Evidence that carnitine palmitoyltransferase I (CPT I) is expressed in microsomes and peroxisomes of rat liver

Distinct immunoreactivity of the N-terminal domain of the microsomal protein

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Abstract Mitochondria, microsomes and peroxisomes all express overt (cytosol-facing) carnitine palmitoyltransferase activity that is inhibitable by malonyl-CoA. The overt carnitine palmitoyltransferase activity (CPTo) associated with the different fractions was measured. Mitochondria accounted for 65% of total cellular CPTo activity, with the microsomal and peroxisomal contributions accounting for the remaining 25% and 10%, respectively. In parallel experiments, rat livers were perfused in situ with medium containing dinitrophenyl (DNP)-etomoxir in order to inhibit quantitatively and label covalently (with DNP-etomoxiryl-CoA) the molecular species responsible for CPTo activity in each of the membrane systems under near-physiological conditions. In all three membrane fractions, a single protein with an identical molecular mass of approximately 88 000 kDa (p88) was labelled after DNP-etomoxir perfusion of the liver. The abundance of labelled p88 was quantitatively related to the respective specific activities of CPTo in each fraction. On Western blots the same protein was immunoreactive with three anti-peptide antibodies raised against linear epitopes of the cytosolic N- and C-domains and of the inter-membrane space loop (L) domain of the mitochondrial enzyme (L-CPT I). However, the reaction of the microsomal protein with the anti-N peptide antibody (raised against epitope Val-14–Lys-29 of CPT I) was an order of magnitude stronger than expected from either microsomal CPTo activity or its DNP-etomoxiryl-CoA labelling. This suggests that the N-terminal domain of the microsomal protein differs from that in the mitochondrial or peroxisomal protein. This conclusion was confirmed using antibody back-titration experiments, in which the binding of anti-N and anti-C antibodies by mitochondria and microsomes was quantified.

Key words: Carnitine palmitoyltransferase; Mitochondrion; Peroxisome; Microsome

1. Introduction

Long-chain fatty acids are converted to their acyl-coenzyme A (CoA) esters within the cytosol. Delivery of the acyl moieties to the mitochondrial matrix and endoplasmic reticular or peroxisomal lumen necessitates conversion into the corresponding acylcarnitines [1] by overt carnitine palmitoyltransferases (hereafter referred to generically as CPTo). Re-formation of acyl-CoA esters within the internal compartments of these membrane systems (after transport of the acylcarnitines through the respective membranes) is performed by a family of latent CPTs [2,3]. All the CPTo activities of the different cell membranes are inhibited by malonyl-CoA [4–6] in contrast to the latent CPT isoforms. Only the protein responsible for the activity of the mitochondrial outer membrane (L-CPT I) is well-characterised [7–9]. Results published in abstract form only [10] have indicated that a peroxisomal protein of M_r 88 000 resembling L-CPT I may be responsible for the CPTo activity of these organelles, but these authors suggested that it is immunologically distinct from L-CPT I. A microsomal protein of M_r 47 000 has previously been postulated to represent the microsomal CPTo on the basis that, when microsomes are incubated with [^{3}H]-etomoxir, CoA and ATP, a protein of this molecular size was labelled most prominently [11]. Similar observations were made by [12] who, however, also observed that under these in vitro conditions at least one other protein of M_r 87 000 was also prominently labelled. More recently, it has been confirmed that microsomes express a malonyl-CoA-sensitive overt CPT which has an apparent molecular size of 300 kDa in detergent extracts of the membranes [13].

In the present study we have measured CPTo activity in mitochondrial, microsomal, peroxisomal and high-speed supernatant fractions prepared from rat liver on a quantitative basis. We have also quantified the relative expression of proteins that bind dinitrophenyl (DNP)-etomoxiryl-CoA (a 2-oxirane carboxylate irreversible inhibitor of hepatic CPT I [14]), in each of these fractions in the intact isolated liver perfused with DNP-etomoxir.

