Acetyl-Coenzyme A Carboxylases: Versatile Targets for Drug Discovery

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Abstract Acetyl-coenzyme A carboxylases (ACCs) have crucial roles in fatty acid metabolism in humans and most other living organisms. They are attractive targets for drug discovery against a variety of human diseases, including diabetes, obesity, cancer, and microbial infections. In addition, ACCs from grasses are the targets of herbicides that have been in commercial use for more than 20 years. Significant progresses in both basic research and drug discovery have been made over the past few years in the studies on these enzymes. At the basic research level, the crystal structures of the biotin carboxylase (BC) and the carboxyltransferase (CT) components of ACC have been determined, and the molecular basis for ACC inhibition by small molecules are beginning to be understood. At the drug discovery level, a large number of nanomolar inhibitors of mammalian ACCs have been reported and the extent of their therapeutic potential is being aggressively explored. This review summarizes these new progresses and also offers some prospects in terms of the future directions for the studies on these important enzymes. J. Cell. Biochem. 99: 1476–1488, 2006. © 2006 Wiley-Liss, Inc.

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Acetyl-CoA carboxylase (ACC) catalyzes the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA [Kim, 1997; Harwood, 2005; Tong, 2005]. This reaction, which proceeds in two half-reactions, a biotin carboxylase (BC) reaction and a carboxyltransferase (CT) reaction (Fig. 1A), is the first committed step in fatty acid biosynthesis and is the rate limiting reaction for the pathway [Kim, 1997; Harwood, 2005; Tong, 2005]. In humans and other animals, ACC activity is tightly regulated through a variety of dietary, hormonal, and other physiological responses that proceed via feed-forward allosteric activation by citrate, feedback inhibition by long-chain fatty acids, reversible phosphorylation and inactivation, and modulation of enzyme production through altered gene expression [Kim, 1997; Harwood, 2005; Tong, 2005; Brownsey et al., 2006].

In addition to its role as a substrate in fatty acid biosynthesis, malonyl-CoA, the product of the ACC-catalyzed reaction, also plays an important regulatory role in controlling mitochondrial fatty acid uptake through allosteric inhibition of carnitine palmitoyltransferase I (CPT-I), the enzyme catalyzing the first committed step in mitochondrial fatty acid oxidation [McGarry and Brown, 1997]. Malonyl-CoA, therefore, is a key metabolic signal for the control of fatty acid production and utilization in response to dietary changes and altered nutritional requirements in animals, for example during exercise, and therefore plays a key role in controlling the switch between carbohydrate and fatty acid utilization in liver and skeletal muscle [Harwood, 2005]. Malonyl-CoA may also act centrally to control food intake through hypothalamic neuropeptide Y production [Lane et al., 2005], and may play an important role in controlling insulin secretion from the pancreas [An et al., 2004; Ruderman and Prentki, 2004], further coordinating the regulation of intermediary metabolism.

In humans and other animals, ACC exists as two tissue-specific isozymes, a 265 KDa isozyme (ACC1) present in lipogenic tissues (liver, adipose) and a 280 KDa isozyme (ACC2) present...
in oxidative tissues (liver, heart, skeletal muscle) [Bianchi et al., 1990; Kim, 1997]. ACC1 and ACC2, which are encoded by separate genes [Widmer et al., 1996; Kim, 1997] and display distinct cellular distributions [Abu-Elheiga et al., 2001, 2003] and $K_m$ values for acetyl-CoA and citrate [Bianchi et al., 1990], share 75% overall amino acid sequence identity except for an extra 114 amino acids in the N-terminus of ACC2, the first 20 of which are thought to direct through the actions of ACC2 acts as an allosteric inhibitor of CPT-I to prevent entry of fatty acids into the mitochondria for oxidation. In the postprandial state, where excess acetyl-CoA formation from dietary sources leads to increases in both ACC1-mediated and ACC2-mediated malonyl-CoA production, simultaneous increases in fatty acid synthesis and reductions in fatty acid oxidation result in a net storage of energy as fat. In the fasted state and during exercise, where acetyl-CoA availability from dietary sources is limited, reductions in both ACC1-mediated and ACC2-mediated malonyl-CoA production lead to simultaneous reductions in fatty acid synthesis and increases in fatty acid oxidation, resulting in a net utilization of stored fat for energy.
ACC2 to the mitochondrial membrane [Abu-Elheiga et al., 2000]. ACC1, which lacks this targeting sequence, is localized to the cytosol [Abu-Elheiga et al., 2000]. In the heart and skeletal muscle, which have a limited capacity to synthesize fatty acids, the malonyl-CoA formed by ACC2 functions primarily as a regulator of fatty acid oxidation through CPT-I inhibition [Harwood, 2005]. In the liver, the malonyl-CoA formed in the cytosol through the actions of ACC1 is utilized for fatty acid synthesis and elongation leading to triglyceride formation and VLDL production whereas the malonyl-CoA formed at the mitochondrial surface by ACC2 acts primarily in the regulation of mitochondrial fatty acid oxidation (Fig. 1B) [Harwood, 2005]. This compartmentalization of malonyl-CoA results from a combination of synthesis proximity [Abu-Elheiga et al., 2005] and the rapid action of malonyl-CoA decarboxylase [Cheng et al., 2006].

