Appl Microbiol Biotechnol (2012) 93:983–992
DOI 10.1007/s00253-011-3796-z

MINI-REVIEW

Structure, function and selective inhibition of bacterial acetyl-coa carboxylase

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Received: 19 October 2011 / Revised: 21 November 2011 / Accepted: 24 November 2011 / Published online: 20 December 2011

Abstract Acetyl-CoA carboxylase (ACC) catalyses the first committed step in fatty acid biosynthesis: a metabolic pathway required for several important biological processes including the synthesis and maintenance of cellular membranes. ACC employs a covalently attached biotin moiety to bind a carboxyl anion and then transfer it to acetyl-CoA, yielding malonyl-CoA. These activities occur at two different subsites: the biotin carboxylase (BC) and carboxyltransferase (CT). Structural biology, together with small molecule inhibitor studies, has provided new insights into the molecular mechanisms that govern ACC catalysis, specifically the BC and CT subunits. Here, we review these recent findings and highlight key differences between the bacterial and eukaryotic isozymes with a view to establish those features that provide an opportunity for selective inhibition. Especially important are examples of highly selective small molecule inhibitors capable of differentiating between ACCs from different phyla. The implications for early stage antibiotic discovery projects, stemming from these studies, are discussed.

Keywords Acetyl-CoA carboxylase · Enzyme · Inhibition · Antibiotics · Fatty acid biosynthesis

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Introduction

De novo fatty acid biosynthesis is an important metabolic process ubiquitously performed throughout the biological world. The products produced from this pathway are required in numerous biological processes such as bacterial quorum sensing and protein modification. Crucially, fatty acids are also required to synthesise and maintain cellular membranes in all living organisms. Consequently, the biosynthetic pathways that supply and replenish membrane components such as fatty acids also share common features between phyla (Chan and Vogel 2010; Cronan and Thomas 2009). For unicellular microorganisms, maintenance of the cell membrane is critical as this structure represents a vital line of defense against environmental factors, host immune systems and antibiotic agents. For this reason, the fatty acid pathway has been proposed as a potential drug target for the development of new antibiotics against certain pathogenic bacteria that require de novo synthesis (Balemans et al. 2010; Parsons and Rock 2011; Chan and Vogel 2010; Wright and Reynolds 2007). This strategy is dependent upon finding chemical agents that are highly selective for bacterial targets over the host enzymes. This novel approach to antibiotic discovery is greatly assisted by the availability of high resolution protein structures along with detailed molecular understanding of the target's biological function. Hence, structural biology can play an important role in

delineating protein structure–function relationships as well as guiding the design of small molecule inhibitors. One interesting example worthy of investigation is acetyl-CoA carboxylase (ACC). This essential metabolic enzyme is required for fatty acid biosynthesis. Here, we review recent structural studies that extend our molecular understanding surrounding the assembly and mechanism of catalysis employed by ACC. Additionally, we also investigate recent reports of small molecule inhibitors that selectively target bacterial ACC over eukaryotic equivalents. By combining the findings of these studies, it is possible to probe the molecular basis of selective inhibition.

Catalytic mechanism of ACC

ACC [EC 6.4.1.2] belongs to a ubiquitous enzyme family that requires biotin as a coenzyme to catalyse the carboxylation, decarboxylation or transcarboxylation of metabolites (reviewed (Polyak and Chapman-Smith 2004)). ACC catalyses the carboxylation of acetyl-CoA to produce malonyl-CoA in the first committed step of the fatty acid synthesis pathway (Tong 2005). Biotin is covalently attached to a single lysine residue within the biotin carboxyl carrier domain (BCCD). Catalysis proceeds through a conserved reaction mechanism whereby the biotin–BCCD complex, attached to a highly mobile "swinging arm", oscillates between two partial reaction sites (Fig. 1). The first step involves carboxylation of biotin at N1 in a reaction requiring ATP and bicarbonate. This first partial reaction occurs within the biotin carboxylase domain (BC). The CO_2–biotin–BCCD complex then moves to the site of the second partial reaction, namely, the carboxyltransferase

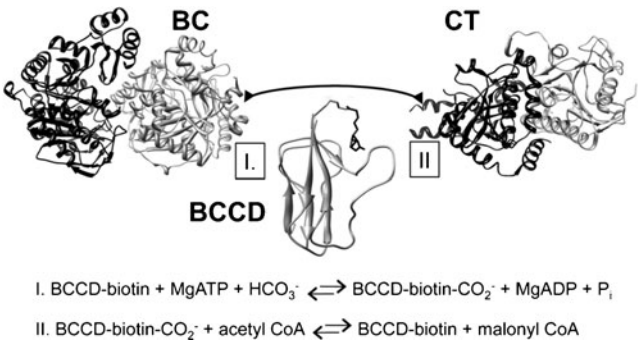

Fig. 1 ACC reaction mechanism. The structures of biotin carboxylase (BC), carboxyltransferase (CT) and biotin carboxyl carried domain (BCCD) from *E. coli* are shown, along with the two partial reactions carried out at each subsite. BC and CT are depicted as dimers with one subunit in *black ribbon* and the other *grey*. The *arrow* represents the movement of biotin–BCCD between the BC and TC proteins where CO_2 is first attached to biotin then transferred to the acetyl-CoA substrate. PDB files employed here were 2J9G for BC (Mochalkin et al. 2008), 1BDO for BCCD (Athappilly and Hendrickson 1995) and 2F9Y for CT (Bilder et al. 2006)

