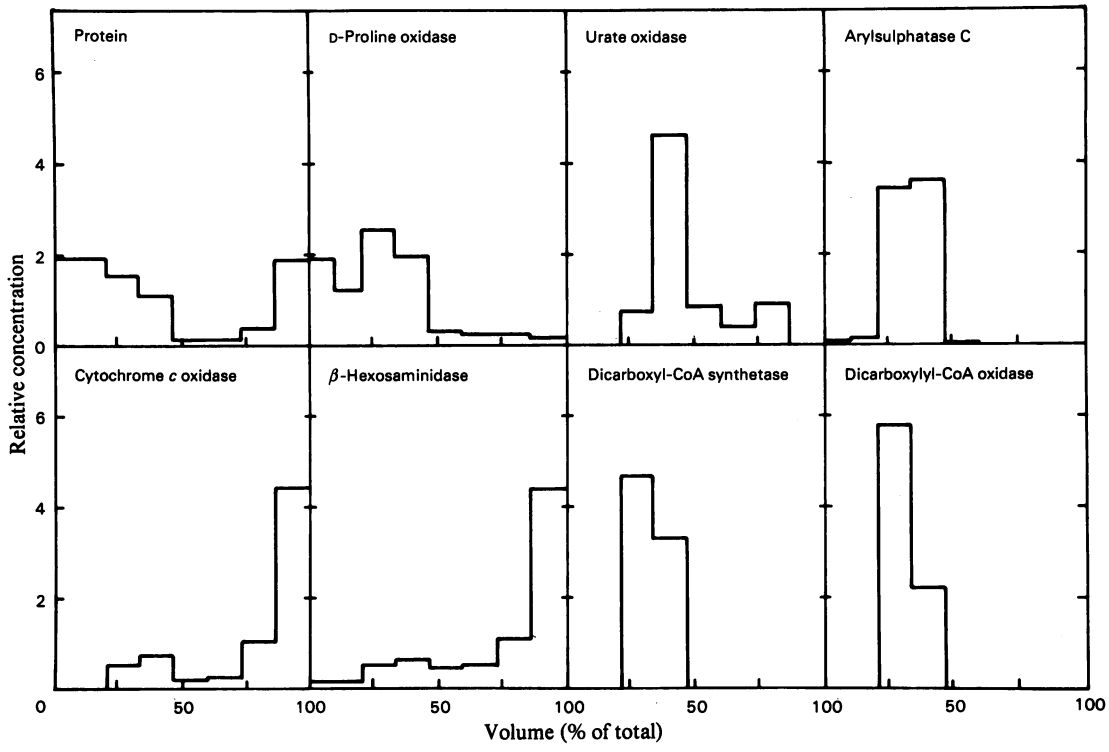


Fig. 7. *Subfractionation of postnuclear supernatants from a rat liver by equilibrium density centrifugation in a self-generating Percoll gradient*

Top to bottom fractions are drawn from left to right on the abscissa. Results are normalized as described by Leighton *et al.* (1968). Recovery of proteins and of enzyme activities in the sum of fractions was between 86% and 109%.

Dicarboxylyl-CoA synthetase and oxidase activities were not detectable in the mitochondrial and lysosomal fractions and were only detected in fractions in which peroxisomes and endoplasmic reticulum were abundant. The latter fractions were then layered on a linear sucrose gradient and centrifuged in a vertical rotor (100000*g* for 90 min). In such conditions peroxisomes migrate to higher equilibration densities than does the endoplasmic reticulum (Fig. 8). The density distribution of dicarboxylyl-CoA synthetase parallels that of microsomal enzymes (arylsulphatase C) and is clearly distinct from that of peroxisomal enzymes (D-proline oxidase and urate oxidase). Isolated peroxisomal membranes and microsomes have similar density properties. However, the density shift between peroxisomal and microsomal enzyme activities on a sucrose gradient appears to be too large to support the hypothesis that the synthetase is linked to peroxisomal membranes. Furthermore, in this gradient no peroxisomal peak of dicarboxylyl-CoA synthetase activity is observed. Dicarboxylyl-CoA oxidase displays the same activity distribution as dicarboxylyl-CoA synthetase. However, it must be stressed that in our assay conditions the oxidase activity is dependent on the activity of the synthetase, which is rate-limiting.

The fact that dicarboxylyl-CoA synthetase belongs to the endoplasmic reticulum was confirmed by studies on fractions obtained by differential centrifugation as described by de Duve (1975). Over 70% of dicarboxylyl-CoA synthetase activity was invariably found in the P fraction, in which arylsulphatase C was also concentrated (Table 1).

