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Isolation of a selenium-containing thiolase from *Clostridium kluyveri*: Identification of the selenium moiety as selenomethionine

(fatty acid synthesis/[^{75}Se]thiolase/acetoacetyl-CoA/amino acid composition/*Se*-adenosyl[^{75}Se]selenomethionine)

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ABSTRACT ***Clostridium kluyveri*** **grown in the presence of 1 μM $Na_2{}^{75}SeO_3$ produces a thiolase that copurifies with ^{75}Se. Based on several criteria, the selenium moiety in this protein is selenomethionine. The ^{75}Se-labeled amino acid in acid hydrolysates of the radioactive protein cochromatographed with authentic selenomethionine on an amino acid analyzer and on TLC plates in acidic and basic solvents. Incubation with *S*-adenosylmethionine synthetase and ATP converted the ^{75}Se-labeled amino acid to a radioactive basic product that was indistinguishable from authentic *Se*-adenosylselenomethionine by ion exchange and TLC. The native selenoenzyme, M_r 155,000–158,000, is composed of four subunits of M_r 38,000–40,000. Thiolase of similar molecular weight that is less acidic and lacks selenium is also produced by *C. kluyveri*. The factors that control the relative levels of the two enzymes in the cell have not been identified.**

During a survey of various microorganisms for the presence of selenium-modified tRNAs (1), an unknown selenium-containing protein was detected in extracts of the fatty acid-producing anaerobe, *Clostridium kluyveri*. This protein, labeled with ^{75}Se during growth of the organism in the presence of $Na_2{}^{75}SeO_3$, was purified to near homogeneity by a relatively simple procedure prior to its identification as thiolase.‡ Crude extracts of *C. kluyveri* proved to contain two proteins of similar molecular weights but different affinities for DEAE-cellulose that exhibited thiolase activity. Multiple forms of thiolases differing in substrate specificity or having different isoelectric points are known to occur in liver (2, 3), in yeast (4), in *Clostridium pasteurianum* (5), and in *Escherichia coli* (6, 7). In the present communication, the purification of one of the *C. kluyveri* thiolases, a selenium-containing protein, and some of its properties are reported.

MATERIALS AND METHODS

Materials. The following products were obtained from commercial sources: $H_2{}^{75}SeO_3$ (100–800 mCi/μmol; 1 Ci = 3.7 × 10^{10} becquerels), Amersham; Ultrogel AcA 44, LKB; Matrex gel green A, Amicon; acetoacetyl coenzyme A, P-L Biochemicals; coenzyme A and calibration protein kit for molecular weight determination, Boehringer Mannheim; DL-selenomethionine and *S*-adenosylmethionine chloride, Sigma; Bio-Rex 70 cation exchange resin, Bio-Rad.

Enzyme Source. *C. kluyveri*§ was grown on ethanol and acetate in a mineral salts medium supplemented with biotin, *p*-aminobenzoic acid, and bicarbonate (8). For the present studies, 1 μM Na_2SeO_3 was added and 1 mM Na_2S or 0.18 mM $Na_2S_2O_4$ was used as the reducing agent. To obtain ^{75}Se-labeled cells, cultures were also supplemented with $Na_2{}^{75}SeO_3$ (0.05–0.5 mCi/liter). The amount of sulfur in these media in the form of added Mg^{2+}, Fe^{2+}, and Mn^{2+} sulfates was 440 μmol/liter. The S/Se (mol/mol) ratio was 1,440:1 for the sulfide media and ≈800:1 for the dithionite media. Harvested cells suspended in 50 mM potassium phosphate, pH 7.1/1 mM 1,4-dithiothreitol (buffer A) were ruptured by sonication.

Protein Determination. Protein was assayed by the biuret reaction or by monitoring absorbance at 280 and 260 nm. Gamma globulin was used as the reference protein.