2. Materials and methods

2.1. Animals

These were female Wistar rats (200–230 g) maintained on a laboratory chow diet [15] and starved for 24 h prior to being used, 2 h into the light phase of a 12 h light-12 h dark regime.

2.2. Preparation of subcellular fractions

Livers were homogenised in an ice-cold medium containing 250 mM sucrose, 10 mM Tris, 1 mM EGTA (pH 7.4) and a cocktail of protease inhibitors (Complete, Boehringer) designed to inhibit a wide spectrum of mammalian cell proteases. Two protocols were used depending on whether quantitative recovery of the fractions was required (protocol 1) or whether highly pure but non-quantitative preparations of mitochondria and microsomes were required (protocol 2). In protocol 1, after low-speed centrifugation of the homogenate ($500\times g$ for 10 min) to remove debris, the supernatant was centrifuged for 10 min at $5800\times g$ to obtain a mitochondrial pellet. Preliminary experiments indicated that, at a centrifugal force of $5800\times g$, $>95\%$ of mitochondria were sedimented, but that $<8\%$ of microsomes were lost from the supernatant. Microsomal pellets were obtained from the $5800\times g$ supernatants by centrifugation at $100\,000\times g$ for 60 min. As expected, most of the peroxisomes ($>90\%$) were sedimented with the crude mitochondrial pellet. Therefore, routinely, purified peroxisomes were prepared from this mitochondrial fraction by gradient centrifu-

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Table 1
Quantification of CPTo activity (measured at 37°C) and total protein (per g wet weight of liver) in the different subcellular fractions obtained from 24 h starved rat livers using protocol 1 (Section 2)

Fraction	Mitochondria	Microsomes	Peroxisomes	Soluble
CPTo activity (μmol/min)	0.40 ± 0.06	0.15 ± 0.04	0.07 ± 0.03	< 0.01
Total protein (mg)	25.1 ± 2.5	22.8 ± 1.9	2.15 ± 1.0	49.8 ± 3.1

Values are means (± S.E.M.) for three liver fractionations, and have been corrected for cross-contamination of fractions as determined by marker enzyme activities.

gation on a Nycodenz gradient, as described previously [16]. Samples of all the fractions were retained for measurement of marker enzyme activities in order to quantify the recovery of the individual membrane populations from the liver and assess cross-contamination.

Protocol 2 was designed to obtain non-quantitative recoveries of highly pure mitochondria and microsomes, in addition to Nycodenz-purified peroxisomes. A crude mitochondrial pellet was obtained by centrifugation of the $500\times g$ supernatant at $12\,000\times g$ for 20 min. One-half of this mitochondrial fraction was subjected to centrifugation on a 33% Percoll self-forming gradient to obtain highly pure mitochondria, as described previously [17,18]. The other half was used to prepare Nycodenz-purified peroxisomes, as described above. Pure microsomes were obtained by centrifugation of the $12\,000\times g$ supernatants at $100\,000\times g$ for 60 min.

2.3. Measurement of enzyme activities

The following enzyme activities were measured as described previously [8,18,19]: cytochrome *c* oxidase (mitochondria), NADPH-cytochrome *c* oxidoreductase (microsomes), uricase (peroxisomes) and lactate dehydrogenase (cytosol). CPTo activities were measured using either an optimal concentration of palmitoyl-CoA (135 μM in the presence of 1% albumin) to obtain maximal activities, or 35 μM palmitoyl-CoA in the presence or absence of 100 μM malonyl-CoA, as described previously [15].

