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Purification and Characterization of Acetyl-CoA Carboxylase from the Diatom *Cyclotella cryptica*[1]

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ABSTRACT

Acetyl-CoA carboxylase from the diatom *Cyclotella cryptica* has been purified to near homogeneity by the use of ammonium sulfate fractionation, gel filtration chromatography, and affinity chromatography with monomeric avidin-agarose. The specific activity of the final preparation was as high as 14.6 micromoles malonyl-CoA formed per milligram protein per minute, indicating a 600-fold purification. Native acetyl-CoA carboxylase has a molecular weight of approximately 740 kilodaltons and appears to be composed of four identical biotin-containing subunits. The enzyme has maximal activity at pH 8.2, but enzyme stability is greater at pH 6.5. K_m values for MgATP, acetyl-CoA, and HCO_3^- were determined to be 65, 233, and 750 micromolar, respectively. The purified enzyme is strongly inhibited by palmitoyl-CoA, and is inhibited to a lesser extent by malonyl-CoA, ADP, and phosphate. Pyruvate stimulates enzymatic activity to a slight extent. Acetyl-CoA carboxylase from *Cyclotella cryptica* is not inhibited by cyclohexanedione or aryloxyphenoxypropionic acid herbicides as strongly as monocot acetyl-CoA carboxylases; 50% and 0% inhibition was observed in the presence of 23 micromolar clethodim and 100 micromolar haloxyfop, respectively.

Acetyl-CoA carboxylase is a biotin-containing enzyme that catalyzes the formation of malonyl-CoA, which is one of the initial steps of fatty acid biosynthesis. Previous research has indicated that changes in the activity of this enzyme may play a role in the accumulation of lipids when the diatom *Cyclotella cryptica* is grown under silicon-limiting conditions (19). It was therefore of interest to investigate the properties of acetyl-CoA carboxylase from this alga. Although acetyl-CoA carboxylase has been purified from several higher plants, the enzyme has not previously been purified from an algal source.

Acetyl-CoA carboxylases from higher plants have several characteristics in common, including an alkaline pH optimum and an absolute requirement for ATP and divalent metal cations. Early studies indicated that plant acetyl-CoA carboxylases had complex subunit structures (three to six peptides having different mol wt), but more recent studies suggest that these earlier attempts to characterize the enzyme were subject to error due to the effects of endogenous proteolytic activity. Recent investigations have suggested simpler subunit structures. High mol wt (200–240 kD), biotin-containing subunits are commonly observed, leading several investigators to suggest that plant acetyl-CoA carboxylases are multifunctional proteins able to catalyze both steps of the reaction (biotin carboxylation and carboxyl transfer to acetyl-CoA) (8).

Little is known about the allosteric regulation of acetyl-CoA carboxylase activity in higher plants. Adenylate nucleotides (AMP, ADP, and free ATP) have been shown to inhibit the enzyme from several higher plants (2, 3, 15, 20). Acetyl-CoA carboxylase from maize is also inhibited by malonyl-CoA and palmitoyl-CoA (15). Free coenzyme A has been shown to inhibit the enzyme from maize (15) but to stimulate the activity of the enzyme from spinach (11). Citrate, free Mg^{2+}, K^+, and glycine have also been reported to stimulate acetyl-CoA carboxylase from various plant sources (6, 12–15).

The research described in this report was carried out in order to further our understanding of acetyl-CoA carboxylases from eukaryotic microalgae and to compare the properties of acetyl-CoA carboxylase from the diatom *Cyclotella cryptica* with those of higher plant acetyl-CoA carboxylases.

MATERIALS AND METHODS

Organism and Growth Conditions

Cyclotella cryptica Reimann, Lewin, and Guillard strain T13L was obtained from the Culture Collection of Marine Phytoplankton at the Bigelow Laboratory for Ocean Sciences (W. Boothbay Harbor, ME). Cells were cultured in 2 L polycarbonate bottles as described previously (18). Cultures were bubbled with 0.5% CO_2 in air (500 mL/min) and maintained at 25°C under constant illumination from fluorescent lamps (photon flux density at the vessel surface averaged over 360° = 85 μmol quanta·m^{-2}·s^{-1}).

