RECENT ADVANCES IN ACETYL COA CARBOXYLASE; A KEY ENZYME OF FATTY ACID BIOSYNTHESIS IN PLANTS

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ABSTRACT: The first committed step in the lipid biosynthesis is the ATP dependent carboxylation of Acetyl-CoA to malonyl-CoA. This reaction is catalyzed by an enzyme acetyl CoA carboxylase, a key regulatory enzyme of fatty acid biosynthesis. Two isoforms of acetyl-CoA carboxylase (ACCase) are present in the plant cells. The prokaryotic form of ACCase exists in plastids except Gramineae family while in cytosol eukaryotic form of ACCase makes available malonyl-CoA for elongation of fatty acids, flavonoid biosynthesis and malonation of amino acids. The ACCase, a rate-limiting enzyme in the fatty acid biosynthesis is down regulated by palmitoyl CoA (end product) and phosphorylation through a glucagon-cAMP cascade. Further regulation of acetyl CoA carboxylase at transcriptional, post transcriptional level, by light/dark, some metabolites and RNA editing is also discussed. The enzyme is very much essential for the survival of the plants. In mammals, ACCases are multifunctional dimeric proteins with two active sites and are regulated allosterically. The expression is tissue-specific and responsive to hormones and nutritional status. Eukaryotic form of ACCase is sensitive to the herbicides (gramineaceides) which inhibit the enzyme activity. Enzyme also plays an important role in defense system of the cell.

Key words: ACCase; Acetyl-CoA; Malonyl-CoA

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INTRODUCTION

Oil seed crops store economically important oils in their seeds in the form of triacylglycerols which are very important plant commodity with economic potential for further nutritional and industrial exploitation. Oil seeds production is important to humans who for thousands of years have tapped into oils and utilized as raw material for foods, animal feeds and industrial feed stocks. Hundreds of plants accumulate oils being used for various purposes but six agronomically recognized crops namely soybean, cottonseed, groundnut, rapeseed, copra and palm kernels contribute more than 90% of 317 million tons of global oilseed (FAO Report, 2015). Over 80% of the plant oils are used for human consumption, notably in the production of cooking oils, margarines and processed foods. They are also used as industrial feed stocks for the production of paints, detergents, surfactants, plasticizers, high temperature lubricants, pharmaceuticals and as novel treatment for aderenolenkodystrophy (Ohlrogge, 1994; Princen and Rothfus, 1984; Van Dyne, 1990). De-novo synthesis of these fatty acids takes place in the stroma of the plastids by stepwise condensation of two carbon units from malonyl-acyl carrier protein to acyl chains with stearoyl-ACP (18:0) as the terminal product (Murphy, 1999). A series of reactions localized in plastids result in the bio assembly of fatty acids. Each reaction is catalyzed by a separate enzyme activity encoded by an individual gene. Each elongation cycle results in the addition of two carbon atoms to the growing chain. The first committed step of the lipid biosynthesis is catalyzed by the tightly regulated enzyme acetyl-CoA carboxylase (ACCase) which largely controls the carbon flux in this pathway.

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Acetyl CoA carboxylase (ACCase) or Acetyl CoA Carbon dioxide ligase (ADP forming, EC 6.4.1.2) catalyzes the ATP dependent carboxylation of Acetyl CoA to form malonyl CoA. This is a two-step reaction (Wood and Barden, 1997).

\[
\text{BCCP} + \text{HCO}_3^- + \text{Mg}^{2+} \cdot \text{A} + \text{TP} \rightarrow \text{BCCP}-\text{CO}_2 + \text{Mg}^{2+} \cdot \text{ADP} + \text{Pi} : \text{biotin carboxylase}
\]

\[
\text{BCCP}-\text{CO}_2 + \text{acetyl-CoA} \rightarrow \text{BCCP} + \text{malonyl-CoA} : \text{carboxyl transferase}
\]

