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# Purification and Characterization of 1-Aminocyclopropane-1-Carboxylate Synthase from Apple Fruits[1]

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## ABSTRACT

**1-Aminocyclopropane-1-carboxylate (ACC) synthase, a key enzyme in ethylene biosynthesis, was isolated and partially purified from apple (*Malus sylvestris* Mill.) fruits. Unlike ACC synthase isolated from other sources, apple ACC synthase is associated with the pellet fraction and can be solubilized in active form with Triton X-100. Following five purification steps, the solubilized enzyme was purified over 5000-fold to a specific activity of 100 micromoles per milligram protein per hour, and its purity was estimated to be 20 to 30%. Using this preparation, specific monoclonal antibodies were raised. Monoclonal antibodies against ACC synthase immunoglobulin were coupled to protein-A agarose to make an immunoaffinity column, which effectively purified the enzyme from a relatively crude enzyme preparation (100 units per milligram protein). As with the tomato enzyme, apple ACC synthase was inactivated and radiolabeled by its substrate *S*-adenosyl-L-methionine. Apple ACC synthase was identified to be a 48-kilodalton protein based on the observation that it was specifically bound to immunoaffinity column and it was specifically radiolabeled by its substrate *S*-adenosyl-L-methionine.**

Ethylene, a regulator of plant growth and development, is biosynthesized from methionine via AdoMet[2] and ACC (1). It has been well documented that the production of ethylene from plants is mainly regulated by the synthesis of ACC synthase (24). Since ACC synthase exists in very low concentrations in plant tissues and is unstable, progress in the purification of this enzyme has been slow. Using a combination of various purification procedures, Bleecker *et al.* (2), Mehta *et al.* (12), and Van der Straeten *et al.* (22) have purified the enzyme from wounded tomato pericarp, Nakajima *et al.* (14) from wounded winter squash fruits, and Tsai *et al.* (21) from auxin-treated mungbean hypocotyls and Sato and Theologis (16) from wound-induced zucchini fruits. A complementary DNA clone encoding ACC synthase from zucchini has been reported recently (16) without revealing the nucleic acid sequence. Most recently, two different cDNA sequences encoding ACC synthase from tomato were reported (23).

Since ACC synthases induced by different developmental, hormonal, and environmental factors differ in their immunological and physicochemical properties, it has been suggested that more than one ACC synthase gene is specifically activated (13).

AdoMet, the substrate for ACC synthase, has been shown to act as a suicide inactivator of ACC synthase, isolated from tomato (4, 18, 19), mungbean (17), and winter squash (Y Lee, unpublished data). Satoh and Yang (18) demonstrated that when a partially purified ACC synthase preparation isolated from tomato was incubated with *S*-adenosyl-L-[3,4-$^{14}$C]methionine and the resulting protein was analyzed by SDS-PAGE, only one radioactive protein was observed. This protein was judged to be ACC synthase based on the observations that its molecular mass was 50 kD and that it was specifically bound to a monoclonal antibody against ACC synthase prepared by Bleecker *et al.* (2). Later work showed that ACC synthase was also radiolabeled with *S*-adenosyl-L-[carboxyl-$^{14}$C]methionine but not with *S*-adenosyl-L-[methyl-$^{14}$C]methionine (19). These results suggest that the AdoMet-induced inactivation of ACC synthase involves a covalent linkage of a fragment of AdoMet, probably 2-aminobutyric acid moiety, into the active site of ACC synthase. Such an AdoMet-dependent radiolabeling has been employed as a tool for the confirmation of ACC synthase (22).

Although apple fruits at the climacteric stage produced ethylene at a much higher rate (10 nmol/gh$^{-1}$) than tomato fruits (1 nmol/gh$^{-1}$), early attempts to isolate active ACC synthase from apple fruits were not as successful as that from tomatoes (26). In 1983, Bufler and Bangerth (6) isolated ACC synthase in the soluble fraction from apple fruits with 0.2% Triton X-100 in the extraction medium. Mansour *et al.* (11) isolated active ACC synthase from apple tissues with an extraction medium containing 2% PVP. In the present study, we have shown that ACC synthase extracted from apple tissue is associated with the pellet fraction and can be solubilized in active form with Triton X-100. After partial purification, monoclonal antibodies were raised against ACC synthase. Using an immunoaffinity column made with the monoclonal antibodies to ACC synthase and radiolabeling of ACC synthase with AdoMet, we have identified apple ACC synthase to be a 48-kD protein.