(CT) domain. The carboxyl group is subsequently transferred from carboxybiotin onto acetyl-CoA to give malonyl-CoA (Tong 2005). To date an X-ray crystal structure of an intact ACC holoenzyme complex has not been reported, most likely due the labile nature of these complexes. However, structures of the BC, BCCD and TC subunits of ACC have proven more accessible, with reports of structures from several bacterial and eukaryotic species. Details of these structures are discussed in this review to provide information for the design of selective small molecule inhibitors of ACC.

Genetic studies into ACC

Genetic studies have established that ACC is essential for growth in vitro in a number of bacteria including *Escherichia coli* (Gerdes et al. 2003), *Staphylococcus aureus* (Forsyth et al. 2002; Payne et al. 2007) *Streptococcus pneumoniae* (Thanassi et al. 2002), *Mycobacterium tuberculosis* (Sassetti et al. 2003), *Helicobacter pylori* (Salama et al. 2004) and *Pseudomonas aeruginosa* (Jacobs et al. 2003) among others. Bacteria require regulated expression of the *acc* genes in order to maintain appropriate cellular concentrations of the enzyme's subunits. The importance of maintaining the correct stoichiometry in vivo has been demonstrated in *E. coli* where overexpression of one gene product inhibits the biosynthesis of both biotin (Abdel-Hamid and Cronan 2007) and fatty acids (Karow et al. 1992). Consequently, *E. coli* can be sensitized to ACC inhibitors by the overexpression of BCCD, which decreases the pool of functional ACC in the cell (Cheng et al. 2009). The genes encoding BC (*accC*) and BCCD (*accB*) often cluster together in the vast majority of bacterial genomes (Abdel-Hamid and Cronan 2007) allowing coordinated expression of the two subunits. This helps to maintain the BC_2–$BCCD_4$ stoichiometery observed in the active holoenzyme complex (Choi-Rhee and Cronan 2003). The genes that encode the transcarboxylase subunits (*accA* and *accD*) are not found in an operon and, therefore, their coordinated expression must be controlled by another mechanism. In *E. coli*, expression of these subunits is controlled at the level of translation as a result of CT binding directly to its own mRNA (Meades et al. 2010). During times of adequate CT supply, the enzyme bound to the transcript represses further protein synthesis in a negative feedback loop.

Comparisons of bacterial genomes have shown that lipid metabolism is not highly conserved between bacterial species (Gago et al. 2011; Cronan and Thomas 2009). For example, actinomycetes have an expanded repertoire of *acc* genes whose products can combine to produce acyl-CoA carboxylases with unique biological properties. *Mycobacterium*

sp. have three *accA* and six *accD* genes that encode the two subunits of CT (Kurth et al. 2009). These combine to produce acyl-CoA carboxylases to utilize various length short chain acyl-CoAs as substrates including acetyl-CoA, propionyl-CoA and butanyl-CoA (Gago et al. 2011; Arabolaza et al. 2010). Carboxylation of these substrates provides metabolites that feed into the fatty acid synthesis and polyketide synthesis pathways, resulting in the production of mycolic acids and multimethyl-branched fatty acids present in the cell envelope (Takayama et al. 2005).

Small molecule inhibition of ACC

Currently, there are no examples of antibacterial ACC inhibitors in clinical use as antibiotics. Such new molecular scaffolds are particularly valuable as they are likely to control infections of otherwise drug-resistant bacterial pathogens. Here, we will discuss recent advances towards discovering small molecule inhibitors of bacterial ACC with clinical potential. Inhibition of the human homologues ACC1 and ACC2, or other off target activities, is likely to produce undesirable side effects for the patient. As such, it is critical to develop selective inhibitors. Differences in the quaternary structures of prokaryotic and eukaryotic ACCs suggest selective inhibition should be possible. For example, in eukaryotes, the BC, CT and BCCD components reside in a single polypeptide chain and are part of the Type I FAS system. ACC activity is dependent upon protein dimerization, and subsequent polymerization of ACC further increases enzyme activity (Kim et al. 2010; Shen et al. 2004; Weatherly et al. 2004). Conversely, in archea and eubacteria ACC is a component of the Type II FAS system that assembles as a multisubunit complex with each of the reactions performed by a separate enzyme. These striking differences in tertiary structure are noteworthy and the following sections of this review bring together recent studies that highlight the importance of the BC and CT subunits as sites for small molecule inhibition.

