Plant Physiol. (1991) 97, 657–662
0032-0889/91/97/0657/06/$01.00/0

Received for publication March 4, 1991
Accepted May 24, 1991

Overexpression of Acetohydroxyacid Synthase from *Arabidopsis* as an Inducible Fusion Protein in *Escherichia coli*

Production of Polyclonal Antibodies, and Immunological Characterization of the Enzyme

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ABSTRACT

Acetohydroxyacid synthase (AHAS, EC 4.1.3.18) is the first enzyme unique to the biosynthesis of valine, leucine, and isoleucine. This enzyme is the target site of several classes of structurally unrelated herbicides. The conventional method of antibody production using purified protein has not been successful with this enzyme. Two separate fragments of a gene encoding a portion of the mature region of AHAS from *Arabidopsis* were fused with the *trpE* gene from *Escherichia coli* using the pATH1 vector. *E. coli* cells transformed with each respective plasmid expressed a fusion protein at levels greater than 10% of the total cell protein. The fusion protein was purified and used to immunize rabbits. Antisera obtained from the immunized rabbits immunoprecipitated AHAS activity from *Arabidopsis* cell free extracts. The anti-AHAS antisera reacted with a 65 kilodalton protein band in electrophoretically resolved extracts of *Arabidopsis*. In cross-reactivity tests, this antibody was able to immunoprecipitate AHAS activity from various plant species. Furthermore, a protein band with a molecular mass of 65 kilodaltons was detected in the crude extracts of all plant species tested on a Western blot. These results indicate that the 65 kilodalton protein represents AHAS in various plant species. The wide spectrum of cross-reactivity for the antisera supports the view that the AHAS enzyme is highly conserved across all plant species.

The first enzymatic step common to the biosynthesis of the branched chain amino acids (valine, leucine, and isoleucine) is catalyzed by AHAS[1] (also known as acetolactate synthase; EC 4.1.3.18). The enzyme catalyzes two parallel reactions: condensation of 2 mol of pyruvate to give rise to acetolactate, and condensation of pyruvate and α-ketobutyrate to yield acetohydroxybutyrate (23, 24). AHAS activity is inhibited by the end products of the pathway, *i.e.* valine, leucine, and isoleucine, which provide a mechanism for regulation of this pathway in higher plants (12, 14). Biochemical and genetic studies have shown that AHAS is the target site of several classes of structurally unrelated herbicides, which include the imidazolinones (18), the sulfonylcarboxamides (4), the sulfonylureas (3, 9, 17), and the triazolopyrimidines (8, 22).

Genes coding for AHAS have been cloned and sequenced from various plant species (2, 11, 26). The AHAS genes are encoded on nuclear DNA and translated on cytosolic ribosomes, and the mature enzyme is localized in the chloroplast (6, 13). As with numerous other chloroplast-localized proteins, AHAS is synthesized as a precursor of higher mol wt than the mature form. These precursors are translocated across the chloroplast envelope and proteolytically processed to their mature form (1). The N-terminal sequence of the mature AHAS protein from plant species has not been directly determined; therefore, the mol wt of the enzyme subunit cannot be correctly predicted from the gene sequence data.

AHAS from plants has been isolated as high mol wt aggregate (18, 19, 27). However, it is not known whether this aggregate is composed of homologous or heterologous subunits of the enzyme. Also, very little information has been published about the developmental regulation, tissue-specific expression, or evolutionary aspects of the enzyme. Immunological techniques are powerful biochemical tools to assist in addressing these issues. However, for the production of antibodies, significant amounts of protein are required. The low abundance and extreme lability of AHAS from plants has hindered purification attempts (5, 15, 27). To obtain sufficient AHAS protein to immunize rabbits, gene fusions were made between the *trpE* gene of *Escherichia coli* and different portions of the *Arabidopsis* AHAS gene. By inducing the *trpE* promoter on these plasmids in the transformed *E. coli* cells, large amounts of *trpE*-AHAS fusion proteins were produced and used to immunize rabbits. Antisera that recognize the AHAS portion of the fusion protein were obtained and used to examine the enzyme from different plant species.

