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Review

Acyl-CoA:cholesterol acyltransferases (ACATs/SOATs): Enzymes with multiple sterols as substrates and as activators

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ABSTRACT

Cholesterol is essential to the growth and viability of cells. The metabolites of cholesterol include: steroids, oxysterols, and bile acids, all of which play important physiological functions. Cholesterol and its metabolites have been implicated in the pathogenesis of multiple human diseases, including: atherosclerosis, cancer, neurodegenerative diseases, and diabetes. Thus, understanding how cells maintain the homeostasis of cholesterol and its metabolites is an important area of study. Acyl-coenzyme A:cholesterol acyltransferases (ACATs, also abbreviated as SOATs) converts cholesterol to cholesteryl esters and play key roles in the regulation of cellular cholesterol homeostasis. ACATs are most unusual enzymes because (i) they metabolize diverse substrates including both sterols and certain steroids; (ii) they contain two different binding sites for steroidal molecules. In mammals, there are two ACAT genes that encode two different enzymes, ACAT1 and ACAT2. Both are allosteric enzymes that can be activated by a variety of sterols. In addition to cholesterol, other sterols that possess the 3-beta OH at C-3, including PREG, oxysterols (such as 24(*S*)-hydroxycholesterol and 27-hydroxycholesterol, *etc.*), and various plant sterols, could all be ACAT substrates. All sterols that possess the *iso*-octyl side chain including cholesterol, oxysterols, various plant sterols could all be activators of ACAT. PREG can only be an ACAT substrate because it lacks the *iso*-octyl side chain required to be an ACAT activator. The unnatural cholesterol analogs epi-cholesterol (with 3-alpha OH in steroid ring B) and ent-cholesterol (the mirror image of cholesterol) contain the *iso*-octyl side chain but do not have the 3-beta OH at C-3. Thus, they can only serve as activators and cannot serve as substrates. Thus, within the ACAT holoenzyme, there are site(s) that bind sterol as substrate and site(s) that bind sterol as activator; these sites are distinct from each other. These features form the basis to further pursue ACAT structure–function analysis, and can be explored to develop novel allosteric ACAT inhibitors for therapeutic purposes.

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Contents

Abbreviations: ACAT, acyl-coenzymeA:cholesterol acyltransferase; DGAT1, diacylglycerol acyltransferase 1; MBOAT, membrane-bound *O*-acyltransferase; LCAT, lecithin: cholesterol acyltransferase; OXY, oxysterol; CHOL, cholesterol; ent-cholesterol, enantiomeric cholestrol; HDL, high-density lipoprotein; MAM, mitochondria associated membrane; PREG, pregnenolone; SREBP, sterol regulatory element-binging protein; SOAT, sterol-*O*-acyltransferase; STAR, steroid-acute regulatory protein; LXR, liver X receptor.

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1. ACAT enzymes

Acyl-CoA:cholesterol acyltransferases (ACATs), also known as sterol *O*-acyltransferases (SOATs), play important roles in cellular cholesterol homeostasis and are drug targets for therapeutic intervention of several diseases including atherosclerosis (reviewed in [1]), Alzheimer's disease [2–5] and cancer [6]. In mammals, two genes that encode two different proteins exist: *Acat1* and *Acat2* [7]. Along with acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1), ACAT1 and ACAT2 are founding members of the membrane-bound *O*-acyltransferase (MBOAT) enzyme family [8]. MBOATs are multi-span membrane enzymes that use long-chain or medium-chain fatty acyl-CoA as the first substrate, and catalyze the transfer of the fatty acyl group to the 3β-hydroxyl moiety of a certain hydrophobic substance as the second substrate. An MBOAT contains two active sites: a histidine within a long hydrophobic peptide region, and an asparagine located within a long hydrophilic peptide region. In humans, there are 11 MBOAT members, with similar catalytic mechanisms but with diverse biological functions. At present, there are no complete crystal structures for any members of the MBOAT family. For ACAT1 and ACAT2, the major sterol substrate is cholesterol; both enzymes use long chain fatty acyl-coenzyme A as the fatty acyl donor to convert cholesterol to cholesteryl esters. For ACAT1, the preferred fatty acyl-CoA is oleoyl coenzyme A [9]. Cholesterol is a lipid molecule; it partitions well within the phospholipid bilayer of various cell membranes. Unlike free (unesterified) cholesterol, the cholesteryl esters do not partition well within the lipid bilayer; instead, cholesteryl esters coalesce in aqueous medium and form cytoplasmic lipid droplets. The over accumulation of free cholesterol in the membranes can be cytotoxic to cells. Thus, a major function of ACATs is to protect against the unnecessary built up of free cholesterol within the cell membranes. ACAT1 is ubiquitously expressed in essentially all tissues examined; it is a resident enzyme at the endoplasmic reticulum (ER). ACAT2 is mainly expressed in the intestines and hepatocytes. It is also expressed in various other tissues, but at much lower levels than ACAT1. In intestines, ACAT2 provides cholesteryl esters for lipoprotein assemblies. In humans, both ACAT1 and ACAT2 are expressed in hepatocytes; the relative roles of ACAT1 and ACAT2 in human hepatic lipoprotein assembly are yet to be clarified. (reviewed in [1]). Homologs of ACAT1 and ACAT2 have been identified in yeast saccharomyces cerevisiae [10,11] and other species.

