

Structure, Mechanism, and Disease Implications of Acetyl CoA Carboxylase: A Mini-review

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Abstract: The ubiquitous acetyl-CoA carboxylase is a pivotal enzyme in the synthesis of fatty acids in both eukaryotes and prokaryotes. The importance of this enzyme is needed in the initiating reaction for synthesizing fatty acids, which are very important when used as fuel molecules and providing the building blocks of biological membranes. Acetyl-CoA carboxylase catalyzes the committed step in making fatty acids by converting acetyl-CoA into malonyl-CoA. With its three functional domains it is a key regulatory enzyme for fatty acid metabolism and its product is key in regulating fatty acid degradation. Its multidomain enzymatic functions are characterized by a biotin carboxylating activity, biotin binding, and a carboxyltransferase activity. At the basic research level, the crystal structures of each of these domains have been determined, and the molecular basis for acetyl-CoA carboxylase inhibition by small molecules is beginning to be understood. Although not much on a deficiency of the enzyme has been reported, there are some cases in which some combinations of the four known human carboxylating enzymes act collectively producing devastating symptoms in neonates and infants.

Introduction

Acetyl-Coenzyme A carboxylase (ACC) has a crucial role in fatty acid metabolism in humans and most other living organisms.¹ The metabolism of carbohydrates to that of fatty acids is biochemically important for several reasons. First, fatty acids are fuel molecules which provide the primary source of energy during rest or moderate exercise. The oxidation of triacylglycerols, which are the storage form of fatty acids, is an important step in meeting certain energy requirements of the cell. Another biochemical reason for the need in fatty acids is that they are building blocks of phospholipids which are important components of biological membranes. Still more basis for the importance of fatty acids include their attachment to proteins to direct them to the right locations within the cell as well as their ability to serve as hormones and other intracellular messengers. It is easy to see that the needs for these long hydrocarbon chains with their terminal carboxylate group, coined fatty acids, are very important for the

cells of an organism, and therefore, the need for ACC.

Acetyl-CoA carboxylase catalyzes the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA, which is the first committed step in fatty acid synthesis.¹ ACC is a multifunctional enzyme in that this conversion is accomplished via sequential half-reactions: the ATP-dependent carboxylation of enzyme-bound biotin followed by the transfer of the carboxy group to acetyl-CoA.² The BC (biotin carboxylase), BCCP (biotin carboxyl carrier protein) and CT (carboxyltransferase) functions are contained on a single, large (~250 kDa) multidomain polypeptide in the ACCs found in mammals, yeast, fungi, and plant cytosols.² These three enzyme domains are fused in eukaryotic ACCs to form one polypeptide and are separated in *Escherichia coli*, and other bacteria. In contrast, there are four polypeptide chains comprising the multisubunit enzyme of prokaryotic ACCs

with BC, BCCP, and two subunits making up the CT.³

ACC is also a key regulatory enzyme for fatty acid metabolism. It reacts to changes in its environment such as phosphorylation and dephosphorylation as well as allosteric regulation by nearby metabolites. This puts it as a pharmaceutical target for the treatment of obesity, Type II diabetes, cancer and microbial infections.³ In addition, the product of the carboxylase reaction plays a role in the regulation of fatty acid degradation.¹ Malonyl-CoA 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.¹

Structural Details

Acetyl-CoA carboxylase has a biotin requirement functioning as a coenzyme to carry CO₂ for the carboxylation reaction. The terminal carboxyl group of biotin is attached to the BCCP domain of the enzyme by an amide bond to the ε-amino group of a Lysine residue. As mentioned before, ACC contains three enzyme domains: biotin carboxylase activity, a biotin carboxyl carrier protein, and carboxyltransferase functionality.⁴ The overall fold of the BC domain can be thought of as three structural subdomains. These include A, B, and C. The N-terminal region, formed by Met 1-Ile 103, adopts a dinucleotide binding motif with five strands of parallel beta-sheet flanked on either side by alpha-helices.⁴ In addition to this major secondary structural element, the C-terminal domain also contains a smaller three-stranded antiparallel beta-sheet and seven alpha-helices.⁴ The active site is located between the B-domain and the A, C domains together, which is where ATP binds.¹ The amino acid residues believed to form the active site in the BC domain include His 209-Glu 211, His 236-Glu 241, Glu 276, Ile 287-Glu 296, and

Arg 338.²⁺⁴ The crystallized structure of the BC domain is shown in Figure 1 complexed as a dimer along with each of the representative subdomains labeled. The binding sites for the biotin and bicarbonate substrates have not been identified.