This reaction represents the first committed step in de novo biosynthesis of malonyl CoA in plants and animals. ACCase is the key regulatory enzyme of fatty acid biosynthetic pathway (Kim et al., 1989). In plants Acetyl-CoA carboxylase also plays a regulatory role in fatty acid biosynthetic pathway which takes place in plastids (Post Beittenmiller et al., 1992; Page et al., 1994; Shitani and Ohlrogge, 1995; Ohlrogge & Browse, 1995). In the cytosol of the plant cell acetyl CoA carboxylase provides malonyl CoA for fatty acid elongation (Harwood, 1988), flavonoid formation (Ebel and Hahlbrock, 1977), polyketides synthesis (Harwood and Sherman, 1990) and several other metabolic pathways (Nikolau et al., 1984) like malonation of amino acids, glycosides and ethylene precursor of aminocyclopropane -1- carboxylate (Kionka and Amerhein, 1984).

**STRUCTURE OF ACCase**

Plants and eukaryotes have multi-subunit ACCases composed of several polypeptides encoded by distinct genes. There are two isoforms of enzymes found in plant kingdom. Heteromeric ACCase, a tetrameric enzyme is usually found in prokaryotes encoded by three nuclear and one plastid genes and composed of four separate proteins i.e. Biotin carboxylase (BC), Biotin carboxyl carrier protein (BCCP), \( \alpha \)-carboxyl transferase (\( \alpha \)-CT) and \( \beta \)-carboxyl transferase (\( \beta \)-CT). Homomeric ACCase composed of a single large polypeptide is found in eukaryotes. The stoichiometry of these subunits in the ACCase holoenzyme differs amongst organisms (Tong et al., 2005). Most plants have both forms, the heteromeric form in plastids, and the Homomeric form in cytosol (Sasgaki et al., 2004). The prokaryotic form of ACCase found in E.Coli consists of three dissociable components, 17 kD BCCP encoded by accB, 49 kD BT encoded by accC, and carboxyl-transferase component is a complex of two non-identical subunits i.e. 35-kDa \( \beta \)-carboxyl transferase encoded by accD and 33-kDa \( \alpha \)-carboxyl transferase encoded by accA (Li and Carnoon, 1992; Tong, 2013; Tran et al., 2015). The Carboxylase transferase component is a heteromeric tetramer with an \( \alpha 4 \beta 4 \) quaternary structure (Guchhait et al., 1974; Li and Cronan, 1992). ACCase functional region starting from the N-terminus to C-terminus are the biotin carboxylase (BC), biotin binding (BB) carboxyltransferase (CT), and ATP-binding (AB) regions. AB region lies within BC. Biotin sare covalently attached through an amide bond to the long side chain of a lysine residue in BB region. As BB is between BC and CT regions, biotin can easily translocate to both active sites where it is required. The accD gene is located in the plastid genome and the other three genes occur in the nuclear genome. The plastids have their bacterial origin and these three genes accB, accC and accA of bacterial origin may have been transferred from the nuclear genes. The plastids have bacterial type of machinery for fatty acid synthesis but only one of the genes of plastidic origin involved in the fatty acid biosynthesis i.e. accD. The accD gene is so far found to be present in all plants except in the gramineae. The chain length of the accD protein varies from 316 amino acids in liverwort (Ohyama et al., 1986) to 590 residues in pea (Nagano et al., 1991). The 300 amino acids at -COOH terminus are conserved among the various plant species. The variability exists at the -NH\(_2\) terminus.
The prokaryotic form of the ACCase in gramineae has been replaced by the homolog of eukaryotic form of ACCase in course of evolution. Various systems in plastids such as, RNA synthesis, protein synthesis and fatty acids biosynthesis are of bacterial origin but in the gramineae the system of fatty acid synthesis is the combination of bacterial and eukaryotic origin (Sasaki et al., 1995; Yu et al., 2010). The eukaryotic form of ACCase has been isolated from many plants (Harwood, 1988). The molecular size is about 500kD with the multimer of a single polypeptide of about 220kD as in mammals.

