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Use of monoclonal antibodies in the purification and characterization of 1-aminocyclopropane-1-carboxylate synthase, an enzyme in ethylene biosynthesis

(tomato fruit development/enzyme regulation)

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ABSTRACT **1-Aminocyclopropane-1-carboxylate (ACC) synthase (EC 4.4.1.14), extracted from tomato pericarp tissue, was purified 6500-fold by conventional and high-performance liquid chromatography. Two-dimensional gel electrophoresis of this preparation indicated that ACC synthase activity was associated with a protein band at 50 kDa, a value consistent with size determinations by gel filtration. Monoclonal antibodies against ACC synthase were obtained from murine hybridoma cell lines. These antibodies recognized the native enzyme, as shown with an immunoprecipitation assay. A monoclonal IgG immunoaffinity gel was used to isolate, from a relatively crude enzyme preparation, a single protein, which migrated at 50 kDa in a NaDodSO$_4$/polyacrylamide gel. *In vivo* labeling of wounded tomato pericarp tissue with [^{35}S]methionine followed by immunoaffinity purification of ACC synthase yielded a radioactive protein of 50 kDa. We conclude that the 50-kDa protein represents ACC synthase in extracts of wounded tomato pericarp tissue.**

Biosynthesis of ethylene, a regulator of plant growth and development, is subject to developmental and environmental regulation (1–3). During the early stages of tomato fruit development, for example, the immature green fruit produces little ethylene. One of the earliest observable events in tomato ripening is a sharp increase in the rate of ethylene formation (4). A number of subsequent ripening-related processes are controlled by ethylene (5). Application of an environmental stress (e.g., wounding) to tomato fruit tissue causes an additional increase in ethylene production (6, 7).

In higher plants, ethylene is synthesized from 1-aminocyclopropane-1-carboxylic acid (ACC) (8, 9). A cell-free preparation of ACC synthase (*S*-adenosyl-L-methionine methylthioadenosine-lyase, EC 4.4.1.14), the enzyme catalyzing the conversion of *S*-adenosyl-L-methionine (AdoMet) to ACC, was first obtained from ripening tomato fruit (10). In most plant tissues, the rate of ethylene synthesis is limited by the activity of ACC synthase. Hence, conditions and chemicals that induce ethylene formation, such as stress or auxin at high concentrations, often enhance the activity of ACC synthase (for review, see ref. 3). In tomato fruits, developmental and wound-induced increases in ethylene production are reflected in the levels of extractable ACC synthase activity (6, 7). Induction of ACC synthase in tomato fruit tissue appears to involve *de novo* synthesis of the enzyme, as indicated by the ability of cycloheximide to inhibit wound-induced increases in the activity of ACC synthase (6, 7) and by an increase in the buoyant density of the enzyme in wounded tomato fruit tissue incubated on ^2H_2O (11).

The activity of ACC synthase often determines the developmental fate of plant tissues; hence, there is considerable interest in understanding how this enzyme is regulated. To reach such an understanding, molecular probes for ACC synthase and its antecedent mRNA and DNA sequences will be required. We report here on the isolation of monoclonal antibodies against ACC synthase and their use in the purification and characterization of the enzyme from tomato fruit tissue.

MATERIALS AND METHODS

Plant Material. Fruits from greenhouse-grown tomato plants (*Lycopersicon esculentum* Mill., cv. Duke) were harvested at the pink-to-red stage. Enzyme induction and preparation of crude homogenates from the pericarp were as described (12). Hollowed-out tomato fruit halves were filled with 50 mM LiCl and incubated at room temperature overnight. Fruit tissue was then sliced into 1-cm pieces and homogenized at low speed with a Polytron (Brinkmann).

ACC Synthase Assay. Appropriate aliquots of enzyme were incubated at 30°C in 20 mM potassium phosphate buffer (pH 8) containing 100 μM AdoMet and 5 μM pyridoxal phosphate. The amount of ACC formed was determined by chemical conversion of ACC to ethylene followed by gas-chromatographic quantitation (13). One unit of enzyme converts 1 nmol of AdoMet to ACC per hr at 30°C.

Enzyme Purification. A 40–95% $(NH_4)_2SO_4$ fraction of the homogenate was obtained as described (11). The protein precipitate was either used immediately or stored at −80°C. After resuspension of the precipitate in dialysis buffer consisting of 10 mM potassium phosphate buffer (pH 8), 0.1 mM dithiothreitol, and 5 μM pyridoxal phosphate, the enzyme was dialyzed overnight.