MATERIALS AND METHODS

Plant Material

The species used in this study included corn (*Zea mays*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), spinach (*Spinacea oler-*

[1] Abbreviations: AHAS, acetohydroxyacid synthase; FAD, flavin adenine dinucleotide; bp, base pair.

acea), *Arabidopsis* (*Arabidopsis thaliana*), pea (*Pisum sativum*), sugar beet (*Beta vulgaris*), and lima bean (*Phaseolus limensis*). Green seedlings of all species used in this study were grown by planting seeds in soil in a greenhouse. Protein extracts were obtained from shoots and leaves from 1-week-old seedlings.

Enzyme Extraction

Plant material was powdered in liquid nitrogen and then homogenized in 100 mM potassium phosphate buffer (pH 7.5) containing 10 mM pyruvate, 5 mM magnesium chloride, 5 mM EDTA, 100 μM FAD, 1 mM valine, 1 mM leucine, 10% (v/v) glycerol, and 10 mM cysteine. The homogenate was filtered through a nylon cloth (53 μM mesh), and the filtrate was centrifuged at 25,000*g* for 20 min. The supernatant fraction was brought to 50% saturation with respect to ammonium sulfate and allowed to stand for 20 to 30 min on ice. After centrifugation at 25,000*g* for 20 min, the ammonium sulfate pellet was used immediately or frozen with liquid nitrogen and then stored at −20°C for later use.

Sephadex G-25 Desalting

The ammonium sulfate pellet collected from the previous step was dissolved in 50 mM phosphate (pH 7), 150 mM NaCl, and 10 μM FAD, and the resulting solution was desalted on a bed of prepacked Sephadex G-25 column according to the manufacturer's protocol (Bio-Rad). The same buffer that was used for dissolving the ammonium sulfate pellets was also used for column equilibration and protein elution.

AHAS Assay

AHAS activity was measured by estimation of the formation of product, acetolactate, after conversion by acid decarboxylation to acetoin (20). Standard reaction mixtures contained the enzyme in 50 mM potassium phosphate buffer (pH 7.0) containing 100 mM sodium pyruvate, 10 mM magnesium chloride, 1 mM thiamine pyrophosphate, and 10 μM FAD. This mixture was incubated at 37°C for 1 h. The reaction was stopped with the addition of sulfuric acid to a final concentration of 0.85%. The reaction product was allowed to decarboxylate at 60°C for 15 min. The acetoin formed was determined by incubating with creatine (0.17%) and 1-naphthol (1.7%) by the method of Westerfeld (25). Appropriate checks of direct acetoin formation during the enzyme assay were made.

Construction of Recombinant Plasmids

The construction of expression vectors is described in Figure 1. A 5.5 kilobase *Xba*I fragment containing the regulatory and coding regions of *Arabidopsis* AHAS gene was previously described (11). Either of two separate restriction fragments from the mature coding region of the gene (Fig. 1) was ligated to the pATH1 vector, which contains the *trpE* regulatory region and a portion of the *trpE* structural gene from *E. coli* (7, 21) (Fig. 1). In the first construct, a 791 bp *Bam*HI fragment containing nucleotides 1257 to 2048 of the *Arabidopsis* AHAS gene was gel-purified and ligated at the *Bam*HI site in the pATH1 vector resulting in plasmid pAB20. To produce the second construct, the gel-purified *Eco*RV-*Bgl*II fragment of the *Arabidopsis* AHAS gene containing nucleotides 972 to 2138 was ligated to *Sma*I-*Bam*HI digested pATH1, yielding plasmid pARB6. In both constructs, the *Arabidopsis* AHAS gene fragments were in phase with the 5′ portion of the *trpE* gene. Transformation of *E. coli*, preparation of plasmid DNA, and other routine procedures were performed according to the protocols of Maniatis *et al.* (10).

