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Review

Regulation of peroxisomal lipid metabolism: The role of acyl-CoA and coenzyme A metabolizing enzymes

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ABSTRACT

Peroxisomes are nearly ubiquitous organelles involved in a number of metabolic pathways that vary between organisms and tissues. A common metabolic function in mammals is the partial degradation of various (di)carboxylic acids via α- and β-oxidation. While only a small number of enzymes catalyze the reactions of β-oxidation, numerous auxiliary enzymes have been identified to be involved in uptake of fatty acids and cofactors required for β-oxidation, regulation of β-oxidation and transport of metabolites across the membrane. These proteins include membrane transporters/channels, acyl-CoA thioesterases, acyl-CoA:amino acid *N*-acyltransferases, carnitine acyltransferases and nudix hydrolases. Here we review the current view of the role of these auxiliary enzymes in peroxisomal lipid metabolism and propose that they function in concert to provide a means to regulate fatty acid metabolism and transport of products across the peroxisomal membrane.

1. Introduction

Peroxisomes are organelles present in yeast, fungi, plants and animals that perform diverse metabolic functions. A common function is the degradation of a variety of carboxylic acids via α- and β-oxidation, a process that has even been proposed to be the driving force for the evolution of peroxisomes [1,2]. The importance of peroxisomes in lipid metabolism and human health is underscored by the severity of disorders associated with impairment of peroxisomal function and there are numerous excellent reviews on peroxisome biogenesis disorders and peroxisomal fatty acid oxidation defects, see Refs. [3–5]. The past two decades have resulted in the elucidation of the metabolic pathways and enzymes involved in peroxisomes for the β-oxidation and α-oxidation of acyl-CoAs, glyoxylate metabolism, ether-phospholipid synthesis, cholesterol and isoprenoid metabolism and bile-acid synthesis. However, this review will focus on four groups of enzymes and their roles in peroxisomal lipid metabolism, particularly in β-oxidation of fatty acids, in regulation of coenzyme A metabolism and transport of metabolites across the peroxisomal membrane. These enzymes are the peroxisomal acyl-CoA thioesterases (ACOTs), carnitine acyltransferases (CRAT and CROT), *N*-acyltransferases (BAAT and ACNAT1 and 2) and nudix hydrolases (NUDTs).

2. An overview of peroxisomal acyl-CoA thioesterases and *N*-acyltranferases

Acyl-CoA thioesterases catalyze the hydrolysis of acyl-CoAs to the corresponding fatty acids and CoA in a seemingly 'energy wasting' futile cycle. However, the ACOTs have now been established as auxiliary enzymes in a number of peroxisomal pathways. ACOTs are divided into two families, which are commonly known as Type-I and Type II thioesterases. The Type-I thioesterases show highest sequence similarity to each other and to the *N*-acyltransferases, and structurally they belong to the α/β-hydrolase superfamily. The Type-II thioesterases are structurally related to each other although they show a low degree of sequence similarity and belong to the HotDog fold family (for reviews see Refs. [6–12]). The majority of the ACOTs identified in peroxisomes in human and mouse are the Type-I thioesterases, although one member of the Type-II ACOT family, the ACOT8, has been identified in peroxisomes and, in addition, the Type-II ACOT12 is cytosolic/peroxisomal in

Abbreviations: ACNAT, acyl-CoA:amino acid *N*-acyltransferase; ACOT, acyl-CoA thioesterase; ACOX3, acyl-CoA oxidase 3; ADP, adenosine diphosphate; AMP, adenosine monophosphate; BAAT, bile acid-CoA:amino acid *N*-acyltransferase; CoASH, coenzyme A; CRAT, carnitine acetyltransferase; CROT, carnitine octanoyltransferase; DMN-CoA, dimethylnonanoyl-CoA; FAAH, fatty acid amide hydrolase; FMN, flavin mononucleotide; NAT, *N*-acyl taurine; NUDT, nudix (nucleoside diphosphates linked to some moiety X) hydrolase; PAP, adenosine 3′,5′-diphosphate; PPAR, peroxisome proliferator activated receptor; PTS1, peroxisomal type 1 targeting signal.

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rodents. Together the Type-I and Type-II ACOTs catalyze the hydrolysis of a broad spectrum of acyl-CoAs. The ACOT-related *N*-acyltransferase enzymes catalyze a similar enzymatic reaction by hydrolyzing bile acid-CoAs and acyl-CoAs but using the amino acids taurine and/or glycine as acceptors (instead of water) to produce the corresponding amidated bile acids and fatty acids. In addition, recently one of the peroxisomal ATP binding cassette transporter proteins, Comatose (CTS), in *Arabidopsis thaliana* was shown to possess intrinsic ACOT activity coupled to uptake of fatty acids into peroxisomes [13].

Initial work in this area involved biochemical analysis of highly purified peroxisomes and identification of various acyl-CoA thioesterase activities in rat [14,15], and further gene cloning studies and bioinformatic work identified ACOTs coding for putative peroxisomal enzymes in the mouse [16]. To date ACOT3, ACOT4, ACOT5, ACOT6, ACOT8 and ACOT12 have been shown to localize in peroxisomes, although ACOT12 is partially localized in peroxisomes only in rodents as discussed below. These ACOTs are targeted to peroxisomes via C-terminal peroxisomal targeting signals of −SKL (PTS1) and variants thereof. It should be mentioned that the ACOTs show an unusually high degree of variation in the PTS1, which likely results in dual localizations between peroxisomes and cytosol (ACOT1 and also for ACOT12 as discussed below) and possibly peroxisomes and mitochondria (for example human ACOT2) for several ACOTs in various species. Table 1 shows the ACOT enzymes identified in peroxisomes to date, and indicates the metabolic pathways where these enzymes function. The role of ACOTs in these pathways will be discussed in the following sections. It should be noted here that ACOT3 and ACOT5 apparently are rat/mouse "inventions" by gene duplications and that ACNATs are present in a limited number of species, e.g. not in humans who still contains the bile-acid/fatty acid conjugating enzyme BAAT. In this review we discuss mainly the functions of the mouse and humans genes.

