Purification and Properties of d-Serine Dehydrase from Escherichia coli*

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SUMMARY

A procedure is described for obtaining crystalline d-serine dehydrase from a mutant of Escherichia coli which produces this enzyme constitutively. The enzyme appears pure by ultracentrifugal and electrophoretic criteria. In sucrose gradients, serine dehydrase sediments to the same position as horseradish peroxidase, indicating a molecular weight of about 40,000. This corresponds closely to its combining weight of 42,800 per pyridoxal phosphate. Its absorption maximum at 410 nm due to bound pyridoxal phosphate is not materially changed by interaction with substrate or by variation in pH between 6.0 and 9.0. d-Serine and d-threonine are the only substrates found; O-methylserine is a potent competitive inhibitor. In the presence of suitable protective agents, the enzyme can be resolved completely by dialysis against D- or L-cysteine, and reactivated by addition of pyridoxal phosphate. Tris buffer inactivates the enzyme; this inactivation is prevented by the presence of sufficient K⁺ or NH₄⁺, and less effectively by Na⁺ or pyridoxal phosphate.

The enzymatic, nonoxidative deamination of serine to pyruvate and ammonia has been known since 1938 (1). Many different enzymes carry out this reaction with L-serine. In addition to the relatively specific L-serine dehydrases of Neurospora crassa (2), Escherichia coli (3, 4), Clostridium aciduriici (5), and sheep liver (6), this reaction is also catalyzed by apparently pure or highly purified preparations of tryptophanase (7, 8), the B protein of tryptophan synthetase (9), cystathionine synthetase (10), and threonine dehydrase (11). Most of these enzymes have been shown to require pyridoxal phosphate as a coenzyme.

Cell-free pyridoxal phosphate enzymes that carry out the corresponding deamination of d-serine to pyruvate and ammonia but do not attack L-serine were first obtained from E. coli by Metzler and Snell (4) and from N. crassa by Yanofsky (12). We report here the preparation of crystalline d-serine dehydrase (d-serine hydrolyase (deaminating), EC 4.2.1.14) from E. coli, and some of its properties. Labow and Robinson (13) report similar findings in a parallel article.

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Table I

Activities of wild-type and mutant strains of E. coli as source of d-serine dehydrase

<table>
<thead>
<tr>
<th>Additions to growth medium*</th>
<th>Wild-type strains</th>
<th>Constitutive mutants† of E. coli W 3828</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>of cell-free extracts</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. coli B</td>
<td>E. coli W 3828</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>d-Serine</td>
<td>0.50</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* d-Serine (2 mg per ml) was added to the growth medium where indicated. The growth conditions are those described in the text except that acid-hydrolyzed casein was omitted from the growth medium.

† We are indebted to Dr. Elizabeth McFall for these constitutive mutants.

Table II

Purification of d-serine dehydrase

<table>
<thead>
<tr>
<th>Purification step*</th>
<th>Volume</th>
<th>Protein</th>
<th>Specific activity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>mc</td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>1. Crude extract</td>
<td>800</td>
<td>56,000</td>
<td>0.85</td>
<td>(100)</td>
</tr>
<tr>
<td>2. Protamine filtrate</td>
<td>1,100</td>
<td>15,500</td>
<td>3.0</td>
<td>96</td>
</tr>
<tr>
<td>3. Treatment at 55°</td>
<td>300</td>
<td>5,400</td>
<td>7.0</td>
<td>68</td>
</tr>
<tr>
<td>4. Hydroxylapatite 1</td>
<td>280</td>
<td>60</td>
<td>4.5</td>
<td>45</td>
</tr>
<tr>
<td>5. DEAE-cellulose</td>
<td>60</td>
<td>300</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>6. Hydroxylapatite 2</td>
<td>3</td>
<td>15</td>
<td>300</td>
<td>8</td>
</tr>
</tbody>
</table>

* The numerals correspond to the description in the text.
FIG. 2. Crystalline n-serine dehydrase (X 2400)

FIG. 3. Migration of purified n-serine dehydrase in starch gel. Each sample contained 400 µg of protein and was subjected to electrophoresis at about pH 8.0 as described by Barrett, Frieson, and Astwood (22). A, crystalline bovine serum albumin; B, crystalline n-serine dehydrase.