## MATERIALS AND METHODS

### Plant Materials

Apples (*Malus sylvestris* Mill., var Golden Delicious) that had been stored at 0 to 4°C were placed at 24°C for about 5 d until their ethylene production rate reached around 10 nmol/gh$^{-1}$, at which time their skin color turned slightly yellow. These fruits were then transferred to 0°C and stored

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[2] Abbreviations: AdoMet, *S*-adenosyl-L-methionine; ACC, 1-aminocyclopropane-1-carboxylic acid; PLP, pyridoxal 5′-phosphate.

for at least 3 d before extraction (Fig. 1). For an unknown reason, extracting ACC synthase directly from apple after warming yields relatively low enzyme activity.

### ACC Synthase Activity Assay

ACC synthase activity was measured by incubating an enzyme sample (1–100 μL) at 30°C for 15 min with 200 μM AdoMet, 10 μM PLP, and 50 mM Hepes-KOH buffer (pH 8.5) in a total volume of 0.5 mL. When assaying ACC synthase activity in the pellet suspension, 1% Triton X-100 was included in the assay mixture. The amount of ACC formed was determined by the method of Lizada and Yang (10). One unit of enzyme is defined as that which converts 1 nmol of AdoMet to ACC per h at 30°C.

### Pellet Preparation and Solubilization

Apples that had been stored at 0°C were peeled, cut into small slices (2 cm × 2 cm), and homogenized at maximum speed for 2 min with a Waring blender in an equal volume of homogenization buffer (w/v) containing 400 mM K-phosphate (pH 8.5), 1 mM EDTA, 0.5% 2-mercaptoethanol, and 10 μM PLP. The homogenate was squeezed through four layers of cheese cloth and centrifuged at 28,000*g* for 30 min. The pellet fraction was collected and stored at −20°C. Thirty-five kilograms of fruits can be processed by a coordinated effort of two workers in 1 d. Pellet preparation stored under −20°C was thawed and resuspended in buffer A (20 mM K-phosphate at pH 8.5, 1 mM EDTA, 1 mM 2-mercaptoethanol, 10 μM PLP, and 30% glycerol). To the pellet suspension (2 mg protein/mL) was added with stirring an equal volume of the solubilization buffer, which consisted of buffer A plus 0.2% (v/v) Triton X-100. After incubation for 30 min, the mixture was centrifuged at 28,000*g* for 20 min. The resulting supernatant (total volume, 10 liters), which contained ACC synthase activity, was collected and further purified. All procedures were carried out at 1°C.

