Carnitine acyltransferase and acyl-coenzyme A hydrolase activities in human liver

Quantitative analysis of their subcellular localization

Miguel BRONFMAN and Federico LEIGHTON
Laboratorio de Citologia Bioquimica, Departamento de Biologia Celular, Universidad Catolica de Chile, Casilla 114-D, Santiago, Chile

(Received 22 May 1984/Accepted 30 August 1984)

The subcellular localizations of carnitine acyltransferase and acyl-CoA hydrolase activities with different chain-length substrates were quantitatively evaluated in human liver by fractionation of total homogenates in metrizamide density gradients and by differential centrifugation. Peroxisomes were found to contain 8–37% of the liver acyltransferase activity, the relative amount depending on the chain length of the substrate. The remaining activity was ascribed to mitochondria, except for carnitine octanoyltransferase, for which 25% of the activity was present in microsomal fractions. In contrast with rat liver, where the activity in peroxisomes is very low or absent, human liver peroxisomes contain about 20% of the carnitine palmitoyltransferase. Short-chain acyl-CoA hydrolase activity was found to be localized mainly in the mitochondrial and soluble compartments, whereas the long-chain activity was present in both microsomal fractions and the soluble compartment. Particle-bound acyl-CoA hydrolase activity for medium-chain substrates exhibited an intermediate distribution, in mitochondria and microsomal fractions, with 30–40% of the activity in the soluble fraction. No acyl-CoA hydrolase activity appears to be present in human liver peroxisomes.

Carnitine acyltransferases and acyl-CoA hydrolases are two important groups of enzymes involved in the metabolism of fatty acids. In the rat and in other mammals, carnitine acyltransferases have a multiple subcellular localization, with different distribution patterns, depending on the substrate used for the assay. The activity is present in mitochondria, microsomal fractions and peroxisomes (Markwell et al., 1973, 1976; Kahonen, 1976; Martin et al., 1979; Bieber et al., 1981). Administration of clofibrate and other hypolipidaemic drugs that produce a marked proliferation of peroxisomes (Hess et al., 1965; Leighton et al., 1975; Lazarow, 1977) increases the activities of the transferases in mitochondria, microsomal fractions and peroxisomes (Kahonen, 1976; Markwell et al., 1977).

The role of carnitine acyltransferases in acylgroup transfer across the mitochondrial inner membrane has been well established [see Hoppel (1982) for a review]. In contrast, the function of these enzymes in microsomal fractions and peroxisomes is not yet known. For peroxisomes it has been suggested that short- and medium-chain acyltransferases function in the transport of acyl residues across the peroxisomal membrane (Tolbert, 1981; Leighton et al., 1982). These acyl residues would be the products of the peroxisomal β-oxidation system known to exist in rat and in human liver (Lazarow & de Duve, 1976; Bronfman et al., 1979).

Short- and long-chain acyl-CoA hydrolases are present in various mammalian tissues (Berson, 1976; Berge & Farstad, 1979; Berge et al., 1980; Hsu & Claghorn, 1981; Andersen & Berge, 1982). Long-chain acyl-CoA hydrolase has been localized mainly in microsomal fractions and mitochondria, both in the rat and in human liver (Berge & Farstad, 1979; Berge et al., 1980).

The physiological function of acyl-CoA hydrolases is not known. However, the complex regulatory properties described for acetyl-CoA hydrolase from rat liver (Prass et al., 1980), together with the fact that long-chain acyl-CoA hydrolase activity is...
increased in rat liver after treatment with peroxiso-
mal proliferators (Kawashima et al., 1982), suggest
a regulatory role for these enzyme activities.

The known differences between enzyme local-
ization in rat and human liver, particularly with
peroxisomal enzymes (Hagen et al., 1979; de Duve
& Baudhuin, 1966; Noguchi & Takada, 1978,
1979), and the fact that little is known about
the subcellular localization of carnitine acyl-
transferases and acyl-CoA hydrolases in human
liver, prompted us to investigate quantitatively
their subcellular localization. Different substrates
were employed, namely saturated acyl-CoA
derivatives (C2-C16 chain length) and oleoyl-CoA.

