Isolation and Properties of a Homogeneous Preparation of Cystathionine Synthetase-1-Serine and 1-Threonine Dehydratase*

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Selim and Greenberg (1, 2) achieved a considerable degree of purification of L-serine dehydratase (L-serine hydro-lyase (deaminating), EC 4.2.1.13) from rat liver and demonstrated that this protein preparation contained the cystathionine-synthesizing activity of the liver (L-serine hydro-lyase (adding L-homocysteine), EC 4.2.1.21). These workers (2) also observed activity of their enzyme preparation on L-threonine. Subsequently, the work of Goldstein, Knox, and Behrman (3) indicated that the L-threonine dehydratase activity of rat liver was a function of the same enzyme protein. Pitot, Potter, and Morris (4) demonstrated a large increase in the threonine and serine dehydratase activities of the livers of rats fed a high protein diet. This observation was confirmed by Goldstein et al. (3).

This ability to increase the content of the enzyme in the liver offers a decided advantage in its purification. From livers of rats fed a high protein diet, we have succeeded in obtaining enzyme preparations apparently consisting of a single homogeneous protein. Various properties of the purified enzyme have been studied. An observation of considerable interest is that the \( \beta \)-hydroxy group is not an essential characteristic for substrate activity. Chloride, for example, can be substituted for the hydroxyl group.

**EXPERIMENTAL PROCEDURE**

*Materials—L-Serine, DL + DL-allocystathionine, DL-homocysteine thiolactone hydrochloride, and pyridoxal phosphate were purchased from Calbiochem. The thiolactone hydrochloride was converted to homocysteine by treatment with alkali immediately before incubation with the enzyme preparation. The material was washed successively with NaOH, 1 NaOH, and finally with water.

DEAE-cellulose was obtained from Schleicher and Schuell. The material was washed successively with 1 NaOH, 1 HCl, ethanol, 1 NaOH, and finally with water.*

*Analytical Methods—The \( \alpha \)-keto acids derived from the enzymatic decomposition of serine, threonine, and the \( \beta \)-chloroamino acids were determined by the direct method of Friedemann and Haugen (7), with the modification described by Savyre and Greenberg (8). Standard curves were prepared from salts of \( \alpha \)-ketobutyrate and pyruvate.

Serine was determined by periodate oxidation by the method of Frisell, Meech, and Mackenzie (9). Homocysteine present in the incubation mixtures in which serine is to be determined tends to react with the formaldehyde to form a thiosalicylic ring derivative and thus decrease the absorbance values. To correct for this, standard curves were prepared with serine solutions containing homocysteine. The absorbance of the colored product of the chromotropic acid and formaldehyde was measured at 570 mp. The indirect estimation of the cystathionine formed (from the difference between the initial serine concentration and the residual serine concentration, less the pyruvate formed by deamination of serine) lacks precision.

An alternate method of cystathionine estimation was developed, therefore, based on the keto acid formed by the catalytic decomposition of this compound by cystathionase. One of the possible sources of error in this procedure was the known activity of cystathionase on L-cysteine and on homocysteine (10). To test the magnitude of these errors, a series of determinations were made on mixtures containing varying cystathionine concentrations, with and without added L-cysteine and DL-homocysteine.

In addition to the use of varying amounts of cysteine and homocysteine in the incubation mixtures, the tests were carried out anaerobically under nitrogen in Thunberg tubes and aerobically in the presence of 1 mivr iodoacetate. The purpose of anaerobiosis was to prevent oxidation of cysteine to cystine, which has been reported to be the actual substrate for cystathionase when cysteine is tested (11). Iodoacetate was shown by Matsuo and Greenberg (12) to alkylate the cysteine amino acid, which was determined by analysis with a spectrophotometer. The results of a typical experiment with DL-cystathionine freed from DL-allocystathionine by chromatography on Dowex 50 Na+ by the method of Blackburn and Schoberl (13) compared with a DL-cystathionine preparation containing an unknown amount of DL-allocystathionine are reported in Table I. Addition of 0.1 m DL-homocysteine to the cystathionine incubations did not affect the results.