Purification by HPLC was performed using an SP 8700 HPLC system (Spectra Physics, San Jose, CA). A protein sample of up to 500 mg was loaded onto a preparative stainless-steel HPLC column (1.5 × 25 cm) packed with Synchroprep AX-300 support (SynChrom, Linden, IN) and equilibrated with dialysis buffer. Protein was eluted from the column over 120 min with a linear NaCl gradient (0–700 mM) at a flow rate of 3 ml/min. Fractions (9 ml) were collected, and those showing highest specific enzyme activity were combined. Enzyme purified by preparative anion-exchange HPLC was dialyzed against dialysis buffer and loaded (up to 40 mg of protein) onto a 1 × 20-cm hydroxylapatite column at a flow rate of 0.5 ml/min. The column was washed with dialysis buffer until the eluate showed no absorbance at 280 nm. ACC synthase was eluted in 100 ml of a potassium

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Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; AdoMet, *S*-adenosyl-L-methionine.

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phosphate gradient (pH 8, 10–300 mM) at 0.5 ml/min. Fractions (4 ml) were collected, and those showing highest specific enzyme activity were combined. Enzyme from this purification step was further purified on a 1 × 20-cm column packed with phenyl-Sepharose, as previously described (11). Additional purification was achieved with a 1-ml Affi-Gel Blue (Bio-Rad) column according to the procedure of T. Boller (personal communication). The column and enzyme were equilibrated with 10 mM potassium phosphate buffer (pH 6.8) containing 5 μM pyridoxal phosphate. The enzyme was loaded onto the column in dilute solution (200 μg of protein per ml) at a flow rate of 0.5 ml/min. The column was washed with starting buffer until the eluate showed no absorbance at 280 nm. The enzyme was eluted with 50 mM potassium phosphate buffer (pH 8) containing 5 μM pyridoxal phosphate, at 0.5 ml/min. This enzyme preparation of relatively high specific activity was further purified on an analytical HPLC anion-exchange column (0.4 × 250 cm, Nugel DE-300, Separation Industries, Metuchen, NJ). Enzyme (≤2 mg of protein) was injected onto this column at a flow rate of 1 ml/min and eluted over 40 min with a NaCl gradient (0–700 mM). Fractions (1 ml) were collected, and those with high specific enzyme activity were combined. A final purification step was performed using an analytical HPLC hydroxylapatite column (Bio-Rad). Enzyme was injected onto the column at a flow rate of 0.4 ml/min and eluted over 40 min with a gradient of potassium phosphate buffer (pH 8, 10–300 mM) at the same flow rate. Buffer compositions were as recommended by the manufacturer of the column.

Size Determination by Gel Filtration. A partially purified preparation of ACC synthase (400 units per mg of protein) or ACC synthase bound to monoclonal antibody was fractionated on a TSK-250 HPLC column (7.5 × 600 mm, Bio-Rad) at a flow rate of 0.4 ml/min, using 10 mM potassium phosphate buffer (pH 7) containing 0.15 mM NaCl as the mobile phase.

Gel Electrophoresis and Fluorography. Gradient (8–14%) PAGE in the presence of $NaDodSO_4$ was performed according to Laemmli (14). Gels were stained with Coomassie blue as described (14). Gels were prepared for fluorography by presoaking in 1 M sodium salicylate. Dried gels were placed onto Kodak SB-5 x-ray film and exposed at −80°C for 6–15 days. Radioactivity was quantified by densitometric scanning using a Response spectrophotometer (Gilford). For two-dimensional PAGE, the first dimension was run in nondenaturing, discontinuous, 8% polyacrylamide tube gels, using a GT 3 apparatus (Hoefer, San Francisco) according to the manufacturer's recommendations. Enzyme activity was located in tube gels by slicing the resolving gel into 0.5-cm segments and incubating the slices in the ACC synthase assay mixture for 1 hr. The second dimension was run in 8–14% gradient polyacrylamide gels.

