Purification, Separation, and Characterization of Two Molecular Forms of D-1-Amino-2-propanol:NAD⁺ Oxidoreductase Activity from Extracts of Escherichia coli K-12*

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D-1-Amino-2-propanol:NAD⁺ oxidoreductase activity, which catalyzes the second step in a pathway where L-threonine is converted to D-1-amino-2-propanol via the intermediate formation of aminoacetone, has been purified 500-fold from Escherichia coli K-12. Although the enzyme catalyzes the oxidation of certain diols as well as 1-amino-2-propanol, it is completely specific for the D-isomer of the amino alcohol and for NAD⁺. Two molecular forms (designated Form L and Form S) of the oxidoreductase, both of which are catalytically active, have been separated by gel filtration on Sephadex G-200; apparently, Form L is converted to Form S by dissociation (Form L → Form S). Molecular weight determinations indicate that the two forms of the enzyme are different not only in size but also in shape; Form L apparently is an asymmetric tetramer of Form S.

The two molecular species have similar catalytic properties. Both exhibit the same pH optimum of 8.6, have nearly identical apparent Km values for substrate and cosubstrate, are equally sensitive to inhibition by p-mercuribenzoate and N-ethylmaleimide, and show the same specificity for cosubstrate. Neither form of the enzyme has an absolute requirement for added thiol compounds or divalent metal ions.

1-Amino-2-propanol serves as a bridge between the corrinoid system and the dimethylbenzimidazole ribonucleotide moiety of the vitamin B₁₂ molecule. Only the D-isomer of the amino alcohol is a component of the vitamin (1). How, on the enzyme level, this piece of the total molecule is biosynthesized remains unknown.

Isotope studies with growing cultures of microorganisms that biosynthesize vitamin B₁₂ have clearly shown that the amino group of the vitamin is derived ultimately from L-threonine (3-5). Neuberger and Tait (6) suggested that D-1-amino-2-propanol might be formed from L-threonine, not by direct decarboxylation as suggested by others (2), but by sequential action of two separate enzymes. The paper describes the purification and characterization of D-1-amino-2-propanol oxidoreductase activity. It has been possible to separate two catalytically active forms of the enzyme; the one form appears to arise by dissociation of the other. The two forms are similar in a number of catalytic properties but are markedly different in molecular size.

EXPERIMENTAL PROCEDURES

Materials

The individual D- and L-isomers of 1-amino-2-propanol were synthesized by chemical decarboxylation of either D- or L-threonine, respectively, in the presence of acetophenone (19) and purified by chromatography on columns of Dowex 50 (H⁺ resin). Ascending paper chromatographic techniques in the following solvent systems established their identity and purity: ethanol/water (7:3), Rf = 0.65; methanol-butanol-1:benzene/water (2:1:1), Rf = 0.56; butanol-1-glacial acetic acid/water (77:6:17), Rf = 0.22; chloroform/methanol/17% aqueous ammonia (2:2:1), Rf = 0.86; butanol-1 saturated with 0.1% aqueous ammonia, Rf = 0.38. Compounds were detected by ninhydrin spray and, in every solvent, migrated as single ninhydrin-positive spots with Rf values identical with reference DL-1-amino-2-propanol. The two isomers were also identified by the melting points of their crystalline hexachloroplatinate salts (20) and by specific rotation values of both the free amino alcohols and corresponding hexachloroplatinate salts: D-1-amino-2-propanol HCl (2.18 g/100 ml of H₂O), [α]D⁰ = -22.8°; L-1-amino-2-propanol HCl (2.92 g/100 ml of H₂O), [α]D⁰ = +28.6°; D-hexachloroplatinate salt (2.5 g/100 ml of H₂O), [α]D⁰ = +28.6°; L-hexachloroplatinate salt (3.0 g/100 ml of H₂O), [α]D⁰ = +12.6°. Literature values are D-1-amino-2-propanol HCl (1% in methanol), -31.5°; L-1-amino-2-propanol HCl (1% in methanol), +35° (21); D-hexachloroplatinate salt, -31.5° (22); L-hexachloroplatinate salt, -12.2° (22). Aminoacetone·HCl was synthesized by the method of Schudy et al. (23). In the calorimetric determination of this aminoacetone, our prepared compound and a commercial sample of aminoacetone·HBr (K & K Laboratories) gave essentially identical standard curves.