Comparison of the indirect chemical and enzymatic methods of estimation in an experiment designed to show a proportionality between enzyme concentration and cystathionine synthetase activity yielded concordant values (Fig. 1).

*Protein—Protein concentrations were determined by a method previously (5, 6).
TABLE I

Recovery of keto acid in enzymatic determination of cystathionine with cystathionase

Determinations were made by incubating 1-ml samples containing 0.1 M potassium phosphate buffer, pH 7.5, \(1 \times 10^{-4}\) M pyridoxal-P, \(1 \times 10^{-3}\) M EDTA, 360 units of crystalline enzyme, and varying amounts of DL-cystathionine at 37° for 20 min. Iodoacetate was used at a concentration of \(1 \times 10^{-5}\) M.

<table>
<thead>
<tr>
<th>DL-Cystathionine added</th>
<th>Keto acid formed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anaerobic incubation</td>
</tr>
<tr>
<td></td>
<td>From chromatographed cystathionine</td>
</tr>
<tr>
<td>(\mu)moles</td>
<td>(\mu)moles</td>
</tr>
<tr>
<td>3</td>
<td>2.55</td>
</tr>
<tr>
<td>10</td>
<td>5.10</td>
</tr>
<tr>
<td>15</td>
<td>7.50</td>
</tr>
</tbody>
</table>

reaction with crude enzyme preparations (cf. Reference 14) and by absorption in the ultraviolet range with purified enzyme (cf. Reference 14).

Enzyme Assay — The assay method of Selim and Greenberg (1) was employed, except that a higher concentration of pyridoxal-P, \(1 \times 10^{-4}\) M, was used. A reaction medium containing concentrations of 8.3 \(\times 10^{-2}\) M \(L\)-homocysteine; 8.3 \(\times 10^{-2}\) M \(L\)-serine; 0.1 \(M\) potassium borate buffer, pH 8.3; \(1 \times 10^{-4}\) M pyridoxal phosphate; \(5 \times 10^{-3}\) M EDTA; and \(1.3 \times 10^{-4}\) M mercaptoethanol in a total volume of 3 ml was used in the measurement of cystathionine synthetase activity. The incubations were run for 30 min at 37° in a Dubnoff metabolic shaker. The rates of decomposition of \(L\)-serine and \(L\)-threonine were essentially the same, except that mercaptoethanol and \(DL\)-homocysteine were omitted from the incubation media.

An enzyme unit is defined as the amount that yields 1 \(\mu\)mole of the respective keto acid (pyruvate or \(\alpha\)-ketobutyrate) per hour under the conditions of the assay.

An exact linear relation between enzyme concentration and the rates of decomposition of \(L\)-serine and \(L\)-threonine was found. Demonstration of the proportionality between enzyme concentration and the estivated cystathionine synthesized is less accurate (Fig. 1), because of the poorer precision of the analytical procedure.

RESULTS

Purification of Enzyme

High activity of the enzyme was found in mouse, rat, and dog livers (Table II). Chicken livers exhibited a lower degree of activity. Beef, horse, hog, and sheep livers contained little or no activity as assayed by our procedure. The low activity may be related to the nature of the diet (cf. Reference 15). Sheep liver catalyzed the deamination of threonine only, with wide variations in the degree of activity.

Taking advantage of the observation that a high protein diet increases the serine and threonine dehydratase activities of rat and mouse livers (4), groups of 50 rats were placed on a high protein diet (50% casein) for 2 weeks. The animals were killed by decapitation and the livers were collected. The specific activity of serine dehydratase was increased 5 to 8 times the normal value by this regimen.

The initial steps in the purification of the enzyme were essentially the same as those described by Selim and Greenberg (1, 2). However, higher specific activities were obtained (Table III). Further purification was achieved by chromatography on DEAE-cellulose.

