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Review

The emerging role of acyl-CoA thioesterases and acyltransferases in regulating peroxisomal lipid metabolism☆

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ABSTRACT

The importance of peroxisomes in lipid metabolism is now well established and peroxisomes contain approximately 60 enzymes involved in these lipid metabolic pathways. Several acyl-CoA thioesterase enzymes (ACOTs) have been identified in peroxisomes that catalyze the hydrolysis of acyl-CoAs (short-, medium-, long- and very long-chain), bile acid-CoAs, and methyl branched-CoAs, to the free fatty acid and coenzyme A. A number of acyltransferase enzymes, which are structurally and functionally related to ACOTs, have also been identified in peroxisomes, which conjugate (or amidate) bile acid-CoAs and acyl-CoAs to amino acids, resulting in the production of amidated bile acids and fatty acids. The function of ACOTs is to act as auxiliary enzymes in the α- and β-oxidation of various lipids in peroxisomes. Human peroxisomes contain at least two ACOTs (ACOT4 and ACOT8) whereas mouse peroxisomes contain six ACOTs (ACOT3, 4, 5, 6, 8 and 12). Similarly, human peroxisomes contain one bile acid-CoA:amino acid *N*-acyltransferase (BAAT), whereas mouse peroxisomes contain three acyltransferases (BAAT and acyl-CoA:amino acid *N*-acyltransferases 1 and 2: ACNAT1 and ACNAT2). This review will focus on the human and mouse peroxisomal ACOT and acyltransferase enzymes identified to date and discuss their cellular localizations, emerging structural information and functions as auxiliary enzymes in peroxisomal metabolic pathways. This article is part of a Special Issue entitled: Metabolic Functions and Biogenesis of Peroxisomes in Health and Disease.

1. Introduction

Peroxisomes are nearly ubiquitous organelles present in yeast, fungi, plants and animals and in the last number of years there has been a huge increase in research into peroxisome biogenesis, peroxisomal lipid metabolism and the role of peroxisomes in human diseases. The involvement of peroxins in peroxisome biogenesis disorders has been widely studied, together with diseases associated with individual enzyme deficiencies and has provided new insights into the functions of peroxisomes in health and disease. There are numerous excellent reviews on peroxisome biogenesis disorders and peroxisomal fatty acid oxidation defects ([1–3] and including two chapters in this current Special Issue). Research including a combination of biochemical techniques, molecular biology and proteomics over the last number of years, has been instrumental in the elucidation of the enzymatic pathways 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.

In recent years, a number of enzymes called acyl-CoA thioesterases (ACOTs) and acyltransferases have been identified and characterized in peroxisomes and distinct roles for these enzymes as auxiliary enzymes in peroxisomal lipid metabolism have now been established. This review will focus on these ACOTs and acyltransferases identified to date and their roles in peroxisomal lipid metabolism.

2. Identification and characterization of acyl-CoA thioesterases and acyltransferases in peroxisomes

In the 1950s, the first identification of acyl-CoA thioesterase (ACOT) activity was published describing the partial purification of a succinyl-CoA thioesterase from pig heart [4]. In the intervening years, advances in techniques led to the identification and

Abbreviations: ACNAT, acyl-CoA:amino acid *N*-acyltransferase; ACOT, acyl-CoA thioesterase; ACOX3, acyl-CoA oxidase 3; BAAT, bile acid-CoA:amino acid *N*-acyltransferase; CA, cholic acid; CoASH, coenzyme A; CDCA, chenodeoxycholic acid; DMN-CoA, dimethylnonanoyl-CoA; FAAH, fatty acid amide hydrolase; FXR, farnesoid X receptor; HAO1, hydroxyacid oxidase 1; HNF-4α, hepatocyte nuclear factor 4 alpha; NAT, *N*-acyl taurine; 4-HNE, 4-hydroxynonenal; PPAR, peroxisome proliferator activated receptor; PTS1, peroxisomal type 1 targeting signal

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characterization of several different families of ACOTs as reviewed in [5–8]. A nomenclature system was introduced in 2005, which is now followed by the scientific community and new ACOT genes identified are assigned the next available number [9]. Within the peroxisome, several ACOTs have been identified and characterized that cleave the thioester bond of acyl groups attached to coenzyme A (CoASH), to release the free acid and CoASH (Fig. 1). The acyl groups have been identified as long-, medium- and short-chain fatty acids, dicarboxylic acids, methyl-branched chain fatty acids and bile acids, depending on the ACOT enzyme involved.

ACOTs are divided into two families, named the Type-I and Type-II thioesterases, where the Type-I thioesterases, together with the acyltransferases, show a high degree of sequence similarity and structurally belong to the α/β-hydrolase superfamily, which is one of the largest superfamilies of proteins. The Type-II thioesterases show a low degree of sequence similarity to each other, however they are structurally related and were found to belong to the HotDog fold family of proteins [10]. While the Type-I thioesterases are only found in (some) bacteria and in the animal kingdom (not in yeast, insects or plants), the Type-II thioesterases are found in all three branches of life. Numerous HotDog domain-containing proteins are fusion proteins in which two separate genes have been fused to give a protein with two functional domains (as is the case for ACOT12 discussed below).

The second group of enzymes identified in peroxisomes is the acyl-CoA:amino acid *N*-acyltransferases, which catalyze the transfer of carboxylic acids from the CoA ester to an amino acid, usually taurine or glycine (Fig. 1). The final step in bile-acid synthesis is a conjugation (or amidation) of the bile acids, producing taurine or glycine conjugated bile acids that are excreted into the bile. This reaction is catalyzed by the well-characterized bile acid-CoA:amino acid *N*-acyltransferase (BAAT). More recently, two further genes have been identified in mouse that code for proteins that are sequence-related to BAAT, called *Acnat1* and *Acnat2* (acyl-CoA:amino acid *N*-acyltransferase 1 and 2), which function in the conjugation of fatty acids to taurine (and possibly other amino acids). The structures and functions of peroxisomal acyl-CoA thioesterases and acyltransferases are the focus of this review.