E. coli K-12 cells were grown at 37°C in a New Brunswick Ferracin Filtermator in 100- to 200-liter volumes with aeration. The medium was modified from that of Fraser and Jerrel (24) and contained the following materials (w/v): 0.84% Na₂HPO₄, 0.45% NaH₂PO₄, 0.15% NH₄Cl, 0.03% MgSO₄, 1.7% glucose, and 0.25% casein hydrolysate. After 18 to 20 h, bacterial cells were harvested in stationary phase with a Sharples centrifuge and washed once by suspending in 0.05 M Tris HCl buffer (pH 8.4). The cells were stored at -20°C.
Purification of 3-Deino-2-propanol: NAD⁺ Oxidoreductase Activity of E. coli

**Results**

All operations were carried out at 4°C unless otherwise noted. All buffers contained 1 mM 2-mercaptoethanol.

**Step 1**—Frozen cells (400 g, wet weight) of *E. coli* K-12 were suspended in 2 liters of 0.05 M Tris-HCl buffer (pH 8.4) and disrupted by sonic oscillation. Sonication was carried out for a total of 4 h, in 12 separate 20-min bursts, using a Branson Sonifier, model 575. The temperature of the mixture was kept below 20°C during this process by cooling in an ice-salt bath following each burst of power. The resulting extract was centrifuged at 21,500 × g for 30 min and the precipitate discarded. DNase I (2.4 mg), RNase A (2.4 mg), and RNase T₁ (20 µg) were then added to the supernatant fluid and the solution stirred for 45 min at room temperature.

**Step 2**—After the enzyme-treated extract was cooled to 4°C, 100-ml portions were transferred to a round-bottomed flask and heated in a 90°C water bath with constant stirring until the solution temperature reached 78°C. The flask was then immediately plunged into an ice-water bath and stirring was continued until the solution temperature dropped to 4°C. The precipitate of denatured protein was removed by centrifuging at 21,500 × g for 1 h and discarded.

**Step 3**—Crystalline ammonium sulfate was added slowly with stirring to the supernatant fluid until the salt concentration was 20% saturation (111 g/liter, initial volume). The mixture was stirred for 30 min and centrifuged at 20,000 × g for 40 min. Crystalline ammonium sulfate was then added with stirring to the supernatant fluid until the salt concentration was 50% saturation (185 g additional/liter of original volume). This mixture was stirred for 1 h and then centrifuged at 20,000 × g for 1 h. The precipitate obtained was dissolved in 30 ml of 0.01 M Tris-HCl buffer (pH 8.4) and the resulting solution dialyzed for 18 h against 5 liters of the same buffer. Any precipitate which formed during dialysis was removed by centrifugation.

**Step 4**—The dialyzed enzyme solution was applied to a calcium phosphate-cellulose column (3 × 22 cm), prepared by the method of Massey (36), which had been equilibrated with 1 liter of 0.01 M Tris-HCl buffer (pH 8.4). The column was first washed with 100 ml of the same buffer. The enzyme was eluted with a nonlinear gradient (0 → 10%) of ammonium sulfate in 0.01 M Tris-HCl buffer (pH 8.4). Fractions (10 ml) were collected and those containing maximum enzyme activity (tubes 58 to 66) were pooled. The protein was concentrated by precipitation with ammonium sulfate (413 g/liter, initial volume). The precipitate was dissolved in 3 ml of 0.01 M Tris-HCl buffer (pH 8.4) and the resulting solution dialyzed for 18 h against 5 liters of the same buffer. Any precipitate which formed during dialysis was removed by centrifugation.

### TABLE I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Total protein</th>
<th>Specific activity</th>
<th>Total units</th>
<th>Recovery</th>
<th>Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Extract treated with DNase and RNase</td>
<td>2.140</td>
<td>46,224</td>
<td>146</td>
<td>6.75</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>II. Heated at 78°C</td>
<td>1,950</td>
<td>8,970</td>
<td>502</td>
<td>4.50</td>
<td>67</td>
<td>3</td>
</tr>
<tr>
<td>III. (NH₄)₂SO₄ precipitate (20 to 50% saturation)</td>
<td>38</td>
<td>585</td>
<td>6,500</td>
<td>3.80</td>
<td>56</td>
<td>45</td>
</tr>
<tr>
<td>IV. Calcium phosphate-cellulose eluate; 0 to 65% (NH₄)₂SO₄ precipitate</td>
<td>2.9</td>
<td>30</td>
<td>69,400</td>
<td>2.08</td>
<td>31</td>
<td>475</td>
</tr>
</tbody>
</table>

**Methods**

Protein was measured by the method of Lowry et al. (26) with crystalline bovine serum albumin as standard. Protein in column eluates was estimated by absorbance at either 280 or 230 nm.

