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Purification and Subunit Composition of Acetohydroxyacid Synthase I from *Escherichia coli* K-12

LILLIAN EOYANG AND PHILIP M. SILVERMAN*

Department of Molecular Biology, Division of Biological Sciences, Albert Einstein College of Medicine, Bronx, New York 10461

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Acetohydroxyacid synthase I from *Escherichia coli* K-12 has been purified to near homogeneity. Analysis of the purified enzyme by sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed the presence of two polypeptides, one with a molecular weight of 60,000 and one with a molecular weight of 9,500. These two polypeptides were present in constant proportion to each other and to enzyme activity. The molar ratio of the two polypeptides (M_r 9,500:60,000), estimated from stained polyacrylamide gels, was 1. Antisera raised against the 60,000 M_r polypeptide precipitated both the 60,000 and the 9,500 M_r polypeptides from extracts of cells labeled with [^{35}S]methionine. The addition of sodium dodecyl sulfate before immunoprecipitation eliminated the smaller polypeptide, and only the larger one was recovered. The hydrodynamic properties of the native enzyme confirmed a previous report that the largest enzymatically active species has a molecular weight of about 200,000; this species contains both the 60,000- and 9,500-molecular-weight polypeptides.

At least three isozymes of acetohydroxyacid synthase can catalyze the first pair of homologous reactions common to the parallel isoleucine and valine biosynthetic pathways in *Escherichia coli* K-12 (6, 15, 20). Genetic evidence suggests that each isozyme is structurally and functionally distinct. Mutations affecting one isozyme have not affected any of the others, indicating that the different isozymes do not share subunits essential for their in vivo functions. Furthermore, the synthesis of each isozyme is subject to a different regulatory pattern, suggesting that each isozyme functions in a different set of physiological conditions. The most unusual of these patterns consists of mutations leading to the synthesis of acetohydroxyacid synthases from otherwise inactive or minimally active genes in *E. coli* K-12 (9, 15) and in *Salmonella typhimurium* (18). The in vivo functions of the different isozymes appear also to be differentially controlled or even to depend on interactions with different cellular compartments (11, 19). Analysis of the structural and regulatory genes for the acetohydroxyacid synthases is beginning to clarify the different patterns of genetic regulation (3, 5, 10, 12, 13). However, the physiology of the different isozymes remains unclear and will be resolved only with biochemical analyses of the enzymes themselves.

We report here the purification and subunit composition of acetohydroxyacid synthase I from *E. coli* K-12. A previous report established that the catalytic activity of this enzyme resides in a multimeric protein containing 60,000 M_r subunits (4). However, several observations suggest that the subunit composition of the enzyme may be more complex. First, acetohydroxyacid synthase III of *E. coli* K-12 contains two kinds of subunits, each encoded by a different gene: a 62,000 M_r catalytic subunit and a 17,500 M_r regulatory subunit (6, 17). Second, Friden et al. (3) suggested that the structural locus for acetohydroxyacid synthase I, *ilvB*, may be an operon, encoding the information for 60,000 and (approximately) 9,000 M_r proteins. As we show here, purified acetohydroxyacid synthase I contains two proteins of just these molecular weights. These two proteins are associated with each other in crude extracts, and their minimum molar ratio (M_r 9,500:60,000) in preparations of purified enzyme is 1. We propose that both proteins are subunits of the enzyme.

* Corresponding author.

MATERIALS AND METHODS

Materials. Blue Sepharose CL-6B, Sephadex G-200, polybuffer exchanger PBE 94, blue dextran, and protein molecular weight standards for gel electrophoresis were purchased from Pharmacia Fine Chemicals Inc., Piscataway, N.J. Flavin adenine dinucleotide (FAD), thiamine diphosphate, and ampicillin were obtained from the Sigma Chemical Co., St. Louis, Mo.; yeast alcohol dehydrogenase was from Boehringer Mannheim Corp., New York, N.Y.; and bovine serum albumin was from the Miles Chemical Co. Bacteriophage fd gene 5 protein was a gift from K. Williams.