Induction of Expression Plasmids and Purification of Fusion Protein

Cell culture and induction conditions were as previously described (21), except that in the present experiments cells were grown for 6 to 8 h. Total cell lysates were prepared by boiling whole cells in SDS-PAGE sample buffer. The fusion protein was purified according to a modification of the previously described procedures (7). Cells were resuspended in 50 mM Tris-HCl (pH 7.5) containing 5 mM EDTA and 3 mg/mL lysozyme and incubated on ice for 2 h. The suspension was made 0.3 M NaCl, 0.2% NP-40, 12 mM $MgCl_2$, and 5 μg/mL of deoxyribonuclease I. The suspension was gently shaken at 4°C for 1 h. The cells were sonicated to shear DNA. The insoluble fraction containing the fusion protein was collected by centrifugation and then resuspended and washed twice with 10 mM Tris-HCl (pH 7.5) containing 1 M NaCl. The insoluble fraction was finally washed with 10 mM Tris-HCl. The fusion protein was purified to near homogeneity by preparative SDS-PAGE. The protein containing bands were visualized by staining a strip of the gel with Coomassie blue. The desired protein band was cut out and protein was recovered by electroelution.

Immunization of Rabbits and Collection of Antisera

Purified fusion protein was emulsified with Freund's adjuvant and injected into New Zealand rabbits according to the protocol previously used (21). Sera collected from these rabbits were used directly without any purification.

Neutralization of AHAS Activity with Antibody

The effect of antisera on AHAS activity was determined in reaction mixtures containing 50 mM phosphate buffer (pH 7.0), 150 mM NaCl, 10 μM FAD, desalted enzyme, and varying amounts of antiserum (0–100 μL). Controls for each reaction series contained the preimmune serum. After incubation at room temperature for 30 min, the mixtures were further incubated on ice for 2 h. Following incubation, an aliquot was taken and assayed for AHAS activity after centrifugation at 10,000*g* for 5 min. The amount of acetolactate formed in the tube containing preimmune serum was designated as 100% AHAS activity. Other reactions were expressed as a percentage of this activity.

Western Blotting

Slab gel electrophoresis (11% acrylamide) was conducted by the use of the SDS-PAGE system of Neville (16). Following electrophoresis, protein was transferred to nitrocellulose

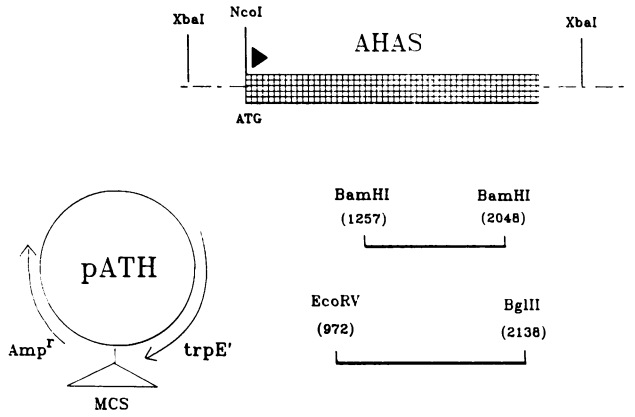

Figure 1. Construction of *trpE*-AHAS gene fusion. A 791 bp *Bam*HI-*Bam*HI fragment and a 1166 bp *Eco*RV-*Bgl*II fragment from the *Arabidopsis* AHAS gene were inserted into the pATH1 vector at the *Bam*HI and *Sma*I-*Bam*HI sites present in the multiple cloning site (MCS), resulting in pAB20 and pARB6, respectively. Amp^r = ampicillin resistance gene; *trpE* = part of *E. coli trpE* gene (21).

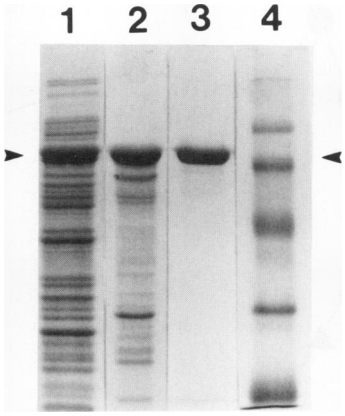

Figure 3. Purification of *trpE*-AHAS fusion protein obtained after transformation of *E. coli* cells with plasmid pARB6. Cells were harvested after 6 h of culture and the fusion protein was purified according to the procedures described in "Materials and Methods." Lane 1, total cell lysate; lane 2, insoluble protein fraction; lane 3, protein isolated by preparative electrophoresis; lane 4, molecular mass standards (as in Fig. 2).

sheets using the protocol provided by the manufacturer (Bio-Rad). The blots were developed using a 1:1000 dilution of the primary antibody, a 1:1500 dilution of goat anti-rabbit immunoglobulin G-alkaline phosphatase conjugate, and nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrate according to the protocol provided by Bio-Rad.