2.4. Identification of proteins that bind DNP-etomoxiryl-CoA in intact rat liver

Quantitative labelling of DNP-etomoxiryl-CoA-binding proteins was performed in the intact liver prior to preparation of the various subcellular fractions. Animals were anaesthetised with pentobarbitone (60 mg/kg) and their livers (weighing 6–8 g) were perfused in situ for 30 min with 250 ml of oxygenated (95% O_2:5% CO_2) recirculating Krebs-Henseleit medium [20] containing 2.5 μmol DNP-etomoxir, at 37°. At the end of the perfusion, the liver subcellular fractions were prepared using protocol 2, described above. Liver perfusion with DNP-etomoxir (which is converted into the effective inhibitory species DNP-etomoxiryl-CoA within the cell) resulted in > 90% inhibition of overt CPT_O activity in all three membranous fractions.

2.5. Back-titration ELISA determination of antibody binding by intact microsomes and mitochondria

This was performed as described previously [8] except that multi-well plates were treated with 4% bovine albumin in phosphate-buffered saline containing 0.1% Tween-20 for 1 h at 20°C to block non-specific sites before applying the samples containing the primary antibody (anti-N or anti-C). Freshly prepared Percoll gradient-purified intact mitochondria [18] and microsomes sedimented from $12\,000\times g$ supernatants (see protocol 2, above) were used. They were incubated, at the protein concentrations indicated, for 16 h at 0°C with anti-N or anti-C antibodies in a total volume of 220 μl. After sedimentation of the membranes, the amounts of antibodies remaining in solution were quantified by ELISA using donkey anti-sheep secondary antibody to detect the amounts of primary antibody that could be bound by an excess (160 ng) of the respective peptide coated on to the multi-well plates. The total binding capacities of the amounts of antibodies added to the incubations were previously determined to be 50 pmol and 80 pmol of peptides N and C, respectively. In preliminary experiments these amounts of antibodies were shown to reside on the linear portions of their respective titre curves. Binding of the antibodies to mitochondria or microsomes could be specifically and completely displaced by the respective peptides against which they were raised.

2.6. Preparation of samples for SDS-gel electrophoresis and Western blotting

The proteins in aliquots of subcellular fractions were precipitated with a ten-fold excess of ice-cold acetone, and the pellets washed with cold 90% acetone. The final pellets were solubilised in a medium containing 62 mM Tris-HCl, 69 mM SDS, 8 M urea, 6 mM dithiothreitol (pH 6.8). The component proteins were separated on 8% polyacrylamide gels and electrotransferred onto nitrocellulose. The resulting blots were probed with rabbit anti-DNP and sheep anti-CPT I peptide antibodies raised against linear epitopes N, L and C of L-CPT I (described in [8]). Appropriate secondary antibodies (anti-sheep or anti-rabbit IgG) conjugated to alkaline phosphatase were used and visualised by using 5-bromo-1-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT) as substrate and chromophore, respectively. The intensity of the resulting bands was quantified densitometrically (Imagequant, Molecular Dynamics).

2.7. Materials

Nycodenz and Percoll centrifugation media and general biochemicals were from Sigma-Aldrich Ltd (Poole, UK). Complete protease inhibitor tablets were from Boehringer Mannheim (Lewes, UK). BCIP and NBT were from Promega UK (Southampton, UK). DNP-etomoxir was from Projekt-Entwicklung GmbH (Allensbach, Germany). Rabbit anti-DNP IgG was from Molecular Probes (Cambridge Bioscience, UK).

3. Results

Mitochondria and microsomes accounted for 65% and 25% of total hepatic CPTo activity, respectively. These estimates are similar to those made by [21] after quantitative prepara-

Table 2
Specific activities of CPTo and marker enzymes, and of the expression of DNP-etomoxiryl-CoA-binding protein (88 kDa) in subcellular fractions prepared from liver of rats starved for 24 h using protocol 2 (Section 2)

