The Subunit Structure of $\alpha$-Acetohydroxyacid Isomeroreductase from Salmonella typhimurium*

(Received for publication, July 31, 1974)

John G. Hoffer,§ Charles J. Decedue,§ Geraldine H. Luginbuhl,§ Jacqueline A. Reynolds,‖ and R. O. Burns

From the Department of Microbiology and Immunology, Duke University School of Medicine, Durham, North Carolina 27710

SUMMARY

$\alpha$-Acetohydroxyacid isomeroreductase from Salmonella typhimurium has a native molecular weight of 220,000. The constituent polypeptide chains exhibit anomalous but unimodal electrophoretic migration on sodium dodecyl sulfate-polyacrylamide gels. The subunit molecular weight, determined by sedimentation equilibrium in 6 M guanidine hydrochloride, is 57,000. The apparent tetrameric nature of the native enzyme was confirmed by determining the types of oligomers formed upon cross-linking with dimethylsuberimidate. Analysis of tryptic peptides suggests that the polypeptide chains have an identical amino acid sequence. Carbohydrate analysis, ultraviolet absorption spectrum, and atomic absorption spectrum are consistent with the lack of cobalamin and cobalt. The Michaelis constants are as follows: $\alpha$-acetolactate, $2.9 \times 10^{-4} \text{M}$; $\alpha$-acetoc-$\alpha$-hydroxybutyrate, $7.8 \times 10^{-4} \text{M}$; NADPH, $1.5 \times 10^{-5} \text{M}$; Mg$^{2+}$, $7.7 \times 10^{-4} \text{M}$. The catalytic constants (molecules of substrate catalyzed per min per molecule of enzyme) for $\alpha$-acetolactate and $\alpha$-acetoc-$\alpha$-hydroxybutyrate are 1,100 and 4,700, respectively. Comparative tryptic peptide analysis and immunological analysis show that $\alpha$-acetohydroxyacid isomeroreductase and biosynthetic L-threonine deaminase bear no structural relationship and therefore rule out a "shared structure" hypothesis for the putative involvement of L-threonine deaminase in the synthesis of $\alpha$-acetohydroxyacid isomeroreductase.

$\alpha$-Acetohydroxyacid isomeroreductase (2,3-dihydroxyisovalerate:NADP$^+$ oxidoreductase, EC 1.1.1.89) is one of the four enzymes common to L-isoleucine and L-valine biosynthesis in Salmonella typhimurium as well as in a variety of other microorganisms (1). This enzyme has been studied with respect to its catalytic mechanism as well as its genetic regulation.

Arfin and Umbarger (1) have shown that the purified isomeroreductase catalyzes the conversion of $\alpha$-acetolactate and $\alpha$-acetoc-$\alpha$-hydroxybutyrate to $\alpha$, $\beta$-dihydroxyisovalerate and $\alpha$, $\beta$-dihydroxy-$\beta$-methylvalerate, respectively. These workers also demonstrated that the enzyme is able to catalyze isomerization and reduction as separate reactions. The synthesis of isomeroreductase is induced by either substrate, $\alpha$-acetolactate or $\alpha$-acetoc-$\alpha$-hydroxybutyrate, and in this respect differs from the other isoleucine-valine biosynthetic enzymes which are multivalently repressed by the branched chain amino acids (2, 3). The present study was undertaken to describe the nature of the substructure of isomeroreductase as it may relate to the genetic and metabolic regulation of L-isoleucine and L-valine biosynthesis as well as to the mechanism of catalysis.

MATERIALS AND METHODS

Growth of Organisms and Preparation of Crude Extracts—Cells enriched for isomeroreductase were obtained by cultivating strain ibhE-281 (obtained from M. Freudlich, State University of New York at Stony Brook, an isoleucine auxotroph of S. typhimurium LT2 (ATCC 15277). This mutant, lacking transaminase B activity, is endogenously limited for L-valine when grown in minimal medium containing relatively high levels of L-isoleucine, and consequently contains derepressed levels of those enzymes under multiv antagonist control by the branched chain amino acids, as well as highly induced levels of isomeroreductase. Cells were grown in a medium containing 0.086 M K$_2$HPO$_4$, 0.047 M KH$_2$PO$_4$, 0.016 M (NH$_4$)$_2$SO$_4$, 1.2 mM MgSO$_4$7H$_2$O, 0.38 mM L-isoleucine, and 23 mM glucose. Eight 12-liter carboys were inoculated with 200 ml of an overnight nutrient broth (Difco) culture of strain ibhE-281 and incubated with aeration at 37°C for 24 hours. The cells were harvested at 4°C by means of Sharples centrifuges.