Separation of dicarboxylyl-CoA synthetase activity from that of dicarboxylyl-CoA oxidase

We were able to separate dicarboxylyl-CoA synthetase activity from oxidase activity with the use of deoxycholate. Postnuclear supernatants from a rat liver homogenate were treated with different concentrations of sodium deoxycholate and were further submitted to high-speed centrifugation.

Both dicarboxylyl-CoA oxidase and dicarboxylyl-CoA synthetase were assayed in the homogenate before centrifugation and in the resulting pellet and supernatant (Fig. 9). Treatment of postnuclear supernatants with 0.1% deoxycholate followed by high-speed centrifugation already efficiently sepa-

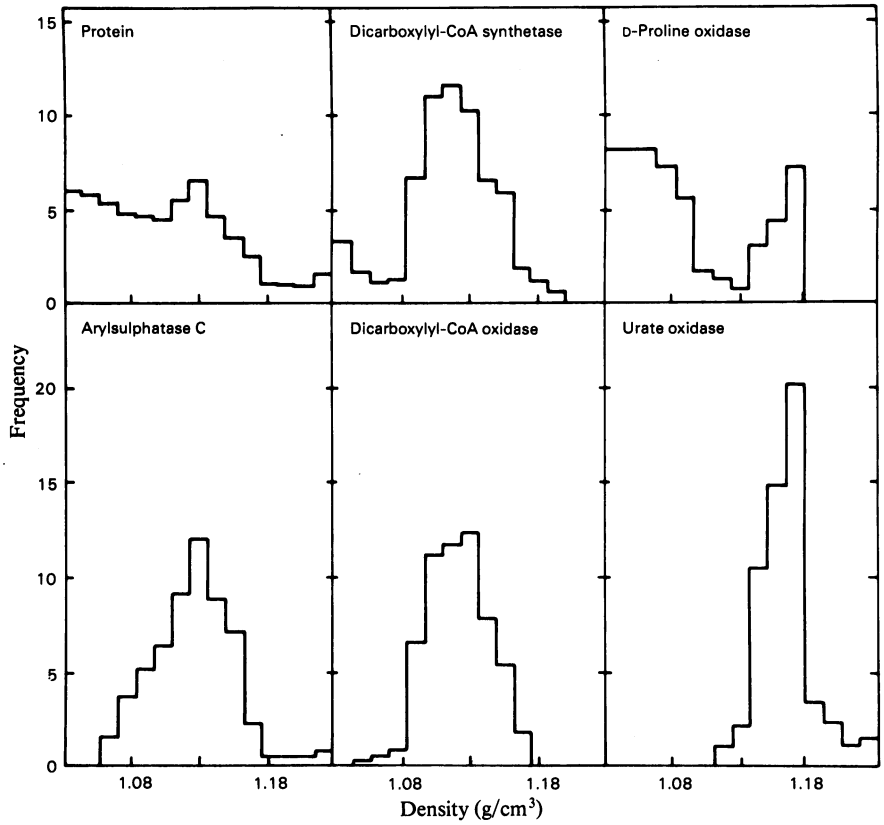


Fig. 8. *Isopycnic centrifugation of three cumulated Percoll fractions (between 9% and 46% of total volume on Fig. 7) on a linear sucrose gradient*

Abscissa and ordinate are expressed as previously reported (Vamecq & Van Hoof, 1984). Recovery of enzyme activity in the sum of fractions was between 80% and 118%. Note that the four heaviest fractions contained accumulated and concentrated Percoll in which peroxisomes (see D-Proline oxidase) do not migrate, in contrast with nucleoids isolated from these organelles (see urate oxidase). The oxidase activity on dodecanedioyl-CoA ester (dicarboxylyl-CoA oxidase) is fully dependent on dicarboxylyl-CoA synthetase activity, which is rate-limiting in our assay conditions.

Table 1. *Enzyme activities and protein concentration in fractions obtained after differential centrifugation of postnuclear supernatants (extract) from rat liver homogenate*

Enzyme activities are expressed as μmol of substrate consumed or product formed per min per ml of fraction. Each fraction corresponds to 200mg of liver/ml.