Chromatography on DEAE-cellulose — The DEAE-cellulose, treated as described in "Experimental Procedure," was equilibrated with 0.02 M potassium phosphate buffer, pH 7.5, containing \(1 \times 10^{-5}\) M EDTA, and was used to fill a column, 3 x 17 cm. The enzyme preparations from the ammonium sulfate fractionation were dialyzed against the same buffer containing \(1 \times 10^{-5}\) M pyridoxal-P. A \(10 \mu\)l aliquot of the dialyzed sample contained 230 to 430 mg of protein. This was pipetted onto the column. After adsorption of the protein, the column was eluted successively with 0.02 M, 0.05 M, and 0.1 M potassium phosphate buffer, pH 7.5, at a rate of 2 ml per min. The eluate was collected in 20-ml fractions.

*Percentage composition of high protein diet: vitamin-free casein, 50; starch, 20; powdered sugar, 8.8; cottonseed oil, 8; brewers' yeast, 2; complete vitamin supplement, 2.2; salt mix (U.S.P.) 4; polyvinylpyrrolidone (binding agent), 5.4

*In a recent experiment, only the 0.02 M and 0.05 M phosphate buffers, pH 7.5, were used for elution and 15-ml fractions were collected. This yielded only the first two protein peaks.
Liver homogenates (5 ml each) were passed through a Sephadex G-25 column (2 x 15 cm) to remove free amino acids. Samples (1 ml) of the homogenate were used for assay. After the incubation (1 hour for cystathionine synthetase determination), the mixtures were heated for 5 min in a boiling water bath to inactivate the enzyme and coagulate the protein. The latter was removed by centrifugation. Serine and keto acid estimations were run on aliquots of the supernatant solutions. To determine the cystathionine formed, 0.5-ml aliquots of the heated solutions were incubated in a total volume of 1 ml with 300 units of cystathionase, 1 X 10^{-3} M pyridoxal-P, and 1 X 10^{-5} M iodoacetate for 20 min at 37°C. The reaction was terminated by addition of 1 ml of 10% trichloroacetic acid. The precipitated protein was removed and the keto acid in the supernatant fluid determined in the usual manner.

TABLE II

Distribution of cystathionine synthetase, L-serine and L-threonine dehydratase activities

<table>
<thead>
<tr>
<th>Liver samples</th>
<th>Cystathionine synthetase activity</th>
<th>L-Serine dehydratase activity</th>
<th>L-Threonine dehydratase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean ± s.d.</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>units/g liver, wet wt</td>
<td></td>
<td>units/g liver, wet wt</td>
</tr>
<tr>
<td>Sheep (8)*</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mouse (8)</td>
<td>300-350</td>
<td>233 ± 16</td>
<td>800-950</td>
</tr>
<tr>
<td>Rat (6)</td>
<td>50-60</td>
<td>55 ± 4</td>
<td>120-210</td>
</tr>
<tr>
<td>Dog (7)</td>
<td>28-120</td>
<td>79 ± 36</td>
<td>86-360</td>
</tr>
<tr>
<td>Chicken (4),† normal diet</td>
<td>9.2-12.9</td>
<td>11.4 ± 1.5</td>
<td>13.7-15.9</td>
</tr>
<tr>
<td>Chicken (4),‡ high protein diet</td>
<td>14-18</td>
<td>15.6 ± 1.6</td>
<td>19-20.6</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are numbers of individual liver samples.
† Chicks, 3 weeks old, maintained in a brooder for 10 days on a diet with 18% casein. Other components of the diet same as in footnote 3.
‡ Chicks, 3 weeks old, maintained in a brooder for 10 days on a diet containing 80% casein. Other components of the diet same as in footnote 3. Casein was substituted for starch. The occurrence of only a slight increase in enzyme activity on the high protein diet may be related to the immaturity of the chickens.