Fraction	Mitochondria	Microsomes	Peroxisomes	Soluble
CPTo activity (nmol/min)	18.6 ± 4.9	8.1 ± 3.3	32.5 ± 2.7	< 0.01
DNP-etomoxir binding (arbitrary units)	1.0	0.45 ± 0.07	1.34 ± 0.30	< 0.01
Cytochrome *c* oxidase (μmol/min)	2.1 ± 0.3	0.2 ± 0.1	0.3 ± 0.1	< 0.01
NADPH-cytochrome *c* oxidoreductase (nmol/min)	10.1 ± 1.1	100.1 ± 9.0	8.9 ± 2.3	< 0.01
Uricase (nmol/min)	20.8 ± 3.8	16.3 ± 3.3	247.5 ± 9.0	< 0.01

Enzyme activities were measured at 37°C. For enzyme activities, values (for four separate fractionations) are expressed per mg protein of the relevant fraction. Expression of DNP-etomoxiryl-CoA- binding protein was measured by densitometric analysis of Western blots (probed with anti-DNP antibody) prepared after SDS-polyacrylamide electrophoresis of aliquots (100 μg protein) of the three fractions. The DNP-etomoxiryl-CoA binding to p88 derived from the mitochondrial fraction has been arbitrarily set at 1.0 and binding to the protein within purified microsomal and peroxisomal fractions isolated from the same liver are expressed relative to this value.

tion of these three membrane fractions from starved rat liver. No CPT activity could be detected in the high-speed supernatant fraction Table 1. Specific activities of CPTo (corrected for cross-contamination) and marker enzyme activities for the different fractions obtained by protocol 2 are given in Table 2. The marker enzyme data show that the fractions were substantially pure. Therefore, the CPTo and Western blot data presented below could not have been affected by any major cross-contamination. The highest CPTo specific activity (expressed per mg protein) was observed in the peroxisomal fraction, whereas microsomes showed the lowest specific activity (Table 2).

3.1. DNP-etomoxiryl-CoA labelling of cell proteins

In order to identify and quantify the relative amounts of CPTo proteins expressed in each fraction, we labelled them in the intact liver in situ prior to the quantitative preparation of subcellular fractions using protocol 2. This ensured that in vitro artefacts due to non-specific binding of 2-oxirane carboxylates, when incubated in large molar excess over the amount of CPTo in isolated microsomes, were avoided. Using the DNP-etomoxir-perfused liver we were able to optimise the specific labelling of p88 in all the three cell fractions prior to homogenisation of the liver under identical conditions to those used in parallel experiments in which CPTo activity was quantified. A minimal quantity of DNP-etomoxir, sufficient to inhibit 90–95% of the activities in all three membrane fractions was used. Preliminary experiments established that recirculating perfusion of rat liver (6–8 g) with 250 ml of medium containing 2.5 μmol of DNP-etomoxir for 30 min at 37°C was sufficient to achieve this.

DNP-etomoxiryl-CoA bound to proteins in each fraction was quantified by SDS-polyacrylamide gel electrophoresis fol-