RESULTS

Over-Expression of Inducible AHAS-*trpE* Fusion Proteins

Two separate gene fusions were constructed to produce AHAS-*trpE* fusion proteins. These constructs contained overlapping fragments of the *Arabidopsis* AHAS gene (Fig. 1). *E. coli* cultures containing plasmids pAB20 or pARB6 were grown in minimal medium, starved for tryptophan, and exposed to indoleacrylic acid to derepress the *trp* operon. Total cell lysates were prepared at various times from the start of inoculation and analyzed by SDS-PAGE. *E. coli* cells transformed with the pATH1 vector showed high-level expression of *trpE* protein (Fig. 2, lane 10). Cells transformed with either pAB20 or pARB6 produced a protein of the molecular weight expected for the respective fusion protein (Fig. 2). Maximum levels of expression of these fusion proteins were reached at 4 to 6 h after inoculation. Fusion proteins were purified to near homogeneity as shown in Figure 3 (results for pAB20 not presented here).

Specificity of Antisera against Fusion Proteins for AHAS from *Arabidopsis*

Antisera obtained against the fusion protein using pAB20, designated antiserum-1, performed better in the immunoprecipitation experiments as compared with the antisera obtained against the fusion protein using pARB6, designated antiserum-2. Therefore, antiserum-1 was used in all immunoprecipitation experiments. There was no difference between the two sets of antisera in the Western blotting experiments.

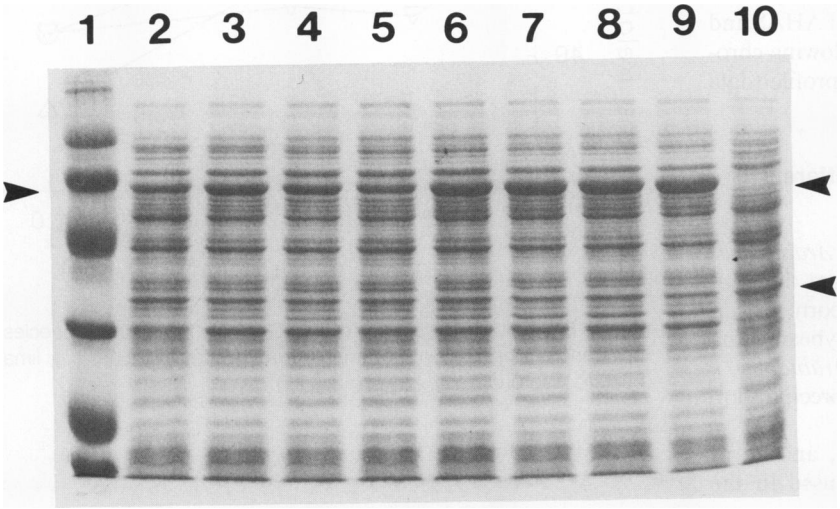

Figure 2. Expression of *trpE*-AHAS fusion protein in *E. coli* cells transformed with plasmid pAB20 or pATH1. Lane 1, pre-stained high molecular mass standards from Bethesda Research Laboratories (200, 97.4, 68, 43, 29, 18.4, and 14.3 kD, respectively). Lanes 2 through 9, cells transformed with pAB20 harvested at hourly intervals at 1 to 8 h from the beginning of culture. Lane 10, cells transformed with pATH1 harvested after 8 h of growth. Growing conditions and procedures for protein sample preparation are described in "Materials and Methods." Proteins were visualized by Coomassie blue staining following SDS-PAGE on a 10% gel.

