Minireview

The Enzymes of Neutral Lipid Synthesis*

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Fueled by fat-rich diets and sedentary lifestyles, atherosclerosis and obesity are major health issues in the Western world. Both diseases involve excessive accumulation of neutral lipids: cholesterol esters in arteries in atherosclerosis and triglycerides in adipocytes in obesity. The study of these diseases has sparked interest in the biochemistry and molecular biology of neutral lipid synthesis.

The discovery of acyl-CoA:cholesterol acyltransferase (ACAT, EC 2.3.1.26), a cholesterol ester synthesis enzyme, dates back to the 1950s (1). ACAT was identified as an intracellular enzyme in the endoplasmic reticulum (ER) that covalently joined cholesterol and fatty acyl-CoA molecules to form cholesterol esters (Fig. 1). ACAT activity was detected in macrophages and in tissues such as liver, small intestine, and adrenal glands (2–4). Another ER enzyme, acyl-CoA:diacylglycerol acyltransferase (DGAT, EC 2.3.1.20), was identified in 1960 (5). The DGAT reaction is similar to that of ACAT except that diacylglycerol is the acyl group acceptor (Fig. 1). In the ensuing 30 years, much was learned about the biochemistry of ACAT and DGAT (2, 3, 6–11). Both enzymes were shown to be involved in the assembly and secretion of lipoproteins, and ACAT was found to be responsible for synthesizing cholesterol esters in arterial macrophage foam cells. However, these hydrophobic proteins proved difficult to isolate, slowing progress in their understanding.

Because of the pioneering work of Chang and colleagues (12), the field of neutral lipid synthesis made great strides during the past decade. These investigators isolated ACAT by using a clever cloning strategy to isolate an ACAT cDNA. Thanks to the resultant molecular probes and to genetic studies in yeast and mice, we now know there are two mammalian ACAT enzymes and probably more than one DGAT enzyme.2 A large number of ACAT- and DGAT-related genes are identifiable in species ranging from plants (13, 14) to yeast (15–17) to humans. Here we review recent advances in understanding the function of the mammalian members of this family, ACAT1, ACAT2, and DGAT.

Gene and Protein Structures

Characteristics of the human and mouse members of the ACAT/DGAT gene family are shown in Table I. The human ACAT1 gene encodes four mRNAs of 7.0, 4.3, 3.6, and 2.8 kilobases (kb) (12, 18–20); all contain the same translational reading frame but differ in the length of the untranslated regions (21). The two shorter mRNAs are products of a proximal ACAT promoter. The 4.3-kb mRNA is derived from an unusual RNA recombination mechanism involving trans-splicing of two discontinuous precursor RNAs produced from chromosomes 1 and 7 (21). The origin of the 7.0-kb transcript is unknown. The human ACAT2 gene encodes a single mRNA of 2.2 kb, and the human DGAT gene encodes two mRNAs of 2.0 and 2.4 kb. Regions of shared sequence identity are present throughout the ACAT and DGAT proteins, but the greatest similarity is found in their C termini (Fig. 2). Human and mouse ACAT2 are ∼40% identical to human ACAT1, and DGAT is ∼20% identical to ACAT1.

Little is known about the relationship between protein structure and function in ACAT/DGAT family members. One motif, FYXDWNN (amino acids 403–409 of human ACAT1), is highly conserved in all family members and may be involved in fatty acyl-CoA binding. Another motif, MMKXXSF (amino acids 265–270 of human ACAT1), is conserved in ACAT family members. The serine of this motif is required for ACAT activity (22, 23). All three family members have a potential tyrosine phosphorylation motif and at least one N-linked glycosylation site, both of unknown significance. ACAT1 and ACAT2 contain an N-terminal leucine zipper motif. Although ACAT1 functions as a homotetramer (24), it is unknown whether this leucine zipper motif participates in tetramer formation or whether ACAT2 and DGAT act as multimers. ACAT1 and ACAT2 do not form hetero-oligomeric complexes (25).

