The Purification and Subunit Structure of Cysteine Desulfhydrase from Salmonella typhimurium*

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SUMMARY

The inducible enzyme, cysteine desulfhydrase, was purified from Salmonella typhimurium to a state of near homogeneity. This enzyme has a very low tryptophanase activity, lacks cystathionase and tryptophan synthetase activities, and appears to be a specific cysteine desulfhydrase. The purified native enzyme has a molecular weight of 229,000 as determined by equilibrium sedimentation. Polyacrylamide electrophoresis in sodium dodecyl sulfate showed a single species of polypeptide chain with a molecular weight of 37,000 while the pyridoxal phosphate content was found to be 1 mole per 37,700 g of protein. These data, together with the results of amino acid analyses, tryptic peptide maps, and an NH2-terminal amino acid analysis, indicate that the native enzyme is a hexamer, containing 6 moles of pyridoxal phosphate, and composed of six identical polypeptide chains with NH2-terminal serines.

The enzymatic desulfuration of cysteine to hydrogen sulfide, ammonia, and pyruvate has been described in a number of different species and tissues (1-8). In spite of a considerable amount of literature on this reaction, a specific cysteine desulfhydrase has never been extensively purified from any source. Guarnieros and Ortega (9) have described certain properties of the inducible cysteine desulfhydrase of Salmonella typhimurium in toluenedized cells and crude extracts, while Collins (10) has studied in considerable detail the kinetic properties of this enzyme using preparations only several-fold purified. We report here the purification of the inducible cysteine desulfhydrase (L-cysteine hydrogen sulfide-lyase (deaminating), EC 4.4.1.1) from S. typhimurium to a state of near homogeneity and present the results of our studies on its physical and chemical properties.

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EXPERIMENTAL PROCEDURE

Materials—L-Cysteine-HCI was purchased from Sigma and recrystallized from 5 N HCl before use. L-(+)-Cystathionine, streptomycin sulfate, and the pyridoxal phosphate used for enzyme purification were also products of Sigma. Pyridoxal phosphate, A grade, from Calbiochem, was used as a standard for analytical studies. Special enzyme grade ammonium sulfate and density gradient grade sucrose were purchased from Mann. Acrylamide and methylenebisacrylamide were products of Eastman, and were recrystallized from chloroform and acetone, respectively, before use. Protein molecular weight standards were kindly donated by M. Schwartz and S. Pizzo. Dansyl chloride, dansyl amino acid standards, and Sequanal grade solvents used for NH2-terminal amino acid analyses were products of Pierce Chemicals. Double-layered polyamide sheets were obtained from Gallard Schlesinger Corp. Calcium phosphate gel was prepared by the method of Swingle and Tiselius (11).

Other materials were obtained as previously described (12).

Assay for Cysteine Desulfhydrase—Enzyme activity can be assayed by measuring the rate of formation of sulfide or of pyruvate by several different procedures. We have found that as the enzyme is purified the ratio of sulfide to pyruvate production increases to a value of approximately 5. Other workers have described similar discrepancies in the stoichiometry of the reaction in crude extracts (5, 9, 10), and for this reason we have chosen the rate of sulfide production as the most convenient and representative measure of enzyme activity. A more careful analysis of the nature of the cysteine desulfhydrase reaction and an explanation for its unusual stoichiometry will be the subject of a subsequent communication.1 For our purposes, 1 unit of cysteine desulfhydrase is defined as that amount of enzyme catalyzing the production of 1 mole of sulfide per min in an assay mixture consisting of 2.0 mM L-cysteine in 0.1 M Tris HCl, pH 8.6.

