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Regulation and structure of the heteromeric acetyl-CoA carboxylase☆

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ABSTRACT

The enzyme acetyl-CoA carboxylase (ACCase) catalyzes the committed step of the *de novo* fatty acid biosynthesis (FAS) pathway by converting acetyl-CoA to malonyl-CoA. Two forms of ACCase exist in nature, a homomeric and heteromic form. The heteromeric form of this enzyme requires four different subunits for activity: biotin carboxylase; biotin carboxyl carrier protein; and α- and β-carboxyltransferases. Heteromeric ACCases (htACCase) can be found in prokaryotes and the plastids of most plants. The plant htACCase is regulated by diverse mechanisms reflected by the biochemical and genetic complexity of this multienzyme complex and the plastid stroma where it resides. In this review we summarize the regulation of the plant htACCase and also describe the structural characteristics of this complex from both prokaryotes and plants. This article is part of a Special Issue entitled: Plant Lipid Biology edited by Kent D. Chapman and Ivo Feussner.

1. Introduction

1.1. Role of acetyl-CoA carboxylase in de novo fatty acid synthesis

Acetyl-CoA carboxylase (EC 6.4.1.2; ACCase) catalyzes the committed step of *de novo* fatty acid synthesis (FAS) in all organisms. The enzyme uses bicarbonate, ATP, acetyl-CoA, and biotin cofactor to produce malonyl-CoA, the building block of fatty acid synthesis (Fig. 1) [1,2]. In nature there are two functional forms of this enzyme, and each contains the four different components required for activity: biotin carboxylase (BC; EC 6.3.4.14); biotin carboxyl carrier protein (BCCP); and α- and β-carboxyltransferases (CTs). One form is heteromeric, for which all four enzymatic components are expressed as individual subunits that associate to form a multienzyme complex [3]. The heteromeric form of ACCase (htACCase) is found in prokaryotes and the plastids of algae, bryophytes, pteridophytes, gymnosperms, non-graminaceous monocots, and dicots [3–5]. A few archaea have been identified to contain a hybrid CT gene that encodes the α and β subunits in tandem, however, these enzymes carboxylate both acetyl-CoA and propionyl-CoA [6–8]. The other form is described as homomeric, whereby all four enzymatic components are concatenated into a single polypeptide. The homomeric ACCase (hmACCase) is the predominant form for *de novo* fatty acid synthesis in the cytosol of animals and fungi, and the plastids of graminaceous monocots. In addition, the cytosol of all plants contains a hmACCase isoform that provides malonyl-CoA for fatty acid elongation as well as polyketide biosynthesis, malonylation reactions, and other specialized metabolic processes [9]. A hmACCase has also been discovered in the plastid of dicot species [10], but its metabolic role is presently unclear.

Due to its complexity and key role in *de novo* FAS, the htACCase is the most extensively studied form of ACCase in plants. There is evidence that increasing htACCase activity leads to increased carbon flux into *de novo* FAS in both microbes [11] and plants [12]. Therefore understanding the regulatory factors that affect htACCase activity will be important in developing successful biotechnology strategies involving lipid metabolism. This review will consequently focus on the recent discoveries related to the regulation and structure of htACCase in both plants and prokaryotes. For a more historical perspective on hmACCase or an in-depth discussion of fatty acid synthesis in plants we recommend the following reviews [13–15].

1.2. The half-reactions of htACCase occur on separate subcomplexes

The htACCase catalyzes two distinct half-reactions and is made up of two separable "subcomplexes", BC–BCCP and α-CT–β-CT [16–18]. These two components catalyze the enzymatic half-reactions noted in Fig. 1. The biotin-containing subunit, BCCP, links the two reactions through an active site coupling mechanism analogous to that of the lipoyl domain of the α-keto acid dehydrogenase complexes [19]. The two subcomplexes readily dissociate during conventional purification techniques, but the subcomplexes themselves remain intact, suggesting that these subcomplexes are more stable compared to the whole htACCase complex [16,18]. The first active site is on the BC subunit, where ATP is bound and hydrolyzed to catalyze the carboxylation of

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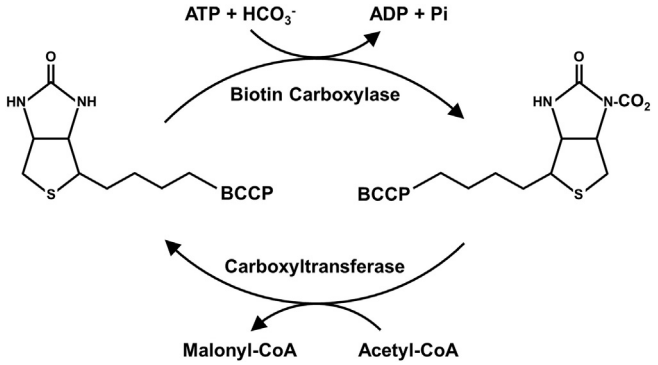

Fig. 1. Reaction mechanism of ACCase. Adapted from [17]. The ATP-dependent carboxylation of the biotinyl moiety with the biotin carboxyl carrier protein (BCCP) is catalyzed by biotin carboxylase (BC). The activated carboxyl group is then transferred to acetyl-coenzyme A by carboxyltransferase (CT) to produce malonyl-coenzyme A. Active site coupling between the BC and CT half reactions is facilitated by the flexible biotinyl-lysine arm within the holo-BCCP.

biotin cofactor covalently bound to a conserved Lys within the BCCP. The carboxylated, biotinyl-Lys arm is then transferred to the active site on the CT subcomplex. This active site is produced by the assembly of both the α- and β-CT subunits, and binds acetyl-CoA as the terminal carboxy acceptor [20].