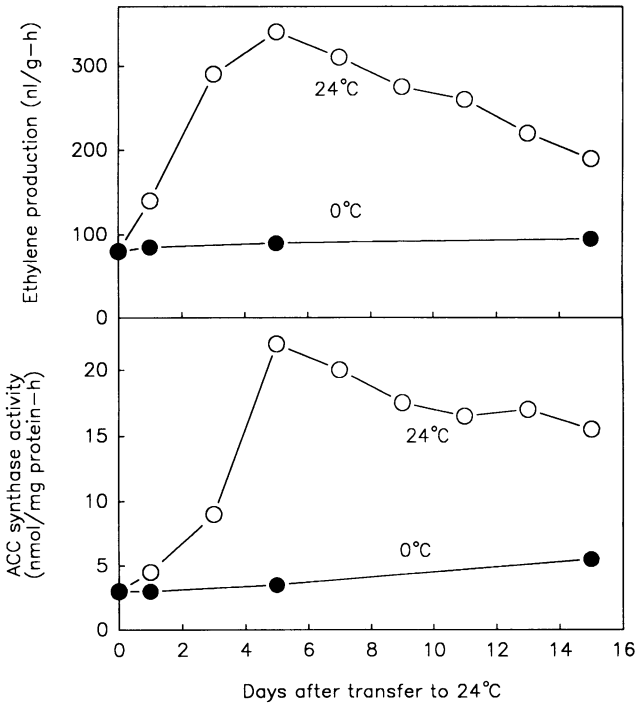


**Figure 1.** The changes of ethylene production rates and extractable ACC synthase activity in apple fruits during cold storage (0°C) or after transfer from cold storage (0°C) to room temperature (24°C). Apple fruits were held either continuously at 0°C (●) or transferred from 0°C to room temperature (24°C) for specified periods (○) after ethylene production rates were determined. These fruits were transferred back to 0°C for 3 d before ACC synthase was extracted and its activity determined.

### Partial Purification of the Solubilized ACC Synthase

Soon after solubilization, batches of about 1.5 liters of the solubilized ACC synthase preparation were poured onto a DEAE-Sepharose (Sigma, fast flow) column (10 cm × 30 cm, bed volume 800 mL) that had been equilibrated with buffer A. The mixture was then stirred with a glass rod and incubated for 30 min to allow the enzyme to be adsorbed. The unadsorbed proteins were removed through filtration. After washing with four bed volumes of buffer A and one bed volume of 75 mM KCl in buffer A at a flow rate of 2 mL/min, one bed volume of 200 mM KCl in buffer A was used to elute ACC synthase from the column also at the same flow rate. The fractions containing ACC synthase were combined (800 mL), diluted with 2.8 liters of buffer B (20 mM K-phosphate pH 8.5, 1 mM EDTA, 1 mM 2-mercaptoethanol, 10 mM PLP, and 20% glycerol), and loaded onto a hydroxylapatite (Calbiochem, fast flow) column (4 cm × 7 cm, bed volume 40 mL), which had been equilibrated with the buffer B. After washing with four bed volumes of 120 mM K-phosphate buffer B at a flow rate of 30 mL/h, the majority of ACC synthase activity was eluted out with four bed volumes of 250 mM K-phosphate buffer B at the same flow rate.

ACC synthase eluted from the hydroxylapatite column (160 mL, 250 mM K-phosphate) was diluted to 200 mM K-phosphate with the buffer B and loaded onto a phenyl-Sepharose (Pharmacia) column (2.5 cm × 10 cm, bed volume 15 mL) that had been equilibrated with 200 mM K-phosphate buffer. The column was washed with four bed volumes of 200 mM K-phosphate, and elution was carried out with three bed volumes of buffer B plus 20% ethylene glycol at a flow rate of 0.1 mL/min. The above enzyme solution (45 mL) was further applied to an aminohexyl-Sepharose (Pharmacia) column (1.5 cm × 8 cm, bed volume 3 mL) that had been equilibrated with the buffer B. After washing with three bed volumes of 300 mM KCl in buffer B, ACC synthase was eluted with three bed volumes of 700 mM KCl in buffer B at a flow rate of 0.1 mL/min. The resulting active fraction (9 mL) was concentrated to about 40 μL in buffer B using a Centricon-30 (Amicon). This partially purified ACC synthase was stored at −80°C until use.

### Inactivation and Radiolabeling of ACC Synthase by AdoMet

The conditions used were similar to those reported by Satoh and Yang (18, 19). For the inactivation assay, a sample of the

partially purified ACC synthase was incubated with 200 μM AdoMet, 10 μM PLP, and 50 mM Hepes-KOH (pH 8.5) in a total volume of 2 mL at 30°C. After incubation for various periods, a 100-μL portion of the reaction mixture was withdrawn, cooled in an ice bath, and applied to a small column of Sephadex G-25 (bed volume 1 mL) that had been equilibrated with buffer B. The column was centrifuged at low speed (2000*g*, 2 min) to remove excess buffer solution. The desalted enzyme solution was collected by low-speed centrifugation and assayed for ACC synthase activity as described above. For radiolabeling, a partially purified ACC synthase preparation in 100 mM Hepes-KOH buffer (pH 8.5) and 10 μM PLP was incubated for 6 h at 30°C with 10 μCi of Ado[carboxyl-$^{14}$C]Met (55 mCi/mmol) in 0.1 N $H_2SO_4$, which had been preheated at 100°C for 7 min (19). To maintain the AdoMet concentration above 200 μM, the reaction mixture volume was kept under 200 μL. After incubation, the reaction mixture was passed through a Sephadex G-25 PD-10 column (Bio-Rad) that had been equilibrated with 50 mM Hepes-KOH buffer (pH 8.5), and the resulting protein fraction was concentrated to a small volume using Centricon-30 (Amicon) before SDS-PAGE analysis and fluorography.