Preparation of Antigen and Immunization. ACC synthase was purified from tomato pericarp by $(NH_4)_2SO_4$ precipitation (40–95% fraction) followed by preparative ion-exchange, hydroxylapatite, phenyl-Sepharose, and analytical ion-exchange chromatography. Enzyme with a specific activity of $1–3 \times 10^4$ units per mg of protein was routinely obtained. The purified enzyme was coupled to lipopolysaccharide (LPS) (15). CNBr-activated LPS (80 μg) was incubated with 100 μg of protein in 20 mM potassium phosphate buffer (pH 8.5) at 4°C for 12 hr. The LPS-protein conjugate was collected by centrifugation in a microcentrifuge (Fisher) for 15 min, and the pellet was resuspended in 0.2 M lysine to block residual cyanate esters. The suspension was centrifuged again, and the pellet was resuspended in the original protein solution. The suspension was adjusted to pH 7.2 (with HCl), diluted 1:10 with sterile phosphate-buffered saline, and sonicated briefly. A 20-week-old female BALB/c mouse was injected i.p. with 20 μg of conjugated LPS and was given booster injections of 20 μg of the conjugate 14 and 40 days later. Two additional i.p. injections (30 μg of conjugated LPS each) were made 3 and 2 days before fusion of the spleen cells (day 70).

Cell Culture and Hybridoma Selection. Murine myeloma cells (10^8) from line SP2/0-Ag14 (16) were fused with 2×10^8 spleen cells from the immunized mouse (17). Hybridomas secreting antibodies against ACC synthase were detected by an immunoprecipitation assay. They were cloned twice by limiting dilution. All cultures were grown in Dulbecco's modified Eagle's medium (GIBCO) with 15% fetal bovine serum (Hazelton Research Products, Denver, PA).

Monoclonal Antibody Production, Purification, and Characterization. Ten-week-old BALB/c mice were injected i.p. with 0.5 ml of pristane and, 2 weeks later, with 3×10^6 hybridoma cells. After an additional 5 days, ascites fluid was removed and either used immediately or frozen at −80°C. Monoclonal antibodies were purified from ascites fluid either by hydroxylapatite chromatography (18) or by protein A affinity chromatography (MAPS II Kit, Bio-Rad). Antibody subtypes were determined using Ouchterlony immunodiffusion plates (Serotec, Blackthorn, UK).

Immunoprecipitation Assay for ACC Synthase. Specified dilutions of either control mouse serum, mouse antiserum, tissue culture supernatant, or IgG purified from mouse ascites fluid were tested for binding to ACC synthase. Fifty microliters of dilute antibody-containing solution was added to 50 μl of a dilute, partially purified ACC synthase preparation (1–5 units/50 μl). All dilutions were made in 50 mM potassium phosphate buffer (pH 8) containing 0.3 M NaCl, 1 mg of bovine serum albumin per ml, and 5 μM pyridoxal phosphate. The mixture was incubated at room temperature for 2 hr. Sufficient rabbit anti-mouse IgG was added in 25 μl to bind 3.5 μg of mouse IgG, and the mixture was incubated for an additional 1 hr. Finally, 25 μl of a 1% (vol/vol) suspension of fixed *Staphylococcus aureus* cells was added and incubated for 1 hr at room temperature. The mixture was centrifuged in a microcentrifuge for 15 min, and the supernatant was transferred to a tube containing ACC synthase assay buffer. ACC synthase was determined as described above. For initial screening of culture supernatants, the entire immunoprecipitation assay was done in 96-well plates.

***In Vivo* Labeling of ACC Synthase.** Wounded tomato disks were prepared from pink tomatoes as described (11). To enhance uptake of label, disks were placed under a stream of air until they had lost 10% of their original weight. An aqueous solution (0.5 ml) containing 0.1–0.2 mCi of $[^{35}S]$-methionine (1127 Ci/mmol; 1 Ci = 37 GBq) was applied to 15 disks (10 g). After incubation for 1.5 hr, the disks were homogenized with a Teflon homogenizer in 10 ml of 100 mM potassium phosphate, pH 8/5 mM EDTA/5 μM pyridoxal phosphate and centrifuged at 18,000 × *g* for 15 min. The supernatant was centrifuged at 100,000 × *g* for 30 min.