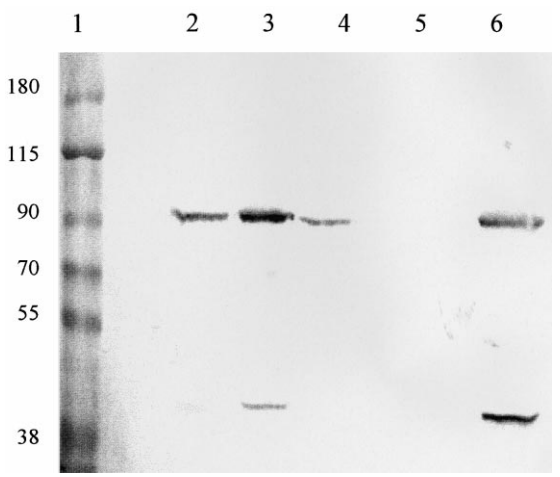

Fig. 1. Detection of DNP-etomoxiryl-CoA-labelled p88 in mitochondria, microsomes and peroxisomes prepared from rat livers previously perfused with DNP-etomoxir. Subcellular fractions were prepared from DNP-etomoxir perfused isolated rat livers, using protocol 2 Section 2. Lane 1: molecular weight markers; 2: $500\times g$ supernatant; 3: mitochondria; 4: microsomes; 5: $100\,000\times g$ supernatant; and 6: peroxisomes. In the experiment shown, a second peroxisomal protein of $M_r \sim 47\,000$ was also labelled. However, labelling of this smaller protein was not always observed and it was not proportional to peroxisomal CPTo activity. In addition, none of the three anti-peptide antibodies detected a protein of this size on immunoblots. When detected, it was also present in the mitochondrial fraction due to minor contamination by peroxisomes (see Table 2).

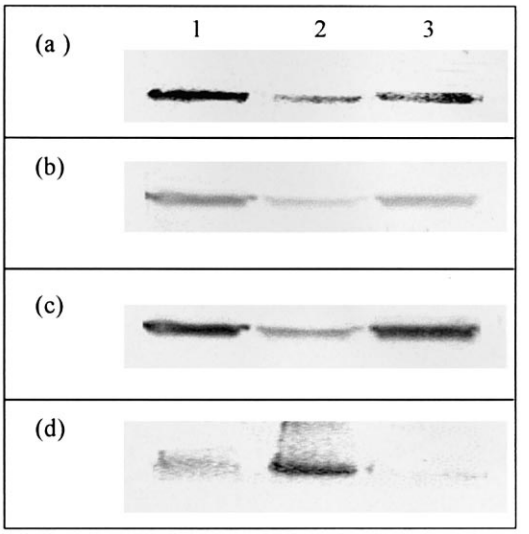

Fig. 2. Immunoreactivity of CPTo in (1) mitochondria, (2) microsomes and (3) peroxisomes with three anti-peptide antibodies raised against linear epitopes of liver mitochondrial CPT I, compared to DNP-etomoxiryl-CoA binding to p88 derived from the same fractions. Subcellular fractions were prepared from DNP-etomoxir-perfused livers as described in the text and 100 μg protein of each was subjected to SDS-PAGE, followed by probing with (a) anti-DNP, (b) anti-L, (c) anti-C, and (d) anti-N peptide antibodies. The Western blots shown are representative of four similar blots on separate preparations.

lowed by electroblotting onto nitrocellulose and probing with anti-DNP antibody. A representative Western blot is shown in Fig. 1. In all three membrane fractions only a protein that migrated with an apparent mass of 88 kDa (p88) was reproducibly labelled by DNP-etomoxiryl-CoA (Fig. 1). A smaller protein (M_r 47 000) was sometimes labelled in peroxisomes but its extent of labelling was very variable (and sometimes absent) and not proportional to peroxisomal CPTo activity. Moreover, by contrast with p88, it was not detected by any of the three anti-peptide antibodies raised against different regions of the mitochondrial CPT I (see below). Consequently, this smaller band was considered to have resulted from non-specific labelling by the highly reactive DNP-etomoxir molecule. Non-specific labelling was much more marked when livers were perfused with higher amounts of DNP-etomoxir.

While for mitochondria and peroxisomes the labelling of the 88 kDa protein was as expected from previous studies [7,10,22,23], the result for microsomes was surprising. This in view of the previous suggestion [11] that CPTo activity in these membranes is associated with a molecular species of M_r 47 000. Evidence that the labelled p88 represents the microsomal overt CPT stems from the observations that (i) the quantitative inhibition of CPTo in all the fractions was achieved under conditions which also labelled this protein quantitatively, and (ii) that the capacity for specific binding of DNP-etomoxiryl-CoA by the different membranous fractions (expressed per mg protein) was positively related to the respective specific activities of CPTo (Table 2). Thus, peroxisomes showed the highest values (per mg protein) for both DNP-etomoxiryl-CoA binding and overt CPTo activity, whereas microsomes had the lowest values for both parameters, with mitochondria having intermediate values.