### Monoclonal Antibody Production and Screening

The details of the production of monoclonal antibodies against apple ACC synthase, to be described elsewhere, essentially followed the standard procedures described in the literature (8). Briefly, a partially purified ACC synthase preparation (60,000 units/mg protein) was injected into two mice, and after two subsequent boosts, sera from both mice were tested for anti-ACC synthase activities. The spleen cells isolated from one of the two mice that demonstrated anti-ACC synthase activity were fused with myeloma cells to generate hybridoma. Hybridoma were then screened by their ability to secret antibody that could functionally precipitate ACC synthase. Monoclonal antibodies were obtained by limited dilution of the positive clones and by repeated subcloning. Finally, eight different monoclonal hybridoma cell lines were selected; two of the eight monoclones recognized ACC synthase in Western blot analysis. The monoclonal line 6A10, which showed positive in Western blot, was chosen to generate anti-ACC synthase ascites fluid *in vivo* for further experiment.

### Immunoaffinity Purification of ACC Synthase

An anti-ACC synthase ascites fluid (monoclone line 6A10) was incubated with protein A-agarose and the adsorbed IgG antibodies were conjugated to the protein A-agarose with coupling reagent dimethyl pimelimidate (15). This immunoaffinity agarose gel had a binding capacity of 200 units/μL and was used to purify ACC synthase from a relatively crude enzyme preparation (specific activity of about 100 units/mg protein). Before loading, the enzyme preparation was dialyzed to remove 2-mercaptoethanol, which would cause the leakage of IgG subunits during the immunoaffinity purification procedures. The loading rate was kept under 0.1 mL/min, at which over 95% of ACC synthase was absorbed to the gel in a small column. After loading and washing, the ACC synthase was eluted with 2% SDS in $H_2O$.

### Gel Electrophoresis and Fluorography

SDS-PAGE was conducted using 10% gel by the methods of Laemmli (9). Gels were stained with Coomassie blue and prepared for fluorography by soaking in Fluoro-Hance (Research Products Inc.). After drying, they were exposed to Kodak XAR-5 x-ray film at −80°C for 3 to 7 d.

### Protein Assay

Protein contents were measured by the method of Bradford (5), using BSA as a standard.

## RESULTS AND DISCUSSION

### Isolation and Subcellular Distribution of ACC Synthase from Apple

Bufler and Bangerth (6) were the first to report that active ACC synthase preparation could be obtained in the soluble fraction when 0.2% Triton X-100 was employed in the extraction medium. These results lead us to speculate that ACC synthase in apple fruits may be associated with an insoluble fraction and can be solubilized into the soluble fraction with Triton X-100. To test this hypothesis, we prepared apple homogenate that was fractionated into two particulate fractions and a supernatant fraction by differential centrifugation. The enzyme activity in each fraction was tested in the presence or absence of Triton X-100. The results of Table I indicate that essentially all of the enzyme activity was associated with pellet fractions, and the enzyme activity was greatly increased in the presence of Triton X-100.

Since ACC synthase activity is associated with the pellet preparation, it is of interest to investigate whether the enzyme is associated with a particular subcellular organelle. Employing differential and sucrose gradient centrifugations, we separated the pellet into mitochondrial, microsomal, and other fractions. Since the enzyme activity was found in all organelle fractions (data not shown), there is no evidence that ACC synthase is a membrane-bound enzyme. The possibility that ACC synthase may bind artificially to particulate fractions during the cell disruption cannot be ruled out.