Bacteria and bacterial growth conditions. *E. coli* K-12 strains MF2000 containing the *ilvB*$^+$ plasmid pTCN4 and PS1567 were obtained from M. Freundlich (3). Cells were grown in 80-liter batches in a New Brunswick fermentor at 37°C in Vogel-Bonner salts (21) containing, per liter, 10 g of yeast extract (Difco Laboratories, Detroit, Mich.), 5 g of glucose, and 25 mg of ampicillin. The cells were harvested by centrifugation at an optical density (660 nm) of 1 and stored at −80°C.

Acetohydroxyacid synthase assay. Enzyme activity was determined by the rate of acetolactate formation from pyruvate as previously described (11). One unit of activity is the amount required to catalyze the formation of 1 μmol of acetolactate per min. Protein was determined as described by Bradford (1). Specific activities are expressed as units per milligram of protein.

Polyacrylamide gel electrophoresis. Samples were concentrated for electrophoresis by acetone precipitation, since recovery of the 9,500-molecular-weight polypeptide chain by acid precipitation was variable. Samples were first diluted with 2 volumes of water to reduce the glycerol concentration. Two to three volumes of acetone were added, and the samples were incubated for at least 30 min at 4°C. Proteins were collected by centrifugation for 15 min in a Fisher microcentrifuge. For routine analyses, slab gels (10 cm by

1.5 mm) contained a 10 to 20% linear gradient of acrylamide but were otherwise run as described by Laemmli (8). For molecular weight determinations, we used the 6 M urea-sodium dodecyl sulfate gel system described by Ito et al. (7). Proteins were stained with Coomassie blue R250.

Enzyme purification. All operations were carried out at 4°C. Frozen cells, usually 50 to 60 g, were thawed and suspended uniformly in 2.5 volumes of standard buffer containing 1 μM FAD and 1 mM $MgCl_2$ (standard buffer is 20 mM potassium phosphate [pH 7.0], 0.5 mM dithiothreitol, 0.1 mM thiamine diphosphate, 20% [vol/vol] glycerol). The cells were disrupted by sonication with a Branson sonifier at 100 W. Power was applied in 15-s bursts with intermittent intervals for cooling until the suspension cleared, usually after a total of 3 min of power. Unbroken cells were removed by centrifugation for 20 min at 30,000 × *g* (crude extract). The crude extract was then subjected to centrifugation at 100,000 × *g* for 90 min, and the supernatant fluid was again retained (high-speed supernatant). Solid ammonium sulfate (0.36 g/ml) was dissolved in the supernatant fluid, and the resultant precipitate was removed by centrifugation at 30,000 × *g* for 20 min. The precipitated protein was dissolved in about 40 ml of standard buffer and dialyzed for 18 h against 4 liters of standard buffer and then for 7 h against 4 liters of column buffer (standard buffer containing 10 mM $MgCl_2$) (ammonium sulfate fraction).

Immediately after dialysis the ammonium sulfate fraction was applied at a flow rate of less than 18 ml/h to a column (2.4 by 20 cm) of blue Sepharose CL-6B equilibrated with column buffer. The column was washed with 500 ml of column buffer. Enzyme was then eluted with 200 ml of column buffer containing 0.2 mM FAD. Active fractions (6 ml each) were pooled, and the protein was precipitated with ammonium sulfate (0.52 g/ml), collected by centrifugation, and dissolved in standard buffer containing 10 μM FAD (blue Sepharose fraction).

The blue Sepharose fraction was applied directly to a column (2.4 by 86 cm) of Sephadex G-200 previously equilibrated with standard buffer containing 10 μM FAD and 1 mM $MgCl_2$. The column was developed in the same buffer. The peak of enzyme activity eluted at approximately 1.2 × V_0. Active fractions (3 ml each) were pooled (G-200 fraction).

The G-200 fraction was applied directly to a column (1 by 3.8 cm) of polybuffer exchanger PBE 94 previously equilibrated with standard buffer containing 10 μM FAD and 1 mM $MgCl_2$. The column was washed with 15 ml of the same buffer, 15 ml of buffer containing 50 mM potassium phosphate, and 15 ml of buffer containing 100 mM potassium phosphate. Enzyme was eluted with 20 ml of buffer containing 200 mM potassium phosphate. Alternatively, the PBE 94 column was developed with a 200-ml linear phosphate gradient from 0.02 to 0.35 M; enzyme eluted at 0.15 M phosphate. Individual fractions (usually 1.3 ml each) from the PBE 94 column were retained as such and not pooled.

