Kinetic Parameters

_Sulfolobus solfataricus_ HMG-CoA reductase exhibits a substrate specificity typical of biosynthetic HMG-CoA reductases. NADH cannot replace NADPH, nor can (R)-HMG-CoA replace (S)-HMG-CoA. Optimal activity for catalysis of the reductive deacylation of HMG-CoA occurs at about pH 5.5. \( V_{\text{max}} \) is 17 eu/mg at 50°. Optimal activity for catalysis of reaction (1) occurs at pH 5.5, well below the optimum pH for the only other characterized archaeal HMG-CoA reductase, that of the halophile _H. volcanii_ (pH 7.3) and of the optimal pH for the hamster enzyme (pH 6.1).

Temperature Profile for Activity and for Stability

Significant features of _S. solfataricus_ HMG-CoA reductase include high thermal stability, high optimal temperature, and low optimal pH. As might be anticipated for an enzyme from a thermophile, concentrated solutions of homogeneous _S. solfataricus_ HMG-CoA reductase are stable at elevated temperatures (\( t_{1/2} = 3.2 \) hr at 90°). The activity of dilute solutions is optimal at 85° and \( \Delta H \) for catalysis of reaction (1) is about 47 kJ (11 kcal)/mol.

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[26] Characterization of 3-Methylcrotonyl-CoA Carboxylase from Plants

By Eve Syrkin Wurtele and Basil J. Nikolau

Introduction

3-Methylcrotonyl-CoA carboxylase (MCCase, EC 6.4.1.4) is a biotin-containing enzyme that catalyzes the ATP-dependent carboxylation of 3-methylcrotonyl-CoA to form 3-methylglutaconyl-CoA.\(^1\) As with all biotin-containing enzymes, the reaction catalyzed by MCCase takes place in two steps (biotin carboxylation and carboxyl transfer), with the enzyme-bound carboxybiotin being an intermediate of the reaction. MCCase was initially purified and characterized from bacterial\(^2\) and animal\(^3\) sources, and was

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in fact the enzyme via which the biochemical function of biotin as a enzyme-bound cofactor was discovered. Relative to this earlier work, the identification and characterization of MCCase in plants is recent.5 Hence, the metabolic role of MCCase in plants is only just starting to be elucidated.

The reaction catalyzed by MCCase is required in a number of metabolic pathways, which may be interconnected (Fig. 1). These include the catabolism of leucine and isoprenoids, and the mevalonate shunt. In animals the catabolism of leucine to acetoacetate and acetyl-CoA is a mitochondrial process,6 as is the subcellular location of MCCase.7 However, in plants,

leucine catabolism occurs via two independent pathways: one pathway catabolizes leucine to acetyl-CoA and acetoacetate, requires MCCase, and is located in mitochondria; the second, MCCase-independent pathway occurs in peroxisomes and catabolizes leucine to acetyl-CoA.

In addition to leucine catabolism, MCCase is also required for the operation of the mevalonate shunt, which diverts mevalonate from isoprenoid biosynthesis to primary metabolism (acetyl-CoA). This shunt appears also to operate in plants. Finally, in some bacteria, MCCase has a role in the catabolism of acyclic isoprenoids, such as geranyl-CoA, to acetyl-CoA. This pathway also requires the biotin-containing enzyme geranyl-CoA carboxylase. The identification of geranyl-CoA carboxylase in plants indicates that this process may also be operating in these organisms.

This chapter discusses the characterization of MCCase from plant sources, which since the discovery of this enzyme in these organisms has led to a clearer understanding of the structure and genetic regulation of this mitochondrial biotin-containing enzyme.

**Purification and Assay Methods**

MCCase has been purified from a number of plant sources by two basic protocols. One of these protocols relies on the specific reaction of avidin with biotin. The other protocol uses more conventional protein purification procedures. Both of these protocols are described below.