Experimental

Liver biopsies were obtained from patients
undergoing surgery for uncomplicated gall-stone or
gastroduodenal-ulcer disease. Informed
consent from the patients was obtained by following the
procedures approved by the Ethics Committee
of the Medical School of the Universidad Catolica
de Chile. Preparation of homogenates and fractiona-
tion on one-step isopycnic gradients of total homogenates was carried out as described in the
preceding paper (Bronfman et al., 1984). Fractiona-
tion by differential centrifugation into nuclear
(N), heavy mitochondria (M), light mitochondria
(L), microsomal (P) and supernatant (S) fractions
was performed as described by de Duve et al.
(1955).

Marker enzymes for peroxisomes (catalase),
mitochondria (glutamate dehydrogenase), micro-
somal fractions (NADPH :cytochrome c reduc-
tase), lysosomes (acid phosphatase) and the soluble
compartment (phosphoglucomutase), as well as
protein, were measured as described by Bronfman
et al. (1984).

Carnitine acyltransferases were assayed as de-
scribed by Leighton et al. (1982). The same
conditions were employed for the assay of acyl-
CoA hydrolase activities, except for the omission of
carnitine from the incubation mixture.

The presentation of enzyme distributions in
metrizamide gradients in the form of normalized
histograms, and the quantitative evaluation of
their subcellular localization by constrained linear
regression, have been described previously (Bronf-

Rat liver homogenates were prepared from fed
male or female Sprague-Dawley rats (200–240 g
body wt.). They were killed by decapitation and
the livers were homogenized (25%, w/v) in cold
0.25M-sucrose with a Teflon-pestle/glass-vessel
homogenizer. Substrates, coenzymes and chemi-
cals were from Sigma Chemical Co., St. Louis,
MO, U.S.A.

Results

Absolute activity values and specificity pattern of
human liver carnitine acyltransferases and acyl-CoA
hydrolases

The absolute activity values for human liver
carnitine acyltransferase and acyl-CoA hydrolase,
determined with saturated acyl-CoA derivatives
and with oleoyl-CoA, are presented in Fig. 1. The
absolute activity values of marker enzymes and
protein are given in the preceding paper (Bronf-
man et al., 1984), pooled together with the data
reported there.

Human liver homogenates contain high carnitine
acyltransferase activity for short-chain acyl
residues, with a maximum at C4. The transferase
activity decreases as the substrate length increases,
but the converse is observed for acyl-CoA hydrol-
ase, which presents the highest activity with
palmitoyl-CoA as substrate. Relatively low activ-
ity towards oleoyl-CoA is detected. For this sub-
strate, as well as for lauroyl- and palmitoyl-CoA,
similar activities are observed for the transferase
and the hydrolase, whereas for the short-chain acyl
derivatives acetyl- and butyryl-CoA the transfera-
se activity is 5–6 times the hydrolase activity.

Very different results have been reported for rat

![Fig. 1. Human liver carnitine acyltransferase and acyl-CoA hydrolase activities](image)

Activities were measured in human liver homog-
genates with straight-chain saturated fatty acyl-
CoA (C2–C16) and oleoyl-CoA (C18:1) as substrates.
Results are means ± s.d. The numbers of inde-
pendent experiments are shown in parentheses.
Key: □, carnitine acyltransferases; ■, acyl-CoA
hydrolases.

1984
liver in carnitine acyltransferase specificity and for the relative proportions of hydrolase activity with different substrates. Carnitine acyltransferase from rat liver is less active with acetyl-CoA than with octanoyl-CoA (Markwell et al., 1977). Moreover, the transferase activity with acetyl-CoA as substrate has been reported to be 5-fold lower than the hydrolase activity (Bieber et al., 1981).

To clarify the point, and for comparative purposes, the specificity patterns of carnitine acyltransferase and acyl-CoA hydrolase from rat liver were determined under the same experimental conditions and with the same saturated substrates as for human liver, except for oleoyl-CoA. Results are shown in Fig. 2.