Materials

Analytical grade haloxyfop and clethodim were kindly provided by J. Secor (Dow Chemical Co., Walnut Creek, CA) and A. Rendina (Chevron Chemical Co., Richmond, CA), respectively.

Analytical Methods

Protein was quantified by the Coomassie blue dye-binding method (Bio-Rad) using bovine γ-globulin as a standard. Radioactivity was determined by liquid scintillation counting in a Beckman model LS9000 scintillation counter, using the

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H# routine for quench correction. Biofluor (Dupont/New England Nuclear) or Optifluor (Packard) was used as the scintillation cocktail for all isotope studies.

Electrophoresis

SDS-PAGE was performed as described by Laemmli (10) with a 7.5% separating slab gel. Proteins were transferred to nitrocellulose membranes with a semidry electroblotting apparatus (American Bionetics) using the buffer system of Shafer-Nielsen *et al.* (23). Proteins were detected by the Bio-Rad Biotin-Blot procedure, using the manufacturer's protocol. Biotin-containing proteins were detected by the same procedure, except that the protein biotinylation step was omitted.

Nondenaturing discontinuous PAGE was performed on 5 mm diameter tube gels ($5\%_{total}/5\%_{bis}$ separating gel) with a Hoefer model DE 102 unit, using the manufacturer's protocol.

Assay for Acetyl-CoA Carboxylase Activity

Acetyl-CoA carboxylase activity was measured by the incorporation of [^{14}C]bicarbonate into acid- and heat-stable material (malonyl-CoA) using a modification of the procedure of Sauer and Heise (20). The reaction mixture (final volume = 0.3 mL) contained 100 mM Tricine buffer (pH 8.2), 0.5 mM acetyl-CoA, 1 mM ATP, 2 mM $MgCl_2$, 10 mM KCl, and 10 mM [^{14}C]$NaHCO_3$ (specific activity = 11.1 MBq/mmol). The reaction was initiated by the addition of enzyme and terminated after 10 min at 30°C by the addition of 0.3 mL of 2 N HCl. A portion (0.5 mL) of the acidified solution was transferred to a scintillation vial and heated at 70°C until dry (3 h). The residue was dissolved in 0.3 mL of 0.2 N HCl prior to the addition of scintillation cocktail. Control assays were carried out in the absence of acetyl-CoA in order to correct for nonspecific radioactivity, which was typically less than 5% of the acetyl-CoA-dependent ^{14}C incorporation. One unit of activity is defined as the amount of enzyme required to catalyze the formation of 1 μmol of malonyl-CoA per minute under standard assay conditions.

The reaction product was analyzed by the procedure described by Thomson and Zalik (24), which includes an alkaline hydrolysis step to cleave the thioester linkage to coenzyme A. The reaction product comigrated with authentic [^{14}C] malonate (Sigma Chemical Co.) on silica gel TLC plates (J. T. Baker Co., Si250) developed in water-saturated diethyl ether:formic acid (7:1, v:v) (24) and on Whatman No. 1 paper developed in isobutyric acid:NH_4OH:H_2O (66:1:33, v:v:v) (25).

Preparation of Cell-Free Extracts

Cells were harvested by centrifugation at 5,000*g* for 5 min and washed with MCD buffer (100 mM Mes containing 10 mM K-citrate and 2 mM DTT, pH 6.5). The cells were then suspended in 10 to 25 mL of MCD buffer and passed through a French pressure cell at 15,000 psi. The pressate was centrifuged at 37,000*g* for 20 min. The supernatant was diluted with MCD buffer so that the total protein concentration was below 5 mg/mL; this is referred to as the crude extract. Cell disruption and all subsequent purification steps were carried out at 4°C.