**Plant Materials**

MCCase has been purified from carrot, maize, soybean, barley, pea, and potato. Studies of the distribution of the enzyme indicate that it

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is prevalent in most plant tissues, but is most abundant in nonphotosynthetic tissues.\textsuperscript{8} However, because of the lack of interfering substances and the ease of obtaining large quantities of tissue, young expanding seedlings (8- to 14-day-old maize seedlings or 5- to 12-day-old soybean seedlings) are a convenient source of plant material for the purification of MCCase.

Soybean seeds (\textit{Glycine max} cv. Corsoy 79) can be germinated in a sterile mixture of 30\% (v/v) black soil, 30\% (v/v) peat moss, and 40\% (v/v) perlite in 50 × 30 × 6 cm flats, in a greenhouse maintained at 22 to 25\°C, under a cycle of 15 hr of illumination and 9 hr of darkness, with a maximum daily irradiance of about 1200 \mu mol photons m\textsuperscript{-2} sec\textsuperscript{-1}. Maize seeds can be germinated in sand in a greenhouse maintained at 25 to 30\°C, under a cycle of 14 hr of illumination and 10 hr of darkness, with a maximum daily irradiance of about 1200 \mu mol photons m\textsuperscript{-2} sec\textsuperscript{-1}. Seedlings are watered daily.

3-Methylcrotonyl-CoA Carboxylase Activity Assay

As with most biotin-containing enzymes MCCase activity is routinely assayed by a radiocchemical assay. MCCase activity is determined as the 3-methylcrotonyl-CoA-dependent incorporation of radioactivity from NaH\textsuperscript{14}CO\textsubscript{3} into the acid-stable product.\textsuperscript{5} The standard assay is carried out in a total volume of 0.2 ml, which is composed of 0.1 M Tricine-KOH (pH 8.0), 5 mM MgCl\textsubscript{2}, 1 mM ATP, 2 mM dithiothreitol (DTT), 5 mM NaH\textsuperscript{14}CO\textsubscript{3}, (5 mCi/mmole), 0.2 mM 3-methylcrotonyl-CoA, and up to 0.01 unit of enzyme activity.

The assay is mixed, on ice, as follows:

- Tricine-KOH (pH 8.0, 0.4 M), 20 mM MgCl\textsubscript{2}, 8 mM DTT
- ATP (10 mM), adjusted to pH 7.0 with KOH
- NaH\textsuperscript{14}CO\textsubscript{3} (5 mCi/mmole), 50 mM: This solution is prepared just before use, by dissolving 3.8 mg of NaHCO\textsubscript{3} in 0.875 ml of water and adding 0.125 ml of NaH\textsuperscript{14}CO\textsubscript{3} (2 mCi/ml; specific radioactivity, 55 mCi/mmole)

Enzyme and water are added to adjust the volume to 180 \mu l, and the mixture is incubated at 37\°C for 30 sec. The assay is then initiated by adding of 20 \mu l of 2 mM 3-methylcrotonyl-CoA, and further incubated at 37\°C for 10 min. The reaction is terminated by the addition of 50 \mu l of 6 N HCl. An aliquot (100 \mu l) is spotted on a strip of Whatman (Clifton, NJ) 3 MM paper, which is dried in a fume hood on a hot plate maintained at about 50\°C. The acid-stable radioactivity is determined by liquid scintillation counting.

This assay is susceptible to interference when used to assay MCCase activity in crude plant extracts. This interference occurs because such extracts contain abundant carboxylases (i.e., ribulose-1,5-bisphosphate car-
bovylase and/or phosphoenolpyruvate carboxylase) and their substrates. This interference can be eliminated by passing the extract through a small gel-filtration column (Sephadex G-25 or Bio-Gel P6), which will remove low molecular weight molecules. These “desalting columns” can be obtained from commercial suppliers or can be made by pouring preswollen matrices. The column bed volume needs to be at least fivefold larger than the volume of extract that is being desalted.