Biotin carboxylase

Structural biology aids to dissect the reaction mechanism

By far, the best characterized BC is that from the model Gram-negative bacteria *E. coli*. The first crystal structure of BC from this bacterium revealed it to contain three domains: a central ATP grasp domain required for nucleotide binding located between N- and C-terminal domains (Chou and Tong 2011; Waldrop et al. 1994). The protein was crystallized as a homodimer, consistent with its oligomeric structure in solution (Janiyani et al. 2001). The dimer interface has two-fold symmetry with the dimer axis between the C domains of each monomer. Importantly, for the reaction mechanism, each subunit contains a complete and independent active site but without the ability to communicate with each other (Chou and Tong 2011; Waldrop et al. 1994). Crystal structures of *E. coli* BC alone or in complex with substrates biotin, Mg^{2+}, ADP and ATP analogues have recently been reported, thereby defining the molecular details of the active site (Chou and Tong 2011; Chou et al. 2009; Mochalkin et al. 2008). Upon ATP binding, the ATP grasp domain undergoes an open to closed conformational change analogous to other enzymes in this superfamily (Schreiber et al. 2009).

The active BC dimer works through a "half-site reactivity" mechanism whereby the two subunits alternate catalytic reactions (Mochalkin et al. 2008; Janiyani et al. 2001). While one subunit binds substrate with subsequent catalysis, the other subunit releases the carboxylated biotin product. Consequently, the two active sites cannot undergo catalysis simultaneously. X-ray crystal structures of BC, in complex with ATP, demonstrate that ligand binding at one active site induces conformational changes in the partner subunit. Amino acids in the dimer interface (Arg 331) and active site (K238) work in concert to facilitate these long distance conformational changes (Mochalkin et al. 2008). This mechanism imparts directionality to the reaction mechanism. Assays using BC hybrid dimers containing one active subunit and one catalytically inactive mutant subunit produced an inactive enzyme (Janiyani et al. 2001). It is unclear whether or not similar mechanisms are at play in eukaryotic isozymes. However, the yeast and mammalian equivalents exist as monomers in solution that are catalytically inactive, suggesting a dimer-dependent mechanism may also be required for activity (Weatherly et al. 2004).

ATP binding

The mechanism of ATP binding imparts another level of control over ACC activity. The ATP analogue, AMPNP, has been cocrystallized with BC in several binding modes. In the presence of biotin, supplied by holoBCCD, the nucleotide triphosphate assumes an extended, productive mode of binding rendering it receptive to ATP hydrolysis (Mochalkin et al. 2008). Alternatively, in the absence of biotin, ATP binds in a nonproductive mode whereby the phosphate chain is curled back upon itself and unable to react with bicarbonate (Mochalkin et al. 2008). The latter mechanism ensures that the nucleotide triphosphate is not exhausted when not required through futile cycles of hydrolysis. This provides an elegant example of substrate-induced synergy where ATP hydrolysis is increased 1,100-fold in the presence of free biotin (Blanchard et al. 1999). Furthermore, at high concentrations, ATP can occupy a second site that

overlaps with the bicarbonate and biotin binding sites (Chou and Tong 2011). Under these conditions, ATP functions as a competitive inhibitor of bicarbonate to regulate ACC activity.

The dimer interface

In addition to the *E. coli* proteins, X-ray structures of bacterial BC proteins are now available from *S. aureus*, *P. aeruginosa* (Mochalkin et al. 2008), *Aquifex aeolicus* (Kondo et al. 2004) and *Geobacillus thermodenitrificans* (Kondo et al. 2007) alongside the BC domains from *Saccharomyces cerevisiae* ACC (Shen et al. 2004) and human ACC1 and ACC2 (Cho et al. 2010; Cho et al. 2008). Details of these works are summarized in Table 1. These assist in understanding the structural differences between the bacterial and eukaryotic isozymes. An analysis of the available structures reveals that they all adopt the same protein fold as *E. coli* BC. In particular, the backbone atoms of the *S. aureus* and human domains (31% primary sequence identity) are essentially superimposable (*S. aureus* vs. ACC1 RMSD of 1.6 Å; *S. aureus* vs. ACC2 1.7 Å) (Fig. 2a). Amino acid alignments show that the greatest diversity between the isozymes is observed in the dimerization interface. The ability to dimerize and form extended oligomers is critical for the catalytic activity of eukaryotic ACCs (Weatherly et al. 2004). The natural product soraphen A, a macrolide polyketide, functions as an inhibitor of eukaryotic ACCs by disrupting dimerization of the BC domains, and subsequently, dimerization of the full-length ACC (Cho et al. 2010; Shen et al. 2004; Weatherly et al. 2004). Mammalian ACC is also regulated via phosphorylation by AMP kinase at Ser 222, which is in the soraphen A binding site (Cho et al. 2010). Phosphorylation disrupts ACC dimerization and, consequently, enzyme activity via an inhibition mechanism analogous to soraphen A. While X-ray crystal structures of the human BC dimer have not been reported, available structures of the phosphorylated BC domain of human ACC2 and the protein in complex with soraphen A help to resolve the dimer interface (Fig. 2b) (Cho et al. 2010). Noteworthy is that soraphen A does not target bacterial ACC due to the absence of key amino acids required for binding. In yeast BC, mutation of Ser 77 (equivalent of 278 in human ACC2) or K73 (274 in human ACC2) renders the enzyme resistant to the inhibitor, highlighting their importance in binding (Fig. 2b) (Vahlensieck et al. 1994). These residues are conserved among eukaryotic species but divergent among bacteria providing an explanation for the selectivity of soraphen A. Currently, there are no bacterial BC inhibitors that target the BC dimer interface.