ACCs are also found in most other living organisms [Tong, 2005]. While most of the eukaryotic ACCs are large, multi-domain enzymes, bacterial ACCs are multi-subunit enzymes. Significant sequence homology can be recognized between the BC subunits of bacterial ACCs and the BC domains of eukaryotic ACCs. In comparison, the sequence conservation of the CT component of these enzymes is much weaker. Biotin is covalently attached to a lysine residue in the biotin-carboxyl carrier protein (BCCP) component in these enzymes.

**RATIONALE FOR ACC INHIBITION AS A THERAPEUTIC TARGET**

**Obesity, Diabetes, Dyslipidemia, and the Metabolic Syndrome**

As a result of its unique position in intermediary metabolism (Fig. 1B), inhibition of ACC offers the ability to inhibit de novo fatty acid production in lipogenic tissues (liver and adipose) while at the same time stimulate fatty acid oxidation in oxidative tissues (liver, heart, and skeletal muscle) and therefore offers an attractive modality for favorably affecting, in a concerted manner, a multitude of cardiovascular risk factors associated with obesity, diabetes, insulin resistance, and the metabolic syndrome. Indeed, several lines of evidence have recently emerged that strongly support the concept of direct inhibition of ACC activity as an important therapeutic target.

Studies by Wakil and coworkers [Abu-Elheiga et al., 2001, 2003; Oh et al., 2005] demonstrated that ACC2 knock-out mice exhibited reduced skeletal and cardiac muscle malonyl-CoA, increased muscle fatty acid oxidation, reduced hepatic fat, reduced total body fat, elevated skeletal muscle UCP3 (indicative of increased energy expenditure), reduced body weight, reduced plasma free fatty acids, reduced plasma glucose, and reduced tissue glycogen, and are protected from diet-induced diabetes and obesity.

Studies by Shulman and colleagues [Savage et al., 2006] using ACC1 and ACC2 antisense oligonucleotides demonstrated stimulation of fatty acid oxidation in isolated rat hepatocytes and in rats fed high-fat diets, and lowering of hepatic triglycerides, improvements in insulin sensitivity, reductions in hepatic glucose production, and increases in UCP1 mRNA in high fat-fed rats that were greater when both ACC1 and ACC2 expression were suppressed than when either ACC1 or ACC2 expression alone was suppressed.

Studies by Harwood et al. [2003] demonstrated that the isozyme-nonselective ACC inhibitor, CP-640186, which equally inhibits rat, mouse, monkey, and human ACC1 and ACC2 (IC_{50} ~60 nM) without inhibiting either pyruvate carboxylase or propionyl-CoA carboxylase, reduced fatty acid synthesis, triglyceride synthesis, and triglyceride secretion in Hep-G2 cells without affecting cholesterol synthesis, and reduced apoB secretion without affecting apoA1 secretion. CP-640186 also stimulated fatty acid oxidation in C2C12 cells and in rat muscle slices and increased CPT-I activity in Hep-G2 cells. In experimental animals CP-640186 acutely reduced malonyl-CoA concentration in both lipogenic and oxidative tissues in both the fed and fasted state, reduced liver and adipose tissue fatty acid synthesis, and increased whole body fatty acid oxidation. In sucrose-fed rats treated with CP-640186 for 3 weeks, CP-640186 dose-dependently reduced liver, muscle and adipose triglycerides, reduced body weight due to selective fat reduction without reducing lean body mass, reduced leptin levels, reduced the hyperinsulinemia produced by the high sucrose diet without changing plasma glucose levels, and improved insulin sensitivity.