**Table I.** *Distribution of ACC Synthase Activity in Different Subcellular Fractions*

Apple tissue (40 g) was used to prepare the homogenate as described in "Materials and Methods." The apple homogenate free from cell debris was fractionated into pellet 1, pellet 2, and supernatant by centrifugations at 5,000*g* for 30 min and at 160,000*g* for 1 h, respectively. Enzyme activities were assayed in the absence or presence of 1% of Triton X-100.

| Fraction | Activity | | Protein |
|---|---|---|---|
| | − Triton X-100 | + Triton X-100 | |
| | *units* | | *mg* |
| Pellet 1 | 41 | 121 | 10.8 |
| Pellet 2 | 20 | 93 | 4.6 |
| Supernatant | <1 | <1 | 2.9 |

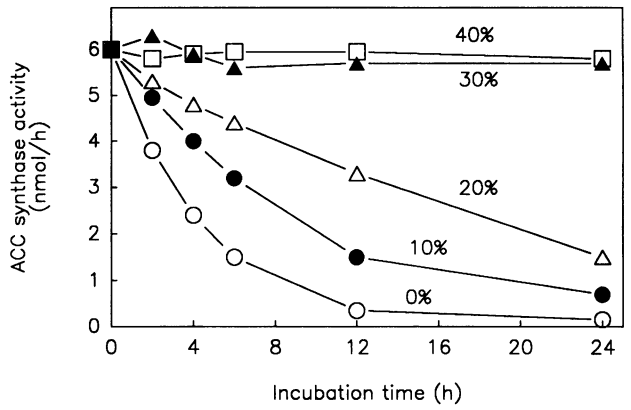


**Figure 2.** Stabilization of ACC synthase with glycerol after solubilization with Triton X-100. Various concentrations of glycerol (0–40%) were added to the solubilized enzyme preparation with 0.2% Triton X-100, and the remaining ACC synthase activities were monitored during the subsequent 24-h incubation period at 0°C.

### Solubilization of ACC Synthase from Pellet and its Stabilization

To test the ability of various detergents and KCl to solubilize ACC synthase in the pellet, a pellet was suspended in a buffer containing one of the reagents for 30 min, the mixtures were then centrifuged, and the resulting pellet and supernatant fractions were separately assayed for their enzyme activity in the presence of 1% Triton X-100. Because Mansour *et al.* (11) have shown that PVP was effective to prepare active ACC synthase in the soluble fraction from apple extracts, the effect of PVP on the solubilization of ACC synthase was also compared. Whereas Triton X-100 and PVP were effective in releasing ACC synthase activity from the pellet, none of the following agents was found to be effective: anionic detergents deoxycholate (0.8%) and cholate (1.0%), zwitterionic detergent 3-([3-cholamidopropyl]dimethylammonio)-1-propanesulfonate (1%), and KCl (0.5 M). PVP at a concentration of 10% (w/v) released only 40% of ACC synthase; an increase in PVP concentration to 30% failed to further increase the release of the enzyme. In contrast, over 90% of the enzyme activity was released by Triton X-100 at a concentration of 0.1% (w/v). Higher concentrations improved the solubilization slightly, but rendered the enzyme more unstable. The presence of glycerol appears to be a critical factor for maintaining the stability of the enzyme following solubilization of the enzyme from the pellet with 0.1% Triton X-100 (Fig. 2). In the absence of glycerol the solubilized enzyme lost most of its activity in 12 h, whereas the enzyme preparation containing 30% glycerol retained its activity over a 24-h incubation period. Therefore, 30% glycerol was used during the solubilization procedures.