Purification Procedure

$(NH_4)_2SO_4$ Fractionation

A saturated solution of ice-cold $(NH_4)_2SO_4$ was added to the crude extract with stirring to yield a 30% saturated solution, which was then centrifuged at 8000*g* for 5 min. The precipitate was discarded and the supernatant solution was subjected to further fractionation by the addition of $(NH_4)_2SO_4$ to 43, 50, and 60% saturation. All precipitates were discarded except for the one obtained at 60% saturation, which was dissolved in 5 to 10 mL of MCD buffer and used for the gel filtration chromatography step.

Gel Filtration Chromatography

The solution obtained after $(NH_4)_2SO_4$ fractionation was loaded onto a 90 x 2.2 cm column of Biogel A-1.5 m (Bio-Rad) and eluted with MCD buffer at a flow rate of 20 mL/h. The fractions containing the highest acetyl-CoA carboxylase activity (numbers 41–57, 4 mL each) were combined and used in the affinity chromatography step.

Affinity Chromatography

The naturally occurring biotin molecules found in acetyl-CoA carboxylase allow affinity chromatography through columns containing covalently bound avidin. Monomeric avidin-agarose was prepared by incubation of 10 mg tetrameric chicken egg avidin (Calbiochem) in 4 mL 50 mM Hepes (pH 8.0) with 2 mL of Affi-gel 10 (Bio-Rad) for 4 h at 4°C, followed by treatment of the gel with 6 M guanidine-HCl (pH 2.15) for 16 h at 20°C. Noncovalently attached avidin subunits were removed from the column by washing with several column volumes of 6 M guanidine-HCl. The column was prepared for use by passing four column volumes of MKD buffer (100 mM Mes buffer with 100 mM KCl and 2 mM DTT) containing 0.5 mg biotin/mL through the column to saturate the biotin-binding sites followed by 10 column volumes of 0.1 M glycine (pH 2) to remove exchangeable biotin (9). Portions of the gel filtration-purified solution were passed through a 2 mL column of monomeric avidin-agarose, followed by washing with 15 mL of MKD buffer. Acetyl-CoA carboxylase was eluted from the column with MKD buffer containing 0.5 mg biotin/mL.

RESULTS

Acetyl-CoA carboxylase was purified from *Cyclotella cryptica* by a simple procedure utilizing $(NH_4)_2SO_4$ precipitation, gel filtration chromatography, and affinity chromatography with monomeric avidin-agarose. This procedure resulted in a nearly homogeneous preparation having a specific activity of up to 14.6 μmol malonyl-CoA formed·mg protein^{-1}·min^{-1}, which represented an increase in specific activity of approximately 600-fold. The results from a typical purification are shown in Table I.

Gel filtration chromatography of *C. cryptica* acetyl-CoA

Table I. *Purification of Acetyl-CoA Carboxylase from the Diatom Cyclotella cryptica*[a]

Step	Total Protein	Total Activity	Yield	Specific Activity	Purification
	mg	*units*	%	*units/mg protein*	*fold*
Crude extract	182	4.45	100	0.0245	1.00
$(NH_4)_2SO_4$ fractionation	54.3	3.15	70.8	0.0581	2.37
Biogel A—1.5 m column	16.6	2.66	59.7	0.160	6.53
Monomeric avidin-agarose column	0.100	1.46	32.9	14.6	598

[a] A 6 L culture containing 1.1 g ash-free dry mass was used for this experiment.