For all the acyl-CoA substrates used, except butyryl-CoA, the acyl-CoA hydrolase activity, on a liver-wet-weight basis, is greater in rat than in human liver. A more striking difference is, however, found for the specificity pattern of human and rat liver carnitine acyltransferase. Whereas human liver has very high activities of carnitine acetyl- and butyryl-transferase and a low activity of carnitine lauroyltransferase, rat liver shows the highest activity for carnitine lauroyl-

![Graph](image)

**Fig. 2. Rat liver carnitine acyltransferase and acyl-CoA hydrolase activities**

Different enzyme activities were measured with straight-chain saturated fatty acyl-CoA as substrates, under the same conditions as in the experiments reported in Fig. 1 for human liver. Results are means ± s.d. of three independent experiments, performed with male rats. Similar results were obtained with female rats. Key: □, carnitine acyltransferases; ■, acyl-CoA hydrolases.

Carnitine acyltransferases and acyl-CoA hydrolases: isopycnic fractionation of human liver homogenates

The density distribution of marker enzymes, of carnitine acyltransferases and of acyl-CoA hydrolases after isopycnic fractionation of total human liver homogenates in metrizamide density gradients is shown in Fig. 3.

As reported in the preceding paper (Bronfman et al., 1984), about 70% of catalase, the peroxisomal marker, remains in the low-density fractions and corresponds to soluble activity. Particulate catalase, equilibrated at 1.20–1.21 g/cm³, is well resolved from the microsomal, mitochondrial and lysosomal markers.

Carnitine acetyl- and butyryl-transferases present a clear bimodal distribution, with a peak that follows that of particulate catalase and the remaining activity in the low-density region of the gradients. The profile of these two enzymes suggests a peroxisomal–mitochondrial localization rather than a peroxisomal—microsomal one. However, the poor resolution between glutamate dehydrogenase and NADPH:cytochrome c reductase does not allow a definite conclusion by simple inspection of the graph.

The density distribution of medium- and long-chain transferases is similar to that of carnitine acetyl- and butyryl-transferases. However, the amount of activity that can be attributed to peroxisomes is much lower than for the short-chain transferases. For carnitine oleoyltransferase, a significant proportion of the activity appears associated with the soluble compartment, judged from the behaviour of phosphoglucomutase.

Most of the activity of acetyl-CoA hydrolase and palmitoyl-CoA hydrolase is found in the low-density fractions. Because of the low resolution in that region of the gradient, it is not possible to visualize the subcellular localization of the enzymes. However, two conclusions can be drawn from the distribution of the hydrolyses. The distribution pattern of acetyl-CoA hydrolase appears different from that of palmitoyl-CoA hydrolase, which has a higher median density. In addition, there is no evidence for a peroxisomal contribution to the hydrolase activities. In contrast, Osmundsen et al. (1980) found that rat liver peroxisomes contain palmitoyl-CoA hydrolase activity, as well as hydrolytic activity towards other acyl-CoA esters.

To estimate quantitatively the fraction of the carnitine acyltransferase and acyl-CoA hydrolase activities present in each subcellular compartment,
the data presented in Fig. 3 were analysed by constrained linear regression (Bronfman et al., 1984) by using marker enzymes as references. The results of the computations are shown in Table 1.

The main proportion of each transferase activity is assigned to mitochondria. The remaining activity is attributed to peroxisomes, except for carnitine octanoyltransferase, of which a significant fraction of the activity is also assigned to microsomal fractions, and for carnitine oleoyltransferase, of which the soluble compartment would contribute almost 20% to the total liver activity. The assignment of no soluble activity to some of the transferases does not mean that they do not have detectable activity in the soluble fractions. The computational method employed uses as reference of peroxisomal distribution the total distribution of catalase, including the soluble and particulate components. As shown below by differential-centrifugation experiments, all the transferases have detectable soluble activity, but this activity is accounted for by the soluble component of catalase. That is to say, the entire soluble fraction can be accounted for by peroxisomes that, apparently,
release part of their content into the soluble fraction. In contrast, for carnitine oleoyltransferase, there is more soluble activity than that justified by the soluble component of catalase.

For all the transferases, a significant proportion of the activity is assigned to peroxisomes, with a maximum for carnitine butyryltransferase.