Electrophoretic and Sedimentation Properties

Crystalline n-serine dehydrase migrates as a single band in starch gel electrophoresis at pH 8.3 (Fig. 3), and sediments as a single component (s_{20,w} = 3.34S) in the analytical ultracentrifuge (Fig. 4). On centrifugation in a sucrose gradient, good separation from yeast alcohol dehydrogenase was achieved; horseradish peroxidase and serine dehydrase sedimented at identical rates (Fig. 5). From the s_{20,w} values reported (23) for alcohol dehydrogenase of 7.6 to 7.2, s_{20,w} values for serine dehydrase of 3.8 to 3.36 may be calculated (17). These values are similar to those found by use of the analytical ultracentrifuge and also with those reported for peroxidase, which vary from 3.85 to 3.48 (24). The approximate molecular weight calculated (25) for serine dehydrase varies with the standard chosen. Because serine dehydrase and peroxidase sediment identically, the value of 40,000 found for the latter enzyme (24) appears most reasonable; this differs substantially from the value of 58,000 calculated on the assumption (23) of an s_{20,w} value of 7.4 and a molecular weight of 150,000 for yeast alcohol dehydrogenase.

Spectrophotometric Behavior and Pyridoxal Phosphate Content

The spectrum of n-serine dehydrase (Fig. 6) shows a pronounced absorption maximum between 405 and 415 nm, indicating that the formyl group of bound pyridoxal-P forms an azomethine link to an amino group of the protein, as in other pyridoxal-P enzymes so far studied. In contrast to glutamate dehydrogenase (26) and to aspartate-glutamate transaminase (27), this spectral band is not affected by variations in pH between 6.0 and 9.0 (Fig. 6), but changes are observed between 6.0 and 3.5 µm. On addition of substrate, an intense absorption maximum near 317 nm appears, a result of pyruvate (cf. Reference 28) formation by enzymatic action; no obvious change in spectrum of the enzyme itself was observed. At pH values below 5.5, denatured protein begins to precipitate and the spectrum of free pyridoxal-P appears in the supernatant solution. Quantitative measurement of pyridoxal-P released by heating the crystalline enzyme for 20 min at 60° with phenylhydrazine reagent (16), or by heating with cysteine and measuring the amount of pyridoxal-P thiazolidine released by its extinction at 330 nm (29), both showed the presence of 0.0233 µmole of pyridoxal-P per mg of protein, corresponding to the presence of 1 mole of pyridoxal-P per 42,800 g of protein. This value corresponds closely with the approximate molecular weight of 40,000 obtained by comparing the sedimentation of serine dehydrase with that of peroxidase in a sucrose gradient. Likely the recently described cystathionine synthetase (10), n-serine dehydrase is unusual among pyridoxal-P enzymes in possessing only a single binding site for pyridoxal-P per molecule. Pyridoxal-P is not fully released from the enzyme by precipitation with trichloroacetic acid or treatment with the phenylhydrazine reagent at room temperature.

Substrate Specificity

n-Serine is the preferred substrate for the enzyme, but n-threonine is attacked at a lower rate (Table III). Through its...
Fig. 4. Sedimentation patterns of D-serine dehydrase after (from 1 to 4) 16, 26, 56, and 80 min at 59,780 rpm in the Spinco model E analytical centrifuge. Photographs were taken at bar angles of 75°, 75°, 70°, and 70°, respectively. The sample cell contained 1.0% protein in 0.1 M potassium phosphate buffer, pH 7.8. The blank cell contained buffer only.

Fig. 5. Separation of yeast alcohol dehydrogenase (ADH, 0.08 mg), horseradish peroxidase (Worthington, 0.68 mg), and D-serine dehydrase (SDH, 0.08 mg) by centrifugation in a sucrose gradient. Centrifugation was performed for 11 hours at 38,000 rpm and 0° in the SW-39 rotor of the Beckman model L centrifuge. The substrate for peroxidase was o-dianisidine. For alcohol dehydrogenase and peroxidase, the plotted values represent the change in absorbance at 340 m
 and at 400 m
, respectively, per 10 μl of sample per 30 sec; for D-serine dehydrase they represent micromoles of pyruvate produced per ml of sample in 10 min.