The reaction is started by adding a small volume of enzyme, diluted in 0.1 M Tris-HCl, pH 7.6, containing 0.5 mg per ml of bovine serum albumin, to 2.0 ml of the assay mixture in a capped test tube (10 × 75 mm). After incubating for 5 min at 23° the reaction is terminated by the addition of 0.2 ml of 0.02 M N,N'-dimethyl-p-phenylenediamine sulfate in 7.2 N HCl, followed by 0.2 ml of 0.2 M N,N'-dimethyl-p-phenylenediamine and 0.2 ml of 0.2 M streptomycin sulfate, and the pyridoxal phosphate used for enzyme purification were also products of Sigma. Pyridoxal phosphate, A grade, from Calbiochem, was used as a standard for analytical studies. Special enzyme grade ammonium sulfate and density gradient grade sucrose were purchased from Mann. Acrylamide and methylenebisacrylamide were products of Eastman, and were recrystallized from chloroform and acetone, respectively, before use. Protein molecular weight standards were kindly donated by M. Schwartz and S. Pizzo. Dansyl chloride, dansyl amino acid standards, and Sequanal grade solvents used for NH2-terminal amino acid analyses were products of Pierce Chemicals. Double-layered polyamide sheets were obtained from Gallard Schlesinger Corp. Calcium phosphate gel was prepared by the method of Swingle and Tiselius (11). Other materials were obtained as previously described (12).

1 The abbreviation used is: dansyl, 1-dimethylaminonaphthalene-5-sulfonyle.2 N. M. Kredich, L. J. Foote, and B. S. Keenan, manuscript in preparation.
allowed immediately by 0.2 ml of 0.03 M ferric chloride in 1.2 N HCl (13). The tube is recapped, vigorously shaken, and, after storage for 15 to 20 min in the dark, the absorbance at 650 nm is determined in a spectrophotometer. Cysteine at this concentration interferes somewhat with the production of the chromophore, and the ε₆₅₀ is 1.56 × 10⁴ M⁻¹ cm⁻¹ rather than the slightly higher values obtained with pure solutions of sulfide (13). Using this assay a plot of sulfide production versus enzyme concentration is not linear due to inhibition of the enzyme by sulfide (10).

Preliminary kinetic data have indicated that under the conditions of our standard assay, sulfide inhibition is of the linear competitive type (14) which can be described by

\[ \frac{1}{v} = \frac{k_a}{v_m} \left[ 1 + \frac{Q}{K_q + A} \right] + \frac{1}{v_m} \]

where \( v \) is the rate of product formation, \( A \) is the cysteine concentration, \( Q \) is the inhibitor (in this case the product, sulfide) concentration, \( V_m \), is the maximum velocity at saturating substrate and zero inhibitor concentrations, \( K_a \) is the Michaelis constant for cysteine, and \( K_q \) is an inhibition constant for sulfide.

Since the change in cysteine concentration is very slight (less than 4%) during the course of our usual assay, the quantity, \( A \), may be treated as a constant, and, using \( v = dQ/dt \), Equation 1 may be expressed as

\[ \frac{dQ}{dt} = \frac{-AV_mK_q}{k_aK_a + K_q + 2K_a + K_qA} \]

where \( t \) is the time measured from the beginning of the reaction. Under the conditions of our assay, where \( Q = 0 \) at \( t = 0 \), the separation of variable technique for solving Equation 2 yields

\[ \int_0^Q (K_aQ + K_qA + K_qA) dt = \int_0^Q Av_mK_q dy \]

Solving for \( V_m \) gives

\[ V_m = \left[ \frac{[K_a + A]}{A} \right] \frac{Q}{1 + \left( \frac{K_q}{2K_a} \frac{Q^2}{1} \right); Q > 0 \]

The initial velocity, \( V_i \), at \( Q = 0 \) is related to \( V_m \) by

\[ V_i = \left( \frac{V_m(1 - A)}{A} \right) \]

Substituting Equation 5 in Equation 4 and rearranging gives

\[ V_i = Q + \left( \frac{k_s}{2K_a[K_a + A]} \right) \frac{Q^2}{1}; Q > 0 \]

Rearrangement of Equation 6 to

\[ Q = \left( \frac{2k_sK_a[A]}{K_a + A} \right) \frac{1}{V_i} \]

yields an equation which shows that, using a constant amount of enzyme and measuring \( Q \) at various times, \( t, \) a plot of \( Q \) versus \( t/Q \) will give a straight line with a reciprocal slope of \( K_a/2K_q \) \( (K_a + A) \). Under the conditions of our standard assay, such a plot is in fact linear with a reciprocal slope of 10 nm⁻². Substituting this experimentally determined value for \( K_a/2K_q \) \( (K_a + A) \) in Equation 6, a plot of \( V_i \) versus enzyme concentration is linear to a sulfide concentration as high as 0.08 mM. All enzyme activities reported here have been calculated using Equation 6.