Purification of the enzyme was followed by absorbance at 340 nm. NAD⁺ was estimated by absorbance at either 280 or 230 nm.

**Step 4**—The dialyzed enzyme solution was applied to a calcium phosphate-cellulose column (3 × 22 cm), prepared by the method of Massey (36), which had been equilibrated with 1 liter of 0.01 M Tris-HCl buffer (pH 8.4). The column was first washed with 100 ml of the same buffer. The enzyme was eluted with a nonlinear gradient (0 → 10%) of ammonium sulfate in 0.01 M Tris-HCl buffer (pH 8.4). Fractions (10 ml) were collected and those containing maximum enzyme activity (tubes 58 to 66) were pooled. The protein was concentrated by precipitation with ammonium sulfate (413 g/liter, initial volume). The precipitate was dissolved in 3 ml of 0.01 M Tris-HCl buffer (pH 8.4) and the resulting solution dialyzed for 18 h against 5 liters of the same buffer. Any precipitate which formed during dialysis was removed by centrifugation.

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The enzyme preparation contains two molecular forms of the oxidoreductase, both catalytically active, which can be separated in the following manner. A sample of the enzyme (from Step 4, Table I) was applied to a column (2.5 x 53 cm) of Sephadex G-200 which had been equilibrated with 0.05 M Tris·HCl buffer (pH 8.4). The same buffer solution was passed through the column using a peristaltic pump running at 8 ml/hr. Fractions of 1.2 ml were collected. The elution pattern of the two forms of the enzyme is shown in Fig. 1. In each instance, those fractions showing highest activity were pooled and the protein concentrated by ultrafiltration with a PM-10 membrane under 30 p.s.i. of pressure (nitrogen gas) in an Amicon diaflow apparatus. Any precipitate that formed during this concentrating procedure was removed by centrifugation and discarded. Typical data received in this procedure for the separation of the two forms of the oxidoreductase are listed in the legend of Fig. 1. For convenience, we have designated the two enzymatically active forms of n-1-amino-2-propanol oxidoreductase; second (R, = 0.21) yields the same two zones of enzyme activity (R, = 0.26 and 0.75) when again electrophoresed with separating gels at pH 9.5. In contrast, the protein corresponding to the lower band (R, = 0.75) on the pH 9.5 gel system maintains its integrity when subjected to electrophoresis a second time at either pH 9.5 or 8.0; only one dehydrogenase-active zone (positioned at R, = 0.71 and 0.59, respectively) is observed in each case.

When Form L of the oxidoreductase (prepared by chromatography on Sephadex G-200 described earlier) is subjected to polyacrylamide gel electrophoresis in the pH 9.5 gel system, two zones of enzymatic activity (R, values = 0.26 and 0.75) are seen. When Form S is prepared and examined in the same manner, however, only one band of dehydrogenase activity (R, = 0.75) is observed. Gel filtration chromatography, therefore, was also used to examine the molecular stability of the two forms of the enzyme. For this purpose, a column (1.6 x 40 cm) of Sephadex G-200 was equilibrated with 0.05 M Tris·HCl buffer (pH 8.4) containing 1 mM 2-mercaptoethanol. A sample (0.5 mg) of the enzyme (from Step 4, Table I), containing a mixture of Forms L and S, was applied to the column and eluted with the same buffer-mercaptoethanol mixture. After the column was exhaustively washed with the buffer mixture, 2 mg of the separated Form L of the enzyme were applied to the same column and eluted as before. As shown in Fig. 3, Form S of the enzyme is not derived from Form L under these experimental conditions; no enzyme activity applied as Form L to the column is eluted in fractions corresponding to Form S. No indication of a conversion of Form S to Form L has been observed in experiments using the techni-
P-mercuribenzoate, whereas N-ethylnalineimide and iodoacetate are considerably less effective. The inhibition caused by 0.315 \( \mu \)m p-mercuribenzoate is completely reversed by the subsequent addition of excess (0.70 mm) 2-mercaptoethanol or dithiothreitol. In tests of this sort, only small differences in the extent of inhibition and the degree of recovery of activity are seen between the two molecular forms of the enzyme.