COMPARTMENTATION OF ISOFORMS OF ACCase
In most plants the prokaryotic form is found in plastids, and the eukaryotic form in cytosol. The presence of prokaryotic form of ACCase in plastids was confirmed by Sasaki et al., in 1993 by purifying a partial DNA fragment of accD gene from pea.

The prokaryotic form of ACCase has biotinylated protein of about 35 kD and eukaryotic form has of about 220 kD. Wheat chloroplast leaves did not contain the prokaryotic form but only eukaryotic form (Konishi and Sasaki, 1994; Rousseau-Gueutin et al., 2013). ACCase in Gramineae was the first example of substitution of a plastid gene by a nuclear gene for a non-ribosomal component. Another member of gramineae family, maize also contains eukaryotic form of ACCase both inside and outside of its plastids (Egli et al., 1993). These facts lead to conclude that all the dicots contain eukaryotic form in cytosol and prokaryotic form in plastids. In gramineae prokaryotic form is absent in both the plastids as well as in cytosol. The eukaryotic form is sensitive to herbicides like sethoxydim (cyclohexanedione) (Alban et al., 1994; Zhang, 2004; Wenlei Guo et al., 2015).

**Table 1: Characteristic features of different forms of ACCases.**

<table>
<thead>
<tr>
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<th>Prokaryotic form of ACCase Heteromeric</th>
<th>Eurokaryotic form of ACCase Homomeric</th>
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<tr>
<td>Polypeptide structure</td>
<td>BC, BCCP, α CT, βCT</td>
<td>Multifunctional ppt.</td>
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<tr>
<td>In gramineae</td>
<td>Absent</td>
<td>Present in plastids &amp; cytosol</td>
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<tr>
<td>In other plants</td>
<td>Present in plastids</td>
<td>In cytosol</td>
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<td>Sensitivity to herbicides</td>
<td>Insensitive</td>
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In animals, including humans two isoforms of acetyl-CoA carboxylase are present. They are ACC1 (Mr 5265 kDa) and ACC2 (Mr 5280 kDa). The predicted amino acid sequence of ACC2 contains an additional 136 aa relative to ACC1, 114 of which constitute the unique N-terminal sequence of ACC2. The hydrophobic profiles of the two ACC isoforms generally are comparable, except for the unique N-terminal sequence in ACC2. The experimental evidences demonstrated that ACC1 is a cytosolic while ACC2 was associated with the mitochondria. The association of ACC2 with the mitochondria is consistent with the hypothesis that ACC2 is involved in the regulation of mitochondrial fatty acid oxidation through the inhibition of carnitine palmitoyl transferase by its product malonyl-CoA (Elheiga et al., 2002).

**ROLE OF ACCase**

Reviewing the role of ACCase shows that prokaryotic form of ACCase in plastids is to provide the precursor, malonyl CoA for fatty acid biosynthesis. ACCase activity controls flux through the de novo fatty acid biosynthetic pathway (Ohlrogge and Jaworski, 1997; Rawsthorne, 2002). The fatty acid biosynthesis in plastids is feasible because ATP and NADPH are produced by photosynthetic electron transfer reactions and are abundantly available. The fatty acid biosynthesis in plastids is widely used not only for the synthesis of thylkoid membranes but also for the synthesis of acyl lipids outside of the plastids. ACCase is more in the young leaves than the mature ones to provide sufficient membrane lipids for developing tissues (Podkowinski et al., 2003). A non-photosynthetic plant beech drop, which has the smallest plastid genome identified so far because of loss of photosynthetic and chloro-respiratory genes still, has the accD gene (Whol FE et al., 1992). This suggests the importance of fatty acid biosynthesis in plastids.