2.1. Functions of ACOT3, ACOT4 and ACOT5 in regulating β-oxidation of medium chain and long chain acyl-CoAs in peroxisomes

One important function of peroxisomes is in chain shortening of various fatty acids followed by transport of the metabolites out of the organelle. Characterization of rat and human ACOX1 showed that both enzymes have maximal activities with C_{12}–C_{18}–CoAs, with a decreasing V_{max}/K_m as chain-length decreases [17,18]. *In vitro* activity experiments with isolated rat liver peroxisomes indicate that e.g. palmitoyl-CoA undergoes 2–5 cycles of β-oxidation, depending on incubation conditions [19]. Further evidence for partial β-oxidation in peroxisomes was obtained from *in situ* studies in heart and liver by the group of Henri Brunengraber. These experiments showed that octanoate undergoes 1–2 cycles of β-oxidation while longer chain fatty acids (palmitate, oleate and dodecanoate) are preferentially chain shortened to C_{12}–C_{14}-CoA [20–22]. These experiments also showed that dicarboxylic acids (at least dodecanedioate) are exclusively oxidized in peroxisomes but the number of cycles was not determined. Interestingly, the acetyl moieties generated in peroxisomes (as acetyl-CoA in heart and acetate in liver) are substrates for acetyl-CoA carboxylase and mainly recovered in malonyl-CoA rather than being transferred to mitochondria. The mechanisms by which fatty acids are only partially oxidized in peroxisomes (in contrast to mitochondria) is not yet clearly understood but considering the spectrum of fatty acids that may be oxidized in peroxisomes producing short-, medium-, and long chain metabolites, we consider the auxiliary enzymes discussed in this review as likely candidates to be involved in such regulation.

ACOT3, ACOT4 and ACOT5 have been characterized from mouse [23,24], with ACOT3 being a long chain acyl-CoA thioesterase (C_{12}–C_{18}–CoAs), whereas ACOT5 is active mainly with medium chain acyl-CoAs (highest activity with C_{10}-CoA) and ACOT4 is only active on short-chain carboxylyl-CoAs. Mouse ACOT4 activity is specific for succinyl-CoA and glutaryl-CoA, so it was surprising that the human ACOT4 possesses activity with medium- to long chain acyl-CoAs in addition to the activity with succinyl-CoA and glutaryl-CoA [25]. It was proposed that ACOT4 through convergent evolution in human "replaced" ACOT3 and ACOT5 in rodents. However, it appears that in fact rat and mouse represent divergent evolution by which they acquired more genes with specialized functions through duplications probably of ACOT1/ACOT2 (cytosolic and mitochondrial respectively), which usually leads to specialization. Comparison of the substrate specificity of human ACOT4, hydrolyzing also C_{10}–C_{20}–CoAs, with the "combined" activities of mouse ACOT3, ACOT4 and ACOT5 shows a striking similarity suggesting that human ACOT4 can catalyze the activities of the three mouse enzymes [25].

Medium-chain fatty acids may be transported across the peroxisomal membrane as the free acid, via PEX11, and are subsequently activated to the corresponding CoA ester inside the peroxisome [26]. One of the functions of ACOT3 and ACOT5 is hypothesized to be in production of long and medium chain fatty acids for transport out of the peroxisome for further β- or ω-oxidation in the mitochondria or endoplasmic reticulum respectively. The characterization of ACOT3 showed very low activity toward the β-oxidation intermediate 3-hydroxy-palmitoyl-CoA, suggesting that it preferentially hydrolyzes the substrate or the product of β-oxidation in peroxisomes, but likely not intermediates (or only with low activity). Alternatively the very long chain acyl-CoA synthetase identified inside peroxisomes [27] would allow

Table 1
Metabolic pathways in peroxisomes involving acyl-CoA thioesterases (ACOTs) and *N*-acyltransferases.

ACOT gene (aliases)	Peroxisomal targeting signal	Species	Acyl-CoA substrate	Pathway	Reference
ACOT3, ACOT4, ACOT5, ACOT8	-AKL (ACOT3 and ACOT5) -SKL (ACOT8) -PKL (ACOT4)	ACOT3 and 5 – mouse. ACOT8 – mouse, human. ACOT4 – human only.	Medium chain, long chain and very long chain acyl-CoAs	β-Oxidation	[23,25,39]
ACOT4 (PTE-Ib, Pte-2b, hPTE-1)	-CRL (mouse ACOT4), -PKL (human ACOT4)	Mouse, human	Succinyl-CoA, glutaryl-CoA	Dicarboxylic acid oxidation	[24,25]
ACOT6 (PTE-Id)	-SKL	Mouse	Phytanoyl-CoA, pristanoyl-CoA	α-Oxidation	[34]
BAAT (BACAT, BAT)	-SQL	Mouse, human, rat	Bile acid-CoAs	Bile acid conjugation	[95,96]
ACNAT1 BAAT	-SKL	Mouse	Long chain acyl-CoAs, very long chain acyl-CoAs	Fatty acid conjugation	[66]
ACOT12 (CACH-I, MGC105114, mCACH-1, CACH)	-SVL	Rat	Short chain acyl-CoAs	β-Oxidation – short chain	[58]
Comatose (CTS; AtABCD1)	(Membrane protein)	*Arabidopsis thaliana*	Medium chain, long chain and very long chain acyl-CoAs	Fatty acid transport	[13]

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