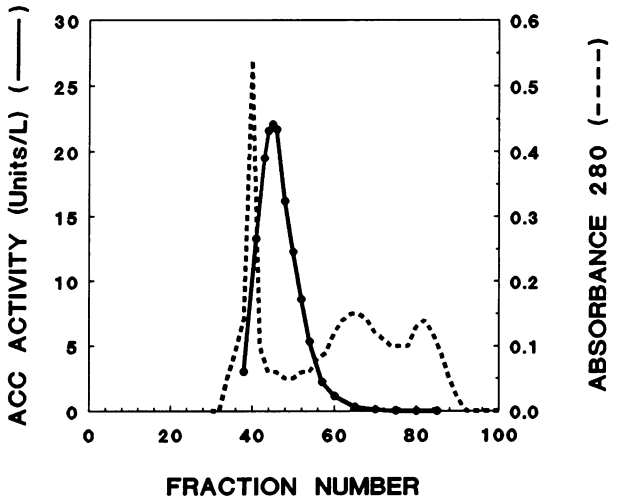

Figure 1. Gel filtration chromatography of *C. cryptica* acetyl-CoA carboxylase. A sample of partially purified acetyl-CoA carboxylase was passed through a 90 x 2.2 cm column containing Biogel A-1.5 m at a flow rate of 20 mL· min^{-1}. Fraction size = 4 mL. Acetyl-CoA carboxylase activity (——); A_{280} (- - -). The protein standards used to calibrate the column were rabbit muscle aldolase (158 kD), bovine liver catalase (232 kD), horse spleen ferritin (440 kD), and bovine thyroglobulin (669 kD).

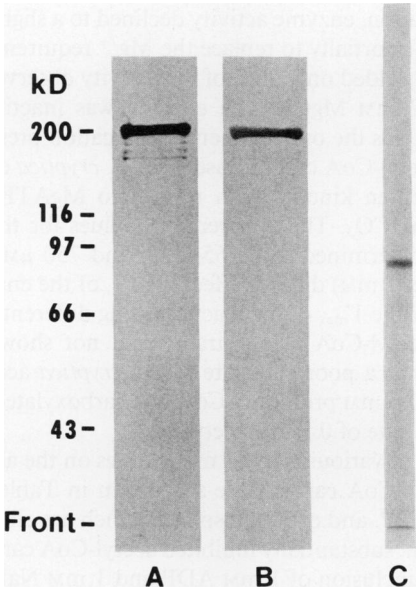

Figure 2. A and B, SDS-PAGE of affinity-purified acetyl-CoA carboxylase from *C. cryptica*. Samples were separated on a $7.5\%_{total}/2.7\%_{bis}$ slab gel (1.2 μg total protein for lane A, 1.8 μg total protein for lane B), transferred to a nitrocellulose membrane, and stained for detection of either total protein (lane A) or biotin-containing proteins only (lane B). The migration distances of protein mol wt standards are indicated on the left. The standards used were chicken egg white ovalbumin (43 kD), bovine serum albumin (66 kD), rabbit muscle phosphorylase b (97 kD), *E. coli* β-galactosidase (116 kD), and rabbit skeletal muscle myosin (200 kD). C, Nondenaturing PAGE of affinity- purified acetyl-CoA carboxylase from *C. cryptica*. Total protein of 2.9 μg was separated on a $5\%_{total}/5\%_{bis}$ tube gel and stained with Coomassie blue.

carboxylase resulted in a single peak of activity (Fig. 1). Based on this chromatographic analysis, the mol wt of native acetyl-CoA carboxylase was estimated to be 740 kD. When analyzed by SDS-PAGE, affinity-purified acetyl-CoA carboxylase migrated as a single major band having an apparent mol wt of 185 kD (Fig. 2A). This peptide was shown to contain biotin, based on its ability to specifically bind avidin-HRP[3] after transfer to a nitrocellulose membrane (Fig. 2B). Some very lightly stained peptides that migrated slightly ahead of the 185 kD peptide were typically observed. These peptides also appeared to contain biotin since they were detected by avidin-HRP and may represent products of limited acetyl-CoA carboxylase proteolysis. Affinity-purified acetyl-CoA carboxylase migrated as a single band when subjected to nondenaturing discontinuous PAGE (Fig. 2C).

Acetyl-CoA carboxylase from *C. cryptica* had a slightly alkaline pH optimum (pH 8.2), which is typical for acetyl-CoA carboxylases from many sources. Enzyme stability, on the other hand, was maximal at pH 6.5. When acetyl-CoA carboxylase purified through the $(NH_4)_2SO_4$ fractionation step was stored at 4°C at pH 6.5, 97% of the activity was retained after 48 h, while only 15% and 4% of the initial activity remained after 48 h when stored at pH 7.5 and pH 5.5, respectively. Acetyl-CoA carboxylase also lost activity rapidly in the absence of a sulfhydryl reductant, and therefore DTT was included in all buffers. Citrate had a slight stabilizing effect on the enzyme and was therefore routinely included in all buffers up to the affinity chromatography step. Enzyme stability also required the presence of NaCl or KCl. When these salts were removed from the affinity-purified prepara-