The *Acat1* gene was identified by functional complementation of a Chinese hamster ovary cell mutant lacking ACAT activity [12]. Unlike most other genes, human *Acat1* is located in two different chromosomes, chromosomes 1 and 7, with each site containing a distinct promoter: chromosome 1 contains exons 1–16, and chromosome 7 contains the optional long exon Xa [13]. The majority of ACAT1 mRNAs is transcribed from exons 1–16; this mRNA translates into a 50-kDa size protein on SDS-PAGE [14]. In addition, the pre-mRNAs produced from exons 1–16 and the pre-mRNAs produced from the optional exon Xa participate in a trans-splicing event to produce an endogenous, chimeric 4.3 kb ACAT1 mRNA. Remarkably, the endogenous 4.3 kb mRNA then undergoes a second trans-splicing event with an exogenous transcript encoded by the antisense strand of Amp(r) (asAmp), which is present in common Amp(r)-plasmids, to produce a novel mRNA species. This novel mRNA species translates into a 56-kDa size protein [15]. The 56-kDa protein has less ACAT enzyme activity than that of the 50-kDa protein [16]. The chimeric 4.3 kb ACAT1 mRNA found in human cannot be found in mouse. In mouse the *Acat1* gene is located solely on chromosome 1, contains 17 exons [17], and produces a single protein of 48-kDa size protein on SDS-PAGE [18]. Human *Acat2* is located in chromosome 12 and produces a single 46-kDa size protein on SDS-PAGE [19]. Meiner et al. [18,20] generated and characterized the *Acat1* and *Acat2* knockout mice. These mice have served as valuable tools in lipoprotein metabolism, atherosclerosis and neurodegenerative disease research.

The recombinant 50-kDa human ACAT1 has been purified to homogeneity with full retention in enzymatic activity [21]. When assayed in reconstituted liposomes or in mixed micelles, the enzyme responds to cholesterol as its substrate in a sigmoidal manner. ACAT2 activity also responds to cholesterol in a sigmoidal manner. Additional kinetic analyses show that both ACAT1 and ACAT2 can use a variety of sterols as substrates, and their activities are significantly activated by a variety of sterols. Among the sterols tested (including oxysterols, plant sterols, yeast sterols, and several synthetic sterol analogs), cholesterol is the best substrate and the best activator [22]. Those sterols that act both as ACAT activators and as substrates all contain a 3β hydroxyl group at steroid ring A. The sterols that contain alterations at the *iso*-octyl side chain, such as the plant sterol sitosterol, are both poor substrates and poor activators of ACAT. In addition, epi-cholesterol, which contains the 3α hydroxyl, is not a substrate and is a very poor activator. Likewise, enantiomeric cholesterol (ent-cholesterol), which is the mirror image of cholesterol and possesses essentially the same biophysical properties of cholesterol [23], is also not a substrate and a very poor activator [24]. These studies implicate that both ACAT1 and ACAT2 are allosteric enzymes, and the structural feature of a given sterol as an ACAT substrate deviates from that as an activator. A shortcoming of these studies was that a sterol-like molecule that is only a substrate but not an activator was not found. In the absence of such molecule, in order to test the structural features of various sterols as activators, sitosterol was chosen as a substrate [24]; however, sitosterol might act as an activator. Thus, by using sitosterol as the substrate in the ACAT activation assay, certain essential structural feature of a given sterol to act as an activator might have been masked.

2. PREG esterification and ACAT

PREG is the obligatory precursor for all steroid hormones. Biosynthesis of PREG occurs in mitochondria, using cholesterol (CHOL) as the precursor [25,26]. Once produced, PREG can be converted by enzymes in the mitochondria and in the ER to various steroid hormones. In addition, PREG can be stored as fatty acyl esters. Steroid fatty acyl esters can provide a means to quickly provide a substrate pool in times of need. Lipoidal conjugates of PREG were first identified in the bovine adrenal [27]. Using bovine adrenal homogenates, Mellon and Hochberg [28] demonstrated that PREG could form lipoidal derivatives *in vitro*. In the adrenals of dog, rat and guinea-pig, PREG esters have been reported to be up to 40% of the total adrenal PREG content, while in human adrenals, PREG esters comprise more than 3 times the amount of free PREG [29].

Lavallee et al. [30] reported that PREG could be esterified by lecithin:cholesterol acyltransferase (LCAT). LCAT uses cholesterol