**Polyacrylamide Gel Electrophoresis**—Analytical polyacrylamide gel electrophoresis was carried out at room temperature, using the system of Reisfeld and Small (15) with 7% acrylamide gels and omitting urea. Proteins were stained with Coomassie brilliant blue by the method of Crambach et al. (16). Polyacrylamide gel electrophoresis in sodium dodecyl sulfate was performed by the method of Weber and Osborn (17). Samples were incubated at 37°C for 2 hours in 0.01 M sodium phosphate, pH 7.0, 1% sodium dodecyl sulfate, 1% 2-mercaptoethanol, and 6 mM urea before being run in gels which were 7.5% in acrylamide and 0.2% in methylenebisacrylamide. The molecular weight standards used were as follows (assumed subunit molecular weights in parentheses): phosphorylase a (92,500), human transferrin (77,000), bovine serum albumin (67,000), catalase (60,000), ovalbumin (43,000), pepsin (35,000), chymotrypsinogen (26,000), cytochrome c (13,700), and ribonuclease (12,700).

**Ultracentrifugation Studies**—Sedimentation coefficients were measured at 50,740 rpm in a Spinco model E ultracentrifuge equipped with schlieren optics, and by sucrose gradient centrifugation in linear 5 to 20% gradients using catalase and O-acetylseryl sulfhydrylase (12) as markers. Molecular weight determinations were performed in the analytical ultracentrifuge by the meniscus depletion method of Yphantis (16) using Rayleigh interference optics. The partial specific volume, \( \bar{v} \), of purified enzyme was calculated from the amino acid content (19).

**Amino Acid Analyses**—Samples of protein were hydrolyzed in sealed, evacuated tubes with 6 N HCl at 110°C for varying periods of time. The half-cystine content was determined as cysteic acid from a separate 24-hour hydrolysatе in which 0.21 ml diethylsulfoxide was included with the 6 N HCl (20). Tryptophan was measured after hydrolysis in 6 N HCl, 4% thioglycolic acid for 24 hours (21). All analyses were performed with a Beckman model 120B amino acid analyzer.

**NH₂-terminal Amino Acid Analysis**—Protein was dansylated by a modification of the technique of Gros and Labouesse (22). A 0.9-mg sample of lyophilized, salt-free enzyme was dissolved in 1.0 ml of 0.1 N sodium phosphate, pH 9.0, containing 5 mM ura, 10 mg per ml of sodium dodecyl sulfate, and 10 mM 2-mercaptoethanol. One milligram of dansyl chloride in 0.1 ml of acetone was added, and the mixture was stirred at 37°C for 30 min. Dansylated protein was precipitated with 5 ml of acetone, collected by centrifugation, and washed with an additional 5 ml of acetone. After drying in a stream of nitrogen the protein was hydrolyzed in 0.5 ml of 6 N HCl in a sealed tube for 4 hours at 105°C. The hydrolysate was dried in vacuo, dissolved in 0.3 ml of dilute HCl, pH 3.5, and extracted with two 0.5-ml portions of ether. The ether extracts were taken to dryness in a stream of nitrogen, redissolved in a small amount of dry pyridine and analyzed by thin layer chromatography on polyamide sheets (10 cm × 10 cm) using the solvents suggested by Hartley (23).

**Other Methods**—Tryptophan synthetase was assayed by a modification of the method which measures the rate of tryptophan synthesis from indole and serine (24). Using L-[U-¹⁴C]serine as substrate, enzyme activity was determined by the appearance of radiolabel in tryptophan after separation from substrate by thin layer chromatography.