[3] Abbreviation: avidin-HRP, avidin-horseradish peroxidase conjugate.

tion by the use of a desalting column, enzymatic activity was completely lost and could not be restored by the readdition of NaCl or KCl. Partially purified acetyl-CoA carboxylase from *C. cryptica* could be stored frozen at -20°C for at least 3 weeks with little loss in activity. However, affinity-purified acetyl-CoA carboxylase was less stable, losing all activity upon freezing and a substantial portion (30%) of the inititial activity after 24 h at 4°C.

The activity of the enzyme was dependent upon the presence of divalent metal cations, with Mg^{2+} being the most effective cation tested. When ATP was included at a concentration of 1 mM, maximal acetyl-CoA carboxylase activity was observed at a Mg^{2+} concentration of 2 mM (Fig. 3). Above this concentration, enzyme activity declined to a slight extent. Mn^{2+} was able partially to replace the Mg^{2+} requirement, but 2 mM $MnCl_2$ yielded only 20% of the activity observed in the presence of 2 mM $MgCl_2$. The enzyme was inactive when CO^{2+} (2 mM) was the only divalent metal cation present.

Purified acetyl-CoA carboxylase from *C. cryptica* exhibited Michaelis-Menten kinetics with respect to MgATP, acetyl-CoA, and $NaHCO_3$. The apparent K_m values for these substrates were determined to be 65, 233, and 750 μM, respectively. Citrate (1 mM) did not affect the K_m of the enzyme for acetyl-CoA or the V_{max} of the reaction using different concentrations of acetyl-CoA as substrate (data not shown). Propionyl-CoA was a poor substrate for *C. cryptica* acetyl-CoA carboxylase; 0.5 mM propionyl-CoA was carboxylated at only one-tenth the rate of 0.5 mM acetyl-CoA.

The effects of various cellular metabolites on the activity of purified acetyl-CoA carboxylase are shown in Table II. Malonyl-CoA, ADP, and orthophosphate, which are all products of the reaction, substantially inhibited acetyl-CoA carboxylase activity. The inclusion of 1 mM ADP and 1 mM NaH_2PO_4 at the same time resulted in a 64% reduction in acetyl-CoA carboxylase activity, while 1 mM malonyl-CoA inhibited the reaction by 84%. Palmitoyl-CoA was a strong inhibitor, decreasing enzymatic activity by 78% when included at a concentration of 100 μM. Free palmitate was also quite inhibitory; 100 μM palmitate (which is approximately three times higher than the solubility limit of palmitate in water) inhibited malonyl-CoA formation by 44%. The photosynthetic/glycolytic intermediates 3-phosphoglycerate, phosphoenolpyruvate, fructose-1,6-bisphosphate, glucose-1-phosphate, glucose-6-phosphate, and fructose-6-phosphate had little effect on acetyl-CoA carboxylase activity, but 1 mM pyruvate was shown to consistently stimulate enzymatic activity by approximately 50%. CoA, which stimulates the activity of certain higher plant acetyl-CoA carboxylases (11) while inhibiting others (15), had no effect on the enzyme from *C. cryptica*. Citrate, acetate, AMP, NADH, NADPH, NAD^+, and $NADP^+$ (1 mM) likewise did not alter acetyl-CoA carboxylase activity substantially.

Since acetyl-CoA carboxylase has recently been shown to be the site of action of monocot-specific aryloxyphenoxypropionic acid and cyclohexanedione herbicides (1, 16, 21), it was of interest to determine the effects of these herbicides on the activity of purified *C. cryptica* acetyl-CoA carboxylase. The cyclohexanedione herbicide clethodim inhibited *C. cryptica* acetyl-CoA carboxylase activity in a dose-dependent manner, with 50% inhibition occurring at a concentration of 23 μM (Fig. 4). Conversely, the aryloxyphenoxypropionic acid herbicide haloxyfop did not affect enzymatic activity at concentrations up to 100 μM.