Preparation of Monomeric Avidin Affinity Column

Although monomeric avidin affinity columns are available from a number of commercial sources, we have not obtained reliable results with these matrices. Synthesis of a monomeric avidin affinity matrix is carried out on the basis of previously published procedures. Twenty grams of cyanogen bromide-activated Sepharose 4B is suspended in 100 ml of 1 mM HCl. This suspension is sequentially washed with 1 liter of 1 mM HCl and 500 ml of 10 mM potassium phosphate buffer (pH 7.0). Avidin (200 mg), dissolved in 200 ml of 10 mM potassium phosphate buffer (pH 7.0), is added to the activated Sepharose suspension (200 ml), and the mixture is allowed to react overnight at 4°C. The reacted Sepharose is then sequentially washed with 1 liter of 1 M ethanolamine hydrochloride (pH 7.0) and 2 liters of 10 mM potassium phosphate buffer (pH 7.0). The resulting Sepharose–avidin conjugate is poured into a 2.5 × 50 cm column (72-ml bed volume). To dissociate the avidin subunits, the column is washed with 5 bed volumes of 6 M guanidine hydrochloride and maintained in the presence of this solution at room temperature for 20 hr. The column is then washed with 2–3 additional bed volumes of 6 M guanidine hydrochloride, followed by 10 bed volumes of 10 mM potassium phosphate buffer (pH 7.0). The elution of the dissociated avidin subunits is monitored by the A280 of this last wash. Once the A280 of the eluate is less than 0.01, the column is washed with 100 ml of 10 mM potassium phosphate buffer (pH 7.0) containing 2 mM biotin. The column is then extensively washed with 0.1 M glycine hydrochloride (pH 2.0) in order to remove biotin bound to exchangeable binding sites. (Note: It is important to wash the column extensively at this stage because the solubility of biotin at pH 2.0 is low and any precipitated biotin that remains on the column will subsequently interfere with enzyme purification.) The column is finally equilibrated with buffer A. Buffer A is composed of 10 mM HEPES–KOH (pH 7.0), 20 mM 2-mercaptoethanol, and 20% (v/v) glycerol.

MCCase is purified by affinity chromatography with such monomeric

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avidin columns. Enzyme preparations are bound to the matrix in the presence of high concentration of salts, and MCCase is specifically eluted with a solution containing biotin. Between uses the affinity matrix can be regenerated by washing the column with 3–5 bed volumes of 0.1 M glycine hydrochloride (pH 2.0), and then equilibrating with buffer A. Once every three or four uses, the column should be regenerated by washing with 6 M guanidine hydrochloride, followed by sequential washes with 10 mM potassium phosphate buffer (pH 7.0) containing 2 mM biotin, and with 0.1 M glycine hydrochloride (pH 2.0). Although such regeneration of a monomeric avidin matrix by denaturation–renaturation cycles extends the life of the matrix, its binding capacity is reduced. Hence, in our hands such columns have a limited lifetime and need to be remade after about 10 uses.

**Purification of 3-Methylcrotonyl-CoA Carboxylase**

Two methods for the purification of MCCase are presented below. The first method (method A) is illustrated by the purification of the enzyme from soybean seedlings (Table I), and the second method (method B) is illustrated by the purification of the enzyme from maize seedlings (Table II). Both methods give similarly pure preparations of MCCase, with approximately similar yields. In the case of the maize MCCase, recovery of the enzyme is somewhat difficult to judge, as activity in the extract is underestimated, probably because of the presence of some interfering substance, which is removed in the first purification step (see Table II).

The long-term stability of MCCase in extracts is enhanced if the enzyme is extracted at neutral pH. MCCase extracted with 0.1 M Tris-HCl (pH 8.0) is relatively unstable; the enzyme is completely inactive 2–3 days after extraction. Either addition of 1% (w/v) bovine serum albumin (BSA) to the extract or changing the pH of the extract fails to stabilize the enzyme.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein (mg)</th>
<th>Total activity (units$^a$)</th>
<th>Specific activity (units/mg protein)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>714</td>
<td>663</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td>PEG</td>
<td>595</td>
<td>595</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Cibacron Blue</td>
<td>106</td>
<td>724</td>
<td>6.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Q-Sepharose</td>
<td>80</td>
<td>549</td>
<td>6.9</td>
<td>7.7</td>
</tr>
<tr>
<td>Monomeric avidin</td>
<td>0.9</td>
<td>303</td>
<td>336.7</td>
<td>374.0</td>
</tr>
</tbody>
</table>

$^a$ Purification from 350 g of soybean seedlings.