The multiple localization of carnitine octanoyltransferase in peroxisomes, mitochondria and microsomal fractions, suggested by the computations of Table 1, is in agreement with results from Markwell et al. (1973, 1977) and Valkner & Bieber (1982) in rat liver. However, the last authors also found that carnitine acetyltransferase, in addition to a mitochondrial–peroxisomal localization, is also present in microsomal fractions, but in human liver this appears not to be the case. Moreover, in rat liver, carnitine palmitoyltransferase has little or no demonstrable activity in peroxisomes (Markwell et al., 1977; Leighton et al., 1982), whereas results presented here suggest that the human liver enzyme has a peroxisomal–mitochondrial localization.

For the acyl-CoA hydrolases, the computations by constrained linear regression suggest a soluble–mitochondrial localization for acetyl-CoA hydrolase, whereas palmitoyl-CoA hydrolase is assigned to the soluble compartment, to microsomal fractions and to lysosomes. No activity was assigned to the peroxisomal marker, catalase.

To verify these results, we studied the subcellular distribution of carnitine acyltransferase and acyl-CoA hydrolases by differential centrifugation and, for the carnitine acyltransferases, also by measuring their activity in peroxisomal fractions purified by density-gradient subfractionation of the L fraction.

Subcellular distribution of carnitine acyltransferases: differential centrifugation and density-gradient subfractionation of L fraction

The results of one experiment of human liver fractionation by differential centrifugation is shown in Fig. 4. In agreement with the computations above, most of the transferase activities are present in the mitochondrial M fraction. For the transferase acting on octanoyl-CoA, the higher relative specific activity in the microsomal P fraction and the lower activity in the mitochondrial M fraction, compared with the other transferases, is consistent with the presence of a fraction of the enzyme located in microsomal vesicles, as already suggested by the computations shown in Fig. 1.

Since the peroxisomal contribution to an enzyme activity is difficult to assess by differential centrifugation when this contribution is low, the L fraction was further subfractionated by metrizamide-density-gradient centrifugation. Results are shown in Fig. 5.

A bimodal distribution is observed for all the transferases, with a clear peak in the peroxisomal region. The remaining activity equilibrates in the lower-density fractions, which contain most of the mitochondrial marker enzyme.

The relative amount of transferase activities that can be attributed to peroxisomes in the L fraction, on the basis of this experiment, ranges from 17 (palmitoyltransferase) to 68 (butyryltransferase)%. These values correlate well, within experimental error, with the data presented above for total homogenate fractionation (Table 1), if they are adjusted to account for the fact that only a fraction of the peroxisomes from the homogenate was
Fig. 4. Subcellular fractionation of human liver by differential centrifugation: distribution of carnitine acetyl- (C2), octanoyl- (C8) and palmitoyl- (C16) transferases

For each distribution pattern, the abscissa represents the protein content of the fraction as a percentage of the total protein of the liver. The ordinate represents the percentage, in the fraction, of the liver content of the marker enzyme divided by the percentage of liver protein in that fraction. Recoveries were 112, 81 and 94% respectively for acetyl-, octanoyl- and palmitoyl-transferase.

present in the L fraction loaded on the gradient (10–15%, on the basis of catalase).

Because of the small amount of mitochondria and microsomal vesicles in the L fraction (as assessed from their marker enzymes in this fraction: 4 and 3% for NADPH:cytochrome c reductase and glutamate dehydrogenase respectively), it was not possible to verify the microsomal contribution to carnitine octanoyltransferase suggested above by the experiments with total homogenates (Table 1).

Subcellular distribution of acyl-CoA hydrolases: differential centrifugation

The subcellular distributions of acyl-CoA hydrolases were further investigated in one experiment by
Carnitine acyltransferases and acyl-CoA hydrolases

Subfractionation of an L fraction in metrizamide density gradient: distribution of carnitine acyltransferases and marker enzymes

The transferases are indicated by the carbon-chain length of the substrate: carnitine oleoyltransferase is indicated as $C_{18:1}$. Recoveries ranged from 84 to 122%.