Zone sedimentation. Enzyme was dialyzed for 18 h against column buffer containing 5% (vol/vol) glycerol, instead of the standard 20%, and 0.1 M NaCl. Yeast alcohol dehydrogenase (1 mg) was added as a size standard, and the enzymes were loaded on a 10-ml linear glycerol gradient (10 to 30%) containing column buffer plus 0.1 M NaCl. Sedimentation was in a Spinco SW41 rotor at 40,000 rpm for 24 h at 4°C. Fractions (0.3 ml) were collected from the bottom of the gradient and assayed for acetolactate formation and for protein by gel electrophoresis. The position of acetohydroxyacid synthase I was indicated by enzyme activity and by the 60,000- and 9,500-molecular-weight subunits; that of yeast alcohol dehydrogenase was indicated by its 37,000-molecular-weight subunit (2). Where indicated, 40 mM pyruvate was included in the gradient, and the sedimentation of the enzyme was followed by the accumulation of acetolactate, as described by Grimminger and Umbarger (4).

Preparation of antiserum against the 60,000 M_r subunit. The 60,000 M_r subunit of acetohydroxyacid synthase I was purified by electrophoresis through a 3-mm thick 10% polyacrylamide gel. Generally, 2 mg of protein (blue Sepharose fraction) was layered across the top of a stacking gel cast with one 8-cm-wide sample well. After electrophoresis, proteins were visualized by the addition of ice-cold 0.25 M KCl–0.5 mM dithiothreitol. A gel slice containing the predominant 60,000 M_r subunit was extracted by 16 h of incubation at 25°C after the gel slice was homogenized in a glass tissue homogenizer with 5 ml of a solution containing 50 mM Trishydrochloride (pH 7.4), 0.1% sodium dodecyl sulfate, and 0.2 M NaCl. Gel particles were removed by sedimentation at 30,000 × *g* and 25°C for 20 min. The gel was washed once by sedimentation with 3 ml of extraction buffer, and the supernatants were combined. Protein was precipitated by the addition of 18 ml of acetone at −20°C. After incubation for 30 min at −20°C, the precipitate was collected by sedimentation at 30,000 × *g* and 4°C for 20 min. Recovery of the 60,000 M_r subunit was about 200 μg. The protein was dissolved in 1 ml of TEN buffer (50 mM Trishydrochloride [pH 7.4], 0.15 M NaCl, 0.1 mM EDTA), emulsified with 1 ml of Freund complete adjuvant, and used to inoculate two New Zealand White rabbits at multiple subdermal sites in the back. The animals were boosted with 50 to 100 μg of protein at twice-monthly intervals by intramuscular injection in the thigh. Blood (usually 30 to 40 ml) was collected from an ear artery and stored at 4°C for 16 h. Serum was collected after sedimentation at 10,000 × *g* for 20 min and stored at −80°C. Sera were routinely examined by immunodiffusion against extracts of strain MF2000, from which the enzyme was purified, and PS1567, similar to MF2000 except lacking the *ilvB*$^+$ plasmid; both strains contain the *ilvB800*::Mu insertion in the chromosome. Although precipitin lines were only detected against extracts of MF2000, we found high backgrounds of other polypeptides in immunoprecipitates of radioactive proteins. These polypeptides were also immunoprecipitated with preimmune sera taken from the same animals. We believe that they are cell envelope proteins precipitated by low levels of antibodies present in the animals before inoculation. To minimize this problem, we routinely absorbed the sera with extracts of PS1567 before use.