1.3. htACCase gene localization

The genes encoding the catalytic subunits for htACCase were first discovered in *Escherichia coli* [2,21–23] and subsequently in plants [24–27]. In the model plant Arabidopsis (*Arabidopsis thaliana*), each catalytic subunit is encoded by a single gene except for BCCP, which is encoded by two genes [25,28,29]. Each of these genes is encoded within the nuclear genome except for *accD* (encoding the β-CT subunit), which resides in the plastid genome. This is the only known FAS-related gene that is encoded in the plastid genome [30]. Presumably, each of the genes for the ACCase subunits initially resided in the plastid genome after the original endosymbiotic event in algae and has undergone sequential transfer to the nuclear genome [5]. It is unclear why the *accD* gene was retained in the plastid. In *E. coli*, the CT subunits have been suggested to regulate their own gene expression by binding the mRNAs that encode the subunits [31]. Perhaps retaining the *accD* gene in the plastid allows the plant to partially maintain this form of transcriptional regulation. However, this function must not be essential, as evidence of stochastic *accD* gene transfer to the nucleus has been observed for some land plant species [32–38].

2. Genetic regulation

2.1. htACCase gene expression in plants

The genes for htACCase subunits are expressed in all plant tissues, though at different levels. In Arabidopsis, steady-state transcript levels are most abundant in developing seed, during which transcript levels of htACCase, as well as many other FAS enzymes, follow a bell-shaped curve that crests between 8 and 11 days after flowering (DAF) [39]. This precedes the maximal accumulation of seed oil, which occurs between 9 and 13 DAF [39].

One of the contributors to the increase in FAS gene expression during seed filling is the transcription factor WRINKLED1 (WRI1). The *wri1* mutant was first isolated in Arabidopsis by Focks and Benning, and was later shown to encode an APETALA2/ ethylene-responsive element-binding protein [40,41]. Further study showed that WRI1 is responsible for increasing the expression of at least 18 genes involved in FAS [39,40]. Of these, only the BCCP2 subunit gene of htACCase is significantly induced [39,42]. Gene expression is directly affected by binding of WRI1 to an 'AW box' in the promoter region of the BCCP2 gene [42,43]. Interestingly, the promoter regions of the BC and α-CT genes also contain this AW box and can be bound by WRI1 [42]. However, BC and α-CT expression is unchanged in the *wri1* mutant and WRI1 overexpression lines, suggesting that other cis-elements might be involved in expression of these genes [42]. It should also be noted that WRI1 can affect htACCase activity by regulating the expression of genes required for biotin synthesis [44].

2.2. Genetic attempts to modulate htACCase gene expression

As the committed step of *de novo* FAS, ACCase is considered the gatekeeper for carbon flow into this pathway. Thus increasing ACCase levels, and ultimately activity, may enhance flux through this pathway. For example, overexpression of all four htACCase subunits in *E. coli* resulted in an approximate 100-fold and six-fold increase in malonyl-CoA production and FAS rate, respectively [11]. This result confirms that increased htACCase activity does increase carbon flux through FAS in a model bacterium. The situation in plants, however, is still unclear.

Attempts to increase fatty acid synthesis by modifying htACCase gene expression have had mixed results. Overexpression of the BC subunit had no significant effect on *de novo* FAS and did not result in a concomitant increase in BCCP protein levels, providing the first clue that up-regulation of any one catalytic subunit does not result in a compensatory increase in other catalytic subunits [45]. In contrast, overexpression of β-CT in tobacco led to increased expression of the other catalytic subunits to the htACCase. However, this change did not significantly enhance seed oil content, suggesting that post-transcriptional regulation might be another important layer governing overall htACCase activity [46]. Interestingly, the β-CT overexpression lines did produce approximately double the amount of seeds per plant.

Both seed-specific overexpression and constitutive reduction of the BCCP2 subunit in Arabidopsis unexpectedly produced similar results. Down-regulation of BCCP2 by antisense silencing led to an average 9% reduction in total seed oil content [47]. In addition, overexpression of the BCCP2 subunit led to a reduction in seed oil content (23%) associated with incomplete biotinylation of overexpressed BCCP2. Over half of the BCCP2 protein present in developing siliques was in the non-biotinylated, apo form, resulting in a 65% reduction in htACCase activity. Quantitative immunoblot analysis of the BCCP2 overexpression lines showed that expression of the other catalytic subunits to htACCase did not significantly change in abundance, while the biotin synthesis pathway was up-regulated at both the transcript and protein levels [48]. Additionally, an uncharacterized protein annotated a "biotin attachment domain-containing" protein was upregulated at both the transcript and protein levels. This uncharacterized protein is discussed further in Section 3.4 on regulatory proteins for htACCase. These observations illustrate the importance of the biotin synthetic pathway in htACCase function and the necessity for a fully biotinylated BCCP for proper htACCase function. In addition, this study confirmed results from Shintani et al., 1997 that upregulation of a single nuclear-encoded ACCase subunit does not produce a compensatory increase in any of the other subunits to the complex.

Analysis of BCCP1 and BCCP2 T-DNA lines in Arabidopsis determined the functional redundancy of the two isoforms to be unidirectional [49]. Lack of BCCP2 expression results in no obvious phenotype, while marginal reduction of BCCP1 expression results in a number of defects including reduced growth and increased seed abortion [49]. Furthermore, complete loss of BCCP1 expression appears to be lethal. The lack of functional redundancy can be explained in part by the seed-specific nature of BCCP2 expression. Additionally, BCCP1 appears to comprise a majority of the total BCCP pool in developing siliques with an estimated BCCP1:BCCP2 ratio of 5:1 [49].

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