Thiolase Assay. Thiolase activity was assayed routinely in the direction of coenzyme A-dependent acetoacetyl-CoA cleavage by measuring the decrease in absorbance at 303 nm. The reaction mixture (1.0 ml), which was a modification of that used by Stern (9), contained 100 mM Tris·HCl, pH 8.2/10 mM $MgCl_2$/5 mM reduced glutathione/20 μM acetoacetyl-CoA/200 μM coenzyme A and enzyme. The reaction was initiated by addition of coenzyme A. Under these conditions, the molar extinction coefficient of acetoacetyl-CoA is 14,000 $M^{-1}\cdot cm^{-1}$ (10). One unit of enzyme catalyzed the coenzyme A-dependent conversion of 1 μmol of acetoacetyl-CoA to products per min.

Molecular Weight Determinations. The molecular weight of thiolase under nondenaturing conditions was estimated by HPLC using a Hewlett-Packard 1084B liquid chromatograph with an Altex Spherogel TSK 3000SW column (7.5 × 600 nm) in 20 mM sodium phosphate, pH 7/100 mM sodium sulfate. For calibration, β-galactosidase, aldolase, sheep IgG, bovine serum albumin, hen ovalbumin, and soybean trypsin inhibitor were chromatographed under the same conditions. Chromatography on a Sephadex G-150 column equilibrated with buffer A and calibrated with bovine serum albumin (M_r = 68,000) and rabbit aldolase (M_r = 158,000) was also used for molecular weight estimation of the native protein. Molecular weights of the thiolase subunits were estimated from their electrophoretic mobilities in $NaDodSO_4$/10% polyacrylamide slab gels by the method of Laemmli (11).

Disc Gel Electrophoretic Analysis. Disc gel electrophoresis of native thiolase was carried out using the method of Davis (12). The gels were first run for 30 min to remove residual ammonium persulfate.

Determination of Radioactivity. ^{75}Se was determined by liquid scintillation techniques using a Beckman LS-250 scintillation spectrometer. For determination of radioactivity in poly-

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‡ Enzymes that catalyze the reaction, acetoacetyl-CoA + CoA-SH ⇌ 2 acetyl-CoA, are known variously as acetyl-CoA acetyltransferase (EC 2.3.1.9), acetoacetyl-CoA thiolase, or simply thiolase.

§ Strain originally isolated by H. A. Barker.

acrylamide gels, gel slices were incubated overnight at 60°C with 0.6 ml of 15% H_2O_2 in tightly capped scintillation vials. The radioactivity was measured after addition of 15 ml of Aquasol (New England Nuclear).

Amino Acid Analyses. Thiolase preparations were hydrolyzed in 3 M thioethanesulfonic acid (Pierce) at 155°C for 20, 40, or 60 min, and amino acid compositions were determined as described by Hare (13) using a Dionex model 300 amino acid component system modified to use either ninhydrin or *o*-phthalaldehyde detection. Values calculated from the averages of timed hydrolyses for the mole fraction of amino acids corrected to one phenylalanine residue were multiplied by 11 and converted to the nearest integer.

For radioactivity measurements, 1-min samples were collected directly from the amino acid analyzer column.

Preparation of *Se*-adenosyl[^{75}Se]selenomethionine. A strain of *E. coli* (HT-378) derepressed for *S*-adenosylmethionine synthetase and harboring a plasmid containing the structural gene for the synthetase was kindly supplied by Herbert and Celia White Tabor. Culture of this strain and partial purification of the synthetase from sonicated extracts of the bacteria were carried out by a modification of the procedure described (14) for strain EWH 205/pcc 27-37. Enzyme activity was assayed (14) by measuring the conversion of [*methyl*-^{14}C]methionine to *S*-adenosyl[*methyl*-^{14}C]methionine or [^{75}Se]selenomethionine to *Se*-adenosyl[^{75}Se]selenomethionine after separation of the product by cellulose TLC in ethanol/2 M $HCOONH_4$, 70:30 (pH 3.5). The R_F values in this solvent are methionine/selenomethionine, 0.75; *S*-adenosylmethionine/*Se*-adenosylselenomethionine, 0.1. Separation of the reaction product by chromatography on a weak cation exchange resin (Bio-Rex 70) was carried out as described (15).