Cystathionase (25) and tryptophanase (26) activities were assayed by coupling pyruvate production to NADH oxidation with lactate dehydrogenase. Pyridoxal phosphate was determined by the method of Warsh and Snell (27) on samples which had been deproteinized with 0.3 N perchloric acid. Spectra were obtained on a Cary model 15 recording spectrophotometer. Protein was assayed by the biuret method (28) or by absorbance at 280 nm.
for relatively pure preparations. Tryptic digests and peptide mapping were done as previously described (12).

**Purification of Cysteine Desulfhydrase**

**Step I. Cells and Crude Extract**—The wild type, LT-2 strain of *S. typhimurium* was grown at 37°C with forced aeration on a modified minimal salts medium containing 5 mg per ml of glucose and 0.5 mM L-cystine as the sole sulfur source (29). Cells were collected by centrifugation and, as a matter of convenience, were usually kept at -20°C for 1 to 5 days before use. Storage for longer periods of time was found to result in a significant loss of enzymatic activity.

Five hundred and twelve grams (wet weight) of bacteria were thawed and suspended in 4 volumes of Buffer A (0.1 mM Tris-Cl, pH 7.6; 10 mM 2-mercaptoethanol). After chilling to 4°C the cells were disrupted by sonication and the debris removed by centrifugation at 40,000 × g for 30 min. All purification steps subsequent to the preparation of the crude extract were done at room temperature.

**Step II. Streptomycin Sulfate Precipitation**—To the crude extract supernatant was added 0.4 volume of 10% streptomycin sulfate, which had been previously adjusted to pH 7.0 with NaOH. After 10 min of stirring, the precipitate was regained after the next step in purification.

**Step III. Ammonium Sulfate Precipitation**—To the streptomycin sulfate supernatant were slowly added 210 mg per ml of ammonium sulfate with stirring. The precipitate, which contained most of the cysteine desulfhydrase activity, was collected by centrifugation and dissolved in a small amount of Buffer A.

**Step IV. Calcium Phosphate Gel Fractionation**—The ammonium sulfate precipitate was diluted with Buffer A to a protein concentration of 10 mg per ml. To this solution was added 220 mg of ammonium sulfate per ml of Buffer A which adsorbed approximately 95% of the cysteine desulfhydrase activity. The exact amount of gel needed was first determined in a small pilot run and in this preparation amounted to 0.5 ml of gel suspension per ml of enzyme solution. After mixing for 10 min, the suspension was centrifuged for 5 min at 1,500 × g, and the supernatant discarded. After re-suspension in 1,400 ml of Buffer A the gel was again collected by centrifugation, and the supernatant discarded. The gel was then eluted with two successive 700-ml portions of a solution of 0.07 M Tris-HCl, pH 7.6; 0.03 M potassium phosphate, pH 7.6; 7 mM 2-mercaptoethanol. The two eluates were pooled and centrifuged at 27,000 × g for 30 min to remove fine particles of calcium phosphate gel. Enzyme activity was then precipitated by the addition of 220 mg of ammonium sulfate per ml of the supernatant.

**Step V. Sephadex G-200 Gel Filtration**—The precipitate from the preceding step was dissolved in a small amount of Buffer A and applied to a column of Sephadex G-200-40 (92 cm × 3.5 cm²). Equilibrated with Buffer A containing 1 mM pyridoxal phosphate. The column was run at a flow rate of 2.2 ml per hour, and 2.2-ml fractions were collected. Fractions containing the highest amounts of enzyme activity were pooled and used in the next step of purification.