### Partial Purification of ACC Synthase

Over 90% of the enzyme activity could be recovered in the pellet after a single centrifugation at 28,000*g*. By taking advantage of this property, we were able to concentrate a large amount of enzyme activity by centrifugation from a crude extract of apples in large scale. The resulting pellet containing ACC synthase can be stored at −20°C without significant loss of activity over a period of 4 months. Although ACC synthase after solubilization with Triton X-100 can be stabilized with 30% glycerol for a few days, it was found desirable to remove Triton X-100 soon after its release by adsorbing the enzyme to DEAE-Sepharose, and carrying out the subsequent purification procedures. Table II summarizes the results of a five-step purification starting with 35 kg of apple fruit. This purification procedure allows us to partially purify the enzyme over 5,000-fold with 30% yield. The resulting ACC synthase was estimated to be about 20% pure based on a Coomassie blue staining of the proteins after SDS-PAGE (data not shown), where the 48-kD band was subsequently confirmed to be ACC synthase as described later. It is to be noted that neither concentration nor dialysis steps was employed during the purification steps (see "Materials and Methods"). Thus, time and labor are greatly reduced. Among the four chromatography steps, hydroxylapatite afforded the most effective purification. Because the effectiveness of hydroxylapatite depends on its aging (20), it is advisable to facilitate aging by continuously washing the column with buffer B (i.e., at least 20 bed volumes) before loading. PLP has been shown to be an important factor to stabilize the enzyme activity (22, 25). Since the Sepharose tends to retain PLP, PLP was routinely added to the collection flasks so that the concentration of PLP in the eluates was maintained at 10 μM.

### Inactivation and Radiolabeling of the Partially Purified ACC Synthase with AdoMet

Two lines of evidence are given that support the notion that the 48-kD protein on SDS-PAGE is ACC synthase. These were based on the specific radiolabeling of ACC synthase with labeled AdoMet, and the specific binding of ACC synthase by an anti-ACC synthase monoclonal antibody. As with tomato (18) and mungbean (17) ACC synthase, a partially purified ACC synthase from apple was inactivated by its substrate

**Table II.** *Purification of ACC Synthase from the Homogenate of 35-kg Apple Fruits*

| Purification Step | Total Activity | Protein | Specific Activity | Recovery | Purification |
|---|---|---|---|---|---|
| | *units* | *mg* | *units/mg* | % | *fold* |
| Pellet | 385,500 | 19,670 | 19.6 | 100 | — |
| DEAE-Sepharose | 258,000 | 2,407 | 107.2 | 66.9 | 5.5 |
| Hydroxylapatite | 195,000 | 110.9 | 1,758 | 50.6 | 90 |
| Phenyl-Sepharose | 172,000 | 9.08 | 18,940 | 44.6 | 966 |
| AH-Sepharose | 146,200 | 1.378 | 106,100 | 37.9 | 5,410 |

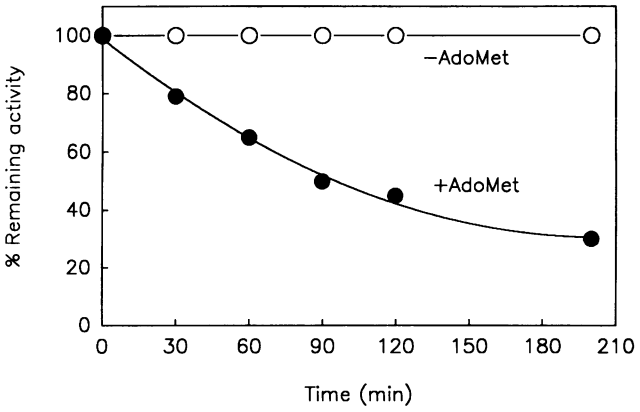


**Figure 3.** Inactivation of ACC synthase with AdoMet. A partially purified ACC synthase (70 units, 20,000 units/mg protein) was incubated at 30°C in a solution containing 200 μM AdoMet, 50 mM Hepes-KOH (pH 8.5), and 10 μM PLP, in a total volume of 2 mL. After incubation for various time periods, a 100-μL portion of the reaction mixture was withdrawn and the remaining ACC synthase activity was assayed.