Recent studies by Ruderman and colleagues [Saha et al., 2006] demonstrated stimulation of
insulin sensitivity in insulin-resistant rat muscle tissue by CP-640186 within 30 min of compound administration, and recent studies by Furler et al. [2006] demonstrated, using dual tracer analysis, that acute (46 min) treatment of rats with CP-640186 resulted in stimulation of fatty acid clearance without decreasing glucose clearance.

Cancer, Fungal Infections, and Bacterial Infections

In addition to its potential to modulate intermediary metabolism and to favorably affect a variety of cardiovascular risk factors associated with obesity, diabetes, insulin resistance, and the metabolic syndrome, ACC inhibitors also have the potential to intervene in the progression of diseases that result from the rapid growth of malignant cells or invading organisms that are dependent on endogenous lipid synthesis to sustain their rapid proliferation. The potential of ACC inhibitors as antifungal agents [Gerth et al., 2003] and as antibacterial agents [Freiberg et al., 2004] is well documented, as is the high degree of expression of both ACC and fatty acid synthase in, for example, human breast cancer cells and in breast carcinomas [Sinilnikova et al., 2004; Brusselmans et al., 2005; Moreau et al., 2006].

ACC Inhibitors in Agricultural Applications

The plastid ACCs of grasses are the targets of two classes of compounds, aryloxyphenoxypropionates (FOPs or APPs) and cyclohexanediones (DIMs or CHDs) [Tong, 2005]. These compounds are potent inhibitors of their target ACC enzymes, and have been in commercial use as herbicides for more than 20 years. Single-site mutations in the CT domain of these enzymes can confer resistance to the herbicides, including an Ile to Leu mutation. This is a subtle change in the side chain of the residue, and suggests that herbicide sensitivity can be controlled by delicate structural details.

MAMMALIAN ACC INHIBITORS

A variety of structurally diverse ACC inhibitors have recently been described in the scientific and patent literature. These can be divided broadly into three classes based on their modes of inhibition and the characteristics of their interaction with the enzyme. The structural, biochemical, and pharmacologic characteristics of these inhibitors have recently been reviewed [Harwood, 2005].

The first class consists of lipophilic fatty acid mimetics whose inhibitory actions may be dependent on their intracellular conversion to their CoA thioesters. Compounds in this class inhibit ACC activity by competing with acetyl-CoA in the CT reaction of the enzyme and include the aryloxyphenoxypropionate and cyclohexanedione herbicides (e.g., haloxyfop and sethoxydim; structures are shown in Fig. 2), TOFA (5-(tetradecyloxy)-2-furancarboxylic acid),

![Chemical structures of selected ACC inhibitors.](Fig. 2)
MEDICA 16 (\(\beta,\beta,\beta',\beta'-\text{tetramethylhexadecanoic acid}\)), ESP-55016 (8-hydroxy-2,2,14,14-tetramethylpentadecanedioc acid), and S2E ((+)-p-[1-p-tert-butylphenyl]-2-oxo-4-pyrrolidinyl methoxybenzoic acid). The biochemical and pharmacological characteristics of these inhibitors are described in detail in a recent review [Harwood, 2005].

The second class of ACC inhibitors consists of the substituted bipiperidylcarboxamides (e.g., CP-640186; structure shown in Fig. 2) [Harwood et al., 2003, 2005; Perry et al., 2004]. These compounds are potent, reversible, isozyme-nonselective inhibitors of the CT reaction [Harwood et al., 2003; Zhang et al., 2004a], interacting within the enzyme active center in a region near the binding site for the (carboxy) biotin moiety [Zhang et al., 2004a; Tong, 2005].

The third class of ACC inhibitors consists of polyketide natural product fungicides (e.g., soraphen A; structure shown in Fig. 2) that inhibit the BC activity of fungal and mammalian ACCs by interacting with the enzyme at an allosteric site, approximately 25 Å from the ATP-binding site of the BC active center [Shen et al., 2004], suggesting that soraphen A may inhibit ACC activity by interfering with dimerization/oligomerization of the BC domain.

A bi-substrate analog CT inhibitor prepared by covalently linking biotin to CoA via an acyl bridge between the thiol of CoA and the 1\(^{0}\)-N of biotin [Levert and Waldrop, 2002] and several series of acylsulfonamides [Harwood, 2005] have also been described. A recent report suggests that pyridoxal phosphate (PLP) may be a weak inhibitor of mammalian ACCs, possibly through competition with the binding of the activator citrate [Lee et al., 2006].