3.2. Immunological cross-reaction with antibodies raised against linear epitopes of mitochondrial CPT I

Blots of the constituent proteins of the three membrane fractions obtained after separation by SDS-PAGE were probed with the three antibodies (anti-N, -L and -C) raised against different regions of the mitochondrial CPT I protein described previously [8].

All three antibodies detected a protein of identical size ($M_r \sim 88\,000$) in mitochondria, microsomes and peroxisomes (Fig. 2). This suggested that in all three membrane fractions, CPTo activity is associated with the same or highly similar molecular species. However, there was one important difference between the microsomal protein and that in the other two membranous fractions. It can be seen from Fig. 2 that, for equivalent amounts of total membrane protein, reaction of p88 with all the antibodies except anti-N was highest for peroxisomes and lowest for microsomes. By marked contrast, reaction with anti-N antibody was by far the highest for the microsomal protein. Thus, as expected from our previous studies [8] the anti-N antibody gave a very weak reaction with L-CPT I in the mitochondrial fraction. This was also the case for the peroxisomal protein. However, the signal obtained with the microsomal protein was much stronger than would have been anticipated from either the relatively low expression of CPTo activity or of the p88 labelled by DNP-etomoxiryl-CoA. Quantification of the bands was performed by densitometry and the results from four separate experiments are presented in Fig. 3, together with those for the relative CPTo activities in each fraction. For each parameter the specific activity or antibody binding (expressed per mg protein), obtained for the mitochondrial fraction has been set at unity. It can be seen (Fig. 3) that, in all three membrane fractions, whereas the relative intensities of interaction of anti N- and anti-C peptide antibodies were proportional to CPTo activity and expression of the p88 DNP-etomoxiryl-CoA binding protein, the intensity of reaction of the microsomal CPTo with anti-N antibody was an order of magnitude higher than expected from either of these parameters. This suggests

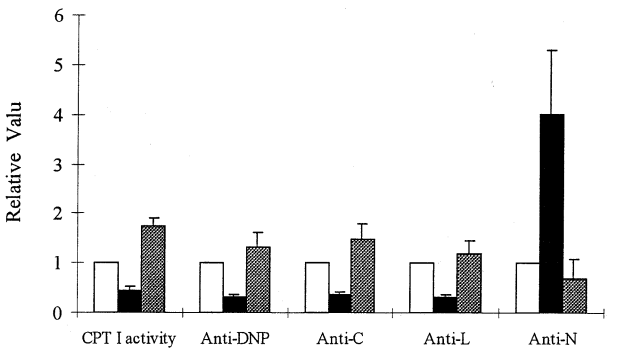

Fig. 3. Quantitative comparison of the immunoreactivity of p88 with anti-CPT I peptide antibodies and the CPTo specific activity and DNP-etomoxiryl-CoA binding in each of the three subcellular fractions. Data for the degree of DNP-etomoxiryl-CoA labelling of p88 obtained as described in Fig. 2 were quantified using densitometry. They are compared with values of the CPTo activity observed in each fraction and the amount of p88 immunoreactive with each of the anti-CPT I peptide antibodies. Expression of the data obtained from four separate experiments was standardised by setting the values of each parameter for the mitochondrial fraction at unity. Those for the other fractions are expressed (means ± S.E.M.) relative to them. Key to bars: mitochondria (empty), microsomes (solid), peroxisomes (stippled).