	Enzyme activity (μmol/min per ml)				
	Extract	Mitochondrial pellet (ML fraction)	Postmitochondrial supernatant (P+S fractions)	S (cytosolic) fraction	P (microsomal) fraction
β-Hexosaminidase (lysosomes)	0.512	0.506	0.043	0.009	0.045
Arylsulphatase C (microsomes)	0.083	0.019	0.053	0.001	0.059
Lactate dehydrogenase (cytosol)	59	12	67	72	5
Tyramine oxidase (mitochondria)	0.250	0.200	0.019	0.001	0.025
Glycollate oxidase (peroxisomal matrix)	0.180	0.200	0.050	0.045	0.010
Urate oxidase (peroxisomal core)	0.400	0.350	0.115	0.005	0.100
Dicarboxylyl-CoA synthetase	0.408	0.096	0.329	0.030	0.352
Protein (mg/ml)	35	21	18	9	8

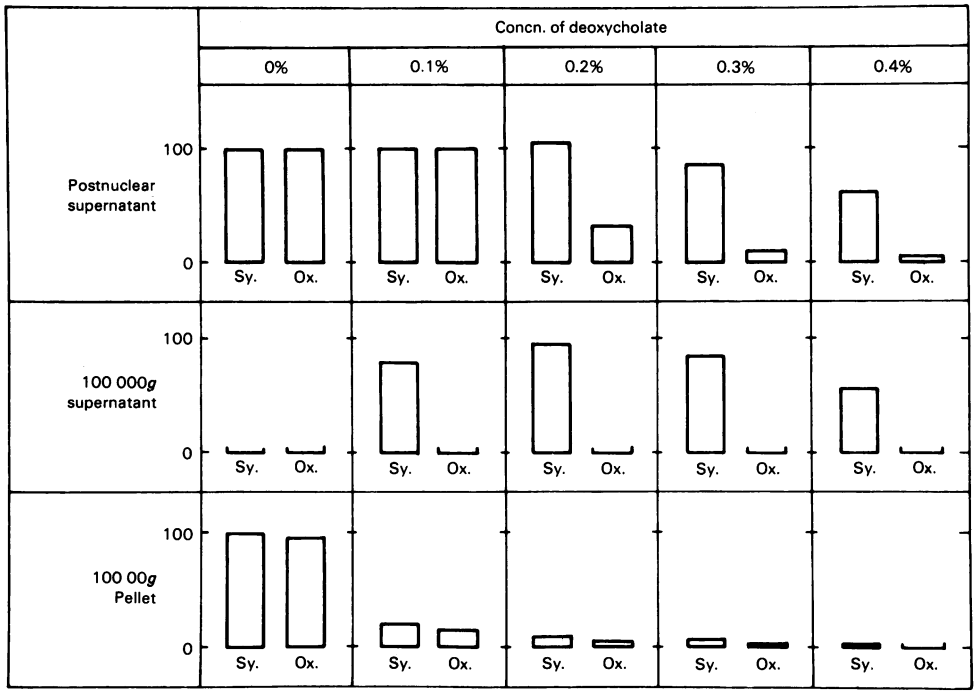


Fig. 9. *Effect of various concentrations of deoxycholate on the activity of dicarboxylyl-CoA synthetase and oxidase in a postnuclear supernatant from a rat liver homogenate*

Deoxycholate was added to the postnuclear supernatant before high-speed centrifugation. Results are expressed as percentages of residual activity after deoxycholate action. Abbreviations: Sy., dicarboxylyl-CoA synthetase; Ox., dicarboxylyl-CoA oxidase. The formation of dodecanedioyl-CoA results from the action of dicarboxylyl-CoA synthetase in the same preparation.

rates synthetase activity from oxidase. Supernatant proteins and accordingly deoxycholate concentrations were diluted 4-fold in the assay mixtures. The highest concentrations of deoxycholate present in the assay of dicarboxylyl-CoA oxidase were without effect on the activity of peroxidase, which is added for the assay of H_2O_2. They were slightly stimulatory on D-proline oxidase activity (1.50-fold activity) and inhibitory on that of glycollate oxidase (0.65-fold activity), these last two enzymes being also assayed by the fluorimetric measurement of H_2O_2. This excludes the possibility that the absence of activity observed for dicarboxylyl-CoA oxidase results from an interference in the assay of H_2O_2.

Using a fraction with dicarboxylyl-CoA synthetase activity and no active dicarboxylyl-CoA oxidase, we have synthesized and isolated dodecanedioyl-CoA as described in the Materials and methods section. This compound was then incubated with a rat liver homogenate. The amount of H_2O_2 produced, by comparison with the appropriate blank, was measured. The ratio of H_2O_2 production to CoA consumption (previously recorded) was about 0.80:1. This confirms the stoichiometrical relationship between the CoA consumption and the H_2O_2 production already illustrated by Fig. 6.

The tissue distribution of dicarboxylyl-CoA oxidase was checked with dodecanedioyl-CoA. In contrast with the synthetase, which is present only in liver, dicarboxylyl-CoA oxidase activity was detected in liver, kidney cortex and jejunal mucosa. This tissue distribution is the same as that of palmitoyl-CoA oxidase and glutaryl-CoA oxidase, which both belong to the peroxisomal matrix (Vamecq & Van Hoof, 1984).