**Step VI. DEAE-Sephadex Chromatography**—The enzyme was adsorbed to a column of DEAE-Sephadex A-50 (5 cm × 3.5 cm²) equilibrated with Buffer A containing 1 mM pyridoxal phosphate. After washing with 20 ml of equilibrating buffer, elution was carried out with 250 ml of a linear, zero to 0.5 M NaCl gradient in equilibrating buffer at a flow rate of 0.15 ml per min. Fractions with the highest specific activities were pooled, and the protein was precipitated with 220 mg per ml of ammonium sulfate. The precipitate was dissolved in a small volume of Buffer A containing 0.1 mM pyridoxal phosphate, filter-sterilized, and stored at room temperature. The total purification was over 1700-fold with a yield of 32%. A summary of the purification appears in Table I.

**RESULTS**

**Stability**—The enzyme is cold-labile at all stages of purification, and in Buffer A with or without pyridoxal phosphate loses 10 to 20% of its activity per day at 4°C or -20°C. The addition of an equal volume of glyceral to enyme in Buffer A permits storage for at least 2 months at -20°C with no appreciable loss of activity. During the final stages of purification the enzyme is very stable at room temperature in Buffer A plus 0.1 mM pyridoxal phosphate, but rapidly precipitates and loses activity in the absence of this cofactor. When sterilized by filtration through a 0.22-µm Millipore membrane, purified enzyme in Buffer A plus 0.1 mM pyridoxal phosphate can be stored for 2 weeks at room temperature with a loss of 10% of activity.

Enzyme used in the studies reported here was not subjected to glycerol treatment, and, except as noted during the initial stages of purification, was never chilled or frozen.

**Purity and Physical Properties**—Analytical polyacrylamide gel electrophoresis of the purified protein over a range of 0.4 to 40 µg per gel showed a single major band staining with Coomassie blue (Fig. 1A). Enzyme activity was located by incubating an unstained gel, containing 1 µg of protein, in a freshly prepared solution of 0.1 mM Tris-HCl, pH 8.6; 0.01 M L-cysteine HCl; 0.5

**Table I**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Protein concentration</th>
<th>Cysteine desulfhydrase</th>
<th>Specific activity</th>
<th>Yield</th>
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<tbody>
<tr>
<td></td>
<td>ml</td>
<td>mg/ml</td>
<td>Per ml</td>
<td>Total</td>
<td>units/mg protein</td>
</tr>
<tr>
<td>I. Crude extract</td>
<td>2,100</td>
<td>33</td>
<td>8.6</td>
<td>18,060</td>
<td>0.26</td>
</tr>
<tr>
<td>II. Streptomycin precipitation</td>
<td>2,750</td>
<td>18.4</td>
<td>3.1</td>
<td>8,560</td>
<td>0.17</td>
</tr>
<tr>
<td>III. Ammonium sulfate precipitation</td>
<td>770</td>
<td>25</td>
<td>18.8</td>
<td>14,400</td>
<td>0.75</td>
</tr>
<tr>
<td>IV. Calcium phosphate gel filtration</td>
<td>5.0</td>
<td>27</td>
<td>2,160</td>
<td>10,800</td>
<td>80</td>
</tr>
<tr>
<td>V. Sephadex G-200 gel filtration</td>
<td>20</td>
<td>1.35</td>
<td>416</td>
<td>8,320</td>
<td>308</td>
</tr>
<tr>
<td>VI. DEAE-Sephadex chromatography</td>
<td>26</td>
<td>0.55</td>
<td>235</td>
<td>6,110</td>
<td>427</td>
</tr>
<tr>
<td>Final ammonium sulfate precipitation</td>
<td>1.2</td>
<td>10.5</td>
<td>4,740</td>
<td>5,690</td>
<td>451</td>
</tr>
</tbody>
</table>
mm lead acetate at room temperature. Within 15 s a single dark brown band, presumably a precipitate of lead sulfide, appeared at a position on the gel identical with that stained by Coomassie blue on a parallel gel. This histochemical assay is dependent upon the presence of L-cysteine and is very sensitive, detecting as little as 0.01 μg of enzyme per gel after a 5-min incubation. We conclude from these data that the enzyme described in these studies is approximately 98% pure by the criterion of disc gel electrophoresis in a single buffer.