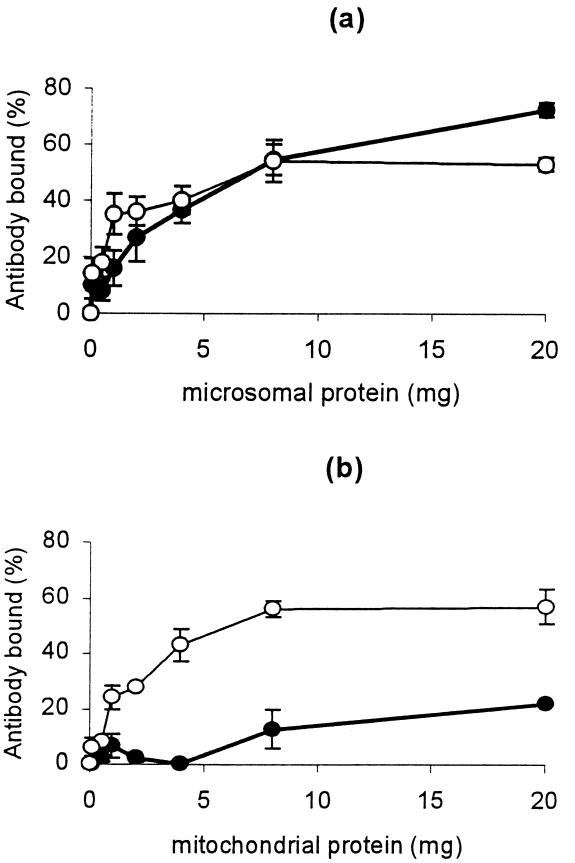

Fig. 4. Binding of anti-N and anti-C antibodies by intact microsomes and rat liver mitochondria. Microsomes (a) or mitochondria (b) were incubated, at the protein concentrations shown, for 16 h at 0°C with anti-N (●) or anti-C (○) antibodies in a total volume of 220 μl. After sedimentation of the membranes, the amounts of antibodies remaining in solution were quantified by ELISA using donkey anti-sheep secondary antibody to detect the amounts of primary antibody that could be bound by an excess (160 ng) of the respective peptide coated on to the multi-well plates. Values are means (± S.E.M.) for three separate determinations. The initial binding capacities of the antibodies in the incubations (100%) were equivalent to 50 pmol and 80 pmol of peptides N and C, respectively. In preliminary experiments these amounts of antibodies were shown to reside on the linear portions of their respective titration curves. Antibody specificity was ascertained by the abolition of binding in the presence of excess peptide (not shown). Values are means (± S.E.M.) for determinations on three separate subcellular fractionations. Where error bars do not show, they lie within the symbols.

that when the proteins are partially refolded on nitrocellulose, the N-epitope (Val-14–Lys-29) of the microsomal CPTo is much more readily available for interaction with the anti-N antibody than that of p88 separated from either the mitochondrial or peroxisomal fractions.

3.3. Binding of anti-N and anti-C antibodies to native CPTo in microsomes and mitochondria

In order to test whether this inference is borne out for the native proteins we performed experiments to determine whether we could detect differential binding of anti-N antibodies by mitochondria and microsomes. After incubation of mitochondria or microsomes with anti-N or anti-C antibodies, the amounts of antibody remaining in solution after sedimentation of the membranes were quantified by back-titration ELISA. As seen in Fig. 4a, microsomal membranes bound

and removed from solution both anti-N and anti-C antibodies efficiently over the same range of membrane protein concentrations. By contrast, and in confirmation of our previous data [8], mitochondria bound anti-C antibody efficiently but reacted very poorly with anti-N (Fig. 4b). These data indicate that even when the mitochondrial and microsomal CPTo proteins are in the native conformations, the N-epitope (Val-14–Lys-29) is much more highly exposed for interaction with the anti-N antibody in the microsomal CPTo than in the mitochondrial protein.