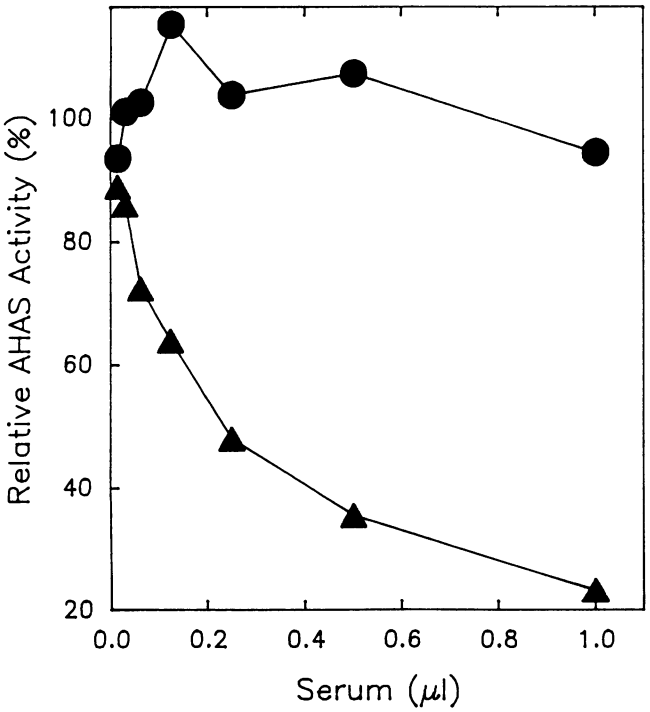

Figure 4. Immunoprecipitation of AHAS activity from *Arabidopsis* seedlings using increasing amounts of antiserum-1. Preimmune serum (●); antiserum-1 (▲).

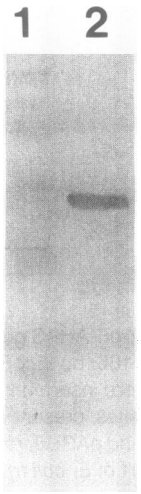

Figure 5. Western blotting using antiserum-1. Lane 1, molecular mass standards; lane 2, following SDS-PAGE of crude extracts of *Arabidopsis* seedlings, proteins were transferred onto a nitrocellulose membrane and probed with antiserum-1 according to the procedures described in "Materials and Methods."

To demonstrate the specificity of antisera for AHAS activity from *Arabidopsis,* immunoprecipitation of the enzyme activity with antisera was performed. Precipitation of the antigen-antibody complex formed upon mixing of antisera with the protein extract resulted in loss of the enzyme activity (Fig. 4). When the precipitated complex was resuspended in buffer, all of the enzyme activity lost from the supernatant fraction was recovered (data not presented here).

The specificity of the antisera for AHAS was also shown by the detection of a protein band on a Western blot of crude extract from *Arabidopsis* (Fig. 5). The protein had a molecular mass of 65 kD. The same protein band was detected by either antiserum-1 or antiserum-2. Furthermore, the same protein band was detected following partial purification of AHAS and the intensity of this band in various fractions following chromatography matched with the enzyme activity profile (data not shown).

Cross-Reaction of Antisera with AHAS from Different Species

Antisera showing specificity for AHAS from *Arabidopsis* were used to test cross-reactivity with AHAS from different species. These species included five monocots (corn, wheat, rice, barley, and sorghum) and seven dicots (soybean, lima bean, pea, spinach, tobacco, sugar beet, and *Arabidopsis*). Tests for cross-reaction were made by immuno-precipitation and Western blotting.