Immunoprecipitation. Strain MF2000 (10 ml) was grown to stationary phase at 37°C in Vogel-Bonner minimal medium containing [^{35}S]methionine (25 μCi/ml; 1,300 Ci/mmol) and carrier methionine (5 μg/ml). Cells were collected by sedimentation, washed once with 2 ml of minimal medium, suspended in 1 ml of standard buffer containing 10 μM FAD, and disrupted by sonication. Insoluble material was removed by sedimentation at 100,000 × *g* for 60 min. The supernatant fraction contained 7 × 10^7 cpm of radioactive protein. Reaction mixtures for immunoprecipitation (1 ml) contained TEN buffer, 0.05% (vol/vol) Triton X-100, 1 mg of bovine serum albumin, 0.02% sodium azide, 0.02 ml of radioactive extract, and 0.025 ml of antiserum. After 16 h at 4°C, immunoprecipitates were collected by 15 min of sedimentation in a microfuge and washed three times by sedimentation through 0.5 ml of Triton-TEN buffer. Radioactive proteins were separated by electrophoresis through a 10 to 20%

polyacrylamide gradient gel and visualized by autoradiography.

RESULTS

Purification of acetohydroxyacid synthase I. The results of a complete purification are shown in Table 1. The most important step is chromatography over blue Sepharose. Thereafter, Sephadex G-200 and PBE 94 chromatography completed the purification. The final specific activity of 25 μmol of acetolactate formed per min per mg of protein is comparable to that reported by Grimminger and Umbarger for their preparations (4) and represents, in the experiment shown, a 260-fold purification. The range of final specific activities in different purifications was 25 to 60 μmol/min per mg of protein. The overall yield of activity was 6%, owing in large part to losses during blue Sepharose chromatography. The yield can be improved somewhat by omitting either Sephadex G-200 or PBE 94 chromatography, only one of which is necessary for most purposes (see below). Acetolactate formation catalyzed by the enzyme at all stages of the purification was inhibited >80% in the presence of 1 mM L-valine.

The proteins at different stages of the purification are shown in Fig. 1. Fractions from the last stage, PBE 94 chromatography (Fig. 1, lanes 3 to 7), were enriched in a 60,000-molecular-weight protein (Fig. 1, upper arrowhead; see below) and a smaller protein whose electrophoretic mobility suggested a molecular weight of less than 14,000 (Fig. 1, lower arrowhead). In the electrophoretic system described by Ito et al. (7) the mobilities of the two proteins corresponded to molecular weights of 60,000 and 9,500 (data not shown). Grimminger and Umbarger, using a different purification scheme, reported a 60,000-molecular-weight protein to be the only component of the enzyme (4). The experiments we describe below indicate that the 9,500-molecular-weight protein is also a subunit of the enzyme.

Constant proportion of the 9,500- and 60,000-molecular-weight proteins in acetohydroxyacid synthase I preparations. Enzyme was prepared as described in Table 1, except that Sephadex G-200 chromatography was omitted. The PBE 94 fractions containing the most activity were then subjected to Sephadex G-200 chromatography. As shown in Fig. 2A, elution of enzyme and protein were congruent, so that the enzyme was purified to constant specific activity. As shown in Fig. 2B, active Sephadex G-200 fractions contained both the 60,000- and the 9,500-molecular-weight proteins. Densitometric analysis of the relative amounts of the two proteins in fractions 38, 40, and 42 indicated mass ratios of 5.9, 7.0, and 7.5, respectively. These values correspond to molar ratios of 0.9, 1.1, and 1.2. Hence, the two proteins appear to be present in constant proportion to both enzyme activity and each other. Owing to possible losses of the small protein during the staining and destaining protocol, we consider the molar ratio measured (M_r 9,500:60,000) to be a minimum value.

TABLE 1. Purification of acetohydroxyacid synthase I

Fraction	Spec act (U/mg)	Total activity (U)	Yield (%)
Crude extract	0.11	800	100
High-speed supernatant	0.07	504	57
Ammonium sulfate	0.08	380	43
Blue Sepharose	6.9	105	12
Sephadex G-200	24	76	9
PBE 94[a]	26	49	6

[a] Computed from individual column fractions; see Fig. 1, lanes 3 to 7.