Targeting the ATP pocket

There is growing interest in the nucleotide-binding site of BC as a potential antibiotic drug target. Over the past decade, considerable effort has been expended exploring a variety of eukaryotic protein kinases as drug targets. Subsequently, large chemical libraries for use in high throughput screening programs have been developed that contain

Table 1 Overview of structural data on acetyl-CoA and Acyl-CoA carboxylases

Species	Gene name	Length[a] (aa)	PDB ID	Tertiary structure	Reference
Biotin carboxylase					
Escherichia coli	accC	449	1DV1	Homodimer	Waldrop et al. (1994)
Staphylococcus aureus	accC	453	2VPQ	Homodimer	Mochalkin et al. (2008)
Pseudomonas aeruginosa	accC	464	2 C00	Homodimer	Mochalkin et al. (2008)
Aquifex aeolicus	pyc	6551	1ULZ	Homodimer (451 aa fragment)	Kondo et al. (2004)
Geobacillus thermodenitrificans	pyc	1147	2DZD	Homodimer (461 aa fragment)	Kondo et al. (2007)
Saccharomyces cerevisiae	hfa1	2273	1 W93	Homodimer (553 aa fragment)	Shen et al. (2004)
Homo sapiens	acc1	2346	2YL2	Monomer (540 aa fragment)	Unpublished
	acc2	2458	2HJW	Monomer (573 aa fragment)	Cho et al. (2008)
Carboxyl transferase					
Escherichia coli	accA, accD	319 304	2F9Y	α2β2 heterotetramer	Bilder et al. (2006)
Staphylococcus aureus	accA accD	314 285	2F9I	α2β2 heterotetramer	Bilder et al. (2006)
Saccharomyces cerevisiae	hfa1	2273	1OD4	Homodimer (805 aa fragment)	Zhang et al. (2003)
Homo sapiens	acc2	2458	3FF6	Homodimer (760 aa fragment)	Madauss et al. (2009)
Acyl CoA carboxylase					
Streptomyces coelicolor	pcc	530	1XO6	Heterohexamer	Diacovich et al. (2004)
Mycobacterium tuberculosis	accD5	548	2A7S	Heterohexamer	Lin et al. (2006)

acc acetyl-CoA carboxylase, *pyc* pyruvate carboxylase, *pcc* propionyl-CoA carboxylase

[a] The number of amino acid residues in the full-length polypeptide chain

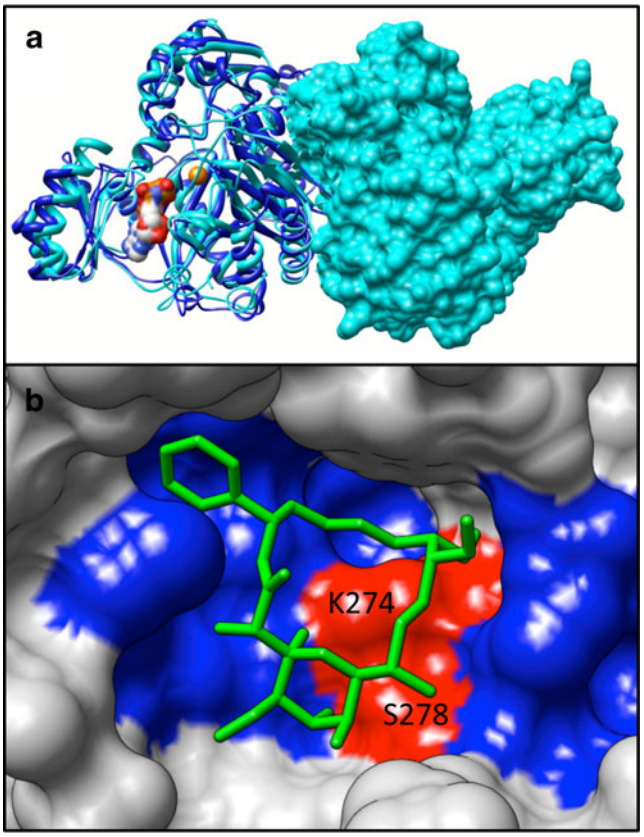

Fig. 2 Biotin carboxylase inhibitors. **a** Overlay analysis of the biotin carboxylase domains from *S. aureus* [*cyan*, PDB 2VPQ (Mochalkin et al. 2008)] and human ACC2 [*dark blue*, PDB 3JRX (Cho et al. 2010)]. The *S. aureus* structure was determined in the presence of an ATP analogue shown in space-filling mode, thus revealing the nucleotide-binding pocket. A chloride ion (*orange ball*) present only in the *S. aureus* protein is also shown. The dimeric form of the *S. aureus* protein is shown with one subunit in space-filled mode and the other subunit represented as a ribbon. Monomeric human BC is shown as a ribbon. **b** The binding mode of the eukaryotic ACC inhibitor soraphen A in the dimer interface of hACC2 (Cho et al. 2010). Amino acids conserved between human ACC2 and *S. aureus* BC are *blue*, whereas the non-conserved residues essential for selective binding are *red*