All ACAT/DGAT proteins possess multiple hydrophobic regions predicted to serve as transmembrane domains, although there is not yet a consensus structural model for the proteins. Investigations of human ACAT1 topology have yielded different results. One study, performed by inserting epitopes at different positions in the expressed protein (26), found seven transmembrane domains. Another study, performed by expressing truncated forms of the protein (22), found five transmembrane domains. In both studies, the N terminus of the protein localized to the cytoplasm and the C terminus to the ER lumen (22, 26). Topologic studies of human ACAT2 performed with the truncation mutant method also found five transmembrane domains with the N terminus in the cytoplasm and the C terminus in the ER lumen (22). The latter study found that a serine residue essential for ACAT activity was located in the cytoplasm for ACAT1 and in the ER lumen for ACAT2, suggesting that the active sites of these enzymes reside on opposite sides of the ER membrane. Several putative transmembrane sequences are conserved in ACAT1 and ACAT2 but not in DGAT (26), suggesting that these regions are involved in cholesterol binding. The topology of DGAT has not been reported, but a putative diacylglycerol binding motif has been identified (27).

Tissue and Cellular Distributions

The tissue distributions of ACAT1 and ACAT2 are largely complementary. ACAT1 mRNA is present in many tissues, with the highest levels in macrophages and in adrenal and sebaceous glands (12, 28, 29), all of which store cholesterol esters in cytoplasmic droplets. ACAT1 is also highly expressed in human atherosclerotic lesions, particularly in macrophage foam cells (30). ACAT2 is expressed predominantly in the liver and small intestine (27, 31, 32). In humans, nonhuman primates, and mice, ACAT2 appears to be the major ACAT in the small intestine (25, 33, 34) and the predominant isozyme in hepatocytes of nonhuman primates and mice (33, 34).

Whether ACAT1 or ACAT2 is the predominant enzyme in human hepatocytes is controversial. Both are expressed in human hepatocytes and HepG2 cells (25). However, immunodepletion experiments found that ACAT1 accounts for ∼90% of ACAT activity in human liver (35), whereas ACAT2 accounts for most of the ACAT activity in adult small intestine and fetal liver but only 10–20% of activity in adult liver (25). It is unclear whether the hepatic ACAT1 activity resides in hepatocytes or macrophage-derived Kupffer cells. ACAT1 expression was detected immunohistochemically in both cell types, with stronger staining in Kupffer cells (36). In

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1 The abbreviations used are: ACAT, acyl-CoA:cholesterol acyltransferase; DGAT, acyl-CoA:diacylglycerol acyltransferase; kb, kilobase(s); ER, endoplasmic reticulum; LDLR, low density lipoprotein receptor-deficient; apo, apolipoprotein.
2 A second mammalian DGAT gene was recently reported (76).
nonhuman primates, ACAT1 was expressed only in Kupffer cells in the liver (33). A significant proportion of the ACAT1 activity in human liver thus may be attributable to Kupffer cells. Another study found little evidence of ACAT2 expression in human hepatocytes (25).

DGAT mRNA and activity are ubiquitous in mouse and human tissues, with the highest levels in liver, small intestine, and adipose tissue (11, 27, 37) and somewhat lower levels in testis and adrenal gland (11). This wide range of tissue expression is consistent with the involvement of DGAT in the glycerol phosphate pathway of triglyceride synthesis, which is common to most cells. DGAT protein expression in tissues has not been studied because of the lack of suitable antibodies.

The intracellular localization of enzymes in this class has been examined only for ACAT1. In human melanoma cells and fibroblasts and in mouse macrophages, ACAT1 is located primarily in the ER (36, 39, 40). In mouse macrophages, a small portion of ACAT1 immunoreactivity localizes to a region near the trans-Golgi network (40, 41). In macrophages, ACAT1 localization may change with different conditions (36, 40).