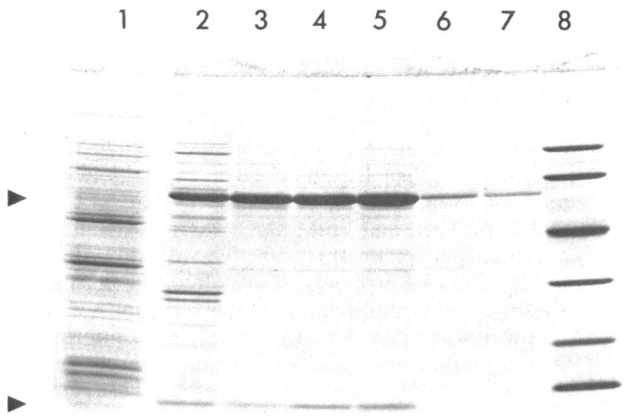

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of fractions from the purification of acetohydroxyacid synthase I. Lane 1, crude extract; lane 2, blue Sepharose fraction; lanes 3 to 7, peak fractions from PBE 94 chromatography. The crude extract proteins (80 μg) were dissolved directly in sample buffer for electrophoresis. All of the other samples, each containing 20 μg of protein, were first concentrated by acetone precipitation as described in the text. Losses during this step may account for the low level of protein in lanes 6 and 7. Lane 8 contains all the molecular weight standards described in the legend to Fig. 2, except bacteriophage fd gene 5 protein.

Coimmunoprecipitation of the 60,000- and 9,500-molecular-weight proteins. To obtain evidence that the two proteins are physically associated in the native enzyme, radioactive proteins in extracts of cells labeled in vivo with [^{35}S]methionine were immunoprecipitated with an antiserum raised against the 60,000-molecular-weight protein (see above). Extracts were prepared by sonication under conditions favorable for enzyme stability. As shown in Fig. 3 (lane 1), both the 60,000- and 9,500-molecular-weight proteins were immunoprecipitated from such extracts. Increasing or decreasing the antiserum concentration had no effect on the amount of either protein in the immunoprecipitates (not shown). To rule out the possibility that the two proteins share antigenic determinants, the extracts were boiled with sodium dodecyl sulfate before immunoprecipitation. In this case the antiserum precipitated only the 60,000-molecular-weight protein (Fig. 3, lane 2). This experiment indicates that the two proteins are physically associated. Moreover, since the smaller protein evidently lacks antigenic determinants common to the larger protein, the one is unlikely to be a degradation product of the other. The experiment does not rule out the possibility that both are derived from a larger precursor.

Hydrodynamic properties of acetohydroxyacid synthase I. The Stokes radius and sedimentation coefficient of the purified enzyme were determined, respectively, by gel filtration chromatography and zone sedimentation as described above. In both experiments active fractions contained the 60,000- and 9,500-molecular-weight proteins. Yeast alcohol dehydrogenase served as a standard (M_r, 148,000; Stokes radius, 46A; $s_{20,w}$, 7.4) (2, 16). The results of these analyses