TABLE III

Summary of purification of cystathionine synthetase-L-serine and L-threonine dehydratase

A portion (200 g) of rat liver was homogenized with 800 ml of 1.2% KCl, containing 5 X 10^{-4} M EDTA for 3 min and then centrifuged at 10,000 X g for 25 min.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Volume</th>
<th>Protein</th>
<th>Total enzyme</th>
<th>Specific activity on serine</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>g</td>
<td>units X 10^{-9}</td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Homogenate</td>
<td>845</td>
<td>31.27</td>
<td>7.6</td>
<td>24.3</td>
<td>100</td>
</tr>
<tr>
<td>(NH4)2SO4 I</td>
<td>146</td>
<td>8.8</td>
<td>7.9</td>
<td>90.0</td>
<td>103.7</td>
</tr>
<tr>
<td>Ca3(PO4)2 gel.</td>
<td>430</td>
<td>1.50</td>
<td>2.4</td>
<td>158.0</td>
<td>31.3</td>
</tr>
<tr>
<td>(NH4)2SO4 II</td>
<td>28</td>
<td>0.83</td>
<td>2.0</td>
<td>237.0</td>
<td>25.8</td>
</tr>
<tr>
<td>Heat treatment</td>
<td>24.5</td>
<td>0.36</td>
<td>1.7</td>
<td>475.0</td>
<td>22.5</td>
</tr>
<tr>
<td>(NH4)2SO4 III</td>
<td>9</td>
<td>0.36</td>
<td>1.9</td>
<td>525.0</td>
<td>24.9</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>0.06</td>
<td>0.1</td>
<td>1800.0</td>
<td>15.0</td>
<td></td>
</tr>
</tbody>
</table>

The fractions were analyzed for protein by ultraviolet absorption at 280 nm and for serine dehydratase activity by the standard assay procedure. The first chromatograms showed the presence of three protein peaks obtained by elution with 0.02 M, 0.05 M, and 0.1 M potassium phosphate buffer, pH 7.5, respectively. The enzyme was present in the second peak emerging with 0.05 M buffer.

For further purification, the most active fractions (specific activity, 1300 to 1500) were combined and precipitated with solid ammonium sulfate at pH 7.0. The precipitate was dissolved in 0.02 M phosphate buffer, pH 7.5, and dialyzed against the same buffer containing 1 X 10^{-4} M pyridoxal-P. The dialyzed sample was adsorbed onto a smaller column of DEAE-cellulose (2 x 17 cm) equilibrated in the same manner as the first column. Elution was performed successively with 100 ml each of 0.02, 0.03, 0.04, and 0.05 M phosphate buffer. Protein was determined colorimetrically (cf. Reference 14) and the serine dehydratase activity was assayed. The correspondence between protein content and serine dehydratase activity in the eluate fractions is shown in Fig. 2. Fractions with a specific activity of 1300 or higher were used for further study of the properties of the enzyme.

The purification and recovery of the enzyme in the individual steps of the purification procedure are recorded in Table III. The ratio of the rate of deamination of L-serine to L-threonine varied from 1.3 during the initial steps of purification to 1 after DEAE-cellulose chromatography.

Unlike cystathionase, the present enzyme is colorless. Thus, its absorption spectrum shows only a slight shoulder at 415 μm.
Properties of Enzyme

The homogeneity and molecular weight of enzyme were assayed by the three procedures described below.

**Sedimentation Velocity**—Ultracentrifugal analysis of the enzyme was performed with a Spinco model E ultracentrifuge in a synthetic boundary cell, at 10°, on samples which were dialyzed against 0.1 M potassium phosphate buffer, pH 7.5. A typical sedimentation pattern is shown in Fig. 3. Sedimentation constants, measured at protein concentrations of 0.85, 0.5, and 0.3%, were extrapolated to zero concentration (Fig. 4) and a value of 1.97 was obtained. This, converted to standard conditions, gave $s_{20, w} = 2.59$.

The molecular weight was estimated from the measured sedimentation and diffusion coefficients (16). The diffusion constant was calculated from the boundary formed between a 1% protein solution and solvent (0.1 M potassium phosphate buffer, pH 7.5) in a synthetic boundary cell operated at 5250 rpm in the ultracentrifuge at a temperature of 20°. The evaluations of the area under the gradient curve and the maximum ordinate were performed by enlarging the photographs of the sedimentation run and tracing these on 1-cm graph paper. The apparent diffusion coefficients were determined by the height-area method (16) and the values obtained were plotted against 1/time and extrapolated to infinite time to correct for any imperfections in the initial boundary. This procedure yielded a diffusion coefficient for the enzyme of $1.085 \times 10^{-6}$ cm² sec⁻¹. Inserting the appropriate data in the formula

\[
M = \frac{RTs}{D(1 - \psi_0)}
\]

yields a figure of 21,900.