The role of eukaryotic form of ACCase in cytosol is to provide malonyl CoA for chain elongation of fatty acid and flavonoids synthesis. Biochemical and molecular studies also provide evidence of occurrence of de novo fatty acid synthesis outside the plastids. The eukaryotic form of ACCase, in fact, is abundant in epidermal tissues (Alban et al., 1994). Where most cuticular waxes and flavonoids are synthesized. Wax and flavonoids are important in interaction of plant with their environment, for the protection against UV light and pathogens. This is evident as ACCase activity increases with UV irradiation (Ebel and Hahlbrock, 1977). The transcript of eukaryotic form of alfalfa ACCase induced by an elicitor (Sorrosth et al., 1994) suggests that ACCase can help to control the synthesis of protective compounds when necessary.

**GENETIC MANIPULATION OF ACCase**

ACCases are important in determining seed oil content and their activity is directly correlated with lipid accumulation as well as many physiological disorders. In soybean, ACCase activity in developing seed was found to have a positive correlation with the seed oil accumulation (Lee, 1995). The peak expression of mRNA for multifunctional ACCase in *Brassica napus* occurs just prior to onset of fatty acid accumulation (Elborough et al., 1994). Decreased plastidic ACCase activity also decreases the fatty acid and thus lipid biosynthesis triggering a series of physiological changes (Hui, 2000). Northern analysis of BCCP and βCT mRNA in rape seed oil showed that their mRNA levels differ in different tissues but their temporal pattern of expression were identical during embryo development (Elborough et al., 1996). To produce functional product, RNA editing of carboxyltransferase (CT) is required in chloroplast. RNA editing is an important feature in in the chloroplast of plants. In chloroplastic CT codon for leucine is not present at the required position so RNA editing takes place to create the codon for leucine to produce functional product. This also indicates that leucine is essential for enzyme activity (Sasaki et al., 2001).

Methods for modifying fatty acid composition and production involve modulation of an ACCase e.g. over expression by stable insertion of at least one copy in plant genome, down regulation by antisense RNA expression, or expression of a sense RNA from a fragment of ACCase gene in the plant cell etc. Vector containing a suitable promoter can be used for using gene in sense or antisense order along with polyadenylation signal to produce transgenic plants. This method can be used to modify the oil production in oil crops like soybean, sunflower, maize, oil palm, or coconut. Increased expression can raise the level of oil produced by plants, and down regulation of oil biosynthesis may be used to divert the substrate (acetyl CoA) in to biosynthesis of alternative storage materials such as starch and polyhydroxyalkaoate. A chloroplast transit peptide and napine promoter were fused with Arabidopsis homomeric ACCase gene and transformed in to Brassica napus. This transformation resulted in 10-20 fold increase in ACCase activity and altered fatty acid composition by increasing oleic acid content. Total lipid content also increased by 5% (Roesler et al., 1997).
Cytosolic ACCase from alfalfa (Medicago sativa) was transformed into soybean and Brassica spp. to increase total oil content of seed (Shorrosh, 2002). Reduction of ACCase by antisense expression reduces the seed lipid content and also affects carbohydrate metabolism (Sellwood, 2000). Chloroplast transformation with accD increases ACCase, leaf longevity and seed yield in tobacco (Madoka et al., 2002). Transformation of two species of diatom i.e. Cyclotella cryptica (CYCLO1) and Navicula saprophyta (NAVICI) was accomplished by introducing chimeric plasmid vectors (pACCNPT10 and pACCNPT5.1) containing regulatory sequences from ACCase from Cyclotella cryptica using microinjectile acceleator. This was used to manipulate the lipid accumulation to get more lipid content replaced with mineral oil as biodiesel (Dunahay, 1998). Ribozyme designed specifically to target ACCase by decreasing the ACCase mRNA level, decreased fatty acid synthesis (Jitta and Kim, 1994). ACCase encoding genes can be used for engineering organisms to be used as bioreactors for fatty acid production. With increasing needs for renewable sources of energy, malonyl-CoA, the direct product of the reaction catalyzed by ACCase, may serve as a universal precursor for a variety of other high-value compounds such as divergent polyketides and flavonoids, which are the center of interest owing to their various biochemical activities widely applied in pharmaceutical industry. The first metabolic engineering application of ACCase was the overexpression of a set of four E. coli genes encoding four bacterial subunits of this enzyme (Davis et al., 2000). All genes were assembled in an artificial operon under bacteriophage T7 promoter. All these genes were put under the same tightly controlled, inducible promoter on a low copy plasmid.

