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The mitochondrial carnitine/acylcarnitine carrier: Function, structure and physiopathology

Cesare Indiveri [a,b,1], Vito Iacobazzi [a,c,1], Annamaria Tonazzi [c], Nicola Giangregorio [c], Vittoria Infantino [a,d], Paolo Convertini [a], Lara Console [a], Ferdinando Palmieri [a,c,*]

[a] Department of Pharmaco-Biology, Laboratory of Biochemistry and Molecular Biology, University of Bari, 70125 Bari, Italy
[b] Department of Cellular Biology, University of Calabria, 87036 Arcavacata di Rende, Italy
[c] CNR Institute of Biomembranes and Bioenergetics, via Orabona 4, 70125 Bari, Italy
[d] Department of Chemistry, University of Basilicata, 85100 Potenza, Italy

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ABSTRACT

The carnitine/acylcarnitine carrier (CAC) is a transport protein of the inner mitochondrial membrane that belongs to the mitochondrial carrier protein family. In its cytosolic conformation the carrier consists of a bundle of six transmembrane α-helices, which delimit a water filled cavity opened towards the cytosol and closed towards the matrix by a network of interacting charged residues. Most of the functional data on this transporter come from studies performed with the protein purified from rat liver mitochondria or recombinant proteins from different sources incorporated into phospholipid vesicles (liposomes). The carnitine/acylcarnitine carrier transports carnitine and acylcarnitines with acyl chains of various lengths from 2 to 18 carbon atoms. The mammalian transporter exhibits higher affinity for acylcarnitines with longer carbon chains. The functional data indicate that CAC plays the important function of catalyzing transport of acylcarnitines into the mitochondria in exchange for intramitochondrial free carnitine. This results in net transport of fatty acyl units into the mitochondrial matrix where they are oxidized by the β-oxidation enzymes. The essential role of the transporter in cell metabolism is demonstrated by the fact that alterations of the human gene SLC25A20 coding for CAC are associated with a severe disease known as carnitine carrier deficiency. This autosomal recessive disorder is characterized by life-threatening episodes of coma induced by fasting, cardiomyopathy, liver dysfunction, muscle weakness, respiratory distress and seizures. Until now 35 different mutations of CAC gene have been identified in carnitine carrier deficient patients. Some missense mutations concern residues of the signature motif present in all mitochondrial carriers. Diagnosis of carnitine carrier deficiency requires biochemical and genetic tests; treatment is essentially limited to important dietetic measures. Recently, a pharmacological approach based on the use of statins and/or fibrates for the treatment of CAC-deficient patients with mild phenotype has been proposed.

* Corresponding author. Address: Department of Pharmaco-Biology, Laboratory of Biochemistry and Molecular Biology, University of Bari, via Orabona 4, 70125 Bari, Italy. Tel.: +39(0) 80 5443374; fax: +39(0) 80 5442770.
E-mail address: fpalm@farmbiol.uniba.it (F. Palmieri).
[1] These authors contributed equally to this work.

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1. Introduction

Oxidation of fatty acids in mitochondria coupled to oxidative phosphorylation is the most important pathway for the production of metabolic energy during fasting. This process occurs in the mitochondrial matrix where the enzymes of fatty acid β-oxidation are located. Fatty acyl groups are transported from the cytosol into the mitochondrial matrix by means of the carnitine shuttle system (Fig. 1). In the cytosol, fatty acid units are transferred from acyl-CoAs to carnitine by the action of the carnitine-palmitoyl-transferase 1 (CPT1) which is located on the external surface of the outer mitochondrial membrane (Lee et al., 2011; Rufer et al., 2009); the formed acylcarnitines cross the outer membrane, which is permeable to small molecules (Zeth and Thein, 2010) and are translocated through the inner mitochondrial membrane by the carnitine/acylcarnitine carrier (CAC) in exchange for intramitochondrial free carnitine; in the mitochondrial matrix, fatty acyl units are transferred from carnitine to matrix CoA by carnitine-palmitoyl-transferase 2 (CPT2) and the formed mitochondrial acyl-CoAs are oxidized by the β-oxidation enzymes. Therefore, the molecular components of the carnitine shuttle system, carnitine acyl transferases and CAC, are essential for the mitochondrial oxidation of fatty acids and, hence, for life (Ramsay et al., 2001). Most of the functional and structural properties of the mitochondrial CAC transporter have been elucidated after the characterization of the carnitine acyl transferases, as usually occurs for membrane proteins with respect to soluble enzymes. This review focuses on the molecular aspects of CAC which are connected with human pathology.