Immunoaffinity Purification of ACC Synthase. An immunoaffinity matrix was prepared by incubating 5–6 mg of purified monoclonal antibody with 1 ml of swollen CNBr-activated Sepharose 4B in 50 mM sodium borate, pH 8.5/0.5 M NaCl for 3 hr at room temperature. Residual cyanate esters were blocked with 1 mM ethanolamine (pH 8.5). Purification of ACC synthase was achieved by adding 30–50 μl of swollen immunoaffinity gel to either 15 ml of crude extract (for the *in vivo* labeling experiments) or to 1.5 ml of partially purified ACC synthase (300–400 units per mg of protein). The suspension was incubated at room temperature for 3 hr, and the gel-bound enzyme was separated from the solution by low-speed centrifugation (100 × *g*, 5 min). The gel was washed twice with 1.5 ml of 100 mM potassium phosphate, pH 8/0.3 M NaCl, once with the same buffer at pH 6, and once again at pH 8. For $NaDodSO_4$/PAGE, the ACC synthase protein was extracted twice from the immunoaf-

Table 1. Purification of ACC synthase from the homogenate of 10 kg of tomato pericarp tissue

Fraction	Total activity, units	Protein, mg	Specific activity, units/mg	Overall purification, -fold	Recovery, %
40–95% $(NH_4)_2SO_4$	10,500	2500	4.2	1	100
Preparative ion-exchange HPLC	9,000	320	28	7	85
Hydroxylapatite column	8,600	48	179	43	82
Phenyl-Sepharose column	4,800	5.1	941	224	46
Analytical ion-exchange HPLC	2,400	0.7	3,428	816	23
Affi-Gel Blue column	1,600	0.15	10,666	2539	15
Analytical hydroxylapatite HPLC	550	0.02	27,500	6547	5

finity gel with 50 μl of electrophoresis sample buffer (0.1 M Tris·HCl, pH 6.8/3% NaDodSO$_4$/4% glycerol). 2-Mercaptoethanol or dithiothreitol were added to this extract to a final concentration of 5–10% (vol/vol) or 5% (wt/vol), respectively, before electrophoresis.

RESULTS

Purification of ACC Synthase. Two major problems had to be overcome in the purification of ACC synthase. Even in the pericarp of ripening tomato fruits, one of the richest known sources of ACC synthase, the level of enzyme activity is very low (0.2 nmol per hr per g of fresh weight; ref. 7). In addition, ACC synthase proved to be very labile during purification (11). The first problem was ameliorated by overnight treatment of pericarp tissue with LiCl, which enhanced enzyme activity more than 100-fold (12). The problem of instability was solved, in part, by using HPLC for purification of the enzyme. This allowed several purification steps to be performed in a short period of time. Losses of activity were also reduced by storing the enzyme at −80°C at any stage of purification, the recovery being >70% upon thawing.

ACC synthase was purified 6500-fold, with 5% recovery, from 10 kg of tomato pericarp tissue by a combination of conventional and high-performance liquid chromatographic methods (Table 1). A major portion of the purified enzyme preparation was further fractionated by two-dimensional PAGE. Nondenaturing conditions were used in the first dimension, and ACC synthase was localized by determining the activity of the enzyme in slices of a second gel run in parallel. NaDodSO$_4$/PAGE was used in the second dimension. One major Coomassie blue-stained protein band at 50 kDa in the second dimension corresponded to the location of ACC synthase activity in the first dimension (Fig. 1). A very faint protein band at ≈95 kDa was also evident.

Monoclonal Antibodies Against ACC Synthase. When the titer of ACC synthase-specific antibodies in the serum of the immunized mouse was sufficiently high to immunoprecipitate 25 units of enzyme per μl of serum, spleen cells were fused with myeloma cells, and the resultant hybridomas were screened for secretion of ACC synthase-specific antibodies. From an initial 26 wells giving positive results, 5 stable hybridoma cell lines (1b–5b) were obtained which produced monoclonal antibodies directed against ACC synthase. Ascites fluid derived from each of these cell lines completely removed ACC synthase activity from enzyme preparations at various stages of purity. Control solutions containing normal mouse serum, ascites fluid obtained from an SP2 myeloma cell line, and ascites containing monoclonal antibodies to