In this study, the 50-times increased activity of the enzyme resulted in near 100-times higher level of malonyl-CoA in the cell (Davis et al., 2000). In some of the E. coli strains, overexpressing ACCase genes with additional modifications are targeted to directly increase fatty acids production efficiency (Lu et al., 2008). The eukaryotic form of ACCase genes present in wheat plastid used for coexpression of yeast ACC1 null mutation enabled the studying of plant genes in a yeast system (Podkowinski et al., 2003; Gornicki et al., 1997; Joachimiak et al., 1997). Zha et al. also made efforts to increase malonyl-CoA level in E. coli cells for the production of its other derivatives by genetic manipulation of ACCase (Zha et al., 2009).

Full-length cDNA sequences encoding multifunctional ACCase have been isolated and characterized from alfalfa (Shorrosh, 1994), wheat (Gornicki et al., 1994), Arabidopsis (Yanai et al., 1995) and maize (Egli, 1995). Further ACCase genes from Arabidopsis (Roesler, 1994), B. napus (Schulte et al., 1994), wheat (Podkowinski, 1996), Brassica juncea (Kishwar Ali et al., 2007), and Avena fatua (Kieth et al., 2014) have been characterized. A full-length cDNA of the carboxyltransferase (accA) gene of acetyl-coenzyme A (acyl-CoA) carboxylase from Jatropha curcas was cloned and sequenced. The gene with an open reading frame (ORF) of 1149 bp encodes a polypeptide of 383 amino acids, with a molecular mass of 41.9 kDa. (Xie et al., 2010). Partial ACCase gene sequences of many other plants like Brassica juncea, B. Oleracea, Avena fatua, Alopecurus japonicus, Lolium rigidum have been cloned and their sequence is available in Genbank.

REGULATION OF ACCase

The ACCase has three important features in carrying out the reaction to convert Acetyl-CoA to malonyl CoA. First, it contains biotin as prosthetic group, second biotin is used as carrier of carbon dioxide from bicarbonate moiety to acyl group and third the carboxylation reaction is driven to completion by hydrolysis of ATP. Biotin is linked to the enzyme by an amide bond between the terminal carboxyl group of biotin side chain and epsilon amino group of lysine residue. The combined biotin and lysine side chain acts as long flexible arm that allows the biotin ring to translocate between two active sites. The regulation of ACCase is complex (Post–Beittenmiller et al., 1995; Shintani and Ohlrogge, 1995; Ke et al., 1997; Roesler et al., 1997; Roughan, 1997; Sunet et al., 1997; Caffrey et al., 1998) and may encompass many different levels of control. Transcriptional regulation during synthesis of fatty acids, membrane and storage lipids, starts at the early stage of cell growth and development and is due to expression of basal level of ACCase. The transcript levels of ACCase increase during early phase of seed development, attain the maximum level, and then decrease during the late phase of development. These changes are mainly caused by transcriptional regulation (Key et al., 2000). Post translational regulatory mechanisms affecting ACCase activity on short time frame include the biotinylation of the BCC subunit (Wang et al., 1995). The targeting, import and assembly of the three nuclear encoded subunits (BCC, BCase and alpha CT) with plastid encoded subunit (beta CT) phosphorylation (Syage and Ohlrogge, 1999) and biochemical modulation of enzyme activity (Estwell and Stump, 1983; Sasaki et al., 1997; Hunter and Ohlrogge, 1998; Kazaki and Sasaki, 1999). In contrast, developmentally induced modulation of ACCase occurs in longer time frame and likely to regulate pre-translational process. Mammalian ACCases are multifunctional dimeric proteins of molecular weight 530-560 kDa especially having the two active sites, essential catalytic biotin, the three-substrate reaction and effects of allosteric ligands. The expression of the two major isoforms and splice variants of mammalian ACCase is tissue-specific and responsive to hormones and nutritional status (Brownsey et al., 2006).
REGULATION BY PHOSPHORYLATION