Cells obtained from 96 liters of growth medium were washed once in 0.05 M potassium phosphate, pH 7.5, containing 0.5 mM MgEDTA$^+$ and 0.5 mM dithiothreitol. The cells were resuspended in 1 liter of the same buffer and disrupted in the cold by means of a 110-watt Branson Sonifier at full power for 10 min. The crude extract was cleared of whole cells and debris by centrifugation at 39,000 x g for 20 min. The debris and remaining intact cells were resuspended in the same buffer and again subjected to sonic vibration for 10 min. The supernatant liquids were combined and used for purification of isomeroreductase.

Enzyme Assay—$\alpha$-Acetohydroxy acid isomeroreductase activity was measured by following the decrease in absorption of NADPH

* The abbreviations used are: MgEDTA, dimagnesium EDTA; TPCK, t-1-tosylamido-2-phenylethyl chloromethyl ketone.
at 340 nm according to the procedure of Arfin and Umbarger (1). Assays were at 50° using a Gilford 2400s recording spectrophotometer. A unit of activity is the amount of enzyme required to oxidize 1 μmol of NADPH per min in the assay; specific activity is expressed as units per mg of protein.

Protein Determination—Protein was measured by the method of Lowry et al. (4). Purified enzyme was measured using an E280 of 8.3 (see “Results”).

Amino Acid Analysis—Carboxymethylated protein was prepared and hydrolyzed according to the method of Pett et al. (5). The amino acid content was quantified with a Beckman 121 amino acid analyzer. The tryptophan content of the protein was determined by the method of Benze and Schmid (6), by the method of Seiffone et al. (7), and following hydrolysis by the method of Liu (8).

Preparation and Analysis of Tryptic Peptides—Purified carboxymethylated isomeroreductase was digested with trypsin and the 50% acetic acid-soluble peptides were analyzed according to the preparative column chromatographic procedure of Herman and Vanaman (9). The peptides eluted from the column were further resolved by chromatography on microcrystalline cellulose plates in 50% acetic acid-water (10:16:3:12).

Polyacrylamide Gel Electrophoresis—Sodium dodecyl sulfate polyacrylamide gel electrophoresis was used to monitor purity of enzyme preparations and to estimate polypeptide chain molecular weight. The procedure of Pett et al. (5) was used with modification of the acrylamide concentration as specified under “Results.”

Cross-Linking Experiments—The constituent polypeptide chains of isomeroreductase were cross-linked by use of the bifunctional reagent dimethylsulfdimide used. Fig. 1, A and B, shows a plot of log molecular weight of 8.3 (see “Results”). Cross-linking was monitored by sodium dodecyl sulfate-gel electrophoresis as described above.

Instrumentation—A Beckman model E analytical ultracentrifuge equipped with a photoelectric scanner was used for sedimentation equilibrium experiments. Ultraviolet absorption spectra were obtained with a Beckman Instruments Acta V spectrophotometer. Atomic absorption spectroscopy at 2102 A was performed with a Perkin-Elmer model 107 spectroscope equipped with a HGA-2000.

Chemicals—The substrates α-acetolactate and α-aceto-α-hydroxybutyrate were prepared by saponification of the methyl esters which were generous gifts from Dr. Frank Armstrong of North Carolina State University. NADPH and dithiothreitol were obtained from P-L Biochemicals. Sodium dodecyl sulfate was purchased from Sigma. TPCK trypsin was obtained from Worthington. Guanidine hydrochloride, o-nitrophenylsulfenyl chloride, p-toluenesulfonic acid, and 3-(2-aminoethyl)indole were obtained from Pierce Chemicals; the latter two reagents were re-crystallized as described by Liu (8). All other chemicals were reagent grade.