Extracts of three monocots (barley, sorghum, and corn) and two dicots (spinach and lima bean) were used in the

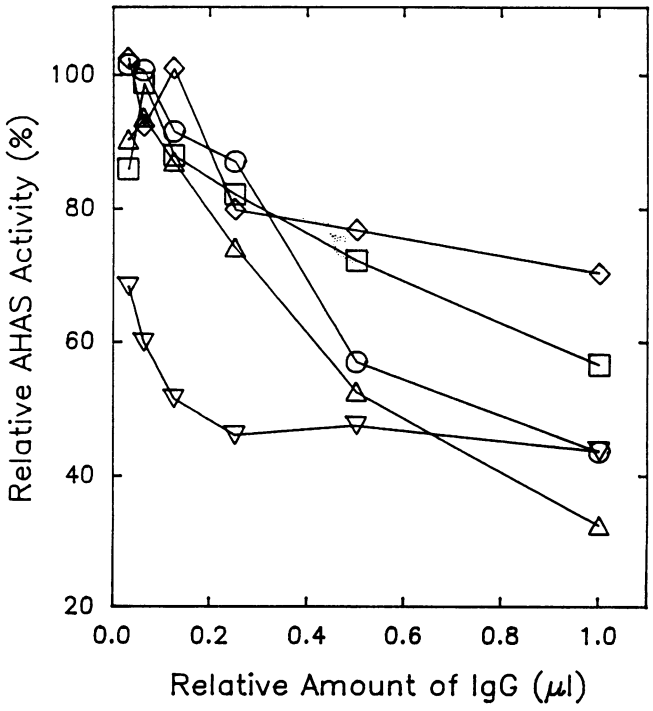

Figure 6. Immunoprecipitation of AHAS activity from various species using increasing amounts of antiserum-1. Barley (▽), corn (◇), lima bean (△), sorghum (○), and spinach (□).

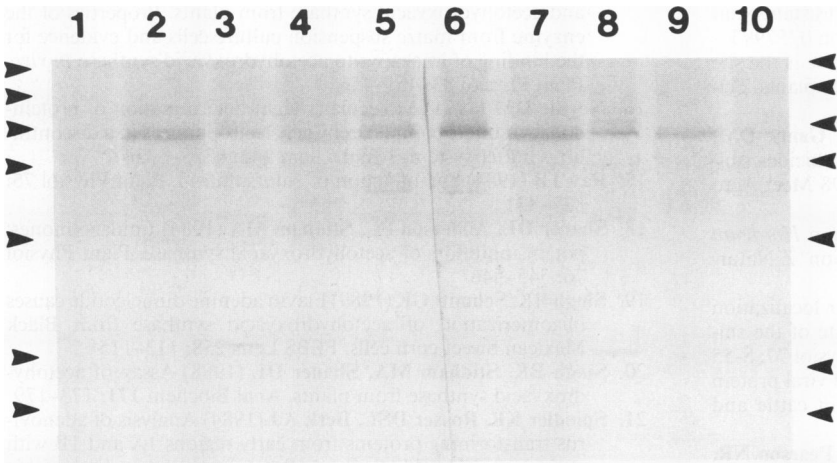

Figure 7. Cross-reaction of antiserum-2 with AHAS from various species on a Western blot. Lanes 1 through 10 contain rice, soybean, sorghum, lima bean, tobacco, spinach, barley, wheat, sugarbeet, and corn, respectively.

immunoprecipitation experiment. AHAS activity from each of these species was immunoprecipitated by the antisera against the fusion protein obtained using pAB20 (Fig. 6). The antisera exhibited the greatest affinity toward the AHAS enzyme from barley and lima bean and the least reaction affinity toward corn AHAS activity.

An additional test for cross-reactivity used crude extracts from different species that were boiled in SDS-PAGE buffer containing 2 mM DTT. Following electrophoresis, Western blots were developed for these extracts. A protein band with an apparent molecular mass of 65 kD was detected in all species (Fig. 7). This protein band corresponds to the protein band detected in the *Arabidopsis* extract (Fig. 5).