RESULTS

In view of the fact that all four previously characterized selenoenzymes (16) have been shown to participate in various types of oxidation–reduction reactions, it seemed likely that the *C. kluyveri* [^{75}Se]protein also would be a member of this class of catalysts. Accordingly, the radioactive protein preparations were assayed for activities of several redox-type enzymes that participate in the overall *C. kluyveri* fermentation pathway [e.g., ethanol dehydrogenase (17), CoA-dependent acetaldehyde dehydrogenase (8), β-hydroxybutyryl-CoA dehydrogenase (18), and also phosphotransacetylase (19) and crotonase (20)]. Although some of these enzyme activities were detectable, they were present only as trace contaminants and none copurified with the [^{75}Se]protein. Another key enzyme of the fermentation pathway, thiolase (acetyl-CoA acetyltransferase), seemed a less likely candidate since it is not a typical redox enzyme but, contrary to expectation, the highly purified [^{75}Se]protein catalyzed the CoA-dependent cleavage of acetoacetyl-CoA at an extremely rapid rate.

Enzyme Purification. A typical procedure for purification of thiolase from *C. kluyveri* cells cultured in the presence of 1 μM $Na_2{}^{75}SeO_3$ and dithionite as reducing agent is as follows.

Step 1. The crude extract was prepared as described in *Materials and Methods.*

Step 2. Crude extract (10.75 ml) containing 492 mg of protein was applied to a DEAE-cellulose column (21 × 90 mm) equilibrated with buffer A. The column was washed with buffer A and total adsorbed thiolase was then eluted with 250 mM potassium phosphate, pH 7/1 mM dithiothreitol.

Step 3. To 26.8 ml of eluate from the DEAE-cellulose column, containing 375 mg of protein, ammonium sulfate was added to 35% saturation. The protein precipitate was separated by centrifugation and discarded, and the supernatant was adjusted to 65% saturation in ammonium sulfate. The mixture was allowed to stand at 4°C overnight, and the protein was collected by centrifugation.

Step 4. The protein pellet from step 3, containing 311 mg of protein, was dissolved in 3.85 ml of buffer A and applied to an Ultrogel AcA 44 column (25 × 900 mm) equilibrated with buffer A. Total thiolase activity was eluted from the column as a single symmetrical peak within the fractionation range of the gel.

Step 5. The fractions from the AcA 44 column containing thiolase activity were pooled and chromatographed on a DEAE-cellulose column as described in Fig. 1. Thiolase activity eluted in two separate peaks. The major peak of enzyme activity, which coincided with the major radioactive peak, eluted with 130 mM phosphate. This peak contained 47% of the thiolase units, 44% of the protein, and 42% of the radioactivity applied to the column. The enzyme peak, which was removed earlier with the buffer A wash, contained ^{75}Se and 26% of the thiolase units applied to the column. In several preparations derived from cells grown in the presence of sulfide instead of dithionite, a protein peak containing 90% of the thiolase activity and little or no selenium eluted with the buffer wash. In these cases, the selenium-containing thiolase was only 5–10% of the total thiolase protein.

Step 6. The pooled fractions (48.5 ml, 3,119 units) of the major thiolase peak from step 5 were diluted to 150 ml and applied to a Matrex gel green A affinity column (15 × 50 mm) equilibrated with buffer A. The column was then washed with buffer A and eluted with a 130-ml linear gradient of 0–0.6 M KCl in buffer A. The peak of thiolase activity from this affinity column coincided with a peak of ^{75}Se and a protein peak (Fig. 2). These fractions contained 35% of the ^{75}Se and 33.5% of the protein applied to the column. Although the specific activity of the enzyme (units/mg of protein) was increased about 1.8-fold by this chromatographic step, the ^{75}Se/protein ratio (50,000 cpm/mg) was essentially unchanged from that of the previous step (48,500 cpm/mg). A second protein peak (fractions 73–80) had a similar radioactivity/A_{280nm} ratio and the same distinctive electronic absorption spectrum as the active enzyme (see below) but exhibited no thiolase activity. This appeared to be a higher molecular weight species as judged by