RESULTS

Enzyme Purification—All purification steps were performed at 4°. The crude extract described under “Materials and Methods” was diluted to contain 10 mg per ml of protein with 0.05 M potassium phosphate, pH 7.5, containing 0.5 mM dithiothreitol and 0.5 mM MgEDTA (standard buffer), and precipitated with ammonium sulfate. The protein precipitating between 35 and 60% ammonium sulfate was dissolved in a minimum amount of standard buffer and desalted by overnight dialysis against 0.25 mg per ml to 2 mg per ml, respectively; the extent of cross-linking was monitored by sodium dodecyl sulfate-urea polyacrylamide gel electrophoresis as described above.

Table I presents a summary of the purification procedure. The final specific activity and the yield of the purified enzyme is considerably higher than that previously reported (1). This difference is probably explained by the stabilizing effect of MgEDTA; isomeroreductase slowly loses activity in the absence of EDTA.

Molecular Weight—The constituent polypeptide chain molecular weight was determined by sodium dodecyl sulfate-urea polyacrylamide gels using 3 gel concentrations. Solutions of enzyme containing 1% sodium dodecyl sulfate and 0.1% β-mercaptoethanol were added to gels containing 5, 7.5, and 12.5% acrylamide and subjected to electrophoresis by standard techniques. The 5 and 7.5% gels contained reovirus proteins X1, X2, X3, X4, X5 and the 12.5% gel contained the catalytic and regulatory subunits of aspartate transcarbamylase (Y- and Z-lactalbumin (obtained from Dr. T. C. Vanaman, Duke University), and cytochrome c as standards. As shown in Table II the chain molecular weight of isomeroreductase determined on the gels was directly related to the concentration of polyacrylamide used. Fig. 1, A and B, shows a plot of log molecular weight versus Rf for the 5 and 12.5% gels. Although no molecular weight can be assigned to the polypeptide chains of isomeroreductase on the basis of these results, nonetheless, they suggest that the constituent polypeptide chains are of equal molecular weight. Native and denatured enzyme was analyzed by sedimentation equilibrium and the molecular weight computed from the slopes of linear plots of -ln A against the radial distance.

<table>
<thead>
<tr>
<th>Step</th>
<th>Total protein</th>
<th>Enzyme retrieved</th>
<th>Specific activity</th>
<th>Recovery</th>
<th>Units discarded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>23,700</td>
<td>1,420</td>
<td>0.900</td>
<td>100</td>
<td>306</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>2,850</td>
<td>1,160</td>
<td>0.405</td>
<td>81</td>
<td>306</td>
</tr>
<tr>
<td>55 to 50% (NH4)2SO4 back extraction</td>
<td>344</td>
<td>912</td>
<td>2.65</td>
<td>64</td>
<td>386</td>
</tr>
<tr>
<td>Sephadex A-50</td>
<td>106</td>
<td>523</td>
<td>4.94+</td>
<td>37</td>
<td>106</td>
</tr>
</tbody>
</table>

* Protein was measured by E280.

878
Table II

<table>
<thead>
<tr>
<th>Method</th>
<th>RPM</th>
<th>Slope</th>
<th>Mol wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native enzyme in standard buffer</td>
<td>10,000</td>
<td>1.293 (linear)</td>
<td>219,700</td>
</tr>
<tr>
<td>Reduced carboxymethylated enzyme in 6 M guanidine HCl</td>
<td>30,000</td>
<td>1.800 (linear)</td>
<td>57,000</td>
</tr>
<tr>
<td>Sodium dodecyl sulfate-polyacrylamide gel electrophoresis 5% acrylamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5% acrylamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.5% acrylamide</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* From a plot of −ln A versus (radial distance)².
* Standard as in Fig. 1A.

Fig. 1. Molecular weight determination by sodium dodecyl sulfate-urea polyacrylamide gel electrophoresis. A, 5% acrylamide with threonine deaminase (TD) (19), and the five major reovirus proteins (23), as molecular weight standards. B, 12.5% acrylamide with the catalytic and regulatory subunits (CSU and RSU) of aspartate transcarbamylase (ACTase) (24), a-ketoglutarate dehydrogenase (a-KGD) (25), and cytochrome c (Cyt C) (26) as molecular weight standards. IR, isomeroreductase.