Sodium dodecyl sulfate polyacrylamide electrophoresis of reduced protein in 7.5% gels showed a major protein band representing approximately 95% of the total Coomassie blue staining material (Fig. 1B). The mobility of this major component is compared to those of standard proteins in Fig. 2 and corresponds to that of a species with a molecular weight of 37,000.

For studies in the analytical ultracentrifuge the enzyme was equilibrated with freshly prepared Buffer A containing 0.1 mM pyridoxal phosphate by gel filtration through Sephadex G-50. During sedimentation of a 2 mg per ml solution at 50,710 rpm, a single peak with a $s_{20,w}$ of 10.4 was noted by schlieren optics. No attempt was made to determine a concentration dependence of the $s_{20,w}$. Sucrose density gradient centrifugation both of a crude extract and of the purified protein gave values of 10 S, indicating that no major change in this property of cysteine desulphhydrase occurred during purification.

When the enzyme was sedimented to equilibrium at 12,500 rpm for 24 hours, a plot of ln fringe displacement versus $r^2$ gave a straight line with a slope of $2.36 \pm 0.02$ cm$^2$ (± standard error of estimate). Using a value for $\phi$ of 0.71, calculated from the amino acid analysis, a molecular weight of 229,000 was calculated for the native enzyme. A $D_{20,w}$ of $3.75 \times 10^{-7}$ cm$^2$ per s and a frictional ratio of 1.41 were derived from these data.

A spectrum was obtained on material dialyzed against Buffer A plus 0.1 mM pyridoxal phosphate using dialysate as a blank. The expected peak at 280 nm was noted (Fig. 3) with a second peak at 415 nm.
The purification of cysteine desulfhydrase to near homogeneity with a good yield has been dependent upon keeping the preparation at room temperature, using a buffer containing pyridoxal phosphate during column chromatography, and assaying the enzyme by sulfide production. Chilling or freezing the preparation or omitting pyridoxal phosphate leads to rapid denaturation of the enzyme, whereas assaying by pyruvate formation leads to an apparent loss of most of the enzyme activity after the first ammonium sulfate step.

The results of the amino acid analyses, tryptic peptide mapping, NH$_2$-terminal amino acid analysis, and polyacrylamide gel electrophoresis in sodium dodecyl sulfate taken together indicate that cysteine desulfhydrase contains a single species of polypeptide chain with a molecular weight of 37,000. The finding of 1 mole of pyridoxal phosphate per 37,700 g of protein is consistent with this conclusion. The molecular weight of 229,000 obtained by equilibrium sedimentation suggests that the native enzyme is a hexamer composed of 6 identical subunits.

The demonstration of significant amounts of cysteine desulfhydrase in purified preparations of cystathionase (25) and the apparently coordinate expression of these two enzyme activities in crude extracts of *Escherichia coli* grown on different sulfur sources have led Rowbury and Woods to suggest that the cysteine desulfhydrase noted in *E. coli* is due to the enzyme cystathionase (33). Collins (10) and Guarneros and Ortega (9) have presented convincing arguments, based on genetic evidence and induction-repression data, for the nonidentity of cysteine desulfhydrase and cystathionase in *S. typhimurium*. The lack of cystathionase activity in our highly purified preparation of cysteine desulfhydrase confirms the conclusions of these investigators.