Effect of clofibrate treatment

Clofibrate is known to stimulate the proliferation of liver peroxisomes and to induce the synthesis of enzymes of the peroxisomal β-oxidation. Dicarboxylyl-CoA synthetase and oxidase activities were measured in liver from control rats and from rats treated for more than 2 months with clofibrate. No increased activity of dicarboxylyl-CoA synthetase was observed in liver of treated animals. The oxidase activity measured was only slightly stimulated (maximum 2-fold) when its assay was coupled with dicarboxylyl-CoA synthe-

tase, but when the oxidase activity was directly measured with dodecanedioyl CoA as substrate a stimulation up to 10-fold was recorded in liver from clofibrate-treated animals.

Effect of stimulation of the endoplasmic reticulum

As dicarboxylyl-CoA synthetase belongs to the hepatic microsomes, it was decided to check the effect on its activity of some microsomal proliferators. Treatment of the animal with 3-methylcholanthrene leads to a slight stimulation (1.5–2-fold) of the activity. Feeding of the animal with a phenobarbital-containing diet was without effect on the activity of liver dicarboxylyl-CoA synthetase.

Discussion

Acyl-CoA synthetase activities

An acyl-CoA synthetase acting on hexadecanedioic acid was described by Pettersen (1973) in rat liver mitochondria prepared in accordance with Myers & Slater (1957). Contamination of these preparations by some microsomal material appears sufficient to account for the dicarboxylyl-CoA synthetase activity observed by Pettersen (1973).

Kornberg & Pricer (1953), working on guinea-pig liver, failed to detect an acyl-CoA synthetase active on dicarboxylic acids (C_8, C_9 and C_{10}). For this study the method of Lipmann & Tuttle (1950) was applied, in which the acyl-CoA synthesized reacts with hydroxylamine to produce CoA and hydroxamic acid, the latter acid being assayed. With respect to a species difference, concerning the hepatic localization of our microsomal enzyme, the negative finding reported by these authors might result from an inhibitory effect of hydroxylamine on dicarboxylyl-CoA synthetase. An alternative explanation could be provided by the fact that Kornberg & Pricer (1953) used freeze-dried liver preparations, and we have observed that repeated freezing of liver homogenates can completely inactivate dicarboxylyl-CoA synthetase.

Since it is associated with the endoplasmic reticulum, dicarboxylyl-CoA synthetase appears to be distinct from the mitochondrial long-chain fatty acyl-CoA synthetase. On the other hand, from the work of Kornberg & Pricer (1953) and from the present data it may be inferred that the microsomal long-chain fatty acyl-CoA synthetase and dicarboxylyl-CoA synthetase are two different enzymes. Thus 0.1% Triton X-100 increased the activity of the former enzyme by 8-fold (Bar-Tana *et al.*, 1971) whereas it is without effect on the latter enzyme. In addition, dicarboxylyl-CoA synthetase activity measured in our assay conditions seems to be restricted to liver. This contrasts with both mitochondrial and microsomal long-chain fatty acyl-CoA synthetase activities, which have been detected in several tissues.

Menon *et al.* (1960) described an enzymic system able to activate glutarate to glutaryl-CoA. For several reasons the latter activity seems different from that of dicarboxylyl-CoA synthetase described in the present paper. It can function with ATP but also with GTP; it has been localized in liver, but also in extrahepatic tissues such as heart and muscle. It is likely that, besides the dicarboxylyl-CoA synthetase acting on short-chain, medium-chain and long-chain dicarboxylic acids and that is restricted to liver, a short-chain dicarboxylyl-CoA synthetase with distinct properties exists.

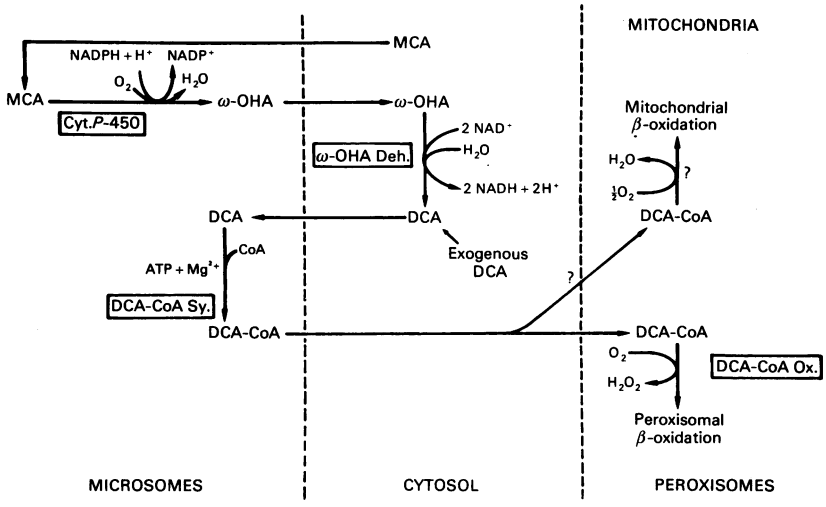
Product of the reaction catalysed by dicarboxylyl-CoA synthetase