**Sedimentation Equilibrium**—The molecular weight of the enzyme was also estimated by the Archibald method according to the procedure described by Klainer and Kegeles (17). Molecular weights were calculated at the top and bottom of the cell after various times of centrifugation and are given in Table IV. The values for mean molecular weight of the enzyme thus obtained were 19,350 and 19,650, respectively. The consistency in the calculated values shows that the protein is homogeneous with respect to molecular weight.

**Sucrose Density Gradient Centrifugation**—The determination of the relative molecular weight by this procedure was carried out by the method of Martin and Ames (18) in 5 to 20% sucrose at pH 7.5 in 0.2 M phosphate buffer. Cytochrome c (mol. wt. = 13,300) was used as a reference protein. The gradients in the plastic centrifuge tubes were stored in a cold room at 3° for 4 to 18 hours before use. The enzyme solution was layered on the sucrose surface and the centrifuge tubes were placed in a swinging bucket rotor (SW-39) fitted to a Spinco model L preparative ultracentrifuge. Centrifugation was continued at 34,000 rpm for 16 hours at 2°. The rotor was decelerated by turning the time knob to zero and allowing the rotor to coast to a halt at 2°. Samples were withdrawn by punching holes in the bottoms of the plastic centrifuge tubes with a syringe needle and collecting 3-drop aliquots (0.05 ml) by a modification of the apparatus of Martin and Ames.

Samples were analyzed for serine and threonine dehydratase activities and for protein content. There was no separation of the serine and threonine dehydratase activities in the gradient tubes (Fig. 5). Estimation of the relative molecular weight by the formula

\[
R = \left( \frac{M}{13,300} \right)^{1/3}
\]

yielded values of 21,600 in two tubes and 18,600 in a third tube in the same experiment.

**Column Electrophoresis**—This experiment was performed in an LKB electrophoresis column (LKB-3340) with an internal diameter of 1 cm. The column was filled with Geon 427 (polyvinyl chloride) generously furnished by B. F. Goodrich. The Geon
powder was suspended in 0.02 M phosphate buffer, pH 7.5, and washed by decantation until the supernatant liquor was clear. The suspension was then de-aerated in a vacuum and the column was packed to a height of 35 cm under hydrostatic pressure, with a simultaneous flow of the buffer through the column. The contents of the column were kept cooled to 0-2°C by circulation from a glycol bath connected to the cooling jacket.

To carry out the electrophoresis, the cathode and anode vessels were filled with the phosphate buffer and 10 mg of the enzyme (specific activity against serine, 1530) in 1 ml of 0.05 M phosphate buffer, pH 7.5, was introduced onto the column and moved 1 cm below the Geon surface by running the buffer down to this level. The whole apparatus was then filled with buffer, the current was connected and the voltage was adjusted to 300. The electrophoresis was continued for 16 hours and the protein was then eluted in 1-ml fractions. The experiment yielded a symmetrical peak of enzyme activity (Fig. 6), with no separation of the serine and threonine dehydratase activities.

**Determination of Pyridoxal-P Binding by Enzyme**—This was performed by the procedure of Schirch and Mason (19). The enzyme solution (40 mg of protein in 4 ml) was dialyzed against 250 ml of 0.05 M phosphate buffer, pH 7.0, containing 1 X 10⁻⁴ M pyridoxal-P, for 18 hours at 0°C. Duplicate aliquots of the dialyzed enzyme containing 5 and 10 mg of protein were pipetted out and 0.5 ml of 1 M cysteine-HCl solution (adjusted to pH 7.0) was added to make a total volume of 1.5 ml. The mixtures were then heated for 5 min each in a boiling water bath and precipitated protein was removed by centrifugation. The absorbance of the thiazoline derivative was measured in a Beckman DU spectrophotometer at 330 mp. To determine the amount of unbound pyridoxal-P present, blank values were obtained on 0.5- and 1-ml aliquots of the dialysate fluid and these were subtracted from the values of the enzyme solutions. Pyridoxal-P content was estimated from a standard curve obtained with known concentrations of the coenzyme. The analysis yielded a value of 0.93 ± 0.03 mole of pyridoxal-P per 20,000 g of enzyme.