The ACCase is a rate-limiting enzyme in the fatty acid biosynthesis and is under tight control. It is down regulated by palmitoyl CoA (end product regulation) and phosphorylation of enzyme through a glucagon-cAMP cascade. It is up regulated by citrate allosterically and dephosphorylation of enzyme under the influence of insulin/glucagon ratio by covalent modification (Travers and Barbers, 1999). In the animals like mammals ACCase is regulated by phosphorylation and by local metabolites like citrate etc. A cAMP cascade activated by hormones glucagon and epinephrine, when blood glucose is low, results in phosphorylation of ACCase by cAMP dependent protein kinase. When phosphorylated, the ACCase filament dissociates in to inactive monomers. ACCase is thus kept available for the production of ketone bodies, the alternate source of metabolic fuel used when glucose in blood is low. Another cAMP-activated kinase also phosphorylates ACCase thus causing inhibition. This is especially important in cardiacl muscles, where ACCase is not significantly involved in FA synthesis and the product of ACCase acts as an inhibitor when AMP is very high (ATP is low). Malonyl CoA is diminished, releasing fatty acids oxidation from inhibition (Munday, 2002; Hardie, Chi-Yuan Chou, 2011; Pan, 2002).

REGULATION BY LOCAL METABOLITES

Palmitoyl CoA, the end product of fatty acid biosynthesis promotes the inactive state of ACCase and regulates by feedback inhibition. Palmitoyl CoA inhibits the translocase that transports citrate from mitochondria to the cytosol, as well as glucose 6-phosphate dehydrogenase, which generates NADPH in the pentose phosphate pathway. Evidence from leaves, isolated chloroplasts and suspension culture cells strongly indicates ACCase as a major point of carbon flux control. ACCase is a valve, which determines the flow of carbon towards fatty acids. In animals and yeast it is partly controlled by feedback on ACCase by long chain acyl CoAs. In vitro study witnessed the inhibition of ACCase at submicromolar concentration (Goodridge, 1972). Citrate activates the enzyme and modulates ACCase activity allosterically (Munday, 2002). This enzyme is allosterically stimulated by citrate. Specifically, citrate partly reverses the inhibition produced by phosphorylation. Citrate facilitates the polymerization of the inactive octamers into active filaments. The level of citrate is high when both acetyl CoA and ATP are abundant. A high level of citrate signifies that two-carbon units and ATP are available for fatty acid synthesis. The stimulatory effect of citrate on the carboxylase is antagonized by palmitoyl CoA, which is abundant when there is an excess of fatty acids.

The experiment to find feedback inhibition was conducted with a variety of Tween esters for their effects on the rate of fatty acid synthesis and it was observed that the maximum inhibition was achieved upon feeding oleic acid (18:1) Tween esters that resulted in the intracellular accumulation of 18:1 free fatty acid, 18:1-CoA, and 18:1-acyl-carrier protein (ACP). Direct, saturable inhibition of ACCase enzyme activity was observed in culture extracts and extracts of developing canola seeds supplemented with 18:1-ACP at physiological concentrations (Andre et al., 2012). Palmitoyl CoA causes the filaments to disassemble into the inactive octamers. The glutamate, an abundant intra cellular amino acid induces ACCase activation through complementary action as a phosphatase activator and as a direct allosteric ligand for dephosphorylated ACCase (Adrienne et al., 2000).
LIGHT AND DARK REGULATION OF ACCase