**STRUCTURAL INFORMATION ON ACC**

**Biotin Carboxylase (BC)**

The structure of BC contains three domains, A, B, and C (Fig. 3A), and has the ATP-grasp fold [Waldrop et al., 1994; Thoden et al., 2000; Kondo et al., 2004; Shen et al., 2004; Tong, 2005]. The eukaryotic BC domain also contains a linker segment between the A and B domains (AB linker) [Shen et al., 2004]. The A and C domains (and the AB linker) form a cylindrical structure, and the B domain is a lid at one end of this cylinder.

The active site of the enzyme is located at the interface between the B domain and the cylinder (A and C domains), as ATP has been observed to bind in this region (Fig. 3A) [Thoden et al., 2000]. The B domain may undergo a large conformational change during catalysis by the enzyme. The free enzymes of bacterial BC subunits are observed in an open conformation for the B domain, whereas the structure in complex with ATP has a closed conformation for this domain. In comparison, the free enzyme of yeast BC domain is observed in the closed conformation for the B domain. The closed conformation, in the presence of substrates, may be catalytically competent, whereas the open conformation for the B domain may be important for substrate binding or product release.

The adenine base of ATP is recognized specifically by BC, through hydrogen-bonding to its N1 and N6 atoms. Amino acid residues in the linker between the B and C domains are involved in this recognition. The bound position of ATP was observed in a mutant of BC, Glu288Lys, which is catalytically inactive [Thoden et al., 2000], although it may be expected that the bound conformation to the wild-type enzyme is similar. On the other hand, the binding sites for the bicarbonate and biotin (BCCP) substrates of BC have not been identified.

The bacterial BC subunits are dimeric in solution, and the crystal structures confirm this dimeric association and show that the active sites in the two monomers are about 25 Å from the dimer interface (Fig. 3B). It has been suggested that dimerization is required for the catalytic activity of the enzyme. The strongest evidence for this comes from studies of hetero-dimers of the *E. coli* BC subunit, where one monomer is wild-type and the other carries an inactivating mutation in the active site [Janiyani et al., 2001]. Such hetero-dimers have only about 1% of the catalytic activity compared to the wild-type dimer, suggesting that catalysis at the two active sites may be coupled and that there may be long-range communication between them (although BC does not exhibit cooperativity).

However, the experiments with the hetero-dimers still examined the catalysis by BC in its dimeric form. To directly address whether the monomeric form of BC is active, single-site mutations were introduced in the dimer interface of the *E. coli* BC subunit [Shen et al., 2006]. Two of these mutants, R19E and E23R, have
greatly increased $K_d$ values for the dimer, such that they are monomeric in solution at micromolar concentrations. Kinetic studies show however that these mutants still have robust catalytic activity, demonstrating that dimerization is actually not absolutely required for the activity of BC. These results lead to a new model for the catalytic activity of BC (Fig. 3C). While there may be long-range communication between the two active sites in the dimer, this
communication can be decoupled and monomers of BC are active. At the same time, the conformation of the dimer interface appears to have an important (indirect) impact on the catalysis. The yeast BC domain has large conformational differences for residues in this region as compared to the bacterial BC subunits (Fig. 3C), which may explain why this domain is monomeric in solution and catalytically inactive [Shen et al., 2004; Weatherly et al., 2004].

The eukaryotic BC domains are the targets of the potent natural product inhibitor soraphen A [Gerth et al., 2003; Weatherly et al., 2004]. The structure of yeast BC domain in complex with this compound revealed that it is bound in an allosteric site, at the opposite end of the cylinder and about 25 Å from the active site of the enzyme (Fig. 3A) [Shen et al., 2004]. Soraphen A is located in a depression on the surface of the BC domain, with half of its surface exposed to the solvent (Fig. 3D). Mutations of residues in the binding site can reduce the affinity of BC for this natural product, confirming the observed binding mode and also explaining the molecular basis for soraphen A resistance mutations [Shen et al., 2004].

Structural and sequence differences between the eukaryotic and bacterial BC are the molecular basis for the specificity of soraphen A towards eukaryotic BC domains. The binding site of soraphen A in yeast BC domain is equivalent to the dimer interface in the bacterial BC subunits. There are significant structural differences between the two enzymes in this region. In addition, residues in the soraphen A binding site are not conserved in bacterial BC subunits. The overall effect of these differences is that the soraphen A binding site does not exist in the bacterial BC subunits.