$^b$ One unit equals 1 nmol of HCO$_3^-$ incorporated per minute.
This problem is overcome by changing the extraction buffer to 0.1 M HEPES-KOH (pH 7.0) and by including 20% (v/v) glycerol. To prevent loss of enzyme activity due to proteolysis, protease inhibitors are added to the extraction buffer [phenylmethylsulfonyl fluoride (PMSF) or trans-epoxysuccinyl-l-leucylamido(4-guanidino)butane]. In addition, to enhance the extractability of the enzyme, Triton X-100 is included in the extraction buffer.

**Purification Method A.** The purification method described below relies on the fact that MCCase contains biotin, which can specifically bind to immobilized avidin. Hence purification of MCCase can be readily achieved via affinity chromatography with immobilized monomeric avidin. Variations of this method have been used to purify MCCase from carrot, barley, pea, and potato.

The method detailed below is that used for the purification of the soybean MCCase. In this method MCCase is purified by sequential use of three chromatographic procedures: Cibacron Blue (Amersham Pharmacia Biotech, Piscataway, NJ) affinity chromatography, Q-Sepharose anion-exchange chromatography, and monomeric avidin affinity chromatography. Although the latter procedure achieves the purification of the enzyme, the first two procedures are required to separate MCCase from the other biotin-containing proteins present in the extract (e.g., acetyl-CoA carboxylase isozymes). In addition, the Q-Sepharose column has the effect of concentrating the enzyme preparation. A summary of a typical purification achieved by this method is presented in Table I.

Five-day-old soybean seedlings (300 g) are pulverized with a mortar and pestle in the presence of liquid N$_2$. The frozen, ground tissue is homogenized with 1 liter of buffer A containing 1 mM EDTA, 0.1% (v/v) Triton X-100, PMSF (100 μg/ml), 10 μM trans-epoxysuccinyl-l-leucylamido(4-guanidino)butane. Subsequent purification steps are performed at 4°C. The

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**TABLE II**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein (mg)</th>
<th>Total activity (units$^a$)</th>
<th>Specific activity (units/mg protein)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>5040</td>
<td>192</td>
<td>0.04</td>
<td>1</td>
</tr>
<tr>
<td>4-16% PEG</td>
<td>1295</td>
<td>723</td>
<td>0.56</td>
<td>14</td>
</tr>
<tr>
<td>Propyl-agarose</td>
<td>438</td>
<td>362</td>
<td>0.83</td>
<td>20</td>
</tr>
<tr>
<td>Cibacron Blue</td>
<td>42</td>
<td>520</td>
<td>12.5</td>
<td>312</td>
</tr>
<tr>
<td>Q-Sepharose</td>
<td>2.8</td>
<td>566</td>
<td>206.2</td>
<td>5155</td>
</tr>
</tbody>
</table>

$^a$ Purification from 1 kg of maize seedlings.

$^b$ One unit equals 1 nmol of HCO$_3^-$ incorporation per minute.
extract is filtered through four layers of cheesecloth, and the filtrate is centrifuged at 22,100g for 15 min. Polyethylene glycol 8000 (PEG 8000) is added to the resulting supernatant to a final concentration of 18% (w/v). Precipitated proteins are collected by centrifugation at 22,100g for 15 min, and the pellet is dissolved in the minimal volume of buffer A.

The enzyme preparation obtained from PEG precipitation is applied to an agarose-Cibacron Blue 3GA column (2 × 10 cm). The column is washed with 1 liter of buffer A, and MCCase is eluted with a linear gradient of 0–1M KCl in buffer A. Peak MCCase activity is eluted at about 0.5 M KCl. All the fractions containing MCCase activity are pooled and dialyzed against 50 volumes of buffer A.