**Biochemical Activity and Regulation**

ACAT and DGAT substrates have been identified by expressing the proteins in insect cells or yeast devoid of sterol esterification activity. In insect cells, mouse, and human ACAT1 and ACAT2 utilize a variety of oxysterols in addition to cholesterol as substrates (31, 42). Ergosterol and plant sterols are poor substrates (31, 42). Ergosterol and plant sterols are poor substrates (31, 42). Moreover, endogenous cholesterol esterification activity is present in the AKR strain of mice (52). A naturally occurring mutation in the mouse ACAT1 gene is present in the AKR strain of mice (53), which are homozygous for the adrenocortical lipid depletion (ald) allele (54).

The analysis of ACAT2-deficient mice (ACAT2−/−) (34) complemented findings from ACAT1−/− mice. ACAT2−/− mice have nearly undetectable ACAT activity and lack cholesterol esters in the liver and small intestine (34). Because they cannot synthesize cholesterol esters in the small intestine, ACAT2−/− mice have a reduced capacity to obtain cholesterol from the diet. As a result, they do not develop hypercholesterolemia or form cholesterol gallstones when fed a diet high in fat, cholesterol, and cholic acid (34). ACAT2 deficiency markedly decreases the cholesterol ester content of apolipoprotein (apo) B-containing lipoproteins in mice fed either a chow diet or the above diet (34). Thus, ACAT2 is the cholesterol esterification enzyme in mouse liver and small intestine and plays a major role in modulating the response to dietary cholesterol.

Gene disruption studies suggest that both ACAT enzymes synthesize cholesterol esters: ACAT1 for storage in cytosolic droplets and ACAT2 for secretion in apoB-containing lipoproteins. However, these results may reflect their different tissue distributions rather than inherent biochemical differences. Both ACAT1 and ACAT2, when expressed in a cell line deficient in cholesterol esterification activity (AC29 cells), synthesize cholesterol esters that are stored in cytoplasmic droplets (25). Also, overexpression of ACAT1 in mouse liver causes both cholesterol ester storage and secretion (55).

**Role of ACAT1 in Atherosclerosis**

Because cholesterol ester synthesis by ACAT1 is involved in macrophage foam cell formation, it has been hypothesized that inhibition of ACAT1 may reduce atherosclerosis. To test this hypothesis that ACAT1 deficiency in macrophages inhibits atherosclerosis, ACAT1−/− mice were crossed with two strains of mice that are susceptible to atherosclerosis: low density lipoprotein receptor-deficient (LDLR−/−) mice and apoE-deficient (apoE−/−) mice (56). ACAT1 deficiency did not prevent atherosclerotic lesions in either model. Because ACAT1 deficiency also reduced serum cholesterol levels in both strains of mice, its effect on lesion size was
design. Ishibashi and colleagues (52) quantified lesion areas by measuring neutral lipid staining even though the cholesterol ester content of atherosclerotic lesions in ACAT1−/− mice was significantly diminished (56, 58). The lesion areas consequently may have been underestimated. In the study by Fazio et al. (58), selective inhibition of macrophage ACAT1 may not have reduced lesion size because ACAT activity was not inhibited in adjacent arterial smooth muscle cells, which can also become foam cells.

Both complete (52, 56) and macrophage-specific (56) ACAT1 deficiencies have deleterious effects in hypercholesterolemic mice. ACAT1 deficiency causes extensive deposition of free cholesterol in the skin (52, 56) and brain (56) of LDLR−/− and apoE−/− mice, suggesting an important role for the enzyme in cholesterol metabolism in these tissues when serum sterol levels are elevated. Although the dramatic effects in these models reflect the extreme condition of complete ACAT1 inhibition in the face of severe hypercholesterolemia, they indicate that selective ACAT inhibition can be detrimental.