Table II. *Effects of Various Compounds on the Activity of Acetyl-CoA Carboxylase Purified from Cyclotella cryptica*

The values shown are the mean values obtained from two to four separate experiments.

Additions	Relative Activity ± SD
None	100[a]
1 mM malonyl-CoA	16.0 ± 1.3
100 μM malonyl-CoA	59.7 ± 6.7
1 mM NaH_2PO_4	53.0 ± 4.2
1 mM ADP[b]	51.3 ± 2.9
1 mM NaH_2PO_4 + 1mM ADP[b]	36.2 ± 0.3
1 mM AMP	94.8 ± 1.1
100 μM palmitoyl-CoA	21.8 ± 4.9
10 μM palmitoyl-CoA	64.7 ± 8.7
100 μM CoA	98.1 ± 3.5
1 mM 3-phosphoglycerate	94.4 ± 0.5
1 mM phosphoenolpyruvate	84.1 ± 8.3
1 mM pyruvate	148.8 ± 10.6
1 mM glucose-1-phosphate	103.1 ± 9.1
1 mM glucose-6-phosphate	100.1 ± 8.2
1 mM fructose-6-phosphate	94.6 ± 7.0
1 mM fructose-1,6-bisphosphate	99.7 ± 8.1
1 mM citrate[b]	107.0 ± 8.3
1 mM acetate	94.1 ± 6.2
1 mM NADH	98.3 ± 5.4
1 mM NADPH	87.3 ± 7.5
1 mM NAD^+	100.0 ± 0.8
1 mM $NADP^+$	94.5 ± 5.0

[a] 100% relative activity ranged from 6.7×10^{-4} to 1.2×10^{-3} units/mL reaction mixture for the different experiments. 2.0×10^{-4} to 3.6×10^{-4} units of affinity-purified enzyme were added per reaction, depending on the experiment. [b] Additional $MgCl_2$ (1 mM) was also included in these assays to overcome the effects of Mg^{2+} chelation.

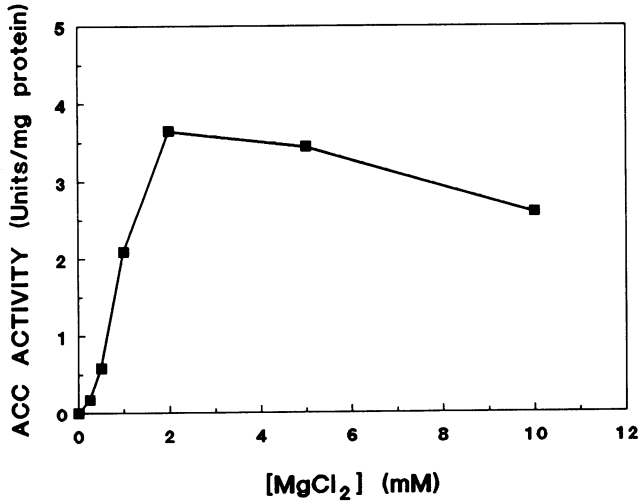

Figure 3. Effect of $MgCl_2$ on the activity of purified *C. cryptica* acetyl-CoA carboxylase. A total of 6.1×10^{-4} units of affinity-purified enzyme were added per reaction.