Table III

<table>
<thead>
<tr>
<th>Amino acid composition of isomeroreductase</th>
<th>Residues per chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>33</td>
</tr>
<tr>
<td>Histidine</td>
<td>8</td>
</tr>
<tr>
<td>Arginine</td>
<td>22</td>
</tr>
<tr>
<td>Carboxymethylglycine</td>
<td>5</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>50</td>
</tr>
<tr>
<td>Threonine</td>
<td>22</td>
</tr>
<tr>
<td>Serine</td>
<td>24</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>66</td>
</tr>
<tr>
<td>Proline</td>
<td>19</td>
</tr>
<tr>
<td>Glycine</td>
<td>49</td>
</tr>
<tr>
<td>Alanine</td>
<td>57</td>
</tr>
<tr>
<td>Valine</td>
<td>33</td>
</tr>
<tr>
<td>Methionine</td>
<td>17</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>28</td>
</tr>
<tr>
<td>Leucine</td>
<td>49</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>17</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>17</td>
</tr>
<tr>
<td>Tryptophan†</td>
<td>6</td>
</tr>
</tbody>
</table>

* The average, extrapolated (threonine and serine), or plateaued (isoleucine and valine) values from 24- and 48-hour hydrolyses in 6 M HCl in vacuo and 24-, 48-, 72-, 96-, and 116-hour hydrolyses in p-toluene sulfonic acid in vacuo. The chain molecular weight was 57,000.
  † Based on a tryptophan to tyrosine ratio of 2.7.

The results of sodium dodecyl sulfate polyacrylamide gel molecular weight determinations, when interpreted in light of the value obtained by sedimentation equilibrium, suggest that the anomalous electrophoretic migrations result from a deviation in the shape of the sodium dodecyl sulfate-isomeroreductase complex from that of most proteins, i.e., it appears as though the radius of gyration of the sodium dodecyl sulfate-isomeroreductase complex is less than expected of a polypeptide chain of this size.

Cross-Linking—The tetrameric nature of isomeroreductase was verified by exposing the native enzyme to the bifunctional reagent dimethylthiosemicarbazide which produces, as do other dimidoesters, amidine cross-links between the polypeptide chains of oligomeric proteins. When the concentration of protein was held constant (0.5 mg per ml) and the dimethylthiosemicarbazide was varied from 0.5 mg per ml to 8 mg per ml and the resulting structures resolved squared. The partial specific volume derived from the amino acid composition (12) used in the molecular weight calculation of native and denatured enzyme is 0.735 ml per g. The molecular weight of the native enzyme is 219,700 which is in excellent agreement with that previously reported (1). The molecular weight of the protein in 0 M guanidine hydrochloride and 0.1 M β-mercaptoethanol is 57,000. In both cases plots of −ln A against radial distances squared yielded straight lines which show that the protein is relatively homogeneous with respect to size, and suggest that native isomeroreductase is composed of four polypeptide chains of equal molecular weight. Table II summarizes the results of the molecular weight measurements.

The results of sodium dodecyl sulfate polyacrylamide gel molecular weight determinations, when interpreted in light of the value obtained by sedimentation equilibrium, suggest that the anomalous electrophoretic migrations result from a deviation in the shape of the sodium dodecyl sulfate-isomeroreductase complex from that of most proteins, i.e., it appears as though the radius of gyration of the sodium dodecyl sulfate-isomeroreductase complex is less than expected of a polypeptide chain of this size.

Cross-Linking—The tetrameric nature of isomeroreductase was verified by exposing the native enzyme to the bifunctional reagent dimethylthiosemicarbazide which produces, as do other dimidoesters, amidine cross-links between the polypeptide chains of oligomeric proteins. When the concentration of protein was held constant (0.5 mg per ml) and the dimethylthiosemicarbazide was varied from 0.5 mg per ml to 8 mg per ml and the resulting structures resolved.
carboxymethylated protein was hydrolyzed for 24 and 48 hours in vacuo in 6 M HCl to which a small crystal of phenol had been added, and for 24, 48, 72, 96, and 116 hours in vacuo with p-toluenesulfonic acid. The values for serine and threonine were obtained by extrapolation to zero time hydrolysis. The values for isoleucine and valine are maximum concentrations. The tryptophan content was determined following 24-hour hydrolysis by the method of Liu (8), by the tryptophan to tyrosine ratio computed from an alkaline spectrum (6), and by reaction of carboxymethylated protein with o-nitrophenylbutyraldehyde chloride (7). The values obtained were 6.5, 6.3, and 4.5, respectively, per polypeptide chain (57,000 mol wt).