Soraphen A may have a novel mechanism for inhibiting the activity of the BC domain, although the molecular details of which are still not completely clear. Soraphen A binding does not appear to cause any significant conformational changes in the BC domain. Instead, soraphen A may stabilize a conformation of the BC enzyme that is incompatible with catalysis (Fig. 3C). Moreover, the soraphen A binding site may also mediate the interactions of the BC domain with other components of the multi-domain ACC enzyme (including possibly homodimerization), suggesting that soraphen A may also be a protein–protein interaction inhibitor. This is supported by the observation that soraphen A promotes the dissociation of oligomers of yeast BC domain and full-length ACC in native gel experiments [Shen et al., 2004].

**Carboxyltransferase (CT)**

The structures of the CT domain of yeast ACC [Zhang et al., 2003, 2004a,b] and the CT subunit of bacterial ACC [Bilder et al., 2006] are now available, as well as the CT component of several related enzymes [Tong, 2005]. The CT structures contain two central domains (N and C domains) with the same backbone fold (the β-β-α superhelix), and may also have additional, smaller modules on the surface (Fig. 4A). CT is a dimer and the two monomers of the dimer are arranged in a head-to-tail fashion, such that the N domain of one monomer is in contact with the C domain of the other monomer (Fig. 4A).

In some related CT enzymes, this dimer unit can be assembled into hexamers. The active site of the CT enzyme is located at the dimer interface. Therefore, CT can only be active as a dimer. There is a canyon in the active site of CT (Fig. 4B), with the walls of the canyon formed by an α-helix from each of the monomers. The acetyl-CoA substrate is mostly associated with the N domain of one monomer, using the bottom half of the canyon, although the acetyl portion is expected to be located right at the dimer interface (Fig. 4B). The biotin substrate is believed to be mostly associated with the C domain of the other monomer in the dimer, and approaches the active site from the top half of the canyon [Tong, 2005].

The CT domain is targeted by the commercial herbicides (FOPs and DIMs) as well as the potent inhibitor of mammalian ACCs, CP-640186 [Harwood, 2005]. The molecular basis for the inhibitory activity of the FOPs and CP-640186 has been established [Zhang et al., 2003, 2004a,b; Tong, 2005]. FOPs (e.g., haloxyfop) bind in a pocket close to the active site of CT, and are competitive with malonyl-CoA. The first aromatic ring of FOPs shows π-stacking interactions with two aromatic side chains of the CT domain, one from each monomer (Fig. 4C). The phenyl ring in the center of the inhibitors is sandwiched between two amide planes of the enzyme, and the propionate carboxylate is hydrogen-bonded to two main-chain amide groups (Fig. 4C). Mutations that confer resistance to the herbicides are located either in this pocket or are close to it. Intriguingly, the formation of the binding pocket for the FOPs
requires a large conformational change for several residues in the dimer interface, as well as a slight reorganization of the dimer [Zhang et al., 2003, 2004a,b]. The N domains of the two monomers are colored in cyan and magenta, and the C domains are colored in yellow and green. The positions of CoA (gray), haloxyfop (black), and CP-640186 (gold) are shown. This suggests that conformational variability in the active site region could also play an important role, in addition to amino acid sequence differences, in determining herbicide sensitivity of various CT (ACC) enzymes. Different ACCs may have distinct propensities for the conformational change that is required for FOPs binding.

In comparison, the anthracene ring of CP-640186 is bound in the canyon at the active site, and the rest of this compound associates with the C domain of the other monomer. This suggests that CP-640186 is probably located in the biotin binding site, consistent with kinetic observations that it is non-competitive versus malonyl-CoA. The two carbonyl oxygens of the inhibitor are both involved in hydrogen-bonding interactions with the enzyme (Fig. 4D). In contrast to the FOPs, binding of CP-640186 does not cause any significant changes in the structure of CT.

Remarkably, the bound positions of haloxyfop, CP-640186 and CoA have essentially no overlap among them [Tong, 2005], although the proximity of the carboxylate residue of haloxyfop and the sulphydryl residue of CoA are consistent with the potent inhibitory properties...
of the FOP CoA thioesters [Harwood, 2005]. Potent inhibitors have been identified against both the FOP and the CP-640186 binding sites, suggesting that there may be several ways of achieving high-affinity interactions with the CT active site. This may hold great promise for the development of new ACC inhibitors targeting this site.

**STRUCTURAL PROSPECTS**

**Biotin Carboxylase (BC)**

To fully understand the catalysis by the BC enzyme, structural information on the substrate (or product) complexes of the enzyme will be crucial. While the ATP binding mode is known, it is not clear how the bicarbonate and the biotin (BCCP) substrate (or the putative carboxyphosphate intermediate) binds to the enzyme.