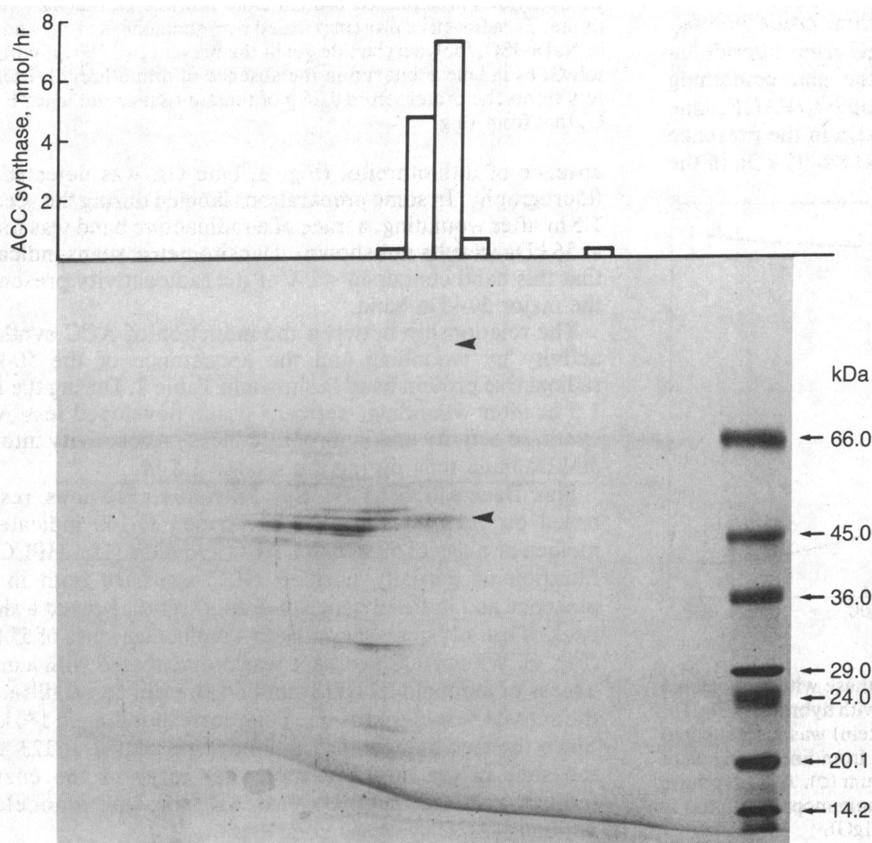

FIG. 1. Two-dimensional gel electrophoresis of a highly purified ACC synthase preparation. Enzyme activity was localized in the first dimension by incubating slices of a parallel-run gel in ACC synthase assay buffer. Arrowheads indicate the locations of protein bands in the second dimension (NaDodSO$_4$/PAGE run with 5% 2-mercaptoethanol) that correspond to the enzyme activity in the first dimension (nondenaturing discontinuous PAGE). Molecular mass standards are at right.

other plant proteins did not immunoprecipitate ACC synthase. An example of a titration curve using monoclonal antibody from line 5b is given in Fig. 2. The monoclonal antibodies from all cell lines were of the IgG1 subclass as determined by the Ouchterlony immunodiffusion assay (data not shown). ACC synthase bound to nitrocellulose could not be detected with any of the five monoclonal antibodies.

Immunoaffinity Purification of ACC Synthase. An immunoaffinity gel was prepared by covalently coupling purified IgG from hybridoma line 5b to Sepharose 4B. Based on the disappearance of enzyme activity from an ACC synthase preparation, the immunoaffinity gel had a binding capacity of 2.5×10^4 units of ACC synthase activity per ml of swollen gel. When the bound enzyme was resuspended in ACC synthase assay buffer, it exhibited 30–40% of the activity removed from the original enzyme solution (data not shown). The immunoaffinity gel (50 μl) was used to isolate ACC synthase from a partially purified enzyme preparation (750 units, 300 units per mg of protein). The gel with bound enzyme was washed in a series of buffers to remove nonspecifically adsorbed protein. When specifically bound protein was extracted from the gel with buffer containing $NaDodSO_4$ and subjected to $NaDodSO_4$/PAGE in the presence of 5% (wt/vol) dithiothreitol, a major polypeptide of 50 kDa, in addition to the two IgG subunits, was detected (Fig. 3, lane B). In the absence of dithiothreitol, the protein migrated as a diffuse band with an apparent molecular mass of 80–100 kDa (data not shown). When a second aliquot of affinity gel was added to the protein solution from which all enzyme activity had been removed, no additional protein became specifically bound to the gel (Fig. 3, lane C). Based on the amount of enzyme activity in the original solution and the amount of protein in the 50-kDa band, a specific activity of 4×10^5 units per mg of protein was calculated.