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**Table II**

**Amino acid composition of cysteine desulfhydrase**

Each value is the result of a single analysis of protein hydrolyzed for the time indicated.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Composition</th>
<th>Average or extrapolated integer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs</td>
<td>48 hrs</td>
</tr>
<tr>
<td></td>
<td>residue/37,000 daltons subunit</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>9.1</td>
<td>9.4</td>
</tr>
<tr>
<td>Histidine</td>
<td>11.9</td>
<td>11.9</td>
</tr>
<tr>
<td>Arginine</td>
<td>22.3</td>
<td>22.8</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>32.2</td>
<td>30.6</td>
</tr>
<tr>
<td>Threonine</td>
<td>20.3</td>
<td>20.3</td>
</tr>
<tr>
<td>Serine</td>
<td>26.2</td>
<td>24.4</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>32.5</td>
<td>31.8</td>
</tr>
<tr>
<td>Proline</td>
<td>10.4</td>
<td>10.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>28.9</td>
<td>27.6</td>
</tr>
<tr>
<td>Alanine</td>
<td>36.1</td>
<td>34.8</td>
</tr>
<tr>
<td>Valine</td>
<td>13.8</td>
<td>14.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>8.0</td>
<td>7.6</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>17.9</td>
<td>20.7</td>
</tr>
<tr>
<td>Leucine</td>
<td>32.5</td>
<td>31.8</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>11.4</td>
<td>11.9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>10.7</td>
<td>11.3</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

* Extrapolated.

† Determined as cysteic acid in a separate hydrolysis (29).

‡ Determined in a separate run using thioglycolic acid in the hydrolysis mixture (21).

The results of the amino acid analyses are shown in Table II. Integer numbers of amino acids are calculated on the basis of a hypothetical 37,000 molecular weight subunit. The partial specific volume (\(\bar{v}\)) calculated from these data is 0.71 cc per g.

The tryptic peptide map of cysteine desulfhydrase is shown in Fig. 4. Arginine spots were located by both the phenanthrenequinone reagent (31) and the Sakaguchi reagent (32). A total of 35 peptides was identified by the ninhydrin stain, 23 of which contained arginine. These numbers agree well with the results of the amino acid analysis, which predicts that a single subunit of molecular weight 37,000 should yield a total of approximately 33 peptides, with 23 arginine-containing peptides.

Using the dansyl chloride method, serine was identified as the only NH$_2$-terminal amino acid. Based on a subjective comparison of fluorescence with known standards, a yield of 0.1 to 0.2 residue per 37,000 daltons was estimated.

Using 5 mM tryptophan as substrate, the tryptophanase activity of the purified enzyme was 0.4 unit per mg, or 0.1% of the cysteine desulfhydrase activity. No cystathionase or tryptophan synthetase activity could be demonstrated.

**Discussion**

The maximum at 415 nm, characteristic of other pyridoxal phosphate-containing proteins. The \(A_{365}/A_{414}\) ratio for our best preparation is 10.8. An \(A_{260}\) of 2.12 liters g$^{-1}$ cm$^{-1}$ was determined by the method of Babul and Stellwagen (30) using the analytical ultracentrifuge with Rayleigh interference optics as a differential refractometer.

Chemical Properties—Pyridoxal phosphate analyses were carried out on enzyme equilibrated with 0.1 M Tris-HCl, pH 7.6, by gel filtration through Sephadex G-50. The \(A_{365}\) was determined immediately after elution from the gel and remained stable for approximately 30 min after which time a slow rise in absorbance occurred due to denaturation and light scattering. Based on the initial \(A_{365}\) the pyridoxal phosphate content was calculated to be 1 mole per 37,700 g of protein. The \(A_{416}\) for the pyridoxal phosphate chromophore is thus \(7.3 \times 10^5\) M$^{-1}$ cm$^{-1}$.

The results of amino acid analyses are shown in Table II. An \(A_{280}\) of 36.1 was determined for the time indicated.

Each value is the result of a single analysis of protein hydrolyzed for the time indicated.

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<tr>
<td>Serine</td>
<td>26.2</td>
<td>24.4</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>32.5</td>
<td>31.8</td>
</tr>
<tr>
<td>Proline</td>
<td>10.4</td>
<td>10.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>28.9</td>
<td>27.6</td>
</tr>
<tr>
<td>Alanine</td>
<td>36.1</td>
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</tr>
<tr>
<td>Valine</td>
<td>13.8</td>
<td>14.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>8.0</td>
<td>7.6</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>17.9</td>
<td>20.7</td>
</tr>
<tr>
<td>Leucine</td>
<td>32.5</td>
<td>31.8</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>11.4</td>
<td>11.9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>10.7</td>
<td>11.3</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

* Extrapolated.