Structural information on the substrate complexes will also help to elucidate the molecular mechanism for the long-range communication between the dimer interface and the active site of BC. It might be possible that there are additional conformational changes in the enzyme upon bicarbonate/biotin binding or during catalysis, which indirectly affect the conformation of residues in the dimer interface. Understanding this mechanism will further clarify why the eukaryotic BC domains are inactive, as well as how soraphen A inhibits the activity of eukaryotic BC.

**Carboxyltransferase (CT)**

Four different classes of inhibitors are currently known against the CT domain, three of which interact with the eukaryotic CT domain (FOPs, DIMs, CP-640186) and one of which interacts with the bacterial CT subunit (moiramide B) (Fig. 2). The binding modes of the FOPs and CP-640186 have been elucidated. It will be desirable to establish the molecular mechanism of action of the other two classes of compounds. The recent publication of the crystal structures of bacterial CT subunits [Bilder et al., 2006] should enable the determination of the moiramide B complex structures.

Although the binding mode of FOPs to the CT domain has been established, the molecular mechanism of how resistance mutations disrupt their binding is still not fully understood. One way to further elucidate this mechanism is to determine the structure of the CT domain of grass ACCs in complex with the compounds. Studying the grass CT domain may also help define the binding mode of the DIMs, as it appears that these compounds have essentially no inhibitory activity against the yeast CT domain.

**Human ACC Structures**

The human ACCs are the direct targets for the development of antiobesity and antidiabetes agents. Therefore, structural information on these enzymes, including the BC and CT domains, will be important for the drug discovery efforts. Unfortunately, no crystals of any of these domains have so far been reported. In the absence of structural information on the human enzymes, yeast BC and CT domains can be used as surrogates for defining the binding modes of newly identified inhibitors.

**Molecular Mechanism for the Acute Regulation of ACCs**

Eukaryotic ACCs are inhibited by phosphorylation [Kim, 1997], although the molecular mechanism for this regulation is not known. The sites of phosphorylation in animal ACCs have been determined [Kim, 1997]. Phosphorylation of a Ser residue just prior to the BC domain in mammalian ACCs can inhibit the enzyme. In addition, phosphorylation of residues between the BC and the CT domains of mammalian ACCs can also inhibit the enzyme. In fact, the primary sequences of eukaryotic ACCs can be divided into three parts. The N-terminal one-third contains the BC and BCCP domains, and the C-terminal one-third contains the CT domain. The middle one-third of the enzyme appears to be unique to ACC, but the function(s) of this region is not known, except that several phosphorylation sites are located here.

Structural studies are also needed to elucidate the molecular mechanism for the activation of animal ACCs by citrate. Such structural information may also help define the mechanism of the inhibitory activity of PLP.

**Holo Enzymes of ACCs**

Bacterial ACC functions as a holo enzyme composed of BC, BCCP, and CT subunits. Therefore, a complete understanding of this enzyme would require structural information on this complex. Eukaryotic ACCs are much
larger in size (250 kDa), and many of them form oligomers. Such a large complex probably excludes its study in sufficient molecular detail by crystallographic methods. At the same time, the large sizes of these proteins make them suitable for cryo electron microscopy (EM) studies. By combining low-resolution information from cryo EM with the atomic models of the various domains from crystallography, a useful model for the eukaryotic ACC enzyme could be constructed.

**PHARMACOLOGICAL PROSPECTS**

*Isozyme-Selective versus Isozyme-Nonselective ACC Inhibition*

It is anticipated that an isozyme-nonselective ACC inhibitor should be superior to either an ACC1-selective or an ACC2-selective inhibitor because of its simultaneous actions in tissues that synthesize and oxidize fat. The efficacy of an ACC2-selective inhibitor in stimulating fatty acid oxidation in liver and muscle tissue would be anticipated to be lessened due to compensatory increases in hepatic and adipose fatty acid synthesis [Abu-Elheiga et al., 2005]. Similarly, the efficacy of an ACC1-selective inhibitor would be anticipated to be lessened due to restriction of its effects to inhibition of fatty acid synthesis and to fatty acid synthesizing tissues [Abu-Elheiga et al., 2005; Oh et al., 2005].