Whereas the monoacyl-CoA derivative of dodecanedioic acid seems the most plausible product of dicarboxylyl-CoA synthetase, our data cannot definitely rule out the formation of a 1,ω-diacyl-CoA derivative of the dicarboxylic acid. We cannot exclude the possibility that the CoA monoester of dodecanedioate could be also itself a substrate of dicarboxylyl-CoA synthetase.

Role of dicarboxylyl-CoA synthetase

All organs containing the ω-oxidative enzymic equipment can produce dicarboxylic acids, but only the liver is able to catabolize them. The hepatic dicarboxylyl-CoA synthetase appears to catalyse an essential step in the degradation of these acids. Indeed, dicarboxylic acids resulting from the ω-oxidation of monocarboxylic acids apparently cannot be further oxidized if they are not previously activated to their CoA esters. The intracellular localization of the catabolism of dicarboxylyl-CoA is as yet unknown, and it is obvious that, in our assay conditions, the measurement of the oxidase activity is dependent on the formation of dicarboxylyl-CoA. An extramicrosomal localization of the oxidase cannot be excluded on the basis of our cell-fractionation data. The fact that this enzyme generates H_2O_2 can, however, be considered as an indication in favour of its peroxisomal nature. It may be proposed that the system responsible for this biochemical pathway belongs to peroxisomes, as already suggested by Mortensen *et al.* (1982) and Gregersen *et al.* (1983), but further investigations on the properties of dicarboxylyl-CoA oxidase, directly assayed, are required to characterize better the catabolism of dicarboxylic acids.

It will also be imperative to check whether or not liver mitochondria are able to catabolize the products of the ω-oxidation of fatty acids. There is no convincing evidence that these organelles



Scheme 2. *Liver metabolism of medium-chain and long-chain dicarboxylic acids*
Metabolic intermediates are: MCA, monocarboxylic acids (non-esterified fatty acids); ω-OHA, ω-hydroxy acids; DCA, dicarboxylic acids; DCA-CoA, dicarboxylyl-CoA. Metabolic reactions are catalysed by: Cyt. *P*-450, cytochrome *P*-450; ω-OHA Deh., ω-hydroxy acid dehydrogenase; DCA-CoA Sy., dicarboxylyl-CoA synthetase; DCA-CoA Ox., dicarboxylyl-CoA oxidase. Exogenous DCA represents dicarboxylic acids from extrahepatic origin, either produced from monocarboxylic acids in peripheral tissues or present in the diet.

metabolize medium-chain and long-chain dicarboxylic acids; in addition, some authors have demonstrated that the latter compounds can strongly depress the O_2 consumption by intact mitochondria (Passi *et al.*, 1984). However, the existence in these organelles of a short-chain dicarboxylyl-CoA dehydrogenase, which is identified with glutaryl-CoA dehydrogenase (Besrat *et al.*, 1969; Vamecq & Van Hoof, 1984; Vamecq *et al.*, 1985), indicate that shorter-chain substrates might be catabolized in this compartment.

Scheme 2 summarizes our account of the liver metabolism of medium-chain and long-chain dicarboxylic acids and raises the following question: does the peroxisomal β-oxidative capacity alone support the degradation of these acids? Whatever the answer to the latter question, the capacity of the handling of fatty acids by the ω-oxidative pathway seems to be limited, since an appreciable proportion of potentially oxidizable substrates (C_6 to C_{10} dicarboxylic acids) is lost in urine when this pathway is overloaded, as for instance upon starvation, in diabetes (Borg *et al.*, 1972; Pettersen, 1972; Pettersen *et al.*, 1972; Björkhem, 1973, 1976; Mortensen, 1981*a*,*b*; Mortensen & Gregersen, 1981, 1982; Mortensen *et al.*, 1982) and in some inherited diseases (Gregersen *et al.*, 1976; Gregersen & Brandt, 1979; Gregersen *et al.*, 1982; Kølvraa *et al.*, 1982; Gregersen *et al.*, 1983).

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