3. Type-I peroxisomal acyl-CoA thioesterases

In the late 1980s and early 1990s the biochemical analysis of highly purified peroxisomes revealed ACOT activity with a wide range of acyl-CoAs, including long-, medium- and short-chain acyl-CoAs (from C_2–C_{20}-CoA) [11,12] and the partial purification of a peroxisomal ACOT identified a protein with long-chain acyl-CoA thioesterase activity [11]. Gene cloning subsequently identified several *Acots* in mouse and human that contained peroxisomal type 1 targeting signals (PTS1) at their carboxyterminal end. These proteins comprise the Type-I family of ACOTs, and mouse contains six closely related genes (that show 66–93% sequence identity to each other), all localized in a gene cluster on mouse chromosome 12 D3, and are named *Acot1* to *Acot6* [13–16]. The human *ACOT* genes (4 genes in total) are all localized in a gene cluster on human chromosome 14q24.3 [17], named *ACOT1*, *ACOT2*, *ACOT4* and *ACOT6*.

In mouse the products of four of the six genes in the gene cluster (ACOT3–6) are localized in peroxisomes, while mouse ACOT1 localizes to cytosol and ACOT2 to mitochondria. ACOT3, ACOT4, ACOT5 and ACOT6 are proteins of about 47 kDa that end with the amino acid sequences -AKL (ACOT3 and ACOT5), -CRL (ACOT4) and -SKL (ACOT6) (see Table 1) and peroxisomal localization was confirmed using green fluorescent fusion protein studies [14–16] and proteomic studies (ACOT3, ACOT4 and ACOT6) in purified mouse kidney peroxisomes [18].

The two first peroxisomal Type-I thioesterases characterized in detail were the mouse ACOT3 and ACOT5. Expression of the recombinant proteins revealed that mouse ACOT3 is a long chain acyl-CoA thioesterase (highest activity with C_{12}–C_{18}-CoA), whereas mouse ACOT5 is a medium chain acyl-CoA thioesterase (highest activity with C_{10}-CoA). Interestingly, ACOT3 and ACOT5 had little or no activity toward 3-hydroxy-palmitoyl-CoA, an intermediate in peroxisomal β-oxidation, suggesting that these enzymes hydrolyze the substrates/products at the beginning and the end of individual β-oxidation cycles, but not the intermediates within the individual cycles [15]. Neither enzyme was active on CoA-esters of bile-acid intermediates (choloyl-CoA or chenodeoxycholoyl-CoA), but both ACOT3 and

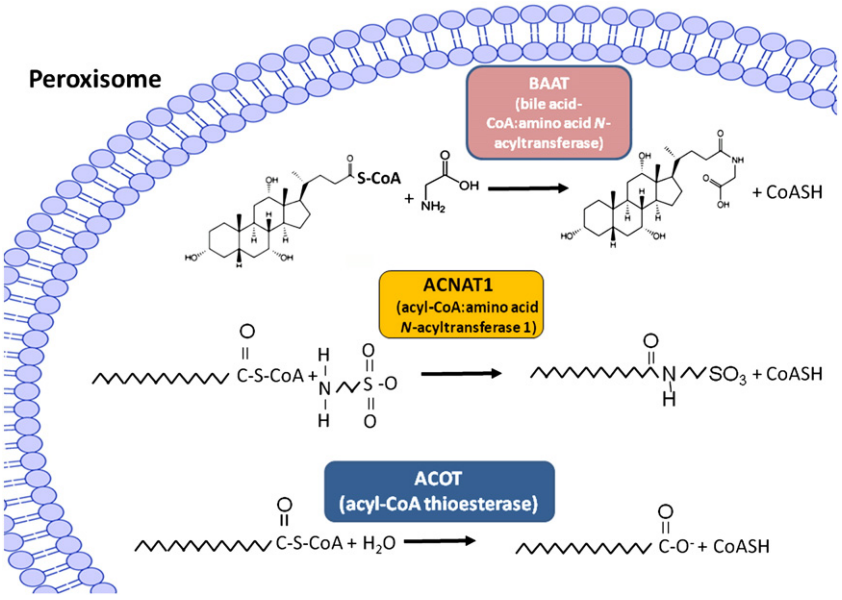

Fig. 1. Reactions catalyzed by peroxisomal acyl-CoA thioesterases and acyltransferases. The bile acid-CoA:amino acid *N*-acyltransferase (BAAT) catalyzes the conjugation (or amidation) of the CoA esters of bile acids to glycine or taurine, resulting in the production of glycine or taurine conjugated bile acids (the figure shows choloyl-CoA with glycine as the acceptor, producing choloylglycine and coenzyme A (CoASH)). The acyl-CoA:amino acid *N*-acyltransferase 1 (ACNAT1) enzyme catalyzes the conjugation of fatty acids to taurine, resulting in the production of *N*-acyl taurines and CoASH (the figure shows stearoyl-CoA ($C_{18:0}$-CoA) and taurine, resulting in the production of *N*-stearoyl taurine). (BAAT can also conjugate fatty acids to taurine and glycine in a similar reaction catalyzed by ACNAT1 but for simplicity only ACNAT1 is shown). Acyl-CoA thioesterases (ACOTs) catalyze the hydrolysis of acyl-CoA esters to produce the free fatty acid and CoASH (the figure shows stearoyl-CoA, resulting in the production of stearic acid and CoASH).