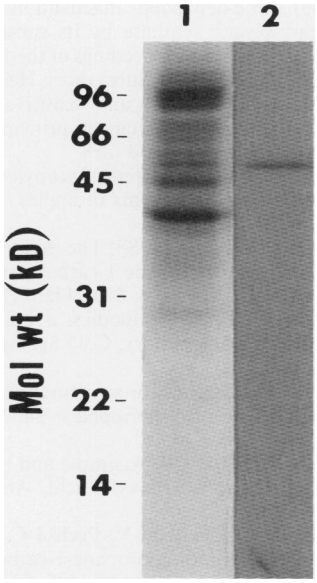


**Figure 4.** SDS-PAGE analysis of a partial purified ACC synthase radiolabeled with AdoMet. A partially purified ACC synthase (specific activity 20,000 units/mg protein, total protein 200 μg) was incubated with 10 μCi of Ado[carboxyl-$^{14}$C]Met as described in "Materials and Methods." Lane 1, Coomassie staining of the radiolabeled preparation; lane 2, fluorogram of lane 1.

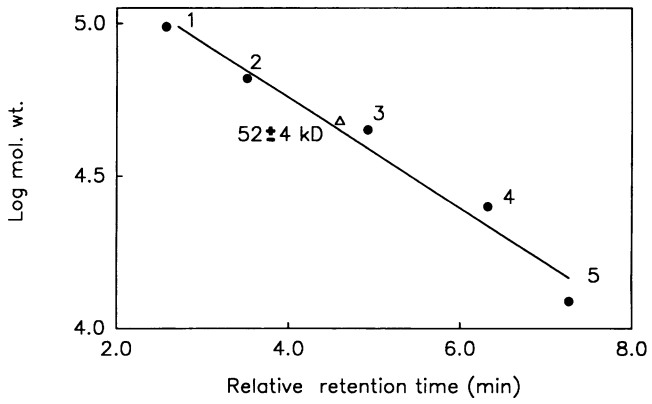


**Figure 5.** Determination of the molecular mass of a partially purified ACC synthase (specific activity, 20,000 units/mg protein) on a Sephadex G-150 column (1 cm × 30 cm, 25-mL bed volume). The column was equilibrated and eluted with 25 mM Hepes-KOH (pH 8.5) containing 10 μM PLP. Protein markers: 1, phosphorylase (97 kD); 2, BSA (66 kD); 3, ovalbumin (45 kD); α-chrymotrypsinogen (25 kD); 5, cytochrome *c* (12.4 kD). Relative retention time was calculated as the difference between retention time of each protein and that of blue dextran. The elution of ACC synthase activity is indicated by Δ.

AdoMet with a half-life of about 70 min (Fig. 3). After a partially purified ACC synthase (20,000 units/mg protein) was incubated with Ado[carboxyl-$^{14}$C]Met at 30°C for 6 h, the resulting proteins were subjected to SDS-PAGE. Fluorography of the gel (Fig. 4) revealed that only one protein band of 48 kD was specifically radiolabeled, a result similar to that reported for tomato ACC synthase (18, 22). However, this radiolabeled protein in apple could not be verified by the tomato monoclonal antibody prepared by Bleecker et al. (2), because tomato enzyme antibody failed to functionally precipitate apple ACC synthase (7). The reciprocal is true also, because monoclonal antibodies against the apple enzyme do not interact with the tomato enzyme. The labeled protein from apple was further verified to be ACC synthase by a specific monoclonal antibody raised against the apple enzyme as will be described later. Gel filtration of the partially purified ACC synthase indicates that the molecular mass of the active enzyme ranged from 48 to 56 kD (Fig. 5). These results indicate that ACC synthase in apple is a monomer as in tomato (2).

### Immunoaffinity Purification of ACC Synthase

Monoclonal anti-ACC synthase IgG (line 6A10) was coupled to protein A-agarose and was used to purify ACC synthase from relatively crude enzyme preparation. The capacity of this immunoaffinity gel was estimated to adsorb 100,000 units of ACC synthase with 0.5 mL of the gel. When an enzyme preparation eluted from DEAE-Sepharose (specific activity of about 100 units/mg) was subjected to immunoaffinity purification, a single 48-kD protein band was eluted with 2% SDS (data not shown). The use of 2% SDS completely eluted all adsorbed protein but resulted in complete inactivation of enzyme activity; less drastic elution conditions such as 10% dioxane (pH 8.0), 50% ethylene glycol (pH 8.0), 0.1