DISCUSSION

The small amount of AHAS present in plant tissue has made characterization at the protein level difficult. The inability of researchers to purify sufficient amounts of this protein to induce antigenic response in rabbits has hindered analysis by serological methods. Our own initial efforts to obtain antibodies against AHAS were unsuccessful at least in part because of the low concentrations of AHAS protein present even in highly purified preparations of the enzyme. The strategy of overexpressing partial sequences of plant AHAS in *E. coli* described here was successful in obtaining high levels of AHAS-*trpE* fusion protein. A portion of the gene encoding *Arabidopsis* AHAS was utilized in both *trpE* fusions instead of the complete gene because the production of *trpE* fusion proteins of >76 kD has not been reported (21). These fusion proteins included expression of two overlapping regions of *Arabidopsis* AHAS: a 791 bp *Bam*HI fragment and a 1150 bp *Eco*RV-*Bgl*II (Fig. 1). The relative ease of purification of these fusion proteins provided us with large amounts of the purified proteins (Fig. 3), which were used to raise AHAS-specific antibodies.

Specific antisera, raised against the two fusion proteins described above, identified a single protein in crude extracts of *Arabidopsis*. This protein has an apparent molecular mass of 65 kD, as determined by SDS-PAGE followed by Western blotting and immunological detection (Fig. 5). The specificity of the antisera for AHAS was confirmed by immunoprecipitation of AHAS activity (Fig. 4). The *Arabidopsis* AHAS gene codes for a protein of mol wt 72,593 (11). The mol wt of the protein detected by these antibodies is equivalent to the predicted mol wt of the mature AHAS protein assuming a transit peptide of about 85 amino acids (11). Therefore, the results presented here provide strong evidence that *Arabidopsis* extracts contain a 65 kD AHAS polypeptide.

A subunit molecular mass of 58 kD has been reported for AHAS from barley (5). However, SDS-PAGE of the purified fractions in that report showed a number of minor bands, one of which had a molecular mass of 65 kD. This particular protein band matches with the mol wt for the barley enzyme determined in this study (Fig. 7, lane 7). Therefore, the band claimed to be AHAS in the work of Durner and Boger (5) may be either a contaminating protein or a breakdown product of the enzyme.

A high degree of conservation at the protein sequence level, deduced from the DNA sequence, has been demonstrated for AHAS from tobacco, *Arabidopsis,* corn, and canola (2, 11, 26). Results presented in Figures 6 and 7 confirm these observations and extend this sequence conservation to other species, based on the cross-reactivity of the antibodies with AHAS from different plant species. An interesting observation here is the close similarity of the mol wts of AHAS from each of the species examined (Fig. 7). The mol wts predicted from known gene sequences from various species (2, 11, 26, our unpublished data) also support this observation. Because AHAS in the native form exists in oligomeric forms, whether or not AHAS is composed of homologous or heterologous subunits remains to be answered.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the invaluable and inspirational contributions to this work of Dr. G. Fink, Whitehead Institute, and Dr. T. Silhavy, Princeton University.

LITERATURE CITED

1. **Bascomb NF, Gutteridge S, Smith JK, Leto KJ** (1987) Import of *in vitro* synthesized acetolactate synthase into isolated pea chloroplasts. J Cell Biochem **11B:** 88
2. **Bedbrook JR, Chaleff RS, Falco SC, Mazur BJ, Yadav NS**

(1987) Nucleic acid fragment encoding herbicide resistant plant acetolactate synthase. European patent application 0257993