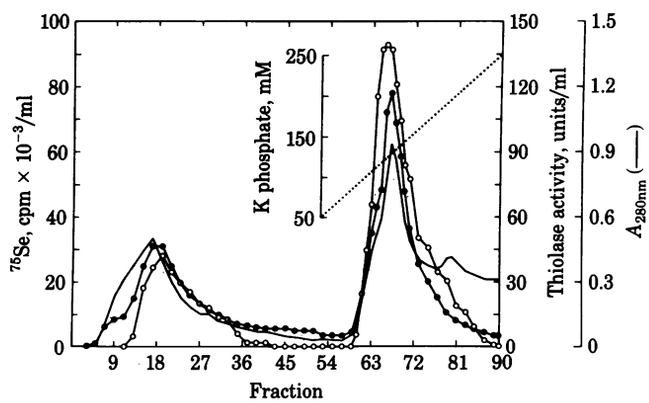

FIG. 1. Chromatography of thiolase on DEAE-cellulose. A thiolase preparation (26 ml, 91 mg of protein, 6,682 enzyme units) eluted from Ultrogel AcA 44 as described in step 4 was applied to a DEAE-cellulose column (25 × 65 mm) equilibrated with buffer A. Thiolase activity (1,728 units, 25 mg of protein) in fractions 13–32 eluted with the buffer A wash of the column. Elution of the column with a 120-ml linear gradient of 50–250 mM potassium phosphate, pH 7/1 mM dithiothreitol removed the major peak of thiolase activity. Fractions (3 ml) were collected at a flow rate of 30 ml/hr at 20°C, and aliquots were assayed for thiolase activity (○) and ^{75}Se (●).

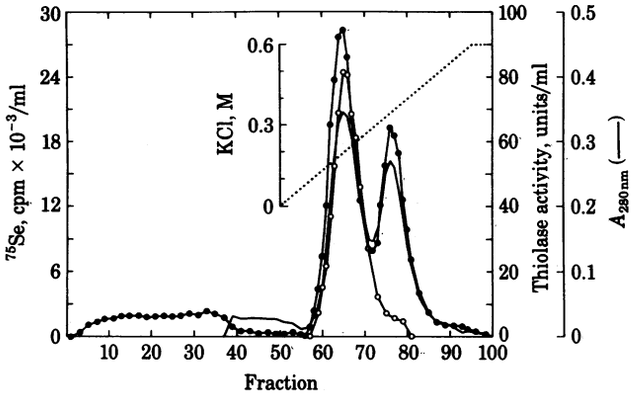

FIG. 2. Affinity chromatography of selenium-containing thiolase on Matrex gel green A. Pooled fractions of the major thiolase peak shown in Fig. 1 were diluted and applied at a rate of 15 ml/hr at 4°C to a Matrex gel green A column as described in purification step 6. Three-milliliter fractions were collected at a flow rate of 32 ml/hr, and aliquots were assayed for enzyme activity (○) and ^{75}Se (●).

HPLC. Since the green A dye of the affinity matrix contains a quinone structure, it is possible that reaction with these groups caused the observed losses of enzyme activity and appreciable separation of selenium from the protein (Fig. 2).

The purification procedure described in steps 1–6 is summarized in Table 1. Since *C. kluyveri* is an extremely rich source of thiolase, an overall purification of about 10-fold is sufficient to give enzyme preparations having activities similar to those from other sources, which require 100- to 700-fold purification.