**Michaelis Constants**—Enzymatic activities of cystathionine synthetase and of L-serine, L-threonine, d,L-allothreonine, and β-chloro-α-amino acid deamination were estimated with enzyme fractions purified by DEAE-cellulose chromatography. The effect of increasing substrate concentration on the reaction rates, for the formation of cystathionine at constant serine and increasing homocysteine concentration, and for the deamination of L-serine, L-threonine, and β-chloro-L-alanine were determined and double reciprocal plots were drawn (Fig. 7). Values of K_m and V_max were estimated from the straight lines fitted to the data in Fig. 7. From the V_max values and the protein concentrations employed in the incubations, molar turnover numbers were calculated, based on a molecular weight for the enzyme of 20,000. The kinetic constants derived from the plots are recorded in Table V.

To give more weight in the extrapolation to the higher substrate concentration values, plots were also prepared of X/V versus S,5. The kinetic constants derived from these plots agreed very well with those from the double reciprocal plots.

The K_m value for L-serine given in Table V is somewhat smaller than the one previously determined (1); the value for the homocysteine agrees with the previous one. The molar turnover numbers are quite low.

In the determination of the kinetic constants for β-chloro-L-alanine, experiments were performed at high substrate concentrations of up to 0.3 M in view of the comparatively large molar value of K_m.

Since even higher molar concentrations of substrate would be required to determine the K_m values of the β-chloro-α-amino butyric acids this was deemed impractical in view of the fact that

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**Fig. 5.** Sucrose density gradient centrifugation of L-serine and L-threonine dehydratase activities. Experimental procedure as described in text. ——, L-serine dehydratase; ——, L-threonine dehydratase; ———, cytochrome c.

**Fig. 6.** Zone electrophoresis profile of L-serine and L-threonine dehydratase activities. Experimental procedure as described in text. ——, serine dehydratase; ———, threonine dehydratase. Protein concentration was not determined because of great dilution of the enzyme. The keto acid formed is a good measure of both threonine and serine dehydratase activities, since the ratio is very close to 1 under the test conditions.

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5 Unpublished experiments.
only the racemic compounds were available. The relative rates of deamination, with respect to the rate of deamination of \( \beta \)-cholor-L-alanine, were determined at several substrate concentrations, with the results shown in Table VI. The velocity of deamination of each of the \( \beta \)-chloro-\( \alpha \)-amino butyrates is about 10% of that of \( \beta \)-cholor-L-alanine.

**Activity on Allothreonine**—Since L-allothreonine was found to be a substrate for sheep liver threonine dehydratase (20), experiments were undertaken to test the activity of the present enzyme on allothreonine. These were performed with \( \beta \)-allothreonine, the \( L \) enantiomorph not being available to us. In an experiment comparing the relative rates of decomposition of L-threonine and DL-threonine at equal concentrations of substrate \( 8.3 \times 10^{-5} \) M and enzyme (78 and 155 units per 3 ml), the rate of decomposition of allothreonine was between 3 and 5% that of L-threonine.

In another experiment, the rate of decomposition of allothreonine was determined as a function of the substrate concentration with 20 times the amount of enzyme used for the other hydroxyamino acids. A double reciprocal plot of the data is included in Fig. 7 (Curve 5). The curve, it is seen, passes through a minimum. This is a consequence of the fact that the reaction rate shows a plateau at the allothreonine concentration of \( 3 \times 10^{-4} \) M and starts decreasing at values of over \( 5 \times 10^{-4} \) M.

**Formation of Cystathionine**—In previous studies from this laboratory (1), the cystathionine synthetase activity was estimated by measuring the amount of serine consumed in the presence of homocysteine over and above the amount converted to pyruvate. We have now isolated and identified the product as cystathionine.