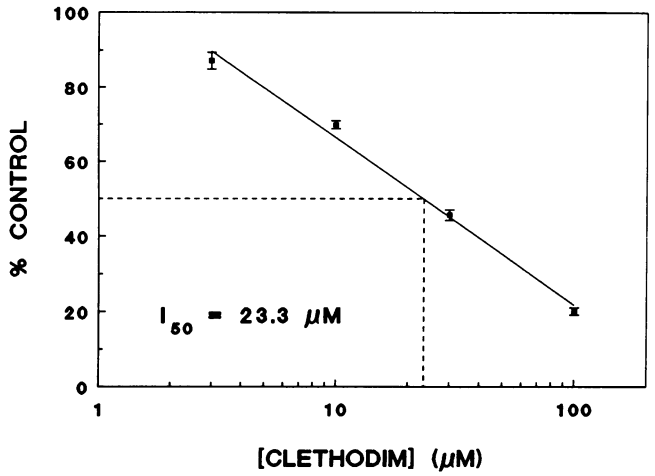

Figure 4. Inhibition of purified *C. cryptica* acetyl-CoA carboxylase by clethodim. A freshly prepared 20 mM clethodim stock solution (in ethanol) was diluted into 50 mM Tricine (pH 8.2) immediately prior to the assay. The mean values (±SD) from two separate experiments are shown. The mean value for 100% relative activity for the two experiments was 6.5 units/mg protein. An average of 6.3×10^{-4} units of affinity-purified enzyme were added per reaction.

DISCUSSION

Many properties of acetyl-CoA carboxylase from the diatom *Cyclotella cryptica* are similar to those of acetyl-CoA carboxylases isolated from various higher plants. Like the enzyme from higher plants, *C. cryptica* acetyl-CoA carboxylase is a large (about 740 kD) protein composed of several subunits. The molecular mass of acetyl-CoA carboxylase from wheat germ (4), avocado (12), castor seed (6), maize (15), and cultured parsley cells (5) was reported to be 700, 650, 528, 500, and 420 kD, respectively. *C. cryptica* acetyl-CoA carboxylase appears to be a tetrameric protein composed of four identical biotin-containing subunits, each having a molecular mass of about 185 kD. This suggests that the subunits are multifunctional peptides containing domains responsible for both biotin carboxylation and subsequent carboxyl transfer to acetyl-CoA. Acetyl-CoA carboxylase from maize is also composed of multiple identical subunits (15), but in this case the subunits are only 60 to 61 kD. Acetyl-CoA carboxylase from cultured parsley cells is composed of two equal subunits, each having a molecular mass of 220 kD (5). Although the mol wt of native acetyl-CoA carboxylase from soybean and oil seed rape are not known, these enzymes have been shown to be composed of identical subunits having molecular mass of 240 and 220 kD, respectively (2, 8). Another variety of soybean ("Wayne") exhibited a more complex subunit structure, however (2). Acetyl-CoA carboxylase from *C. cryptica* and the majority of acetyl-CoA carboxylases from higher plants therefore appear to be similar in that they are composed of multiple, but identical, subunits. The number of subunits in the various holoenzymes can vary substantially, however. It is rather surprising that so much diversity in the structure of this ubiquitous enzyme exists among different plants. It is interesting to note that the subunit structure of *C. cryptica* acetyl-CoA carboxylase is more similar to acetyl-CoA carboxylase from brewer's yeast (which is composed of four identical 150 kD subunits [22]) than to the acetyl-CoA carboxylases described thus far from higher plants.

The activity of all acetyl-CoA carboxylases studied to date has been shown to be dependent upon the presence of divalent metal cations. *C. cryptica* acetyl-CoA carboxylase also exhibited this characteristic (Fig. 3). It has been demonstrated by several researchers (6, 12, 13, 20) that MgATP is the actual substrate for acetyl-CoA carboxylase, and it is assumed that this is also the case for acetyl-CoA carboxylase from *C. cryptica*. In addition, free Mg^{2+} stimulates acetyl-CoA carboxylase activity in several higher plants (6, 12, 13, 20). Based on the increase in activity of *C. cryptica* acetyl-CoA carboxylase due to concentrations of Mg^{2+} that exceeded the ATP concentration in the assay mixture (Fig. 3), it appears that free Mg^{2+} also stimulates the activity of the *C. cryptica* enzyme.

The K_m values obtained for *C. cryptica* acetyl-CoA carboxylase for acetyl-CoA, MgATP, and bicarbonate (233, 65, and 750 μM, respectively) are similar to the K_m values reported for higher plant acetyl-CoA carboxylases. A survey of K_m values for acetyl-CoA carboxylases from several higher plants, (including spinach [12, 20], avocado [12], maize [15], wheat [7], parsley [5], soybean [2], barley [16], and castor seed [6]) yielded ranges of 26 to 320 μM for acetyl-CoA, 21 to 460 μM for MgATP, and 0.86 to 8 mM for bicarbonate. Unlike the case for acetyl-CoA carboxylase from avocado and spinach (12), citrate did not affect the V_{max} of the reaction when the enzyme was supplied with different concentrations of acetyl-CoA.