A representative gel filtration profile of a radioactive enzyme preparation from the second DEAE-cellulose chromatographic step (step 5) shows the coincidence of a peak of ^{75}Se with the peak of thiolase activity (Fig. 3). A similar preparation subjected to gel electrophoresis under nondenaturing conditions exhibited a single protein band that comigrated with both thiolase activity and ^{75}Se (Fig. 4). In this and several similar experiments, no special precautions were taken to remove all traces of oxidizing agents from the gels by prior treatment with thioglycolic acid. The fact that 44% of the radioactivity in the gel was coincident with the protein band indicated the presence of a

Table 1. Purification procedure

Step	Procedure	Total protein,* mg	Total enzyme, units	Specific activity, units/mg	Total ^{75}Se,† cpm × 10^{-6}
1.	Crude extract	492	8,525	17.3	29.5
2.	First DEAE-cellulose chromatography	375	8,415	22.4	14.6
3.	$(NH_4)_2SO_4$ fractionation	311	8,250	26.5	11.7
4.	AcA-44 gel filtration	91	6,682	73.4	4.6
5.	Second DEAE-cellulose chromatography	40	3,119	78.3	1.93
6.	Matrex gel green A chromatography	13.4	1,861	139‡	0.67

* Determined by the biuret method.
† The crude extract was prepared from unwashed cells and contained protein and nonprotein selenium.
‡ Thiolase activity of the peak tube from this step was 162 units/mg of protein.

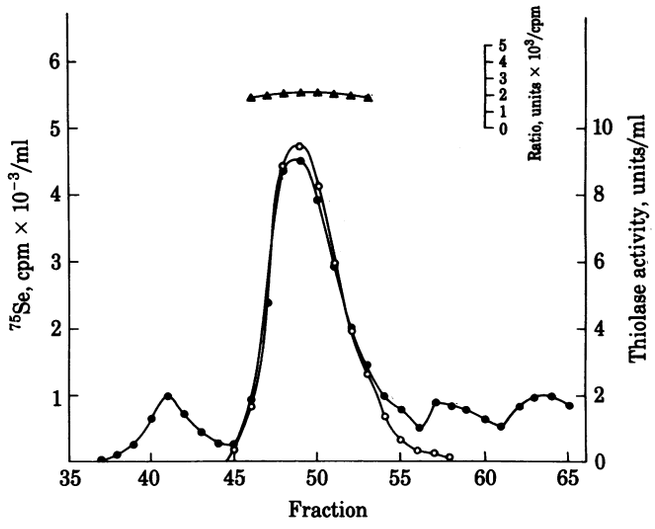

FIG. 3. Cochromatography of thiolase activity and ^{75}Se on Ultrogel AcA 44. A ^{75}Se-labeled thiolase preparation that had been purified by chromatography on DEAE-cellulose as described in step 5 of the purification procedure was concentrated by precipitation with ammonium sulfate at 0.35–0.65 saturation. The precipitate containing 3 mg of protein, 145 thiolase units, and 90,000 cpm of ^{75}Se was dissolved in 2.5 ml of buffer A and applied to an Ultrogel AcA 44 column (1.6 × 90 cm) equilibrated with buffer A. Fractions of 1.8 ml were collected at a flow rate of 11 ml/hr at 25°C, aliquots of each were assayed for thiolase activity (○) and ^{75}Se (●), and the thiolase activity/^{75}Se ratio (▲) was calculated. The thiolase peak emerged in the position expected for a M_r 80,000 protein rather than a M_r 160,000 species. Fractions 1–39, exclusion volume; salt emerged at fraction 95.

relatively stable selenium moiety, and this is consistent with its subsequent identification as selenomethionine (see below).

Molecular Weight Estimation. Under nondenaturing conditions, the selenothiolase was coeluted with aldolase (M_r, 158,000) from a Sephadex G-150 column equilibrated with buffer A. A molecular weight of approximately 155,000 was estimated by HPLC. This is in contrast to a value of 80,000 estimated from the elution position of the native enzyme from an Ultrogel AcA 44 column (Fig. 3). The anomalous behavior of the thiolase as compared with the proteins used for calibration of this column suggests an abnormal binding of the enzyme to the acrylamide-agarose gel support.

Under denaturing conditions, the purified thiolase migrated as a single protein band during electrophoresis in NaDodSO$_4$/polyacrylamide gels. When radioactive thiolase preparations were examined under the same conditions, a peak of ^{75}Se was coincident with the protein band. By comparison with the mobilities of known proteins under the same conditions, an apparent M_r of 40,000 was calculated for this polypeptide.