abundant pharmaceutical entities for testing against bacterial drug targets. Recent studies validate the feasibility of this approach with the nucleotide-binding pockets being sufficiently divergent to obtain desirable specificity. Antibiotic targets that have been explored include histidine kinase (Schreiber et al. 2009) and D-alanine–D-alanine ligase (Triola et al. 2009). BC has also been the subject of promising studies. Miller et al. recently identified a series of pyridopyrimidine derivatives with broad spectrum antibacterial activity from Pfizer's 1.6 million compound library assembled for protein kinase inhibitor screening programs (Miller et al. 2009). The specificity of the lead compound (shown in Fig. 3a) towards bacterial BC was demonstrated via in vitro assays using purified enzymes from *E. coli*, *Haemophilus influenzae* and *P. aeruginosa* alongside a panel of 30 eukaryotic protein kinases. The lead compound was potent for the bacterial targets (K_d 800 pM) and showed no activity against the eukaryotic enzymes. The bacteria most susceptible to the pyridopyrimidine derivatives were fastidious Gram-negative pathogens *H. influenzae* and *Moraxella catarrhalis*. The compound's in vivo efficacy as an antibacterial agent was also demonstrated in a murine infection model using *H. influenzae* (Miller et al. 2009). This project provided a platform for further inhibitor discovery (Mochalkin et al. 2009). The X-ray crystal structure data of *E. coli* BC in complex with the lead pyridopyrimidine facilitated an in silico screening approach upon a library of 2.2 million compounds for structures with similar shapes and surface electrostatics as the lead inhibitor. In vitro testing of 525 hits identified 48 compounds with an IC_{50} of <10 μM against *E. coli* BC. In a separate experiment, a library of 5,200 compounds was screened using a high throughput biochemical assay to reveal 142 hits. Superimposition of these structures produced a pharmacophore defining the features important for binding in the ATP pocket, particularly hydrogen-bonding interactions with key amino acid residues in the adenine binding site and hydrophobic contacts in the ribose-binding site. Iterative cycles of virtual docking, library design, parallel medicinal

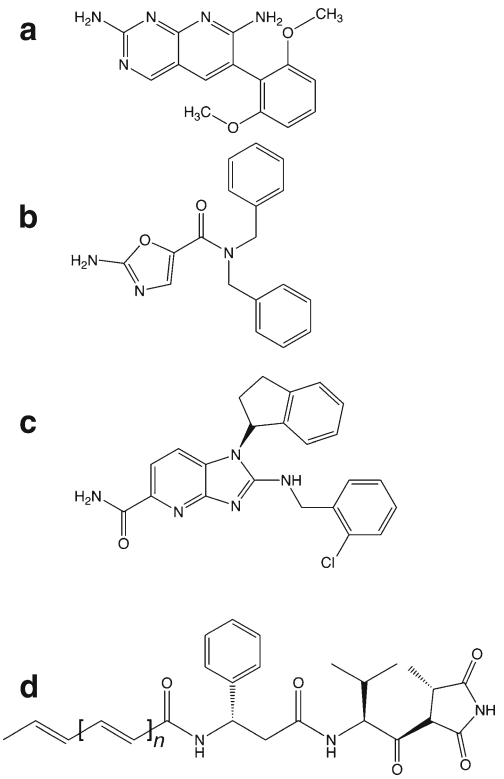

Fig. 3 Bacterial-specific ACC inhibitors. Chemical structures are shown for representative members of various classes of bacterial-specific ACC inhibitors. **a** A pyridopyrimidine from the Pfizer compound library (Miller et al. 2009), **b** an aminooxazole developed by fragment evolution from Pfizer (Mochalkin et al. 2009), **c** an optimised axo-benzimidazole from Schlering-Plough (Cheng et al. 2009) and **d** the natural products moiramide B (n=1) and andrimid (n=2) (Freiberg et al. 2004; Freiberg et al. 2006)

chemistry synthesis, in vitro testing and X-ray crystallography revealed several new chemical series. Improvements in potency of 3,000 fold over the initial starting fragment was observed for the amino-oxazole shown in Fig. 3b. The most potent inhibitors exhibited antibacterial activity against Gram-negative pathogens and efflux deficient strains of *E. coli*. Specificity of the optimized lead compound 3b towards bacterial BC was established by testing the lead structure for inhibitory activity using in vitro enzyme assays for 40 purified human protein kinases as well as rat liver ACC (Mochalkin et al. 2009). Here, X-ray crystallography was employed to understand the molecular basis of specificity. Subtle differences in the primary sequence and/or structure of the binding sites can have significant implications for inhibitor binding. For example, single amino acid changes can impede binding of small molecules by modifying the physicochemical properties of the binding site or shape complementarities resulting in ACC variants that are enzymatically active but resistant to binding the inhibitor. This is highlighted in Fig. 4, which shows the mechanism of binding for a bacterial-specific pyridopyridine ACC inhibitor. The amino acids Ile 437 and His 438 within the ATP-binding pocket enclose the hydrophobic binding site, thereby impeding dissociation of the inhibitor (Miller et al. 2009). The human isozyme is a more open pocket that is not receptive to inhibitor binding.