The activity of *C. cryptica* acetyl-CoA carboxylase can be modulated by several metabolites. As is the case with acetyl-CoA carboxylase from several higher plants (2, 3, 15, 20), the diatom enzyme is inhibited by ADP. For the higher plants examined, this inhibition appears to be competitive with respect to ATP. *C. cryptica* acetyl-CoA carboxylase is also inhibited by orthophosphate and malonyl-CoA. It is not clear whether the inhibitory effects of ADP, phosphate, and malonyl-CoA are due to true allosteric mechanisms or simply to shifts in the thermodynamic equilibrium of the reaction. Nonetheless, it is clear that acetyl-CoA carboxylase activity would be higher during periods of photosynthesis due to the combined effects of increased pH and Mg^{2+} levels and decreased ADP and orthophosphate levels within the chloroplast (the presumed location of the enzyme).

The strongest inhibitor of *C. cryptica* acetyl-CoA carboxylase activity tested was palmitoyl-CoA, which inhibited the reaction by 35 and 78% at concentrations of 10 and 100 μM, respectively. It appears that the acyl component is at least partially responsible for this inhibition since free palmitate also inhibited enzymatic activity quite strongly. Palmitoyl-CoA was also reported to inhibit maize leaf acetyl-CoA carboxylase activity (15), with nearly complete inhibition occurring at a concentration of 37.5 μM. The low concentration of acyl-CoA (or free fatty acids) required to inhibit acetyl-CoA carboxylase suggests that this inhibition may be physiologically relevant under conditions when acyl chains are not incorporated into membrane lipids or exported from the chloroplast at rates comparable to their rates of synthesis.

The only metabolite tested that stimulated the activity of

C. cryptica acetyl-CoA carboxylase was pyruvate, which increased the rate of malonyl-CoA formation by nearly 50% when included at a concentration of 1 mM. The increased ^{14}C incorporation was not due to the activity of contaminating pyruvate carboxylase, since no radioactivity was incorporated into acid-stable products in the presence of pyruvate when acetyl-CoA was omitted from the assay mixture. It is not known whether this slight stimulation has physiological significance. It should be noted, however, that the acetyl-CoA used in this reaction *in vivo* may be derived from plastidial pyruvate, as appears to be the case with certain higher plants. Allosteric activation of acetyl-CoA carboxylase may therefore play a role in increasing fatty acid biosynthetic rates under conditions of pyruvate abundance.

Acetyl-CoA carboxylase from *C. cryptica* is not strongly inhibited by cyclohexanedione and aryloxyphenoxypropionic acid herbicides. In this respect, the diatom enzyme more closely resembles acetyl-CoA carboxylase from dicotyledonous plants than the enzyme from monocotyledonous plants. Although clethodim inhibited *C. cryptica* acetyl-CoA carboxylase in a dose-dependent manner, the I_{50} value determined (23 μM) is one to two orders of magnitude higher than the values reported when monocot acetyl-CoA carboxylases were treated with cyclohexanedione herbicides (1, 16). Likewise, haloxyfop inhibits several monocot acetyl-CoA carboxylases by 50% at concentrations less than 1 μM (1, 17), and yet 100 μM haloxyfop had no effect on *C. cryptica* acetyl-CoA carboxylase activity. The structure of the *C. cryptica* acetyl-CoA carboxylase active site apparently differs greatly from those of monocot acetyl-CoA carboxylases.

The results of this investigation suggest that acetyl-CoA carboxylase from *C. cryptica* has many properties in common with higher plant acetyl-CoA carboxylases. Efforts are currently underway to determine a partial amino acid sequence for the enzyme and to produce antibodies against the enzyme in order to be able to examine the properties of the acetyl-CoA carboxylase-encoding gene.

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