4. Discussion

CPTo activity in microsomes and peroxisomes is accompanied by the presence of only one molecular species (M_r 88 000) that reproducibly binds DNP-etomoxiryl-CoA. Several lines of evidence suggest that this protein is very similar, if not identical, to the mitochondrial outer membrane CPTo, i.e. L-CPT I [7]. Thus (i) their sizes appear to be identical after separation by SDS-polyacrylamide gel electrophoresis, (ii) their relative specific expression in the three membrane fractions varies in parallel with the specific activity of CPTo, and (iii) anti-peptide antibodies raised against three different linear epitopes of mitochondrial CPT I cross-react with the same protein in all three fractions. A similarity between the molecular sizes of mitochondrial and peroxisomal CPTo was observed previously [10]. It is also noteworthy that in previous work in which microsomes were incubated with [^{3}H]etomoxir, ATP and CoA [12] although the most highly labelled protein was of M_r 54 000, very substantial labelling of a protein of M_r 88 000 also occurred. Moreover, contrary to the M_r 54 000 protein, the labelling of the M_r 88 000 species could be attenuated by prior incubation of the microsomes with malonyl-CoA, suggesting that, in spite of its lower degree of labelling under in vitro conditions, this 88 000 Da protein represented microsomal CPTo. However, this possibility was discounted by those authors because of their contention that it was a regulatory subunit of CPTo (a theory which has since been shown to be untenable, at least for mitochondrial CPT I, see [7,24]). In view of the above data, we suggest that CPTo in all three membrane fractions is associated with a protein of approximate $M_r = 88\,000$ and that the current views about the identity of CPTo in mitochondria, microsomes and peroxisomes need to be re-evaluated.

The differential reactivity of the Val-14–Lys-29 epitope with the anti-N antibody was observed both in Western blots and when intact microsomes and mitochondria were used, thus excluding the possibility that differential antibody binding may have resulted from artefacts arising during electrophoretic separation and electrotransfer of the proteins onto nitrocellulose. We conclude, therefore, that the epitope against which the antibody was raised is sterically masked within the N-terminal domain of the mitochondrial (and peroxisomal) CPTo, but not of microsomal CPTo. That the epitope of the mitochondrial protein is fully capable of interacting with the antibody when unmasked was evidenced by our previous observation [8] that the proteolytic cleavage of the extreme N-terminus of the protein markedly increases (by 10-fold) its immunoreactivity with anti-N antibody.

Interaction between the N-terminal and C-terminal domains is crucial in determining the malonyl-CoA sensitivity of mitochondrial CPT I [8,25]. It is plausible that the molecular mechanism that results in the masking of the Lys-14–Val-29 epitope in the mitochondrial protein may be related to the two-fold higher sensitivity of the microsomal enzyme to malonyl-CoA inhibition [21]. The results of Western blots probed with anti-N antibody indicate that no such differences occur between the N-terminal domains of mitochondrial and peroxisomal CPTo. In this respect, it is noteworthy that the malonyl-CoA sensitivity of the mitochondrial and peroxisomal CPTo activities are identical, as is their response to 48 h starvation of the donor animals [26].

The suggestion that CPTo in different cell membrane systems could be due to the same, or very similar, molecular species has parallels. Thus, long-chain acyl-CoA synthase, which catalyses the formation of the acyl-CoA esters utilised by CPTo, exists in all three membrane systems as an apparently identical molecular species [27]. Similarly, Bcl-2 the anti-apoptotic protein which may interact with CPT I [28] is located not only in the mitochondrial outer membrane, but also in the endoplasmic reticular and nuclear membranes [29,30]. Targeting of such widely distributed proteins to different cytosol-facing membranes may involve the use of multiple [31] or hierarchical [32,33] targeting sequences, co-translational import directly from cytosolic polysomes [34] or the use of splice variant mRNA species that differ only in their 5′-untranslated regions but which are important in directing site-specific translation (e.g. for long-chain acyl-CoA synthetase [35]). Whether the molecular mechanism that gives rise to the distinctive N-terminal domain conformation of the microsomal CPTo also affects the membrane targeting specificity of the nascent protein will require further study.

Finally, the binding of both anti-N and anti-C antibodies by intact microsomes also confirms that the topology of CPT in the endoplasmic reticular membrane is the same as that in the outer membrane of mitochondria [8] namely, a polytopic one which results in both the N- and C-domains being exposed on the cytosolic aspect of the membrane.

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