Using kinase inhibitors for antibacterial discovery was also performed by Schering-Plough to identify an azobenzimidazole series of ACC inhibitors (Cheng et al. 2009; Annis et al. 2007). Chemical structures were identified that directly interact with the *E. coli* BC drug target by employing affinity selection techniques coupled with mass spectroscopy. Subsequent chemical modification of the initial hits gave rise to structures with an in vitro IC_{50} of 6–260 nM. The authors did not address the specificity of their optimized compounds against mammalian ACC or kinases, but the potency of the compounds is encouraging. An example of a potent inhibitor is shown in Fig. 3c. This structure shows antibacterial activity against *E. coli* (MIC 0.2–12.5 μg/ml) with efficacy through the drug target being established using an *E. coli* strain sensitized to ACC inhibitors by overexpression of apo-BCCP (Cheng et al. 2009). These independent reports of nonrelated chemical classes specifically targeting the ATP pocket of BC provide proof of concept data for selective inhibition of ACC.

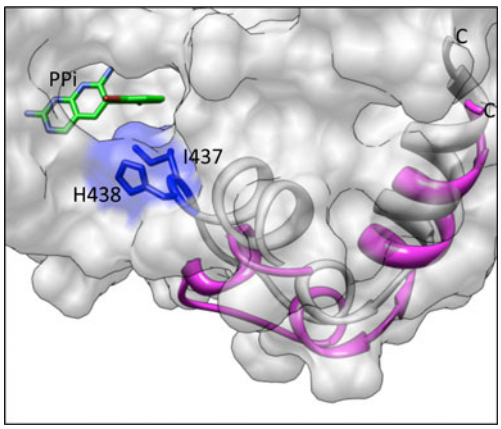

Fig. 4 Selective binding to bacterial BC. The crystal structure of a pyridopyridine inhibitor (PPi) bound in the ATP pocket of *E. coli* BC [PDB 2 V58 (Miller et al. 2009)]. The protein, represented in transparent space-filling mode (*grey*), contains an enclosed pocket that contributes to inhibitor binding. Residues conferring resistance are highlighted (*blue*), as are ribbon structures corresponding to C-terminal regions of *E. coli* BC (*grey*) and human ACC2 (*magenta*; [PDB 3JRW (Cho et al. 2010)]. The absence of appropriate amino acids in the human isozyme contributes to an open ATP pocket that is resistant to binding the bacterial-specific inhibitor

Carboxyltransferase

The CT domain has also been investigated by structural biology and small molecule inhibitor studies. X-ray crystal

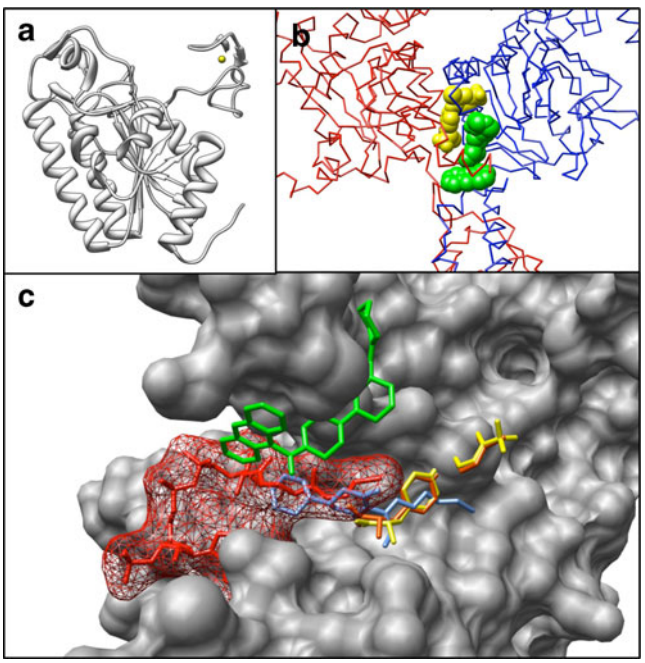

Fig. 5 Carboxyltransferase inhibitors. **a** Monomeric *E. coli* CT [PDB 2F9Y, (Bilder et al. 2006)] containing a zinc-binding motif unique to the bacterial isozymes. The zinc atom is shown in *yellow*. **b** Inhibitors of eukaryotic ACC bind in the dimer interface of *S. cerevisiae* CT. Haloxyfop [*yellow*, 1UYS (Zhang et al. 2004b)] and CP-640186 [*green*, 1W2X (Zhang et al. 2004a)] are shown in space-filing mode located between two subunits shown in *red* and *blue* wire representation. **c** The binding mode for acetyl-CoA (*red mesh*) is shown [PDB 1OD2 (Zhang et al. 2003)] together with inhibitors haloxyfop (*yellow*), diclofop [*orange*, PDB 1UYR] and tepraloxydim [*cyan*, 3K8X (Xiang et al. 2009)] that are competitive with acetyl-CoA. Compound CP-640186 (*green*) binds at a discrete site proposed to accommodate carboxybiotin