2. Function and structure of CAC

2.1. Functional properties of CAC and regulation of its transport activity

Mitochondrial carnitine transport has been preliminarily studied using isolated intact mitochondria (Murthy and Pande, 1984 and references therein). However, most of the available knowledge on the function of the mitochondrial CAC derives from studies performed in liposomes reconstituted with CAC purified from rat liver mitochondria (Indiveri and Palmieri, 1989; Indiveri et al., 1990) or recombinant CAC proteins obtained by gene expression in *Escherichia coli* or *Saccharomyces cerevisiae* (Indiveri et al., 1998; Palmieri et al., 1999; De Lucas et al., 2008). In these studies the purified CAC was inserted into the lipid bilayer of liposomes (Palmieri et al., 1995). In particular, the procedure of cyclic detergent removal adopted for the reconstitution of CAC allowed the insertion of the purified protein into the artificial membrane with the same orientation displayed by CAC in the native membrane. This procedure is based on a slow removal of the detergent from micelles constituted by purified CAC, detergent and phospholipids. The resulting proteoliposomes are used to study the CAC-mediated transport of carnitine and its acyl derivatives following the flux of [^{3}H]carnitine from the outside to the inside of the vesicles or vice versa (Fig. 2). Besides carnitine, the transporter accepts acylcarnitines with carbon chain length from 2 to 18 carbon atoms (Indiveri et al., 1990). The mammalian CAC has a higher affinity for long chain acylcarnitines, whereas the fungal transporter for short chain acylcarnitines (De Lucas et al., 2008). CACs from different sources catalyze both an exchange (antiport) and an uniport (i.e. unidirectional transport) of substrates, which are driven by the concentration gradient of the substrates across the membrane and not by the membrane potential or the pH gradient, as in the case of other mitochondrial carriers (Palmieri and Pierri, 2010a). Furthermore, detailed kinetic studies have shown that CAC follows a ping–pong mechanism, which implies that binary carrier-substrate complexes are formed before the transport reaction occurs (Indiveri et al., 1994), in contrast to a sequential mechanism which would involve a ternary complex of two substrates with the carrier protein (Palmieri et al., 1993).

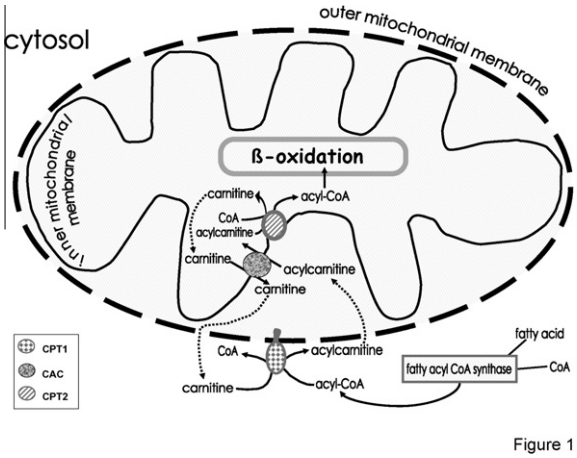

Figure 1

Fig. 1. The mitochondrial carnitine shuttle. *Abbreviations:* CPT1, carnitine-palmitoyl-transferase 1; CPT2, carnitine-palmitoyl-transferase 2; CAC, carnitine/acylcarnitine carrier.