The effect of metal ions and chelating agents was studied with enzyme (8 \( \mu \)g of Form L and 6 \( \mu \)g of Form S) that was first exhaustively dialyzed against 0.05 M Tris-Cl buffer (pH 8.4) containing 1 mM 2-mercaptoethanol plus 0.1 mM EDTA, and then exhaustively dialyzed against the same buffer solution containing no EDTA. Preliminary incubation of the two dialyzed samples of the enzyme with the following cations (0.063 M or 3.2 M, final concentrations, all tested as their chloride salts) has no stimulatory effect on catalytic activity: Mg\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\), Ca\(^{2+}\), Co\(^{2+}\), Cu\(^{2+}\), Ni\(^{2+}\), Fe\(^{3+}\), K\(^{+}\), or Na\(^{+}\). Rather than stimulating activity, several cations caused significant inhibition of both forms of the enzyme when added at the higher concentration (i.e. Zn\(^{2+}\), 100%; Ni\(^{2+}\), 90%; Mn\(^{2+}\), 70%; Co\(^{2+}\), 65%; Cu\(^{2+}\), 60%). Furthermore, direct addition of metal-chelating agents to the dialyzed enzyme samples caused considerable inhibition of activity. When reaction mixtures containing enzyme (9.6 \( \mu \)g of Form L or 12 \( \mu \)g of Form S) were subject to a prior incubation at 37\( ^\circ \)C for 10 min with 0.63 mM EDTA, 8-hydroxyquinoline, or o-phenanthroline before substrates were added and Assay I performed in the normal manner, 70%, 90%, and 90% inhibition, respectively, is observed. These effects are not reversed by adding an excess of various metal ions (such as Mg\(^{2+}\) or Ca\(^{2+}\)). Hence, although a rather complex pattern of inhibitory effects is seen with metal-chelating agents as well as some metal ions, no definitive evidence could be obtained suggesting that either molecular form of the oxidoreductase requires a metal ion for activity.

### Table II

Inhibition of the two molecular forms of D-L-amino-2-propanol oxidoreductase activity by sulfhydryl-reacting reagents

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Final Concentration</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu )M</td>
<td>Form L</td>
</tr>
<tr>
<td>None (dialyzed enzyme)</td>
<td>3.52</td>
<td>5.97</td>
</tr>
<tr>
<td>p-Mercuribenzoate</td>
<td>0.063</td>
<td>3.41</td>
</tr>
<tr>
<td></td>
<td>0.032</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>0.063</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>0.315</td>
<td>0.21</td>
</tr>
<tr>
<td>N-Ethylmaleimide</td>
<td>0.63</td>
<td>3.03</td>
</tr>
<tr>
<td></td>
<td>6.30</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>31.5</td>
<td>0.18</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>6.300</td>
<td>3.03</td>
</tr>
<tr>
<td></td>
<td>31.500</td>
<td>1.90</td>
</tr>
</tbody>
</table>
TABLE III
Substrate specificity of the two molecular forms of D-1-amino-2-propanol oxidoreductase activity

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Form L</th>
<th>Form S</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol NADH/2 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL-1-Amino-2-propanol</td>
<td>6.1</td>
<td>6.4</td>
</tr>
<tr>
<td>D-1-Amino-2-propanol</td>
<td>5.1</td>
<td>5.8</td>
</tr>
<tr>
<td>L-1-Amino-2-propanol</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DL-1,2-Propanediol</td>
<td>18</td>
<td>42</td>
</tr>
<tr>
<td>2,3-Butanediol</td>
<td>7.1</td>
<td>9</td>
</tr>
<tr>
<td>Glycerol</td>
<td>4.8</td>
<td>14</td>
</tr>
<tr>
<td>3-Amino-1,2-propanediol</td>
<td>0.8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Including ethanol, 1-propanol or 2-propanol, or the diols, 1,3-propanediol and 1,3-butanediol. Similarly, the amino alcohol analogs, L-2-amino-1-propanol or 3-amino-1-propanol, are not utilized as substrates by either form of the oxidoreductase. Earlier studies (17) showed that DL-lactate, L-serine, L-threonine, β-hydroxybutyrate, DL-homoserine, DL-γ-amino-β-hydroxybutyrate, and 2-aminoethanol are not substrates. Only NAD⁺ functions as cosubstrate for either form of the enzyme; using 1-amino-2-propanol as substrate, no activity is observed with NAP⁺ or 3-acetylpyridine-NAD⁺.