From the currently available data, it appears that the selenothiolase from *C. kluyveri*, like many other thiolases, is a tetramer of M_r 38,000–40,000 subunits.

Electronic Absorption Spectrum. The UV absorption spectrum of the purified thiolase is shown in Fig. 5. The absorbance of the enzyme at 1 mg/ml (estimated by the biuret reaction) is 0.68 at 280 nm and 0.69 at 277 nm. These values reflect the low tryptophan content of the protein. Purified thiolase preparations are transparent in the visible range of the spectrum.

Amino Acid Composition. A minimum residue weight of 38,121 per subunit was calculated from analysis of the amino acid composition of the native selenothiolase (Table 2). One cysteine residue per subunit, determined as *S*-carboxymethylcysteine, was detected in hydrolysates of [1-^{14}C]carboxamidomethylated native thiolase. Coelution of this compound

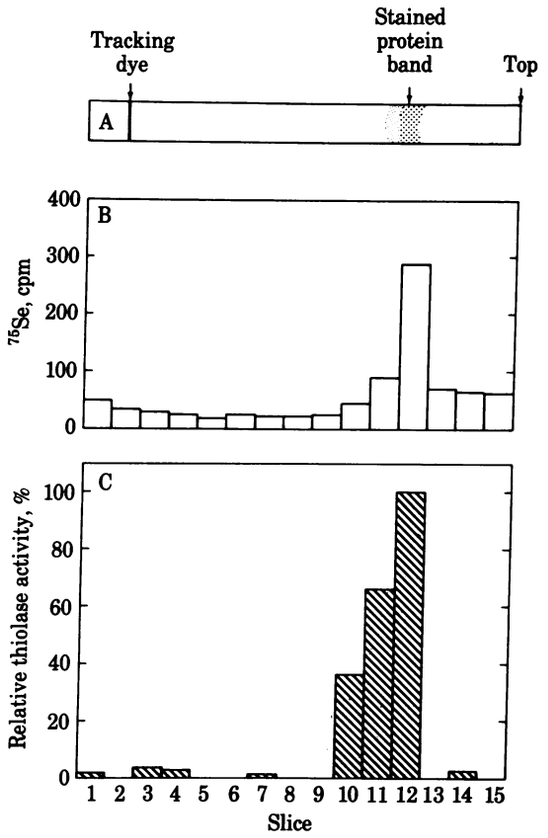

FIG. 4. Disc gel electrophoresis of [^{75}Se]thiolase under nondenaturing conditions. Aliquots of a purified sample of the [^{75}Se]thiolase (0.9 enzyme unit; 1,050 cpm) were subjected to electrophoresis on 7.5% polyacrylamide gels. (*A*) Protein band stained with Coomassie brilliant blue G-250. (*B*) ^{75}Se profile from gel sliced and examined for radioactivity. There were 380 cpm coincident with the protein band and a total of 870 cpm were found throughout the gel. The gel shown in *A* was removed, sliced, and assayed for radioactivity. (*C*) Thiolase activity profile. Slices of an unstained gel were incubated in the standard thiolase assay mixture for 10 min and then the decrease in A_{303nm} of the solution was measured.

at 11 min with a single radioactive peak further established its identity. Determination of cysteine as cysteic acid after performic acid oxidation of thiolase gave a value of four residues per subunit. The presence of a single tryptophan, seven tyrosine, and 11 phenylalanine residues per subunit accounts for the electronic absorption spectrum of the native protein (Fig. 5).

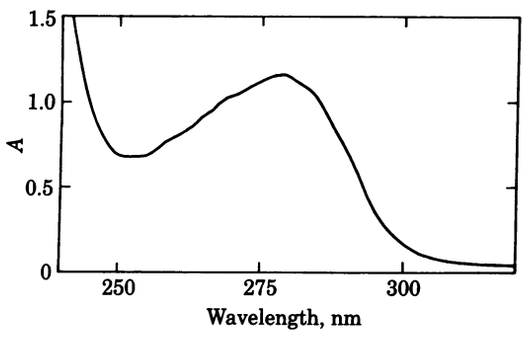

FIG. 5. Electronic absorption spectrum of selenium-containing thiolase. The absorbance of a peak thiolase fraction eluted from Matrex gel green A as described in step 6 was determined for a solution containing protein (biuret) at 1.7 mg/ml of buffer A/0.2 M KCl, pH 7. The reference cuvette contained buffer A (pH 7).