The resulting preparation is applied to a Q-Sepharose column (3 × 20 cm), equilibrated with buffer A. The column is then washed with 500 ml of buffer A to remove proteins that do not bind to the column. MCCase is then eluted with a linear gradient of 0–0.8 M KCl in buffer A. Peak MCCase activity is eluted at about 0.6 M KCl. All the fractions containing MCCase activity are pooled and dialyzed against 50 volumes of buffer A.

The resulting preparation is applied to an agarose-monomeric avidin affinity column (3 × 20 cm), equilibrated with buffer A. The column is washed with 300 ml of buffer A containing 0.25 M KCl and MCCase is then eluted with buffer A containing 0.25 M KCl and 0.4 mM biotin. MCCase activity elutes as a sharp peak immediately on the addition of biotin to the column. This preparation is homogeneous and can be stored at −20°C (although we routinely store these preparations at −70°C). Stored in buffer A, under these conditions, purified MCCase is stable for at least 1 year with minimal loss of activity.

Purification Method B. Purification of MCCase from maize seedlings has been achieved by using the method outlined below. This method achieves purification of MCCase by the sequential application of three chromatographic procedures: hydrophobic chromatography on propylagarose, Cibacron Blue affinity chromatography, and Q-Sepharose anion-exchange chromatography. A summary of the purification achieved by this method is presented in Table II.

Maize leaves (1 kg) are frozen in liquid N2 and pulverized with a mortar and pestle. The resulting powder is homogenized at 4°C in a stainless steel Waring blender for 1–3 min with 3 liters of buffer A containing PMSF (0.1 mg/ml), 0.1% (v/v) Triton X-100, and 1 mM EDTA. The mixture is filtered through several layers of cheesecloth, and the filtrate is immediately centrifuged at 12,200g for 30–40 min at 4°C. The supernatant (extract) is retained, and the pellet discarded.

Finely powdered PEG 8000 is slowly added to the crude extract to a final concentration of 4 g of PEG/100 ml. The solution is stirred until the PEG is completely dissolved and the mixture is then centrifuged at 12,200g
for 30 min at 4°. The supernatant is retained, and more PEG 8000 is dissolved, to a final concentration of 16 g of PEG/100 ml. The precipitate (4–16% PEG fraction) is collected by centrifugation and resuspended in buffer A.

The 4–16% PEG fraction is applied to a propyl-agarose column (2.5 × 45 cm) previously equilibrated with buffer A. The column is washed with buffer A until the A280 of the eluate is less than 0.05. Elution of MCCase is achieved with a linear gradient of 0–0.5 M KCl in buffer A, at a flow rate of 0.5–1 ml/min. The fractions containing MCCase activity are pooled.

The pooled MCCase-containing fractions are applied to a column of Cibacron Blue-agarose (2.5 × 10 cm) previously equilibrated with buffer A. The column is washed with 5 column volumes of buffer A to remove unbound proteins. Elution of MCCase is achieved with an 800-ml linear gradient of 0–1.5 M KCl in buffer A at a flow rate of 1–1.5 ml/min. Fractions containing MCCase activity are pooled.

The pooled MCCase preparation obtained from the Cibacron Blue-agarose column is dialyzed against 4 liters of buffer A for 3–6 hr. The dialyzed sample is then applied to a Q-Sepharose column (2.6 × 18 cm) that has been equilibrated with buffer A. The column is washed with 10 volumes of buffer A. The enzyme is then eluted by using an 800-ml linear gradient of 0–0.75 M KCl in buffer A. Fractions containing MCCase are pooled and stored at −20°. Under these conditions, the purified maize MCCase can be stored for up to 6 months with minimal loss of activity.