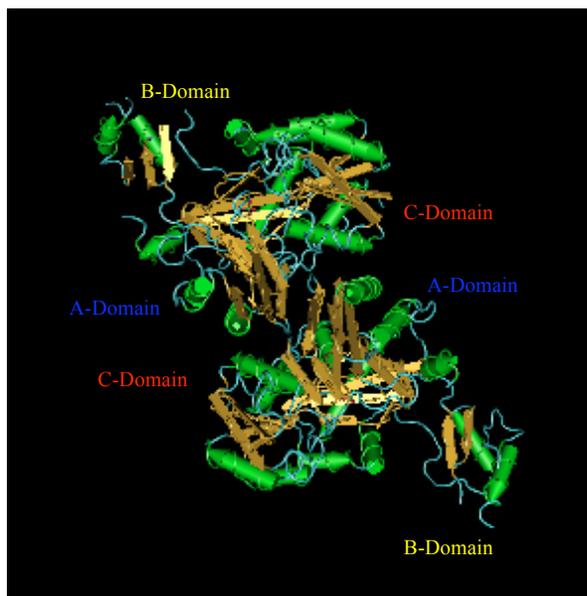


Figure 1. Three-dimensional structure of the biotin carboxylase (BC) subunit of acetyl-CoA carboxylase in *Escherichia coli*.⁴

The CT domain of ACC contains two central subdomains (N and C) with the same backbone fold, β - α superhelix, as that of the BC domain. CT is actually organized as a dimer but is shown as a single monomer in Figure 2. In most enzymes the two monomers align in a head-to-tail fashion so that the C-domain of one monomer meets the N-domain of the other but in other enzymes they may be arranged as a hexamer.¹ The active site of CT is located at the dimer interface which limits CT to only be in its active form as a dimer.¹ The acetyl-CoA substrate is mostly associated with the N domain of one monomer, although the acetyl portion is expected to be located right at the dimer interface. 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.¹

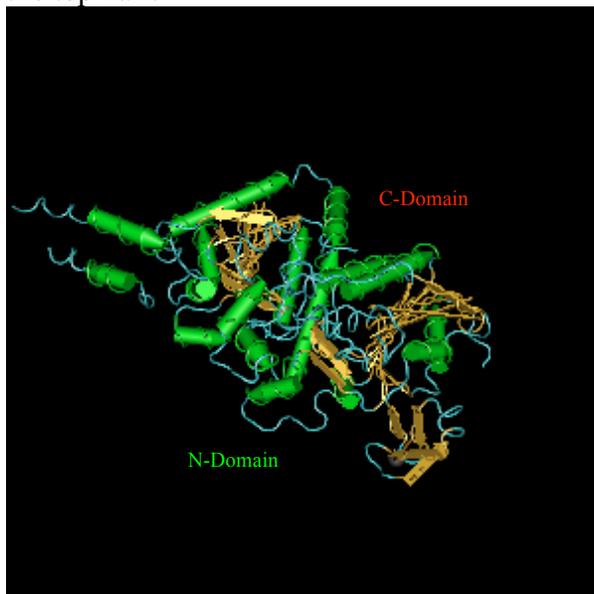


Figure 2. The structure of the carboxyltransferase (CT) component of acetyl-coA carboxylase in *Escherichia coli*.⁵

Although the structure of individual domains of ACC is known, the structure of the entire complex, ≈ 800 kDa is not. In humans, there are two isoforms of acetyl-CoA carboxylases, ACC1 and ACC2 of molecular mass 265 kDa and 280 kDa, respectively.⁶ ACC1 and ACC2 display distinct tissue distribution and are encoded by different genes as well. The cytosolic ACC1 is predominately the isoform present in lipogenic tissue such as liver, adipose tissue, and lactating mammary glands while the mitochondrial ACC2 is highly expressed in the skeletal muscle, heart, and liver.⁷ The cDNAs encoding the human ACC1 and ACC2 have been cloned and sequenced, and the predicted amino acid sequences revealed high homologies between the two isoforms except for the extra 114 amino acids present in the N terminus of ACC2. The first 20 amino acids of this extra peptide are highly hydrophobic, and they are responsible for guiding the ACC2 to the mitochondrial membrane. ACC1, on the other hand, lacks

the hydrophobic N-terminal peptide and was shown to be located in the cytosol.⁸

Enzyme Kinetics

ACC has the typical double-displacement kinetics (or ping-pong kinetics) as other biotin-dependent enzymes. It undergoes a two site mechanism in which the binding of a substrate is followed by release of that substrate only to be bound again by the enzymes second domain, then subsequently released as the new product. The enzyme exhibits K_m values of 0.14 ± 0.013 mM and 0.19 ± 0.041 mM for acetyl-CoA and ATP respectively.²

There have been a variety of diverse ACC inhibitors identified that fall under three broad classes. The first consists of lipophilic fatty acid mimetics which compete with acetyl-CoA in the CT reaction.¹ The second class of ACC inhibitors consists of the substituted bipiperidylcarboxamide which are isozyme-nonspecific inhibitors of the CT reaction.¹ The third class of ACC inhibitors consists of polyketide natural product fungicides like soraphen A, which is an allosteric inhibitor.¹ It has been suggested that soraphen A may inhibit ACC activity by interfering with dimerization/oligomerization of the BC domain.