Fig. 6. Variation in spectrum of D-serine dehydrase with pH. Cells contained 3.5 mg of protein (specific activity, 230) per ml of 0.1 M potassium phosphate buffer at the indicated pH. The curves have been offset slightly to show the identity in shape of the absorption curve above 400 m
. The absorbance at 410 m
 was identical in the three cases.

The optimum pH for action of D-serine dehydrase is 7.8 to 8.0 (Fig. 7A). As reported previously (4), Tris buffer inhibits the enzyme (Fig. 7A), but simultaneous additions of sufficient K+ or NH4+ were found to prevent this inhibition (Fig. 7B) for lactate dehydrogenase, the error in determination of the Kᵢ value for L-threonine is small (cf. Reference 30).
almost completely. Na⁺ is much less effective. The nature of this protective effect of monovalent cations is not understood; tryptophanase (7, 31), the B protein of tryptophan synthetase (9), and the l-serine and l-threonine dehydrases of mammalian livers (11)—all of which are pyridoxal-P enzymes and catalyze α,β elimination or addition reactions—all are activated to a greater or lesser extent by K⁺ or NH₄⁺. With d-serine dehydrase the effect may be related in some way to the binding of pyridoxal-P, for immediate addition of pyridoxal-P to a Tris-enzyme preparation partially protects against inhibition, although less effectively than K⁺. If the enzyme is incubated in

<table>
<thead>
<tr>
<th>Table III</th>
<th>Substrate specificity of d-serine dehydrase*</th>
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</thead>
<tbody>
<tr>
<td>Substrates</td>
<td>D-Threonine</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------</td>
</tr>
<tr>
<td>D-Serine</td>
<td>Kₘ</td>
</tr>
<tr>
<td>-----------</td>
<td>-----</td>
</tr>
<tr>
<td>D-Threonine</td>
<td>1.3</td>
</tr>
<tr>
<td>O-Methyl-l-serine</td>
<td>3.4</td>
</tr>
</tbody>
</table>

* The following compounds were found to be inactive: L-serine, L-threonine, D-cysteine, D-l-homoserine, D-tryptophan, D-alanine, and D-aspartate. These compounds were neither substrates nor inhibitors of d-serine degradation when assayed under standard conditions at concentrations up to 13.2 mM. At higher concentrations (0.1 M), several amino acids (glycine, D,L-allo-threonine, L-alanine, D,D-alanine, D-methyl-D,L-serine, D,L-homoserine) inhibit; such inhibitors are more effective when D-threonine replaces D-serine as substrate.

† Tested with d-serine (1.32 mM) as substrate.

![Figure 8](http://www.jbc.org/) Reactivation of d-serine apodehydrase by pyridoxal-P (PLP). The enzyme was dialyzed for 54 hours against 0.04 M cysteine plus 0.02 M mercaptoethanol, and then assayed. Standard assay conditions were followed except for addition of graded amount of pyridoxal-P.

**Resolution and Reconstitution of d-Serine Dehydrase**

In the absence of added pyridoxal-P, both L- and D-cysteine at moderate concentrations (0.005 to 0.02 M) and pH 7.8 rapidly inactivate d-serine dehydrase, even though potassium phosphate is present. This inactivation is prevented by added pyridoxal-P. After dialysis against potassium phosphate buffer, such inactivated preparations no longer exhibit the spectrum of a pyridoxal-P protein, showing that the prosthetic group has been removed. However, no reactivation by added pyridoxal-P is obtained. This inactivity appears to result from sensitivity of the resolved enzyme to denaturation and oxidation, for if small amounts (2 mg per ml) of bovine serum albumin are added, the enzyme can be completely resolved by dialysis for several days against 0.04 M D,L-cysteine plus 0.02 M mercaptoethanol, and activity can be fully restored by addition of pyridoxal-P (Fig. 8). Half-maximum activity under these conditions is restored by 0.0014 μM pyridoxal-P, but this figure probably underestimates the true affinity of apoenzyme for its coenzyme because of the presence of cysteine.

**REFERENCES**

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