Table 2. Amino acid analysis of selenothiolase

Residue	No.	Residue	No.
Asx	34	Tyr	7
Thr	17	Phe	11
Ser	15	His	4
Glx	31	Lys	31
Gly	39	Trp	1
Ala	49	Arg	12
Val	31	Pro*	11
Met	11	Cys†	4
Ileu	25	SeMet‡	0.25
Leu	29		

Results are calculated as nearest integer values per M_r 38,000 subunits and are means of values obtained from duplicate analyses of samples hydrolyzed for 40 and 60 min in 3 M thioethanesulfonic acid at 155°C.

* Determined in a separate analysis using ninhydrin detection.

† Determined as cysteic acid after performic acid oxidation. In a separate experiment, an enzyme sample was reduced with KBH_4, alkylated with $ICH_2{}^{14}CONH_2$, separated from reagents and hydrolyzed for 20 min at 155°C with 3 M thioethanesulfonic acid. A single radioactive peak that corresponded to the elution position of carboxymethylcysteine (CM-Cys; 11 min) was collected from the amino acid analyzer column. The amount of CM-Cys was equivalent to one residue per subunit.

‡ SeMet, selenomethionine. Originally detected in hydrolysates of [^{75}Se]thiolase as a radioactive peak that emerged at 36 min together with leucine. For direct quantitation, the elution buffer was changed from 67 mM sodium citrate·HCl (pH 3.27) to 67 mM sodium citrate·HCl, pH 3.5/0.25 M NaCl. Under these conditions, leucine emerged at 31.6 min and selenomethionine was detected as a discrete peak at 35.7 min.

The low content of selenomethionine, the selenium-containing moiety present in thiolase (see below), is equivalent to one residue per enzyme tetramer. This value, determined by direct analysis, is consistent with even lower values calculated from ^{75}Se contents of other purified thiolase preparations.

Identification of Selenium Moiety in Thiolase. In initial attempts to identify the selenium moiety in ^{75}Se-labeled thiolase, the protein was reduced and alkylated under conditions suitable for the preparation of derivatives of selenols such as selenocysteine (21) prior to acid hydrolysis. In no case was any radioactivity detected at the elution position at which authentic carboxymethylselenocysteine (13 min) emerged from the amino acid analyzer column. The attractive possibility that selenium might be present in the enzyme in the form of the selenium analog of pantetheine also appeared to be excluded because neither carboxymethylselenocysteamine (44.7 min) nor β-alanine were detected in the hydrolysates. Instead, the radioactive profiles of most of the hydrolysates showed three or four small peaks suggestive of the presence of breakdown products. In a few instances, a radioactive peak that eluted at 36 min in the same position as leucine was the predominant species. In acid hydrolysates of native [^{75}Se]thiolase, this was the major radioactive component, indicating that its presence did not depend on prior alkylation of the protein. The known amino acid, selenomethionine, also eluted precisely at 36 min, and the major radioactive peaks from both native and alkylated [^{75}Se]thiolase hydrolysates were superimposible with the peak of the authentic compound. The radioactive amino acid in these fractions, after separation from the citrate buffer, cochromatographed with authentic selenomethionine in ethanol/*tert*-butanol/88% $HCOOH/H_2O$, 60:20:5:15 (vol/vol), R_F 0.75; in $CHCl_3/CH_3OH$/15% NH_4OH, 40:40:15 (vol/vol), R_F 0.71; and in ethanol/2 M $HCOONH_4$, 70:30 (pH 3.5; vol/vol), R_F 0.74.