Carbohydrate Content—Isomeroreductase contains no carbohydrate as determined by the phenol-sulfuric acid test (15).

Tryptic Peptide Patterns—The column chromatographic elution profile of tryptic peptides of reduced, carboxymethylated isomeroreductase is shown in Fig. 3. Those peptides soluble in 50% acetic acid were analyzed as described under “Materials and Methods”; the small amount of insoluble material was not analyzed. A portion of the effluent from the column was diverted from the hydrolysis coil and fractions were collected. The pooled fractions, designated by the notations in Fig. 3, were concentrated and chromatographed on microcrystalline cellulose plates. The chromatographic patterns from each pool also are shown in Fig. 3; the hatched spots are the histidine-containing peptides. The total number of peptides resolved by this technique is 33. There are 216 lysine and arginine residues per molecule of isomeroreductase (220,000 mol wt). If the four subunits comprising the native enzyme are identical, approximately 56 peptides should be generated by digestion with trypsin. Amino acid analysis of the tryptic digest revealed 6 nmol of free lysine and 6 nmol of free arginine per nmol of polypeptide chain (57,000 mol wt). These results account for 15 of a possible 56 peptides, suggesting that the constituent polypeptide chains of isomeroreductase are identical in amino acid sequence.

These results were confirmed by analyzing the tryptic peptides with a conventional chromatographic-electrophoretic system. Peptides soluble in 0.2 M pyridine acetate, pH 6.5, were spotted on microcrystalline cellulose plates and chromatographed in pyridine butanol acetic acid water (10:15:3:12) followed by electrophoresis in 0.2 M pyridine acetate, pH 6.5. Thirty-five ninhydrin positive spots were detected; among these were four histidine-containing spots. The small amount of insoluble material was not resolvable by standard techniques, but amino acid analysis of a hydrolysate of this material yielded 6 lysine and 4 arginine residues per chain (57,000 mol wt), and together with the quantification of the free arginine and lysine content of the tryptic digest provides an excellent balance (55 of 56) of the arginine and lysine content of the polypeptide chains.

Ultraviolet Absorption Spectrum—The ultraviolet absorption spectrum of native isomeroreductase is shown in Fig. 4. The absorption maximum of the enzyme is 275 nm. The $E_{275}$ is 9.1 and the $E_{268}$ is 8.3; the protein concentration used for calculation of these values was determined by amino acid analysis.

Lack of Cobalt—Because of the presence of cobalt in the isomeroreductase reaction, which involves an alkyl migration, cobalamin or a cobalamin-like compound could be a participating cofactor. However, lack of carbohydrate, as well as failure to observe the characteristic absorption in the ultraviolet spectrum, including the alkaline cyanide spectrum (16), indicates the absence of such a cofactor. The possibility remained, however, that the enzyme required the participation of cobalt for catalysis. The cobalt atomic adsorption spectrum of 10 µg of enzyme revealed the total absence of this metal. These results suggest that the isomerization of the α-acetohydroxyacids proceeds by a mechanism which involves neither cobalamin, nor a primitive cobalamin-like structure which contains cobalt.

Kinetic Parameters—The kinetic parameters of purified isomeroreductase are presented in Table IV. The $K_m$ values for substrates are consistent with those previously published (1). The $K_m$ values for NADPH and Mg$^{2+}$ are the same regardless of the substrate used in the assay.

**DISCUSSION**

The characterization of the subunit structure of α-acetohydroxyacid isomeroreductase serves to augment a growing body of knowledge.
of information relevant to the genetic organization and regulation of the isoleucine and valine biosynthetic system. For example, it is known that mutations within a segment of the isoleucine-valine region of the S. typhimurium and Escherichia coli chromosome cause loss of α-acetohydroxyacid isomeroeductase activity and consequent isoleucine-valine auxotrophy. This segment has been termed ilv C (17). The foregoing results show that native α-acetohydroxyacid isomeroeductase is composed of four identical polypeptide chains and therefore it is concluded that the ilv C region is a single gene which codes for a polypeptide chain of 57,000.