The change in the subcellular localization of the hydrolases depending on the chain length of the substrate is in agreement with observations in guinea-pig small intestine (Andersen & Berge, 1982). In this tissue, decanoyl-CoA hydrolase has a higher activity in the mitochondrial fraction, whereas palmitoyl-CoA hydrolase is mainly present in the microsomal fraction. The mitochondrial localization of acetyl-CoA hydrolase has also been observed in sheep and rat tissues (Knowles et al., 1974).

differential centrifugation. Results are presented in Fig. 6. In agreement with the previous computations shown in Table 1, acetyl-CoA hydrolase presents a dual localization, in the mitochondrial and in the soluble fraction. A similar distribution is presented by butyryl- and octanoyl-transferase. This distribution markedly changes with increasing length of the acyl-CoA chain of the substrate. For lauroyl-, palmitoyl- and oleoyl-CoA activity is present mainly in the microsomal and soluble fractions.
Fig. 6. Subcellular fractionation of human liver by differential centrifugation: distribution of acyl-CoA hydrolase activities
The different activity profiles are identified by the carbon-chain length of the substrate: oleoyl-CoA hydrolase is indicated as C\textsubscript{18:1}. Recoveries ranged from 84 to 124%. The presentation is the same as in Fig. 4.

Discussion

Computations

The use of constrained linear regression allows determination of the subcellular distribution of enzymes directly from the results of total-homogenate fractionation in metrizamide gradients. The reliability of the method was confirmed for the analysis of the subcellular distribution of both carnitine acyltransferases and acyl-CoA hydrolases. For the transferases, the suggested mainly mitochondrial localization was confirmed by differential centrifugation. Moreover, the peroxisomal contribution to the transferase activities that can be calculated from the isopycnic-gradient-subfractionation experiment of an L fraction are within the statistical limits of the peroxisomal contributions calculated by constrained-linear-regression analysis of fractionation of total homogenates.
Carnitine acyltransferases and acyl-CoA hydrolases

For the acyl-CoA hydrolases, in spite of the poor resolution of mitochondria, microsomal vesicles, lysosomes and the cell sap, in the isopycnic gradients, the constrained linear-regression method succeeded in differentiating a mitochondrial-soluble distribution (acytely-CoA hydrolase) from a microsomal-soluble one (palmitoyl-CoA hydrolase), as confirmed by differential centrifugation. In fact, the success of the method depends more on the number of experiments analysed, and on the number of fractions in each experiment, than on the resolution of the gradient (M. Bronfman & E. Feytmans, unpublished work).

In two cases we do not provide experimental confirmation for a subcellular localization calculated by constrained linear regression. The first is the microsomal contribution to carnitine octanoyltransferase, and the second is the proposed lysosomal contribution to a long-chain acyl-CoA hydrolase activity. However, in this and in the preceding paper (Bronfman et al., 1984), each time that a constrained-linear-regression analysis of fractionation of total homogenates was verified by either differential centrifugation or isopycnic-gradient fractionation of purified subcellular fractions, the method was found to be correct. In all cases, the observed contributions were consistent with the values obtained by constrained linear regression.

Further fractionation of P and L fractions by isopycnic gradient would be needed to confirm the microsomal contribution to carnitine octanoyltransferase and the lysosomal contribution to palmitoyl-CoA hydrolase. Because of restrictions in the availability of liver tissue, these experiments were not done.

Subcellular distribution of carnitine acyltransferases and acyl-CoA hydrolases

The results presented here demonstrate that human liver contains, both in mitochondria and in peroxisomes, carnitine acyltransferase with a broad substrate specificity.

In contrast with rat liver, in which carnitine palmitoyltransferase is localized in mitochondria and is absent, or almost so, from peroxisomes (Markwell et al., 1977; Leighton et al., 1982), human liver peroxisomes contain a significant proportion of transferase acting on palmitoyl- and oleoyl-CoA. If fatty acids enter the peroxisome as carnitine derivatives, as we have previously proposed on the basis of the existence of a CoA pool in the rat liver peroxisome and also on the assumption that the peroxisomal membrane is impermeable to CoA and acyl-CoA and permeable to carnitine and its derivatives (Leighton et al., 1982), human liver peroxisomes would contain a higher capacity than the rat liver organelle for long-chain acyl-CoA generation inside the peroxisome, and, in consequence, for β-oxidation.

It has also been proposed that a carrier might exist in the peroxisomal membrane allowing the direct entry of acyl-CoA species into peroxisomes (Mannaerts et al., 1982). This hypothesis is based on experimental evidence suggesting that, in rat liver, peroxisomal long-chain acyl-CoA synthetase is localized at the cytoplasmic side of the peroxisomal membrane. If this were the case, human liver peroxisomes contain also a higher proportion of long-chain acyl-CoA synthetase compared with the rat (Bronfman et al., 1984).