M glycine (pH 2.8), or 2 M urea were all ineffective. However, the native ACC synthase bound on the immunogel exhibited approximately one-third of its catalytic activity, a result similar to that reported for tomato enzyme (3). These results suggest that the active site of the enzyme is not involved in the interaction between the enzyme and the antibody. In a separate experiment, we noted that this immunoaffinity gel was equally effective to bind the AdoMet-radiolabeled, inactive ACC synthase. Thus, it is possible to examine whether the radiolabeled ACC synthase is also specifically bound to the immunoaffinity column. After a crude ACC synthase preparation purified through DEAE-Sepharose step was incubated with radioactive AdoMet, the protein fraction was separated by Sephadex G-25 and passed through an immunoaffinity column. The protein fractions, which were not bound to and which were eluted from the column, were analyzed by SDS-PAGE. Coomassie blue staining on the gel of the fraction eluted from the immunoaffinity gel revealed a major protein of 48 kD, which was also radiolabeled based on fluorography, whereas the fraction that was not bound exhibited many protein bands but retained no radioactivity (Fig. 6). Occasionally, we observed a minor 38-kD protein, which was also labeled by radioactive AdoMet, and co-eluted with the major 48-kD protein from the immunogel. The relationship between this minor 38-kD protein and the major 48-kD protein is not known. Based on the observations that this 48-kD protein was enriched during purification (data not shown), was specifically bound to a monoclonal anti-ACC synthase antibody, and was specifically radiolabeled with AdoMet, this protein was judged to be ACC synthase.

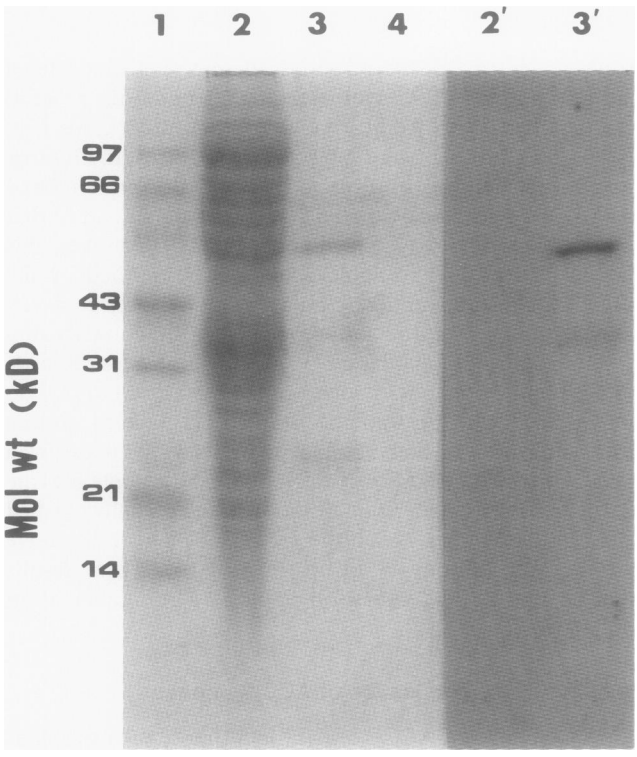


**Figure 6.** SDS-PAGE analysis of an ACC synthase preparation that was incubated with radioactive AdoMet followed by immunoaffinity purification. Lane 1, Coomassie blue staining of the molecular markers: lane 2, Coomassie staining of the protein fraction not retained by the immunoaffinity column to which a relatively crude enzyme (100 units/mg protein) preparation that had been incubated with Ado[carboxyl-$^{14}$C]Met was applied; lane 3, Coomassie staining of the protein eluted from the immunoaffinity column with 2% SDS; lane 4, Coomassie staining of the protein eluted with 2% SDS from the control immunoaffinity column to which no enzyme had been applied; lane 2′, fluorogram of lane 2; lane 3′, fluorogram of lane 3.

This article is dedicated to the memory of Dr. Jacob Biale, who pioneered studies on the biosynthesis and regulatory role of ethylene in fruit ripening. Dr. Biale was a dear mentor to one of us (S.F.Y.). His departure will be felt deeply by his colleagues and many investigators in fruit biochemistry and physiology.

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