structures of CT from a number of species including *E. coli* and *S. aureus* (Bilder et al. 2006), *Streptomyces coelicolor* (Diacovich et al. 2004), the *Mycobacterium* AccD5 subunit (Lin et al. 2006) as well as *S. cerevisiae* (Zhang et al. 2003) and human ACC2 (Madauss et al. 2009) have been reported. These works are summarized in Table 1. The *E. coli* and *S. aureus* paralogues are composed of two AccA and two AccD subunits that assemble into $\alpha 2\beta 2$ heterotetramers via a dimer of $\alpha\beta$ dimers (Bilder et al. 2006). Each monomeric unit adopts two homologous structures, both belonging to the crotonase superfamily of protein folds (Fig. 5a). Conversely, eukaryotic CTs are encoded by a single polypeptide chain that assembles into a dimer in solution and also during crystallography (Zhang et al. 2003). Each polypeptide contains two copies of the crotonase module, suggesting duplication of an ancestral gene sequence during evolution. The two domains within the yeast CT polypeptide dimerize in a head-to-tail fashion, consistent with the $\alpha 2\beta 2$ organization observed in the prokaryotic isozymes. The crystal structure of yeast CT in complex with acetyl-CoA revealed that the ligand-binding site resides between the N-terminal domain of one monomer and the C-terminal domain of another monomer in the dimer interface (Zhang et al. 2003). Interestingly, the bacterial proteins from *E. coli* and *S. aureus* possess a zinc-binding domain that is absent in the eukaryotic enzymes (Bilder et al. 2006). The function of this domain is unclear, but it is proposed to serve as a lid to close over the substrate acetyl-CoA in the active site (Bilder et al. 2006).

Pesticides provide proof of concept for selective inhibition

Selective small molecule intervention of ACC was first demonstrated with haloxyfop and diclofop that bind to the CT domain of susceptible plastid ACCs from monocot but not dicot plants [reviewed (Alban et al. 2000)]. These agents have been employed as herbicides for over 20 years and, by analogy, provide proof of concept for selective inhibition of ACC from a pathogen over its host. While these herbicides and later generation products such as pinoxaden and tepraloxydim (Yu et al. 2010; Xiang et al. 2009) have not been shown to specifically target bacterial ACC, they do provide new insights into inhibitor design. X-ray crystallography has been used to show that these compounds bind in the dimer interface of yeast CT and preclude binding of acetyl-CoA (Fig. 5) (Zhang et al. 2004b). The mammalian ACC inhibitor CP640186 also binds in the dimer interface, but its mechanism of action is proposed to be via inhibition of carboxybiotin binding (Zhang et al. 2004a) (Fig. 5). The first prokaryotic-specific ACC inhibitors Mioramide B and andrimid (Fig. 3d) (Freiberg et al. 2004) and subsequent pyrrolidine dione