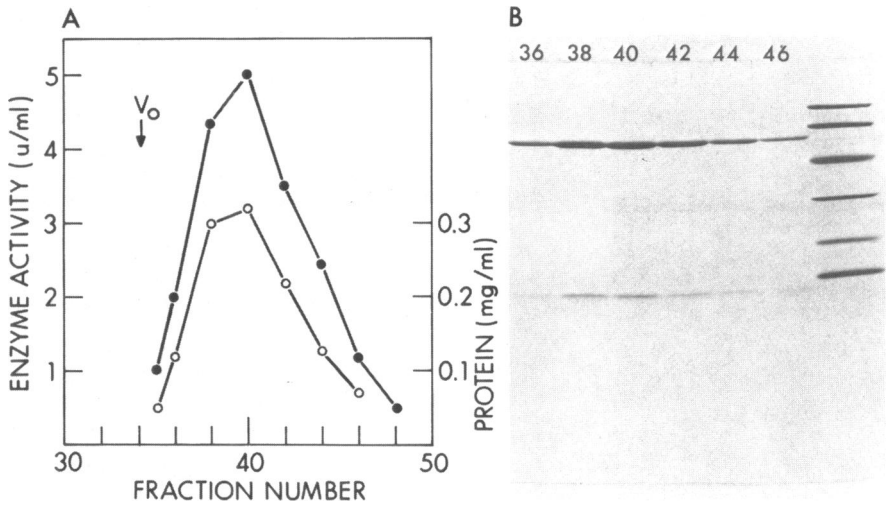

FIG. 2. Constant proportion of the 60,000- and 9,500-molecular-weight proteins in preparations of acetohydroxyacid synthase I. Enzyme was prepared and chromatographed over Sephadex G-200, as described in the text, except that the elution buffer contained 0.1 M NaCl. (A) Enzyme activity (●) and protein concentration (○) in active fractions (3 ml). (B) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the indicated Sephadex G-200 fractions. The lanes corresponding to fractions 38, 40, and 42 were scanned with a densitometer, and the masses of the 60,000- and 9,500-molecular-weight subunits in each fraction were determined with a Numonics digital planimeter. The right lane contains the molecular weight markers shown in Fig. 1, except the fd gene 5 protein.

(not shown) indicated that in solution acetohydroxyacid synthase I is essentially globular, with a Stokes radius of 47A, an $s_{20,w}$ of 8.1, and a frictional coefficient of 1.3. The Stokes radius and sedimentation coefficient suggested an apparent molecular weight of 157,000, comparable to the value of 168,000 estimated by Grimminger and Umbarger from sedimentation data obtained under the same conditions (4). However, Grimminger and Umbarger also found that the sedimentation properties of the enzyme depended on the conditions of the experiment (4). Specifically, they observed, and we have confirmed (data not shown), that when pyruvate was added to the zone sedimentation buffer, in addition to all the other components essential for enzyme activity, the apparent molecular weight of the enzyme increased from about 160,000 to about 200,000. For this reason it is not possible to use a molecular weight estimated from the hydrodynamic properties of the enzyme, along with its subunit composition, to suggest a specific structure. In both conditions of sedimentation (with and without pyruvate) the small and large subunits cosedimented with each other and with active enzyme. We have not examined the subunit composition of enzyme subjected to other conditions of sedimentation.

DISCUSSION

Our experiments confirm a previous report by Grimminger and Umbarger that acetohydroxyacid synthase I from *E. coli* K-12 contains 60,000-molecular-weight subunits (4). However, our data show the presence of a second kind of subunit, a protein of 9,500 molecular weight. Our purification of the enzyme is very different from the one developed by Grimminger and Umbarger, and it is possible that their preparations lacked the small subunit. However, the sedimentation properties of the enzymes prepared by the two methods are similar, and we believe that both methods yield the same species. In any event, our evidence indicates that the 60,000- and 9,500-molecular-weight proteins are physically associated in extracts and in purified enzyme, and on the basis of this evidence we propose that they are both enzyme subunits.

The structure of acetohydroxyacid synthase I in solution appears complex. Hydrodynamic measurements of the purified enzyme in the presence of all of the cofactors necessary for enzyme activity, but in the absence of substrate, suggest a globular protein with a molecular weight of 160,000 (±5%). However, the sedimentation properties of the enzyme in the presence of substrate suggest a molecular weight closer to 200,000. A more extensive analysis by Grimminger and Umbarger indicated molecular weights as low as 130,000 when sedimentation was in the absence of FAD and substrate or in the presence of L-valine, a negative effector of the enzyme (4). It should be emphasized that when substrate is present in the gradient, the accumulation of product as the enzyme sediments reflects unambiguously the molecular weight of the most rapidly sedimenting species of active enzyme (4). Although enzyme protein sediments more slowly under other conditions, it is not clear what structures form in the reaction mixture when enzyme activity in different gradient fractions is measured. A molecular weight of 200,000 is consistent with a structure containing three molecules each of the large and small subunits (expected M_r, 208,500). This structure is also consistent with the measured mass ratio of the two subunits in preparations of purified enzyme, although this ratio is a minimum value owing to our uncertainty about the recovery of the small subunit. Finally, preliminary experiments with the reversible cross linker methyl-4-mercaptobutyrimidate confirm that 30 to 40% of the large subunit exists in solution as a trimer; the remainder is recoverable as a monomer (unpublished observations). Using this reagent, we were unable to cross link the 9,500 M_r subunit to itself or to the 60,000 M_r subunit.