***In Vivo* Labeling of ACC Synthase.** When wounded tomato pericarp disks (10 g) were fed 200 μCi of [^{35}S]methionine between 1.5 and 3 hr after wounding, $\approx 3 \times 10^5$ cpm was incorporated into $(NH_4)_2SO_4$-precipitable material. ACC synthase activity was purified directly from crude homogenates, using immunoaffinity gel with IgG from hybridoma line 2b or 5b. $NaDodSO_4$ eluates of the gel, containing 200–300 cpm, were fractionated by $NaDodSO_4$/PAGE. One major radioactive band, migrating at 50 kDa in the presence of 5% dithiothreitol (Fig. 3, lane F) and at 80–95 kDa in the absence of dithiothreitol (Fig. 3, lane G), was detected by fluorography. In some preparations labeled during the second 1.5 hr after wounding, a trace of a radioactive band was found at 56 kDa (results not shown). Densitometric scans indicated that this band contained <1% of the radioactivity present in the major 50-kDa band.

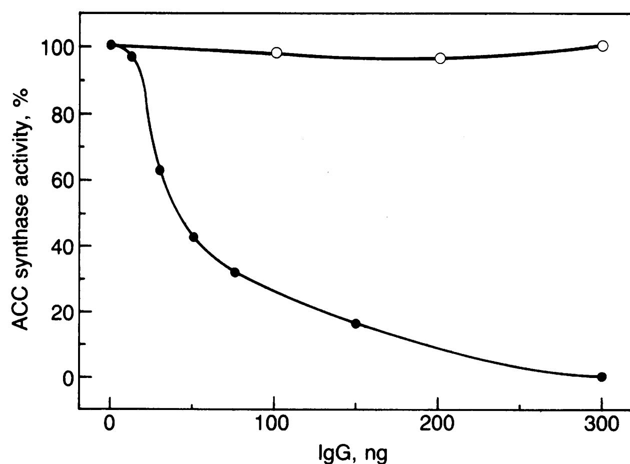

FIG. 2. Immunoprecipitation of ACC synthase with monoclonal antibody purified from ascites fluid produced with hybridoma line 5b. Two units of enzyme (40 units per mg of protein) was preincubated in 100 μl with the indicated amount of IgG1 from line 5b (●) or of mouse IgG1 in dilutions of control mouse serum (○). ACC synthase activity remaining in the supernatant after immunoprecipitation is expressed as % of control (no added mouse IgG).

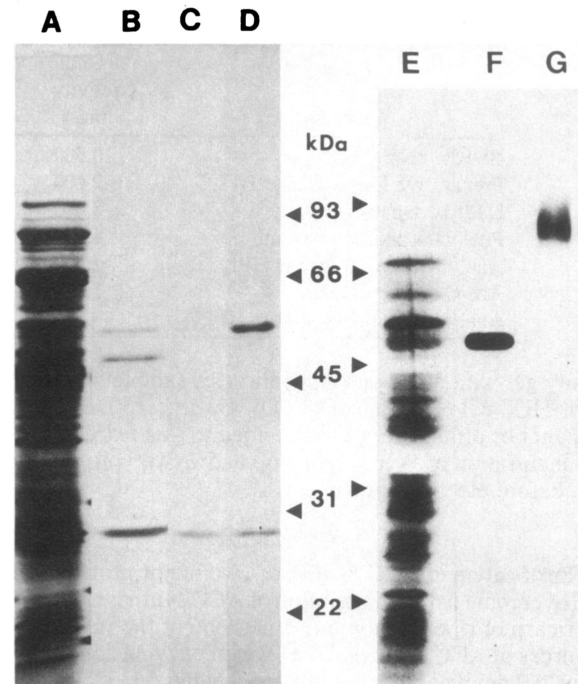

FIG. 3. (*Left*) $NaDodSO_4$/PAGE of tomato pericarp proteins stained with Coomassie blue. Lanes: A, ACC synthase-enriched protein fraction (300 units per mg of protein) after purification by preparative anion-exchange HPLC; B, proteins eluted from the immunoaffinity gel (line 5b IgG) after incubation of the gel with 2.5 mg of the protein preparation used in lane A; C, proteins eluted from a second aliquot of immunoaffinity gel added to the protein preparation shown in lane A after all enzyme activity had been removed by the first aliquot of the immunoaffinity gel; D, purified IgG1 from hybridoma line 5b. (*Right*) Fluorograph of [^{35}S]methionine-labeled protein extracted from wounded tomato pericarp tissue and fractionated by $NaDodSO_4$/PAGE. The tissue was labeled with 200 μCi of [^{35}S]methionine during the second 1.5 hr after wounding. Lanes: E, $(NH_4)_2SO_4$-precipitated protein from a crude extract of tomato tissue; F, radioactive protein purified by immunoaffinity gel and run in $NaDodSO_4$/polyacrylamide gel in the presence of 5% dithiothreitol; G, as in lane F but run in the absence of dithiothreitol. Lane E represents the protein from 0.25 g of tomato tissue, and lanes F and G, that from 10 g.