Moreover, the amounts of peroxisomal acetyl-, butyryl- and octanoyl-transferases that can be estimated from our data are 2.5–4-fold higher, on a wet-weight-of-liver basis, than those of rat liver peroxisomes as estimated from our data and from results of Markwell et al. (1973) and Bieber et al. (1981). Short- and medium-chain transferases are believed to shuttle acyl residues out of the peroxisomes as acylcarnitines (Tolbert, 1981; Leighton et al., 1982), since fatty acids are not completely converted into acetyl-CoA by peroxisomal β-oxidation. On this basis, human liver peroxisomes would contain a higher capacity than the rat liver organelle, both for input of substrates of β-oxidation and for output of β-oxidation products.

No activity of carnitine acyltransferase was found in human liver microsomal fractions, in contrast with the rat (Markwell et al., 1977). Only for carnitine octanoyltransferase do we present evidence for a microsomal localization. Its function is unknown, but it could provide a mechanism to convert peroxisomally generated octanoylcarnitine into octanoyl-CoA, which could be elongated or modified, as proposed by Valkner & Bieber (1982), since in rat liver the transferase is associated with the cytosolic face of the endoplasmic reticulum.

The acyl-CoA hydrolase activity of human liver is low compared with rat liver. Its subcellular distribution depends on the chain length of the substrate, but in all cases 30–40% of the activity is soluble. Particulate acetyl-CoA hydrolase is almost exclusively mitochondrial, whereas microsomal fractions contain larger amounts of palmitoyl- and oleoyl-CoA hydrolase. The rather sharp change from a mitochondrial to a microsomal distribution, when the substrate chain length increases from 8 to 12 carbon atoms, suggests the presence of at least two particulate hydrolases in human liver: one acting on short-chain substrates and localized in mitochondria, and the other a long-chain hydrolase present in microsomal fractions. A second particulate long-chain hydrolase in lysosomes is suggested by the computational analysis of the results. The peroxisomal localization previously
reported for rat liver acyl-CoA hydrolase (Osmundsen et al., 1980) is not confirmed for human liver. Whether the soluble hydrolase activities represent one or more different enzymes is unknown. In the rat, two soluble long-chain acyl-CoA hydrolases, inducible by clofibrate, have been reported (Kawashima et al., 1982). In the hepatic cytosol of the guinea pig, no hydrolase was induced under the same conditions (Kawashima et al., 1983). A soluble acetyl-CoA hydrolase, cold-labile and modulated by ATP and ADP, has been found in rat liver (Prass et al., 1980). Although our homogenates were not assayed for acetyl-CoA hydrolase activity immediately after homogenization, and a quantitative analysis of cold-inactivation was not performed, we did not observe any decay in the hydrolase activity when maintained at 0–4°C. Moreover, recoveries from both density gradients and differential centrifugation were good. This observation suggests that this enzyme does not exist or has low activity in human liver, confirming previous reports that limit the enzyme to rodents (Prass et al., 1980). If this is so, this potential regulatory mechanism for the concentration of cytosolic acetyl-CoA would not exist in human liver.

Heterogeneous and similar complex subcellular distributions have been found for acyl-CoA hydrolase activities from different tissues and mammals (see Andersen & Berge, 1982). To our knowledge, this is the first report of their specificity and subcellular localization with a broad spectrum of chain-length substrates.

Our results stress the importance of assaying and determining the subcellular localization of enzymes in human tissues rather than to assume their presence and amount in specific subcellular compartments on the basis of investigations in other mammals. It is possible that the differences detected at the level of the liver activity and subcellular localization of enzymes in human and other mammals, in this and other reports, reflect a different specificity pattern for fatty acid oxidation as well as different regulatory mechanisms.

This work was supported by the Fondo de Investigaciones de la Universidad Católica de Chile. The skilful participation of Cecilia Necochea and Maria Nelly Morales is gratefully acknowledged. We thank Dr. Flavio O. Nervi for the clinical monitoring of the human volunteers.

References

M. Bronfman and F. Leighton

1984