Characterization of 3-Methylcrotonyl-CoA Carboxylase

Electrophoresis and Western Blot Analyses

Polyacrylamide gel electrophoresis (PAGE), performed under denaturing20 and nondenaturing21,22 conditions, is used to characterize the purified MCCase preparations. After electrophoresis, proteins can be stained with Coomassie blue or can be silver stained. Alternatively, proteins can be electrophoretically transferred to a nitrocellulose membrane, using a semi-dry transfer apparatus.23 Biotin-containing proteins are specifically detected with 125I-labeled streptavidin.24 Immunological detection can be performed by sequential incubation of the nitrocellulose filter with polyclonal antisera.

(diluted about 1:1000), and with $^{125}$I-labeled protein A [100 ng/ml, at a specific radioactivity of $1 \times 10^{10}$ disintegrations per minute (dpm)/mg]. Antiserum directed against the biotin-containing $^{25,26}$ (MCC-A) and nonbiotinylated $^{27,28}$ (MCC-B) subunits of MCCase have been prepared against recombinantly expressed proteins.

**Structural Characterization of 3-Methylcrotonyl-CoA Carboxylase**

Sodium dodecyl sulfate (SDS)–PAGE analyses of purified MCCase indicate that the enzyme is composed of two subunits (Fig. 2). The larger, MCC-A subunit contains covalently bound biotin and has a molecular mass of about 80 kDa. The smaller, nonbiotinylated MCC-B subunit has a molecular mass of about 60 kDa. The holoenzyme appears to be composed of an equal molar ratio of the two subunits.

The quaternary organization of MCCase has been inferred from the molecular weight of the holoenzyme. Gel-filtration chromatography on Superdex 200 yields a molecular weight of about 500,000 for the pea and potato enzyme, $^{18}$ which has been interpreted as indicating an octomeric enzyme with an $A_2B_4$ subunit stoichiometry, analogous to the bacterial MCCase.$^{2,3}$

However, because of the dissociation of MCCase on chromatography through Sephacryl S400, the molecular weight of the carrot,$^{14}$ maize,$^{15}$ soybean,$^{16}$ and tomato$^{29}$ MCCase could not be ascertained by gel-filtration chromatography. Rather, the molecular masses of these latter enzymes were determined to be between 800 and 900 kDa by nondenaturing PAGE.$^{15,16,29}$ These molecular weight determinations indicate that the MCCase holoenzyme is dodecameric, with an $A_4B_4$ subunit stoichiometry; such a quaternary organization is analogous to the animal MCCase.$^4$

The amino acid sequences of the MCC-A$^{25,26,30}$ and MCC-B$^{27,28}$ subunits have been determined by translation of the nucleotide sequence of the cloned cDNAs. Sequence similarities between MCC-A and other biotin-containing enzymes indicate that the MCC-A subunit is composed of two functional domains, which are sequentially arranged in the primary se-

Fig. 2. SDS-PAGE analysis of the purification of MCCase from soybean seedlings. Samples from the fractions obtained during the purification of the soybean MCCase (see Table I) were subjected to SDS-PAGE and the resulting gel was stained with Coomassie blue. Each lane was loaded as follows: 1, extract; 2, PEG fraction; 3, Cibacron Blue fraction; 4, Q-Sepharose fraction; 5, monomeric avidin fraction. Positions of molecular weight standards are indicated. The two MCCase subunits (MCC-A and MCC-B) are arrowed.

quency: the biotin carboxylase domain and the biotin carrier domain. Hence, the MCC-A subunit contains the active site for the catalysis of the first half-reaction catalyzed by MCCase. The sequence of the MCC-B subunit is most similar to carboxyltransferase subunits of the biotin-containing enzymes methylmalonyl-CoA decarboxylase (35% identity), propionyl-CoA carboxylase (30% identity), and transcarboxylase (33% identity). These findings imply that the MCC-B subunit contains the active site for catalysis of the second half of the reaction catalyzed by MCCase.