The relationship between the induction of ACC synthase activity by wounding and the appearance of the 50-kDa radioactive protein band is shown in Table 2. During the first 1.5 hr after wounding, pericarp tissue developed less ACC synthase activity and incorporated less radioactivity into the 50-kDa band than during the second 1.5 hr.

Size Determinations by Gel Filtration. Previous results based on gel filtration using Sephadex G-100 indicated a molecular mass of 57 kDa for ACC synthase (11). HPLC gel filtration of partially purified ACC synthase both in the presence and in the absence of dithiothreitol showed a single peak of activity corresponding to a molecular mass of 55 kDa (Fig. 4). When ACC synthase was preincubated with a molar excess of monoclonal IgG from line 5b prior to gel filtration, the activity was eluted as one peak corresponding to 170 kDa. Since the free monoclonal antibody was eluted at 123 kDa, the shift in the apparent molecular mass of the enzyme indicates a stoichiometry of 1:1 for the monoclonal antibody–ACC synthase complex.

Table 2. Level of ACC synthase activity and of radioactivity in the immunoaffinity-purified fraction from wounded tomato pericarp disks

Source of antibody	Labeling time*	Enzyme activity, units/10 g of tissue	Radioactivity, densitometer integral
Line 5b†	0 –1.5 hr	16.6	0.21
Line 5b†	1.5–3 hr	91.2	3.66
Line 5b‡	1.5–3 hr	7.4	0.16
Line 2b†	1.5–3 hr	86.5	3.36

*Tomato pericarp disks were fed [^{35}S]methionine for the first or second 1.5 hr following wounding.

†Enzyme activity was determined in a dialyzed crude extract prior to the addition of immunoaffinity gel. Immunoaffinity-purified protein was fractionated by $NaDodSO_4$/PAGE. Radioactivity was localized by fluorography and quantitated by densitometry. Line 5b IgG and line 2b IgG recognize different epitopes on the enzyme.

‡Residual enzyme activity and radioactivity associated with the 50-kDa protein band were determined once more in the extract from row 2 (line 5b; 1.5–3 hr labeling time) after the initial removal of ACC synthase with the immunoaffinity gel.

DISCUSSION

Our results represent strong evidence that ACC synthase in extracts of tomato pericarp tissue consists of a 50-kDa polypeptide. Two independent means of purification based on chromatographic and immunological methods led to the isolation of a single major protein of this molecular mass. Additionally, a 50-kDa protein isolated with an immunoaffinity gel became labeled when ACC synthase activity was induced by wounding of tomato tissue. The estimated specific activity of the immunopurified enzyme (2–4 × 10^5 units per mg of protein) and the turnover number (k_3) derived therefrom (3–6 sec^{-1}) fall within the range observed for a number of enzymes (19) and are consistent with the values reported previously for ACC synthase (20). From these values, we calculated that ACC synthase represents <0.0001% of the total protein in the pericarp of ripening tomato fruits.

The behavior of the immunopurified protein on $NaDodSO_4$/PAGE was drastically altered when dithiothreitol was omitted from the sample buffer. Both Coomassie blue-stained and metabolically radiolabeled protein migrated as a diffuse band with an apparent molecular mass of 80–100 kDa, indicating the presence of at least one disulfide bond in the molecule. When $NaDodSO_4$/PAGE was performed in the presence of 2-mercaptoethanol instead of dithiothreitol, both the high- and low-molecular-mass bands were detected, indicating incomplete reduction of the protein. These disulfide bonds may be intramolecular, preventing complete denaturation of the enzyme and causing anomalous behavior during $NaDodSO_4$/PAGE, or intermolecular, in which case the slower migrating band may represent a dimer of two 50-kDa polypeptides. The latter possibility appears unlikely because the size determinations by gel filtration in the absence of reducing agent indicate that the native enzyme in tomato extracts exists as a single protein of ≈50 kDa (Fig. 4). The shift in apparent size of ACC synthase bound to monoclonal antibody is additional evidence that the active enzyme exists as a monomer possessing only one epitope for the antibody.