Table 3. Conversion of ^{75}Se-labeled amino acid from thiolase to *Se*-adenosyl[^{75}Se]selenomethionine by *S*-adenosylmethionine synthetase

Reaction mixture components*	Determined by TLC, cpm	Determined by ion exchange chromatography, cpm
Product	15,600	15,400†
Residual substrate	75,500	73,500‡

* ^{75}Se-Labeled amino acid (92,000 cpm) purified from hydrolysates of native thiolase by chromatography on the amino acid analyzer column and unlabeled DL-selenomethionine (2 μmol) were added as substrates.

† Peak of radioactivity coincident with an A_{260} peak that was eluted with 2 M CH_3COOH in the same position as authentic *Se*-adenosylselenomethionine. After concentration and removal of solvent, the radioactive compound cochromatographed with *Se*-adenosylselenomethionine in the TLC system.

‡ Radioactivity in the effluent from the weak cation exchange resin (Bio-Rex 70); selenomethionine and methionine are not retained under these conditions.

To more precisely establish the identity of the selenoamino acid in thiolase, the isolated radioactive compound was tested for conversion to *Se*-adenosylselenomethionine by reaction with ATP and S-adenosylmethionine synthetase since, in earlier studies, it had been shown that the selenium analog of methionine is an even better substrate for this enzyme than is methionine (14, 22). In controls, it was shown that authentic [^{75}Se]selenomethionine was converted in an enzyme- and ATP-dependent reaction to a ^{75}Se-labeled product (1.5–4% yield) that absorbed at 260 nm and exhibited the expected chromatographic properties. The ^{75}Se-labeled amino acid isolated from the thiolase was incubated with carrier unlabeled DL-selenomethionine under similar conditions in a scaled-up reaction mixture that was supplemented with additional ATP after 60 min and then incubated for an additional 90 min to achieve greater conversion to product (15). The results of ion exchange and TLC analyses of this reaction mixture are shown in Table 3. By both methods of analysis, 17% of the added ^{75}Se was recovered in a product that by several criteria was determined to be *Se*-adenosylselenomethionine. The appearance of radioactivity in this product depended on the presence of S-adenosylmethionine synthetase in the reaction mixture. Similar analysis of a small-scale control sample in which the ^{75}Se-labeled amino acid (11,000 cpm) and carrier selenomethionine were incubated in the absence of enzyme showed that essentially all of the radioactivity (10,700 cpm) was present in the selenomethionine reisolated at the end of the experiment.

DISCUSSION

The finding that selenium in the form of a selenomethionine residue is specifically associated with an enzyme raises the question of (*i*) the biochemical function of this selenoether and (*ii*) its relative advantage over the corresponding thioether—i.e., methionine. Although the essential nature of methionine residues in many proteins has long been recognized, little is known about their specific functions. Consequently, the background for speculation concerning the role of selenomethionine in the *C. kluyveri* thiolase is minimal. Whether the apparent stoichiometry of one residue per tetramer is normal or is instead a reflection of the particular growth conditions or metabolic state of the cells at the time of harvest is currently unknown. The high sulfur/selenium ratio in the culture medium together with the fact that numerous other enzymes produced by the organism lack selenium indicates that nonspecific substitution of selenomethionine for methionine is unlikely to be the explanation of its occurrence in the thiolase. The attractive assumption, early in this work, that the selenium in the thiolase might be in the form of selenocysteine residues proved to be incorrect. Instead, the cysteine residues, as in other thiolases (23, 24), presumably are the catalytically active components that undergo acetylation. The cysteine content of *C. kluyveri* thiolase (16 per tetramer) determined in this study is similar to the values reported for a thiolase from pig heart (20 per tetramer; 25) and for a thiolase from *E. coli* (16 per tetramer; 24). Only four of the cysteine residues (one per subunit) appear to be available in the native protein for reaction with iodoacetamide. Extracts of *C. kluyveri* are exceptionally rich in thiolase, 17 units/mg of protein (Table 1), as compared with other sources that contain 0.01–0.1 times as much (4, 5, 23, 26).

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