**Functional Analysis of DGAT**

Because DGAT catalyzes the final step in the major pathway of triglyceride synthesis (8, 10), it was speculated that inactivation of the encoding gene in the mouse (Dgat) would cause severe morbidity or early lethality. Surprisingly, DGAT-deficient mice are viable and appear healthy although adult mice develop dry fur and female mice have a lactation defect (38). Even though these animals lack triglyceride synthesis in most tissues examined, Dgat−/− mice have normal serum triglyceride levels and triglycerides in white adipose tissue (38). These results indicate the existence of alternative pathways of triglyceride synthesis (11).2 Although DGAT deficiency does not prevent triglyceride synthesis, Dgat−/− mice have ~50% reduction in fat pad content and are resistant to weight gain when fed a high fat diet (38). Unexpectedly, these findings are associated with an ~15% increase in daily total energy expenditure, part of which is mediated by increased physical activity when the mice are fed a high fat diet (38). The mechanism for the increased energy expenditure is unknown, but the Dgat−/− phenotype indicates that the disruption of triglyceride synthesis significantly affects energy metabolism.

**ACAT and DGAT Enzymes as Pharmaceutical Targets**

Because ACAT enzymes participate in cholesterol ester synthesis for assembly of atherogenic apoB-containing lipoproteins (59, 60) and in macrophage foam cell formation, ACAT inhibitors have been developed as potential therapeutics (61–64). These agents produced mixed results in several animal models. In cholesterol-fed rabbits and hamsters, ACAT inhibitors reduced atherosclerotic lesion development independently of their effects on serum cholesterol levels (57, 65–67). A study of genetic models of hypercholesterolemia in mice and rabbits suggested that ACAT inhibitors either had no effect or increased aortic sinus lesion size (68). One inhibitor (avasimibe) reduced atherosclerosis in several animal models (57, 69) and is being evaluated in clinical trials. This compound, like most currently available ACAT inhibitors, is not selective for one of the two ACAT enzymes (31, 69). This pan-specificity may be desirable because pharmacologic (68) and genetic studies (56, 58) in animals suggest that specific inhibition of macrophage ACAT1 may increase lesion size, possibly because of toxicity from

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**Table I**

*Properties of ACAT/DGAT enzymes*

| Enzyme | Gene | Chromosome | Number of exons | Number of amino acids | Substrates | Tissues with high levels of expression |
|--------|------|------------|----------------|----------------------|------------|--------------------------------------|
| ACAT1  | Scao1 | 1q25       | 16             | 550                  | Cholesterol and various oxysterols | Wide range (includes 16:0, 18:1, 18:2, and 20:4) | Adrenal gland, steroidogenic tissues, macrophages, preputial gland (mouse), sebaceous glands, liver (human) |
| ACAT2  | Stat1 | 1          | NR            | 540                  | Cholesterol and various oxysterols | Wide range (includes 16:0, 18:1, and 18:2) | Liver, small intestine |
| DGAT   | Dgat | 8qter       | 15             | 522                  | Cholesterol and various oxysterols | Wide range (includes 16:0, 18:1, and 18:2) | Liver, small intestine, adipose tissue, mammary gland, many others (lower mRNA levels) |

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* NR, not reported.

* R. V. Farese, Jr., unpublished observations.

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**Fig. 2. Alignment of human ACAT1, ACAT2, and DGAT proteins.** Conserved residues are shown in color. Asterisks indicate single, fully conserved residues; colons indicate strongly conserved residues; periods indicate weakly conserved residues.
excess free cholesterol. Selective inactivation of ACAT1 in hypercholesterolemia may also have detrimental consequences (see above). Therefore, selective ACAT1 inhibition should be approached cautiously in humans (70). Partial inhibition of both ACAT enzymes may be a preferable strategy (71). A selective inhibitor of ACAT2 may be useful for preventing diet-induced hypercholesterolemia (34, 72).

DGAT inhibitors as possible therapeutic agents for obesity. Several recent findings in Dgat−/− mice (38) have sparked interest in DGAT inhibitors as possible therapeutic agents for obesity. Several naturally occurring compounds have been identified (73–75).

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