The present results aid in interpretation of previously reported aspects of the genetic regulation of the α-acetohydroxyacid isomeroeductase gene. Pledger and Umbarger (18) have described an isoleucine-valine auxotrophic strain of E. coli in which a mutation within the structural gene for L-threonine deaminase (ilv A) causes this enzyme to be noninhibitable by the end product L-isoleucine, and isomeroeductase to be noninducible. Calhoun and Hatfield (19) have provided evidence from in vivo experiments with S. typhimurium that they interpreted to mean that α-acetohydroxyacid isomeroeductase synthesis requires the formation of holothreonine deaminase. Both of these groups suggest that L-threonine deaminase is directly involved in the synthesis of α-acetohydroxyacid isomeroeductase. These results could be interpreted in terms of L-threonine deaminase and α-acetohydroxyacid isomeroeductase containing shared structures. The results of comparative substructure analyses of these two enzymes strongly support that a shared structure hypothesis is inadequate to explain the results cited above. For example, the fact that an ilv A mutation pleiotropically curtails ilv C expression, if interpreted in the context of shared structure, implies that α-acetohydroxyacid isomeroeductase must be derived from the primary translation product of the ilv A gene. However, the polypeptide chain weight of α-acetohydroxyacid isomeroeductase is larger (57,000) than that of threonine deaminase (48, 500). This fact, together with the observation that α-acetohydroxyacid isomeroeductase contains less L-histidine than L-threonine deaminase (20), suggests that isomeroeductase does not arise by a simple cleavage of the threonine deaminase gene product and vice versa. Although isomeroeductase and threonine deaminase could arise by cleavage of a super polypeptide chain, this possibility is made less likely by results of genetic analysis, which indicate that the operator region for the ilv ADE operon lies between the structural genes for isomeroeductase and threonine deaminase (17). Further evidence for distinctness of the two types of polypeptide chains are provided in Figs. 5 and 6. Fig. 5 shows the elution profiles of the tryptic peptides of isomeroeductase and threonine deaminase from preparative peptide columns described under "Materials and Methods" and "Results," and illustrates the dissimilarity in the pattern of tryptic peptides. Fig. 6 is an Ouchterlony double diffusion plate which shows that antibody prepared against purified threonine deaminase does not cross-react with the purified α-acetohydroxyacid isomeroeductase. Collectively, these results strongly suggest that the primary structures of α-acetohydroxyacid isomeroeductase and threonine deaminase are distinct, and it is concluded that a shared structure hypothesis does not apply to the observations cited above.

The regulation of expression of the structural gene for α-acetohydroxyacid isomeroeductase by substrate induction provides a cascade mechanism whereby the formation of this enzyme responds to the activity of α-acetohydroxyacid synthetase and allows genetic regulation of isomeroeductase apart from the remaining enzymes of L-isoleucine-L-valine biosynthesis. This separation in regulation may be related to the low catalytic constant of isomeroeductase. The results presented here show that isomeroeductase constitutes greater than 1% of the cell's protein even under conditions of suboptimal induction; presumably the low rate of catalysis by this enzyme is compensated by...
its rapid differential rate of synthesis. The low catalytic constant also may reflect the intrinsic ability of an enzyme to effect an alkyl migration without the mediation of cobalmine or cobalt. The lack of cobalmine in α-acetohydroxyacid isomeroreductase is not surprising. Even though S. typhimurium has been reported to contain cobalmine (21), the related organism, E. coli, is incapable of synthesising the corrin ring (22); it would be surprising if the α-acetohydroxyacid isomeroreductase of E. coli differed from that produced by S. typhimurium.

Acknowledgments—The authors wish to acknowledge Alan C. Herman for his aid in the operation of the peptide chromatographic columns, Dennis Winge for the atomic absorption spectral analysis, and Dorothy Thompson for her technical assistance.

REFERENCES
The subunit structure of alpha-acetohydroxyacid isomeroreductase from Salmonella typhimurium.
J G Hofler, C J Decedue, G H Luginbuhl, J A Reynolds and R O Burns


Access the most updated version of this article at http://www.jbc.org/content/250/3/877

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/250/3/877.full.html#ref-list-1