Both the MCC-A and MCC-B subunits are initially synthesized as pre-
Enzymological Characterization of 3-Methylcrotonyl-CoA Carboxylase

The enzymological properties of MCCase have been investigated with the purified enzyme from carrot, maize, pea, potato, barley, and soybean. These investigations indicate that the kinetic behavior of MCCase can be described by the Michaelis–Menten equation. Detailed studies of the initial velocities and inhibition patterns of competitive inhibitors of the soybean MCCase indicate that the kinetic mechanism of the reaction is as shown in Fig. 3. In this mechanism ATP and bicarbonate react as the first two substrates to generate the first two products (ADP and P_i). Subsequently, 3-methylcrotonyl-CoA reacts to form 3-methylglutaconyl-CoA.

The Michaelis constants (K_m) for the substrates methylcrotonyl-CoA and bicarbonate are in the range of 0.01–0.05 and 0.8–2 mM, respectively. As with most other biotin-containing enzymes that require ATP, ATP reacts with MCCase as MgATP, and the K_m for this substrate is about 0.02 mM. The optimum pH for MCCase activity is between 8.0 and 8.5. Tricine is used to buffer MCCase assays. It is important to use a buffer that does not complex Mg^{2+} ions (i.e., do not use Tris, phosphate, etc.), because MCCase requires free Mg^{2+} ions for activation (in excess of that required to complex ATP). The requirement for free Mg^{2+} ions can be partially replaced by Mn^{2+} or Co^{2+} ions, but not by Zn^{2+}. The monovalent cations K^+, Cs^+, Rb^+, and NH_4^+ are activators of MCCase, but Li^+ and Na^+ are inhibitors.

In addition to carboxylating methylcrotonyl-CoA, MCCase will also carboxylate crotonyl-CoA, but the latter is a much poorer substrate. In contrast to bacterial and animal MCCases, which can also carboxylate acetoacetyl-CoA, MCCase from plant sources cannot do so. In fact, acetoacetyl-CoA is a potent inhibitor of plant MCCases.
Physiological Role and Regulation of 3-Methylcrotonyl-CoA Carboxylase in Plant Metabolism

In animals and bacteria, the primary role of this enzyme is considered to be in leucine catabolism. In addition, MCCase has been implicated in the metabolism of mevalonate via the mevalonate shunt, and in the catabolism of noncyclic isoprenoids, such as geranoyl-CoA (Fig. 1). Whether these metabolic processes are also operating in plants is still unclear. Hence, the discovery of MCCase in plants has opened a new avenue of research into plant metabolism. It is now becoming clear that plants catabolize leucine via at least two physically separated pathways: an MCCase-requiring pathway in mitochondria, and an MCCase-independent pathway in peroxisomes. How the operation of these two catabolic pathways is coordinated and regulated must still be explored.

Interestingly, MCCase activity in plants can be regulated by a mechanism that is specific to biotin-containing enzymes, namely biotinylation. Although biotinylation is obviously essential for the function of every biotin-containing enzyme, biotinylation has not been extensively studied as a potential regulatory mechanism by which the activity of such enzymes can be controlled. In the case of MCCase in tomato plants the developmental pattern of MCCase distribution can be regulated by the biotinylation status of the enzyme. Specifically, whereas the MCC-A subunit accumulates to equal levels in leaves and roots of tomato plants, and this subunit is completely biotinylated in roots, only 10% of the MCC-A subunits that accumulate in leaves are biotinylated. Hence, the 10-fold difference in MCCase activity between leaves and roots of tomato plants is due to the differential biotinylation of the enzyme between these two organs. The biochemical mechanism that leads to the differential biotinylation of MCCase in roots and leaves requires additional research.

However, the presence of a pool of apo-MCC-A in tomato leaves leads to a number of questions as to the nature of the apoenzyme. MCCase is present in plant mitochondria; therefore, it will be interesting to ascertain where in the cell the apo-MCC-A subunit accumulates. If it accumulates within the mitochondria, does it occur within assembled MCCase molecules? In this latter case, a mixture of apo and holo subunits may be present in tomato leaf mitochondria. Studies to ascertain the effect of this subunit heterogeneity on MCCase will undoubtedly lead to new and interesting information about the regulation and structure of biotin-containing enzymes.