L-Serine-\( \text{\textsuperscript{3}} \)H (200 \( \mu \)moles, 2500 cpm per \( \mu \) mole) and 250 \( \mu \)moles of \( \text{\textsuperscript{3}} \)H homocysteine were incubated with 300 units of enzyme in a total volume of 3 ml of 0.1 M potassium borate buffer, pH 8.3; containing \( 5 \times 10^{-4} \) M pyridoxal phosphate, \( 1 \times 10^{-4} \) M EDTA; and \( 0.1 \) mg of enzyme (specific activity, 1650) and the concentrations of the amino acid chlorides shown. Incubations were run for 30 min at 37°. The reaction was stopped by the addition of 2 ml of 20% trichloroacetic acid and the keto acid formed was determined on suitable aliquots.

<table>
<thead>
<tr>
<th>Substrate concentration</th>
<th>( \beta )-Chloro-L-alanine</th>
<th>Erythro-( \beta )-chloro-( \alpha )-amino butyrate</th>
<th>Threo-( \beta )-chloro-( \alpha )-amino butyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu )mole</td>
<td>Turnover number: ( \mu )moles per 20,000 g of enzyme</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>15</td>
<td>1.23</td>
<td>1.26</td>
</tr>
<tr>
<td>40</td>
<td>19</td>
<td>1.98</td>
<td>1.02</td>
</tr>
<tr>
<td>80</td>
<td>35</td>
<td>3.51</td>
<td>3.45</td>
</tr>
<tr>
<td>160</td>
<td>54</td>
<td>6.04</td>
<td>6.04</td>
</tr>
<tr>
<td>400</td>
<td>65</td>
<td>9.48</td>
<td>9.60</td>
</tr>
</tbody>
</table>

The pooled fractions containing serine and cystathionine were evaporated to dryness in a vacuum to remove HCl. The dry residue was dissolved in water, decolorized, and crystallized from 95% ethanol. The crystalline samples were dissolved in water...
and 2 mg of each were plated on separate aluminum planchets by evaporation. Counting was performed in a gas flow Geiger-Müller counter. Counts of the samples were repeated five times and average values were taken and corrected for background. The isolated serine had 2500 cpm per pmole and the cystathionine had 2020 cpm per pmole. The specific radioactivity in the cystathionine represents 80% of that in the serine. Serine and cystathionine were identified by paper chromatography in butanol-acetic acid-water and phenol-water solvent systems, respectively, and found to be pure. Furthermore, the enzymatic product was decomposed by crystalline cystathionase. The \(\alpha\)-ketobutyric acid formed in the latter reaction, isolated as the 2,4-dinitrophenylhydrazone, had very little radioactivity; and the cystine, after separation by high voltage electrophoresis, was radioactive.

**DISCUSSION**

The great increase in the enzyme content of the livers of rats fed a high protein diet simplified the task of isolating the cystathionine-synthetase-L-serine dehydratase in a homogeneous state. This phenomenon is important from the standpoint of the inducibility of the synthesis of specific proteins in the mammalian organism. Some of the factors regulating the activity levels of the present enzyme have recently been reviewed by Pitot (21).

Goldstein, Knox, and Behrmann (3) were unable to confirm the report of Sayre, Jensen, and Greerburg (22) that \(L\)-threonine dehydratase of the sheep liver enzyme to form cystathionine.

1. The enzyme cystathionine synthetase-serine dehydratase has been isolated in a homogenous state from the livers of rats fed a high protein diet.

2. The molecular weight of the enzyme has been estimated to be about 21,000 from its sedimentation and diffusion coefficients and from ultracentrifugal equilibrium measurements by the Archibald method. This was confirmed by comparative density gradient centrifugation.

3. The enzyme has been found to catalyze the deamination of \(L\)-threonine, allothreonine, and of certain \(\beta\)-chloro-\(\alpha\)-amino acids, in addition to its previously reported activities in the synthesis of cystathionine and the deamination of \(L\)-serine.

4. Certain physicochemical properties of the enzyme are reported.

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