Three genetic loci specifically affect acetohydroxyacid synthase I. One of these is *ilvB* at 81 to 82 min on the *E. coli* K-12 linkage map (14). This locus codes at least for the 60,000-molecular-weight subunit and possibly for the 9,500-molecular-weight subunit as well (3). Coordinate synthesis of both subunits from a single operon would explain in a simple way the regulation of acetohydroxyacid synthase I levels by attenuation and by cyclic AMP (3, 5).

The other two loci that affect the function of

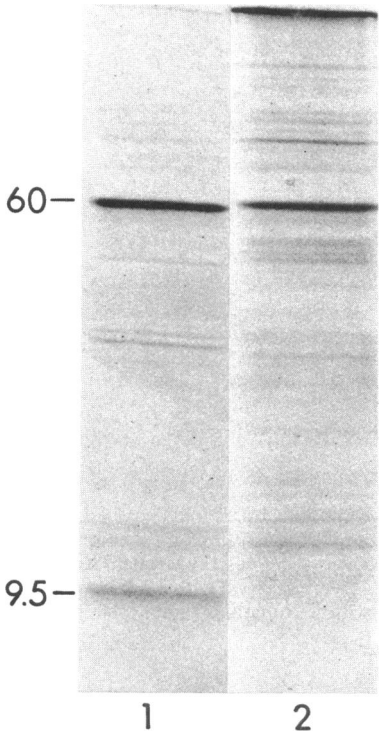

FIG. 3. Immunoprecipitation of acetohydroxyacid synthase I subunits from crude extracts. Cells of strain MF2000 containing the *ilvB*$^+$ plasmid pTCN4 were labeled with [^{35}S]methionine, extracts were prepared, and immunoprecipitations were carried out with antiserum raised against the 60,000 M_r polypeptide, as described in the text. Radioactive proteins were separated by electrophoresis through a 10 to 20% polyacrylamide gradient gel and identified by autoradiography of the dried gel. The exposure was for 72 h on Kodak X-Omat film. Lane 1 contained the polypeptides in immunoprecipitates from native extracts (50,000 cpm); lane 2 shows the polypeptides in immunoprecipitates after the extract was heated to 95°C for 5 min in the presence of 0.1% sodium dodecyl sulfate (80,000 cpm). Immunoprecipitates obtained from extracts treated with sodium dodecyl sulfate reproducibly had a high background, presumably owing to the exposure of epitopes by the detergent that were recognized by antibodies present in the animals before inoculation (see the text). The subunits of acetohydroxyacid synthase I were identified by their mobilities as compared to standards and are indicated by their molecular weights (in kilodaltons).

acetohydroxyacid I are *cpxA* at 87 to 88 min and *cpxB* at 41 min (11). Mutations at both these loci together indirectly alter the function of the enzyme in vivo such that it is unable to function in the isoleucine and valine biosynthetic pathways (18). Mutant cells can maintain substantial quantities of enzyme that are active in vitro, at least in catalyzing acetolactate formation (19). Minicells containing *cpxA* cloned in a multicopy plasmid do not synthesize detectable quantities of a 60,000- or a 9,500-molecular-weight polypeptide (R. Albin and P. M. Silverman, unpublished observations). Since a *cpxB* mutation alone does not affect acetohydroxyacid synthase I function in vivo (11), it seems unlikely that either *cpx* locus codes for a subunit of the enzyme.

We have little information regarding the roles of the two subunits in enzyme function. Preliminary experiments suggest that the 9,500-molecular-weight subunit is not required in stoichiometric amounts for acetolactate formation or for the sensitivity of acetohydroxyacid synthase I to valine inhibition. We have not examined the formation of acetohydroxybutyrate from pyruvate and α-ketobutyrate in the presence and absence of the 9,500-molecular-weight subunit or a possible role for this subunit in interactions with other cellular components required for acetohydroxyacid synthase I function in vivo, as suggested by the effects of the *cpx* mutations on enzyme function (11, 19). These interactions remain as one of several unusual and unexplained features of branched chain amino acid synthesis in enteric bacteria.