De novo fatty acid synthesis in chloroplasts increases in the light and decreases in the dark. It was demonstrated that the isolated chloroplasts incorporate acetate into malonyl-CoA within minutes when exposed to light and the incorporation decreases when exposure ends (Saure et al., 1983). This pattern of change has been partly explained by changes in ACCase activity via the pH, Mg\(^{2+}\) and adenine nucleotide levels of the chloroplast stroma. Fatty acid synthesis in chloroplasts is regulated by light. Synthesis of malonyl CoA by ACCase is modulated by light/dark. The possible involvement of redox cascade in light/dark modulation of ACCase, effect of DTT, a known reductant of S-S bonds was examined in vitro. Study on isolated plastidic ACCase from pea showed that only plastidic ACCase was activated by DTT and thioredoxin in vitro. The cascade is also activated by thioredoxin reduced enzymatically with NADPH and NADP-thioredoxin reductase.

These findings suggest that reduction of ACCase is needed for activation of enzyme and a redox potential generated by photosynthesis is involved in activation of ACCase as for the enzymes of pentose phosphate cycle (Kozaki et al., 1999). The catalytic activity of ACCase was found maximum at pH 8.0 and 2 mM Mg\(^{2+}\), indicating that light produced changes in stromal pH and Mg\(^{2+}\) conc. modulate the ACCase activity. These facts reveal that light directly modulates the activity of plastidic or prokaryotic form of ACCase via signal transduction of redox cascade and indirectly modulates the activity via pH and Mg\(^{2+}\) conc. A redox cascade is likely to link between fatty acid synthesis regulations of ACCase (Sasaki et al., 1997). Savage et al demonstrated that in isolated chloroplasts, β-carboxyl transferase was phosphorylated on serine residue by illumination, and the phosphorylation decreased when the chloroplasts were transferred to dark conditions (Savage et al., 1999; Danon et al., 1994). At molecular level, light dependent regulation is by reduction of carboxyl transferase a subunit of ACCase (Kazaki et al., 2000).

REGULATION BY RNA EDITING

RNA editing has been detected in some organisms, such as trypanosomes and mammals. In chloroplasts, RNA editing creates start and stops codons and changes the coding sequence mostly due to a cytosine-to-uracil change at the second nucleotide position of the triplet (Hoch et al., 1991; Kunda et al., 19920; Mairetal, 1902; Wakasugi et al., 1996; Frever et al., 1997). In transcripts of the tobacco plastid genome, 0.13% of cytosine is changed to uracil (Sugiura et al., 1998). Comparing the accD gene sequence in the pea with its cDNA sequence, it is found that a cytosine-to-uracil change: the second nucleotide of UCG (serine) is converted to a U and the resultant UUG triplet encodes a leucine (Sasaki et al., 2001). Multiple alignments of the amino acid sequences deduced from the accD gene of 15 land plants suggest the occurrence of similar changes in 6 plants. In such plants that do not have a leucine codon at the position, editing was shown to take place so as to create the leucine codon. The requirement of a leucine codon at a specific position suggests that accD editing is necessary for several plants. This was also verified by Sasaki et al., 2001.

The carboxyl transferase activity of recombinant enzymes containing edited subunit was found to be active whereas one with unedited subunit was found to be inactive (Zhang et al., 2003). These experimental evidences suggest that the editing of ACCase is essential. In the case of the accD, however, the biological roles are not yet clear (Sasaki et al., 2004).