Indeed, studies with CP-640186 [Harwood et al., 2003, 2005] and studies with combined ACC1 + ACC2 antisense oligonucleotides [Savage et al., 2006] confirm the hypothesis that isozyme-nonselective ACC inhibition can lead to reduced fatty acid synthesis in lipogenic tissues that express ACC1 and simultaneously to increased fatty acid oxidation in oxidative tissues that express ACC2, and can ultimately lead to reductions in tissue fat content, thereby fostering weight loss and improvements in insulin sensitivity. Furthermore, studies in ACC2 knockout mice that retain a fully functional ACC1 gene locus do demonstrate a propensity for compensatory increases in fatty acid synthesis that could attenuate ACC2 inhibition-mediated efficacy [Abu-Elheiga et al., 2001] and studies in which either ACC1 or ACC2 expression alone was suppressed demonstrated a substantially lesser degree of efficacy than when both ACC1 and ACC2 expression were simultaneously attenuated [Savage et al., 2006].

However, studies in ACC2 knockout mice [Abu-Elheiga et al., 2001, 2003; Oh et al., 2005] and in mice treated with either ACC1 or ACC2 antisense oligonucleotides [Savage et al., 2006] did confirm efficacy of ACC1-selective and ACC2-selective inhibition, indicating the potential of ACC isozyme-selective inhibitors also to favorably affect the cardiovascular risk factors associated with obesity, diabetes, insulin resistance, and the metabolic syndrome. Whether an ACC1-selective or an ACC2-selective inhibitor would be sufficiently efficacious to be useful therapeutically remains to be determined. However, the potential does exist to use isozyme-selective inhibitors as therapy to target specific tissues to the exclusion of others if the need would arise.

**Can Isoform Selectivity Be Achieved?**

Although the actions of isozyme-selective inhibitors have not yet been disclosed, isozyme-specific differences in K_m values for acetyl-CoA and citrate [Bianchi et al., 1990] suggest that identification of such inhibitors may be possible and should allow the full potential of such inhibitors to be evaluated experimentally. At the same time, the two isozymes share substantial amino acid sequence identity, especially in the active site regions of the BC and CT enzymes, and therefore the development of such isozyme-selective inhibitors could be a demanding process. However, observations from the herbicide resistance mutations, where a subtle change from Ile to Leu is sufficient to confer resistance, suggest that inhibitor sensitivity could be determined by delicate structural differences. These differences could be due to amino acid substitutions directly in the binding site or to conformational changes effected by amino acid changes in other parts of the enzyme. Inhibitors that can recognize such differences could gain selectivity between the two isozymes.

**Can Inhibitors Be Developed that Target the Active Site of BC?**

While several classes of inhibitors have been identified that target the active site of the CT enzyme, currently no potent inhibitors of the BC active site are known. The structural information suggests that such inhibitors could be possible, targeting either the ATP or the biotin-binding site (or both at the same time). One potential concern of inhibitors targeting
the ATP binding site could be specificity versus enzymes and receptors that contain nucleotide-binding sites (e.g., kinases, dehydrogenases, G protein-coupled receptors, etc). However, the fact that none of the large collection of protein kinase inhibitors currently available has been reported to inhibit BC suggests that achieving such specificity might not be insurmountable.

**Can the Soraphen A Binding Site Be Used for Drug Development?**

The potency and mechanism of ACC inhibition by soraphen A [Harwood, 2005] suggests that targeting the soraphen A binding site pharmacologically may be a useful avenue for identifying potent, efficacious ACC inhibitors. In this regard, recently, Elich [2006] described the identification of a small molecule ACC inhibitor, CS1880 (IC\(_{50} = 9 \mu M\)), that interacts with the BC domain of yeast ACC in the same manner as soraphen A to prevent subunit dimerization.

**Prospects for Development of ACC Inhibitors for Human Therapy**

Although the clinical efficacy profile of an ACC inhibitor is unprecedented, demonstrating clinical efficacy of an ACC inhibitor should be relatively straightforward. Based on demonstrated tissue fat reductions, weight loss, improved insulin sensitivity, and improvements in dyslipidemia observed in ACC2 knockout mice [Abu-Elheiga et al., 2001, 2003; Oh et al., 2005] and in experimental animals following treatment with isozyme-nonselective ACC inhibitors [Harwood et al., 2003, 2005] and ACC antisense oligonucleotides [Savage et al., 2006], efficacy evaluation in the clinic could focus initially on approval endpoints for obesity and dyslipidemia with demonstration of improvements in insulin sensitivity and reductions in hyperinsulinemia and hypertension giving a more complete picture of the therapeutic potential of an ACC inhibitor. Initial indication that a compound was inhibiting ACC could be obtained by measuring acute reductions in skeletal muscle malonyl-CoA from biopsy samples. Indirect calorimetry, oral glucose tolerance tests, improvements in lipid profiles, and decreased blood pressure could be assessed in 2–4 week Phase II trials. Longer-term clinical studies could assess weight loss, percentage body fat, and regional fat distribution. In late development, primary prevention of coronary heart disease (CHD) and type 2 diabetes (T2DM) or other significant health outcomes related to metabolic syndrome also could be assessed.