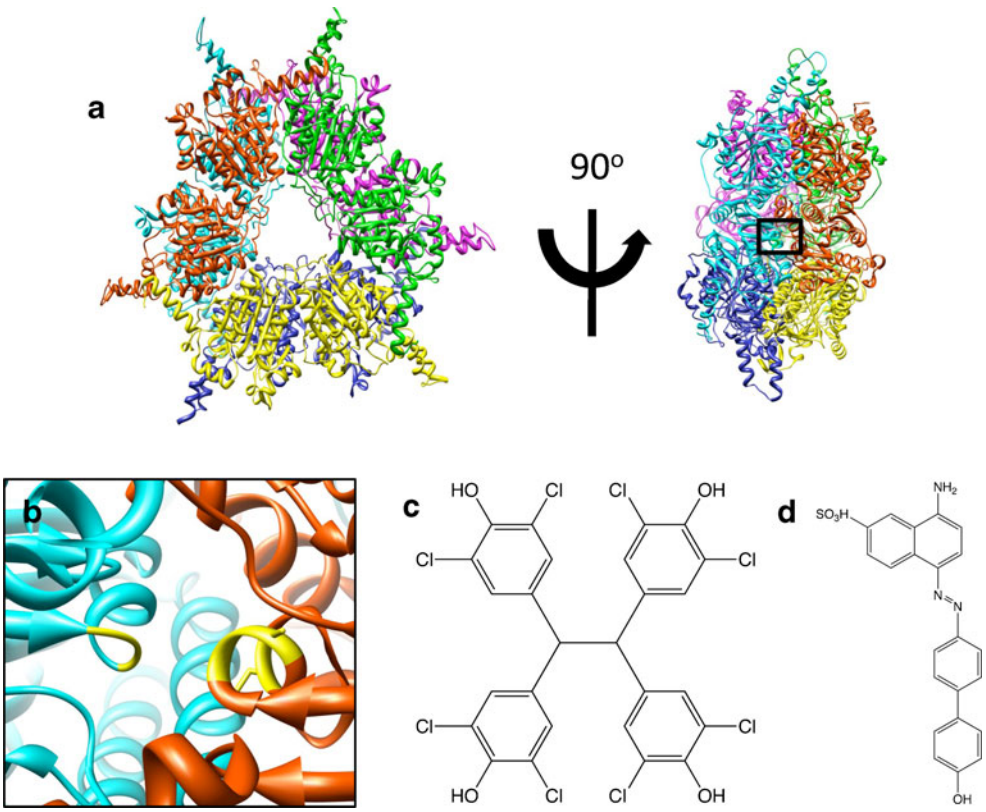

Fig. 6 Acyl-CoA carboxylase from *M. tuberculosis*. **a** The X-ray crystal structure of the AccD5 biotin carboxyltransferase is shown [PDB 2A7S (Lin et al. 2006)] with each subunit of the homohexamer colored differently. Rotation of the ring structure depicts how the protein assembles as a two-layered stack of trimers. **b** An expanded view of the *boxed area* highlights the proposed active site in the interface between subunits. Amino acids that contact ligands in the analogous propionyl-CoA carboxylase from *S. coelicolor* are shown in *yellow*. The chemical structures of hits are shown from in silico screening studies using either **c** a model of AccD6 (Kurth et al. 2009) or **d** the X-ray crystal structure of AccD5 (Lin et al. 2006)

derivatives (Freiberg et al. 2006) are also competitive inhibitors of acetyl-CoA. This suggests inhibitor binding occurs in the dimer interface, but no crystallographic data has been reported to confirm this.

M. tuberculosis CT

Small molecule inhibition of CT has been reported from studies on the *M. tuberculosis* acyl-CoA carboxylase. As mentioned earlier, the *M. tuberculosis* genome contains three *accA* and six *accD* genes (Kurth et al. 2009). The crystal structure of AccD5, resolved to 2.9 Å, revealed the enzyme to be a 360-kDa ring shaped homohexamer consisting of two tightly interacting trimers packed on top of each other (Fig. 6a) (Lin et al. 2006). This architecture is similar to the hexameric structure of *S. coelicolor* CT (Diacovich et al. 2004), as opposed to the heterotetramers of *E. coli* and *S. aureus* or the yeast homodimer. Each AccD5 monomer consists of an N- and C-terminal domain, each adopting a crotonase fold. A comparison of the crystal structures of *S. coelicolor* CT in complex with biotin and propionyl-CoA helped define the active site for *Mycobacterial* AccD5 in the dimer interface between two subunits with amino acid residues from the two chains contributing to the active site (Fig. 6b) (Diacovich et al. 2004). The substrate-binding pockets of both AccD5 and AccD6 have been the targets of in silico drug screening studies. The AccD5 crystal structure was initially employed to screen the UC Irvine ChemDB database. A panel of 100 compounds were tested in vitro for inhibitory activity to identify compound NCI 65828 (Fig. 6c) as a modest in vitro inhibitor of AccD5 (K_i 13.1 μM). A similar approach using a model of AccD6 identified NCI 172033 (Fig. 6d) with in vitro inhibitory activity (K_i 8 μM) and broad anti-*Mycobacterium* activity (Kurth et al. 2009). This compound has been shown to compete with the malonyl-CoA substrate with its antibacterial action occurring via inhibition of the synthesis of fatty and mycolic acids. Growth of a *M. smegmatis* strain overexpressing AccD6 was not affected by the inhibitor, demonstrating its mode of action was specifically through the desired target. While further work is required to define the selectivity of these compounds toward *Mycobacterium*'s acyl-CoA carboxylases over human equivalents, two observations suggest that this is achievable. Firstly, the least conserved regions of the available CT structures are observed in the dimer interface (Zhang et al. 2003) providing further scope to chemically optimize these chemical scaffolds for greater potency and selectivity. Secondly, *M. tuberculosis* AccD5 cannot be inhibited by the herbicides diclofop and haloxyfop (Lin et al. 2006) again highlighting divergence in the binding sites between species. These studies are welcomed given the critical clinical need for new tuberculosis treatments.

Implications for antibiotic drug discovery

The increasing prevalence of bacteria resistant to many, if not all, of the current arsenal of antibiotics has resulted in a serious global health care concern especially given the increasing mortality rates and associated dramatically increased health care costs (Deleo et al. 2010; Graves et al. 2010; Turnidge et al. 2009). To better combat these multidrug resistant bacteria, new molecular scaffolds with novel mechanisms of action are required (Donadio et al. 2010; Fischbach and Walsh 2009; Payne 2008). As government and philanthropic funding agencies begin to respond to this looming crisis, it is timely to reconsider the previous preoccupation for drug targets that have no equivalents in the mammalian host. The most obvious of these have now been extensively investigated. With only three new classes of antibiotics receiving FDA approval in the past 30 years, alternative strategies must be considered. An important new frontier for early stage antibiotic discovery is to investigate bacterial protein targets that have mammalian isozymes. This requires the development of small molecule inhibitors that are selective for the bacterial target with a safe therapeutic window. The success of this approach with ACC as reviewed here provides some level of optimism for this approach.

Acknowledgements The authors wish to thank the National Health and Medical Research Council of Australia for funding (applications 565506 and 1011806).

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