POST-TRANSCTRSCRIPTIONAL REGULATION

Madoka et al., (2004) showed that in case of ACCase, there is coordinated synthesis of four subunits partly caused by post-transcriptional regulation at the level of assembly. Chloroplast transformation experiments provide a method of modifying plastid genes (Svab et al., 1993). The effect of over-expression of a plastid encoded subunit on the expression of a nuclear-encoded subunit and ACCase level, were investigated by replacing the promoter of the tobacco accD operon with the promoter of the tobacco plastid rRNA operon by means of chloroplast transformation. The results showed an increase in the accD transcript, all four subunits, and the ACCase level (Madoka et al., 2002). It was proposed by Sasaki el al that a close coordination of nuclear- and plastid-encoded subunit synthesis is not necessary and that the synthesized subunits assemble into ACCase and excess subunits are rapidly degraded at the assembly site. These findings suggest that three nuclear-encoded subunits increased without a corresponding increase in their transcripts and also showed the involvement of post-transcriptional regulation. Further the experimental proofs reveal that the expression of ACCase in most cases is controlled at the level of transcription of each gene and further finely controlled by protein degradation, at the last step, i.e. assembly.

REGULATION BY HERBICIDES

The eukaryotic type ACCases present in plastids are characteristic of Poaceae family where the aryloxyphenoxypropionates (AOPP), cyclohexanediones (CHD) and phenyl pyrazolines like herbicides block the whole fatty acid synthesis pathway, and consequently cause death of the plant. Some monocot crops, such as wheat, are resistant to herbicides, although their plastids ACCases are still sensitive due to the rapid metabolism of the inhibitors.
The isolation of a eukaryotic type plastid ACCase gene from wheat and using for complementation of yeast ACC1 null mutation enabled the studying of plant genes in a yeast system (Gornicki et al., 1997; Joachimiak et al., 1997; Podkowinski et al., 2003). An analysis of a series of chimeric ACCases composed of fragments of plastid and cytosolic enzymes of different herbicide sensitivity, is due to presence of the specific amino acids within the conserved region of carboxyl transferase domain (Nikolskaya et al., 1999; Zagnitko et al., 2001). The comparison of natural, herbicide-resistant maize plastid ACCase mutants with a susceptible enzyme and an engineered mutant of an altered, specific single amino acid proved that the resistance to herbicide depends on leucine substituting isoleucine in a position equivalent to 1705 aa of yeast ACCase. So far, 35 weed species with such mutants have been reported (Devine and Skula, 2000). The analysis of mutants showed that plastid ACCases resistance to herbicides is associated with the alterations of five amino acids from carboxyl transferase domain (Delye et al., 2005; Nikolau et al., 2003). Since herbicide resistance originates from natural, spontaneous mutations and spreads relatively easily, there is an urgent need for new herbicides active against the resistant weeds. According to this model, the carboxyl transferase domain is composed of two: N- and C-sub domains which correspond to prokaryotic carboxyl transferase -alpha and beta subunits, respectively, and both have β-β-α super helix fold. Plants carboxyl transferase domains are expected to form head-to-tail dimers, which are the active form of enzyme capable of binding and processing the substrate - acetyl-CoA. The interface of the carboxyl transferase domains within dimer is composed of highly conserved amino acids, which also create an active site, and are involved in interactions with thiol group of CoA. The studies on carboxyl transferase domain interactions with herbicides are also the most advanced for the yeast enzyme. The results obtained from computational techniques supported by a functional analysis and experimental approaches allow designing novel ACCase inhibitors for agriculture (Podkowinski et al., 2011).

CONCLUSIONS

Acetyl-coenzyme A carboxylase is a key regulatory enzyme in the fatty acid path way and provides malonyl-CoA for the synthesis of variety of biomolecules which open the door for many biotechnological projects that include, isolation and design of new transgenes for overexpression of the oils used for industries, biodiesel, new drugs, antibiotics and herbicides which address the main needs of the human population like improvement of health care and increased food production. The in depth understanding of the gene and its structural data can enable rationale, computer-aided modeling, prediction of interesting interactions and designing compounds targeted against specific regions of the enzyme. Study on ACCases function, structure, regulation of genes, their promoters and transcription factors controlling their expression need better understanding and their application in biotechnological projects.

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