However, several potential hurdles will need to be overcome in the development of an ACC inhibitor for human therapy. First, recent studies have implicated hypothalamic malonyl-CoA in the control of feeding behavior through its actions as a negative regulator of food intake [Lane et al., 2005], suggesting that reduction in malonyl-CoA levels in the hypothalamus may be undesirable. Consistent with these observations, ACC2 knockout mice consumed more food even though they weighed less than wild-type animals [Abu-Elheiga et al., 2001, 2003] and ob/ob mice (but not chow-fed or high carbohydrate-fed rats) treated with CP-640186 consumed more food concomitant with their weight loss [Harwood, 2005]. Thus, the potential exists for weight reduction in clinical trials with an ACC inhibitor to occur together with increased food consumption. Whether this partially abrogates the positive metabolic effects of an ACC inhibitor, especially for one that crosses the blood-brain barrier, remains to be determined.

Second, it has been suggested that malonyl-CoA plays an important role in controlling insulin secretion from the pancreas [An et al., 2004; Ruderman and Prentki, 2004] and that reducing malonyl-CoA levels via ACC inhibition in islets exposed to high levels of free fatty acids could offset beneficial effects on glucose-stimulated insulin secretion induced by ACC inhibition-mediated reduction in pancreatic \(\beta\)-cell fat content [Ruderman and Prentki, 2004]. Whether direct reduction of pancreatic \(\beta\)-cell malonyl-CoA levels after ACC inhibition adversely influences insulin secretion and whether it offsets beneficial effects of \(\beta\)-cell fat reduction on insulin secretion in the clinic remain to be determined.

Third, while ACC inhibition may favorably affect cardiac function in the aerobic, triglyceride-laden heart through reduction in triglyceride stores that have been associated with depressed contractility, arrhythmias, hypertrophy, and heart failure [Atkinson et al., 2003], a variety of reports, mostly from studies in ex vivo working hearts, have suggested that increased fatty acid oxidation during and after ischemia may contribute to contractile dysfunction and increase ischemic injury [Sambandam and
Lopaschuk, 2003]. Whether ACC inhibition could potentially lead to an increase in myocardial injury after an ischemic event and whether myocardial triglyceride reduction as a consequence of ACC inhibition would prevent the adverse effects associated with fatty acid oxidation enhancement in ischemia remains to be determined experimentally.

Finally, although studies outlined above in ACC2 knockout mice [Abu-Elheiga et al., 2001, 2003; Oh et al., 2005] and in experimental animals following treatment with isozyme-nonselective ACC inhibitors [Harwood et al., 2003, 2005; Furler et al., 2006] and with ACC antisense oligonucleotides [Savage et al., 2006] demonstrate the potential of an ACC inhibitor to favorably impact, in a concerted manner, the multitude of metabolic abnormalities associated with the metabolic syndrome and to limit the development of CHD and T2DM in these patients, metabolic syndrome is not currently a registerable endpoint. As a consequence, it will be necessary to gain registration for a currently approvable indication, (e.g., diabetes, obesity, dyslipidemia, or hypertension), and will likely also require cardiovascular outcomes and progression to diabetes trials to gain approval. Furthermore, the heterogeneity of the metabolic syndrome patient population and the absence of established guidelines regarding approval endpoint criteria for agents simultaneously affecting multiple aspects of metabolic syndrome will pose developmental challenges for initial market entries.

However, during the course of clinical evaluations, it is likely that prototypical ACC inhibitors will play critical roles in determining the metabolic syndrome patient sub-classification that is at the greatest risk of developing CHD and/or T2DM, and therefore is in the greatest need of therapeutic intervention, in establishing treatment criteria for individual patients within this heterogeneous patient population, and in defining the approvable endpoint parameters required for initial as well as subsequent perspective therapeutic modalities when more than one of the disorders associated with metabolic syndrome are treated simultaneously.

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REFERENCES