3. **Chaleff RS, Mauvais CJ** (1984) Acetolactate synthase is the site of action of two sulfonylurea herbicides in higher plants. Science **224:** 1443–1445
4. **Crews AD, Alvarado SI, Brady TE, Doehner RF, Gange DM, Little DL** (1989) Discovery of a new class of herbicides: sulfonylcarboxamides. Abstr Pap Am Chem Soc 198 Meet Agro No 39
5. **Durner J, Boger P** (1988) Acetolactate synthase from *Hordeum vulgare* L.: purification and partial characterization. Z Naturforsch **43c:** 850–856
6. **Jones AV, Young RM, Leto KJ** (1985) Subcellular localization and properties of acetolactate synthase, target site of the sulfonylurea herbicides (abstract No. 293). Plant Physiol **77:** S-55
7. **Kleid DG, Yansura D, Small B, et al** (1981) Cloned viral protein vaccine for foot-and-mouth disease: responses in cattle and swine. Science **214:** 1125–1129
8. **Kleswick WA, Ehr RT, Gerwick BC, Monte WT, Pearson NR, et al** (1984) New 2-arylamino-sulphonyl-1,2,4-triazolo-(1.5-a)-pyrimidine(s): useful as selective herbicides and to suppress nitrification in soil. European patent application 0142152
9. **La Rossa RA, Schloss JV** (1984) The herbicide sulfometuron methyl is bacteriostatic due to inhibition of acetolactate synthase. J Biol Chem **259:** 8753–8757
10. **Maniatis T, Fritsch EF, Sambrook J** (1982) Molecular Cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
11. **Mazur BJ, Chui CF, Smith JK** (1987) Isolation and characterization of plant genes coding for acetolactate synthase, the target enzyme for two classes of herbicides. Plant Physiol **85:** 1110–1117
12. **Miflin BJ** (1971) Cooperative feedback control of barley acetohydroxyacid synthase by leucine, isoleucine, and valine. Arch Biochem Biophys **146:** 542–550
13. **Miflin BJ** (1974) The location of nitrite reductase and other enzymes related to amino acid biosynthesis in the plastids of roots and leaves. Plant Physiol **54:** 550–555
14. **Miflin BJ, Cave PR** (1972) The control of leucine, isoleucine, and valine biosynthesis in a range of higher plants. J Exp Bot **23:** 511–516
15. **Muhitch MJ, Shaner DL, Stidham MA** (1987) Imidazolinones and acetohydroxyacid synthase from plants. Properties of the enzyme from maize suspension culture cells and evidence for the binding of imazapyr to acetohydroxyacid synthase *in vivo*. Plant Physiol **83:** 451–456
16. **Neville DM** (1971) Molecular weight determination of protein-dodecyl sulfate complexes by gel electrophoresis in a discontinuous buffer system. J Biol Chem **246:** 6328–6334
17. **Ray TB** (1984) Site of action of chlorsulfuron. Plant Physiol **75:** 827–831
18. **Shaner DL, Anderson PC, Stidham MA** (1984) Imidazolinones: potent inhibitors of acetohydroxyacid synthase. Plant Physiol **76:** 545–546
19. **Singh BK, Schmitt GK** (1989) Flavin adenine dinucleotide causes oligomerization of acetohydroxyacid synthase from Black Mexican Sweet corn cells. FEBS Letts **258:** 113–115
20. **Singh BK, Stidham MA, Shaner DL** (1988) Assay of acetohydroxyacid synthase from plants. Anal Biochem **171:** 173–179
21. **Spindler KR, Rosser DSE, Berk AJ** (1984) Analysis of adenovirus transforming proteins from early regions 1A and 1B with antisera to inducible fusion antigens produced in *Escherichia coli*. J Virol **49:** 132–141
22. **Subramanian MV, Loney V, Pao L** (1989) Mechanism of action of 1,2,4-triazolo[1,5-a]pyrimidine sulfonamide herbicides. *In* LG Coping, J Dalziel, AD Dodge, eds, Prospects for Amino Acid Biosynthesis Inhibitors in Crop Protection and Pharmaceutical Chemistry, Monograph No 42. The British Crop Protection Council, Surrey, pp 97–100
23. **Umbarger HE** (1969) Regulation of amino acid metabolism. Annu Rev Biochem **38:** 323–370
24. **Umbarger HE** (1987) Biosynthesis of branched chain amino acids. *In* FC Neidhardt, JL Ingraham, KB Low, B Magasonik, M Schaechter, HE Umbarger, eds, *Escherichia coli* and *Salmonella typhimurium:* Cellular and Molecular Biology. American Society of Microbiology, Washington, pp 352–367
25. **Westerfeld WW** (1945) A colorimetric determination of blood acetoin. J Biol Chem **161:** 495–502
26. **Wiersma PA, Schiemann MG, Condie JA, Crosby WL, Moloney MM** (1989) Isolation, expression and phylogenetic inheritance of an acetolactate synthase gene from *Brassica napus*. Mol Gen Genet **219:** 413–420
27. **Wittenback VA, Erbes DL** (1986) Partial purification and properties of acetolactate synthase from pea (abstract No. 350). Plant Physiol **80:** S-66