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LITERATURE CITED

1. **Bradford, M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
2. **Darnall, D., and I. Klotz.** 1972. Protein subunits: a table (revised edition). Arch. Biochem. Biophys. **149**:1–14.
3. **Friden, P., T. Newman, and M. Freundlich.** 1982. Nucleotide sequence of the *ilvB* promoter-regulatory region: a biosynthetic operon controlled by attenuation and cyclic AMP. Proc. Natl. Acad. Sci. U.S.A. **79**:6156–6160.
4. **Grimminger, H., and H. Umbarger.** 1979. Acetohydroxyacid synthase I of *Escherichia coli*: purification and properties. J. Bacteriol. **137**:846–853.
5. **Hauser, C., and G. Hatfield.** 1983. Nucleotide sequence of the *ilvB* multivalent attenuator region of *Escherichia coli* K-12. Nucleic Acids Res. **11**:127–140.
6. **Iaccarino, M., J. Guardiola, M. De Felice, and R. Favre.** 1978. Regulation of isoleucine and protein biosynthesis. Curr. Top. Cell. Reg. **14**:29–73.
7. **Ito, K., T. Date, and W. Wickner.** 1980. Synthesis, assembly into the cytoplasmic membrane, and proteolytic processing of the precursor of coliphage M13 coat protein. J. Biol. Chem. **255**:2123–2130.
8. **Laemmli, U.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
9. **Lawther, R., D. Calhoun, C. Adams, J. Hauser, J. Gray, and G. Hatfield.** 1981. Molecular basis of valine resistance in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **78**:922–925.
10. **Lawther, R., and G. Hatfield.** 1980. Multivalent translation and control of transcription termination at attenuator of *ilv* GEDA operon of *Escherichia coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **77**:1862–1866.
11. **McEwen, J., and P. Silverman.** 1980. Mutations in genes *cpxA* and *cpxB* of *Escherichia coli* K-12 cause a defect in isoleucine and valine synthesis. J. Bacteriol. **144**:68–73.
12. **Nargang, F., C. Subrahmanyam, and H. Umbarger.** 1980. Nucleotide sequence of *ilv* GEDA attenuator region of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:1823–1827.
13. **Newman, T., P. Friden, A. Sutton, and M. Freundlich.** 1982. Cloning and expression of the *ilvB* gene of *Escherichia coli* K-12. Mol. Gen. Genet. **186**:378–384.
14. **Newman, T., and M. Levinnthal.** 1980. A new map location for the *ilvB* locus of *Escherichia coli*. Genetics **96**:59–77.
15. **Robinson, C., and J. Jackson.** 1982. New acetohydroxyacid synthase activity from mutational activation of a cryptic gene in *Escherichia coli* K-12. Mol. Gen. Genet. **186**:240–246.
16. **Siegel, L., and K. Monty.** 1966. Determination of molecular weights and frictional ratios of proteins in impure systems of gel filtration and density gradient centrifugation. Application to crude preparations of sulfite and hydroxylamine reductases. Biochim. Biophys. Acta **112**:346–362.
17. **Squires, C., M. De Felice, J. Devereux, and J. Calvo.** 1983. Molecular structure of *ilv*HI and its evolutionary relationship to

*ilv*G in *Escherichia coli* K-12. Nucleic Acids Res. **11**:5299–5313.
18. **Squires, C., M. De Felice, C. Lago, and J. Calvo.** 1983. *ilv* HI locus of *Salmonella typhimurium*. J. Bacteriol. **154**:1054–1063.
19. **Sutton, A., T. Newman, J. McEwen, P. Silverman, and M. Freundlich.** 1982. Mutations in genes *cpxA* and *cpxB* of *Escherichia coli* K-12 cause a defect in acetohydroxyacid synthase I function in vivo. J. Bacteriol. **151**:976–982.
20. **Umbarger, H.** 1978. Amino acid biosynthesis and its regulation. Annu. Rev. Biochem. **47**:533–606.
21. **Vogel, H., and D. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.