† Determined as cysteic acid in a separate hydrolysis (29).

‡ Determined in a separate run using thioglycolic acid in the hydrolysis mixture (21).

The demonstration of significant amounts of cysteine desulfhydrase in purified preparations of cystathionase (25) and the apparently coordinate expression of these two enzyme activities in crude extracts of *Escherichia coli* grown on different sulfur sources have led Rowbury and Woods to suggest that the cysteine desulfhydrase noted in *E. coli* is due to the enzyme cystathionase (33). Collins (10) and Guarneros and Ortega (9) have presented convincing arguments, based on genetic evidence and induction-repression data, for the nonidentity of cysteine desulfhydrase and cystathionase in *S. typhimurium*. The lack of cystathionase activity in our highly purified preparation of cysteine desulfhydrase confirms the conclusions of these investigators.
The tryptophan synthetase B protein (34) and tryptophanase (35) from E. coli are also known to possess cysteine desulfhydrase activity. In E. coli the tryptophan synthetase B protein has approximately 3% of the ability of the whole complex to synthesize tryptophan from indole and serine (36). Since this amount of activity would have been easily detectable by our assay, it seems unlikely that our purified protein is related to tryptophan synthetase activity.

The finding of a small amount of tryptophanase activity in purified cysteine desulfhydrase is not inconsistent with the fact that S. typhimurium is indole-negative (lacking tryptophanase), since the activity due to cysteine desulfhydrase in an induced crude extract would only be about 0.2 nmole per min per mg of protein. The inducibility of the desulfhydrase by cysteine and its 1000-fold greater reaction rate with cysteine as substrate suggest that its physiologic role is that of cysteine catabolism rather than tryptophan degradation. Thus the existence of a specific cysteine desulfhydrase in S. typhimurium seems firmly established.

Acknowledgments—We are grateful to L. Siegel and J. Huston for their advice and encouragement and to T. Vanaman for his assistance in the NH2-terminal amino acid analysis.

REFERENCES

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5. Smythe, C. V. (1942) J. Biol. Chem. 142, 367-400
Additions and Corrections

Vol. 247 (1972) 7157–7162

In KREDICH, NICHOLAS M., BRUCE S. KEENAN, AND LINDA J. FOOTE. The Puriﬁcation and Subunit Structure of Cysteine Desulﬁhydrase from Salmonella typhimurium.

The ﬁnal paragraph of the section “Assay for Cysteine Desulﬁhydrase” under “Experimental Procedure,” Equation 7 should read:

Rearrangement of Equation 6 to:

\[
Q = 2K_q V_t (K_a + A)/K_a Q - 2K_q (K_a + A)/K_a
\]

(7)

yields an equation which shows that, using a constant amount of enzyme and measuring Q at various times, t, a plot of Q versus \( t/Q \) will give a straight line with an intercept on the y axis equal to the negative reciprocal of \( K_a/2K_q (K_a + A) \). Under the conditions of our standard assay such a plot is in fact linear with an intercept of \(-0.2 \text{ mm}\). Substituting a value of \( 5 \text{ mm}^{-1} \) as the value for \( K_a/2K_q (K_a + A) \) in Equation 6, a plot of \( V_t \) versus enzyme concentration is linear to a sulfide concentration as high as \( 0.08 \text{ mm} \). All enzyme activities reported here have been calculated using Equation 6.

Vol. 247 (1972) 4447–4452

In DOPHEIDE, THEO. A. A., PAULINE CREWTER, AND BARRIE E. DAVIDSON. Chorismate Mutase-Prephenate Dehydratase from Escherichia coli K-12. II. Kinetic Properties.

The caption along the right-hand horizontal axis of Fig. 1 should read:

\[ \log [\text{Pre}] \]

The caption along the vertical axis of Fig. 5 should read:

\% Dehydratase Activity

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.
The Purification and Subunit Structure of Cysteine Desulfhydrase from Salmonella typhimurium

Nicholas M. Kredich, Bruce S. Keenan and Linda J. Foote


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