Our work affirms the power of monoclonal antibody technology when applied to the isolation and characterization of low-abundance proteins. Using partially purified enzyme, the immunoaffinity gel allowed a >2000-fold purification of ACC synthase in a single step. Use of monoclonal antibodies will also permit investigations into the role of protein synthesis and turnover in the regulation of ACC synthase activity *in vivo*. We hope that these studies will lead to an understanding of the mechanisms by which ethylene-mediated processes are regulated during plant development and stress.

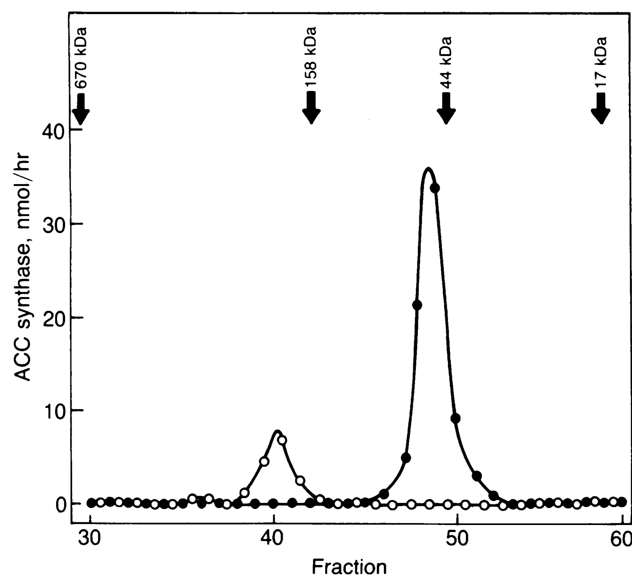

FIG. 4. Gel-filtration HPLC of an ACC synthase preparation (300 units per mg of protein), without dithiothreitol in the elution buffer, in the presence (○) or absence (●) of IgG from hybridoma line 5b. The elution profiles of ACC synthase with dithiothreitol in the elution buffer or with control mouse serum added to the enzyme preparation were very similar to the one obtained in the absence of IgG (●). The center of the ACC synthase peaks were determined by plotting the data points on Gaussian graph paper. Elution positions of standard proteins used to calibrate the column are indicated by arrows.

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1. Abeles, F. B. (1973) *Ethylene in Plant Biology* (Academic, New York), pp. 30–102.
2. Lieberman, M. (1979) *Annu. Rev. Plant Physiol.* **30**, 533–591.
3. Yang, S. F. & Hoffman, N. E. (1984) *Annu. Rev. Plant Physiol.* **35**, 155–189.
4. Workman, M. & Pratt, H. K. (1957) *Plant Physiol.* **32**, 330–334.
5. Jeffery, D., Smith, C., Goodenough, P., Prosser, I. & Grierson, D. (1984) *Plant Physiol.* **74**, 32–38.
6. Boller, T. & Kende, H. (1980) *Nature (London)* **286**, 259–260.
7. Kende, H. & Boller, T. (1981) *Planta* **151**, 476–481.
8. Adams, D. O. & Yang, S. F. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 170–174.
9. Lürssen, K., Naumann, K. & Schröder, R. (1979) *Z. Pflanzenphysiol.* **92**, 285–294.
10. Boller, T., Herner, R. C. & Kende, H. (1979) *Planta* **145**, 293–303.
11. Acaster, M. A. & Kende, H. (1983) *Plant Physiol.* **72**, 139–145.
12. Ramalingam, K., Lee K.-M., Woodard, R. W., Bleecker, A. B. & Kende, H. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7820–7824.
13. Lizada, M. C. C. & Yang, S. F. (1979) *Anal. Biochem.* **100**, 140–145.
14. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
15. Lange, M., Guern, C. L. & Casenave, P.-A. (1983) *J. Immunol. Methods* **63**, 123–131.
16. Shulman, M., Wilde, C. D. & Köhler, G. (1978) *Nature (London)* **276**, 269–270.
17. Galfe, G. & Milstein, C. (1981) *Methods Enzymol.* **73**, 3–46.
18. Stanker, L. H., Vanderlaan, M. & Juarez-Salinas, H. (1985) *J. Immunol. Methods* **76**, 157–169.
19. Stryer, L. (1981) *Biochemistry* (Freeman, San Francisco), p. 114.
20. Kende, H., Bleecker, A. B., Kenyon, W. H. & Mayne, R. G. (1986) in *Plant Growth Substances 1985*, ed. Bopp, M. (Springer, Berlin), pp. 120–128.