Regulation

ACC, as mentioned above, is regulated by its immediate environment. It is inhibited by phosphorylation of a Serine residue just before the BC domain as well as at the site located between the BC and CT domains.⁹ AMP-dependent protein kinase is the kinase that inactivates ACC thus diminishing the production of fatty acids.¹⁰ Contrary to the inactivation of ACC, dephosphorylation activates ACC. This is done by a protein phosphatase. In addition,

ACC is also allosterically stimulated by citrate, a nearby metabolite. Citrate acts by polymerizing the inactive form of ACC thereby decreasing the inhibition opposed on the enzyme.¹¹ Counteracting citrate is palmitoyl CoA, which is abundant in excess of fatty acids. Palmitoyl CoA acts to disassemble the active form of ACC, thus diminishing the production of fatty acids.¹²

Hormones such as epinephrine, insulin and glucagon also regulate ACC.¹³ Glucagon and epinephrine switch off fatty acid synthesis by keeping ACC in its inactive state during periods of low energy.¹³ Fatty acids can't be synthesized when energy is low because it takes high energy concentrations to make them. Insulin, on the other hand, is present at high energy status and stimulates ACC by activating the phosphatase required to remove the phosphate off of inactive enzyme, thus increasing Malonyl-CoA synthesis.¹³

General Mechanism

ACC produces malonyl-CoA by using bicarbonate as a source for CO₂ and ATP as a source for energy.¹⁴ The two step reaction is first carried about by the BC domain in which the prosthetic biotin is carboxylated at the expense of a molecule of ATP.¹⁴ Biotin is covalently bound to the ACC enzyme at the terminal amino group of a lysine residue to the terminal carboxyl group of the biotin side chain. A carboxybiotin intermediate is formed in which the CO₂ is carried on active nitrogen. The second half of the reaction is carried out by the CT domain of ACC in which an acetyl-CoA carbanion performs nucleophilic attack on the carboxybiotin-enzyme.¹⁴ Once the CO₂ has been placed onto acetyl-CoA, the biotin prosthetic group on the ACC is regenerated and malonyl-CoA is formed. The reaction scheme for this simplified mechanism is shown in Figure 3.

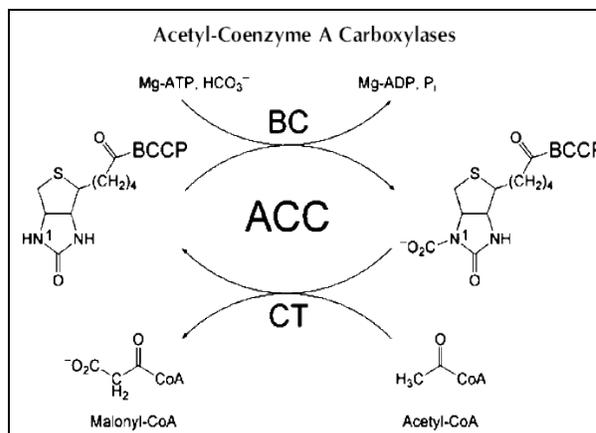


Figure 3. Reaction mechanism of Acetyl-CoA carboxylase from Tong and colleagues.¹

Disease Implications

There are four known human biotin-dependent carboxylase enzymes. These enzymes include acetyl-CoA carboxylase, pyruvate carboxylase (PC), propionyl-CoA carboxylase (PCC), and β -methylcrotonyl CoA carboxylase (β -MCC). There have been numerous case reports on metabolic disorders caused by deficiencies in the latter 3 mentioned above, but only one known report for ACC.¹⁵ The patient, a newborn female with hypotonic myopathy and neurologic damage, excreted urinary metabolites of hexanoic acid, including 2-ethyl-3-keto-hexanoic acid, 2-ethyl-3-hydroxy-hexanoic acid, and 2-ethyl-hexanedioic acid.¹⁵ The diagnosis was confirmed by finding deficient ACC activity in her liver compared to that in rat liver, and about 10% of normal ACC activity was found in her fibroblasts.¹⁵ In addition to deficiencies in each of the four individual known carboxylase enzymes, a disorder known as multiple carboxylase deficiency can also be discriminated in neonatal or infantile form.¹⁵

PCC, PC, and multiple carboxylase deficiencies are well characterized clinical diseases, whereas the delineations of isolated β -MCC and isolated ACC deficiency await

subsequent case studies. Although patients with the exact same enzyme deficiency may have variable clinical features, generally all of the carboxylase deficiencies are manifested in neonates, infants, or young children by feeding difficulties, hypertonia and lethargy associated with metabolic acidosis and excess serum and urine concentrations of respective organic acids.¹⁵ Most cases have improved clinically upon pharmacological doses of biotin.¹⁵

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