The concentration effects of 1-amino-2-propanol and NAD⁺ on enzyme activity were measured and apparent Kₐ values were calculated by the method of Lineweaver and Burk (37). Dehydrogenase activity was measured colorimetrically by Assay I wherein 200 μmol of sodium glycylglycinate (pH 8.4) served as buffer. Initial velocities were calculated as the nmol of aminoacetone formed in 30 min at 37°C in 1 ml of incubation mixture. As is evident in the following values, only small differences are observed when the apparent Michaelis constants for the individual substrates are compared for the two molecular forms of the oxidoreductase: Form L, Kₐ = 2.5 × 10⁻³ M (DL-1-amino-2-propanol), Kₐ = 1.4 × 10⁻³ M (NAD⁺); for Form S, Kₐ = 4.0 × 10⁻² M (DL-1-amino-2-propanol), Kₐ = 1.1 × 10⁻² M (NAD⁺).

Molecular Weight Determinations—Electrophoretic studies, noted earlier, indicated that the two forms of D-1-amino-2-propanol oxidoreductase activity from E. coli K-12 might be the result of an aggregate (Form L) dissociating into a smaller molecular form (Form S), with both species still maintaining catalytic activity. Examination of the relative molecular size of each of the two forms (L and S) of the oxidoreductase was relevant in this regard. The methods of gel filtration, sucrose density centrifugation, and polyacrylamide gel electrophoresis were chosen for this purpose.

For gel filtration (38), a column (2.6 × 50 cm) of Sephadex G-200 was equilibrated at 4°C with 10 mM potassium phosphate buffer (pH 7.5) containing 0.1 M potassium chloride and 1 mM 2-mercaptoethanol; a flow rate of approximately 10 ml/h was maintained. Fig. 5 shows a linear relationship between the elution volumes and molecular weights for several reference proteins and the two forms of the oxidoreductase. By this procedure, the molecular weight of Form L is estimated to be 380,000 while that of Form S is 87,100.

The method of Hedrick and Smith (39) was used to estimate molecular weights by polyacrylamide gel electrophoresis. Fig. 6B shows the lines and slopes obtained for the two forms (L and S) of the oxidoreductase when the log of the R₅₀ value (migration relative to the dye front) is plotted against the percentage of acrylamide monomer present in the gel employed. Fig. 6A presents the type of calibration curve one obtains by use of a number of standard proteins. By this method, the molecular weight of Form L is estimated to be 426,000 while that for Form S is 86,000.

Three samples of D-1-amino-2-propanol oxidoreductase were examined by sucrose density gradient centrifugation (30). One sample contained a mixture of Forms L and S (Fig. 7A) whereas the other samples consisted of one of the two separated forms of the enzyme (Form L in Fig. 7B; Form S in Fig. 7C). The sedimentation profiles of the three enzyme samples and of internal reference proteins of known molecular weights are shown in Fig. 7, A, B and C. By this technique, the average molecular weight for Form L is estimated to be 291,000 while
the amino alcohol moiety of vitamin B12. The two dehydro-
genase activities required in such a pathway have also been
synthesizing vitamin B12 de novo catalyzes the second step in the proposed pathway) 500-fold
D-I-amino-2-propanol:NAD+ oxidoreductase activity (which
corrinoids from precursors simpler than cobinamide (which
already contains the D-1-amino-2-propanol moiety) (42-44),
contrariwise Muller et al. (5) showed that cell suspensions of
beef liver catalase, and 150 ag of rabbit muscle lactate dehydrogenase
2-mercaptoethanol, were carefully layered on the gradient solutions.
Moreover, the molecular weight of each of the two forms of the oxidoreduc-
tase are molecularly stable under certain conditions after they
are separated from each other; only one peak of enzyme
activity is seen for each of the two forms of the enzyme after
being sedimented. Moreover, the molecular weight of each of
the two forms of the oxidoreductase (Gradient B, 289,500; Gradient C, 81,200) are nearly identical with those values
obtained when both forms (L and S) of the enzyme are
centrifuged as a mixture (Gradient A; 293,000 and 84,850,
respectively). The following amounts of oxidoreductase were used in Gradients
A, B, and C, respectively: 1.2 mg of a mixture of Forms L and S; 0.48 mg of Form L; 0.29 mg of Form S. The distance of sedimentation of each
protein from the meniscus was measured graphically. Catalase
activity (M) was determined by Assay I.

DISCUSSION

Whether the pathway, L-threonine \( \rightarrow \) aminoacetone \( \rightarrow \) D-1-
amino-2-propanol (first suggested by Neuberger and Tait (6)),
actually operates in corrinoid biosynthesis remains to be
determined. Some results suggest it does not (see Ref. 40) but
contrariwise Muller et al. (5) showed that cell suspensions of
Propionibacterium shermanii incorporate D-1-amino-2-[U-\( ^{14} \)C]propanol as well as L-[U-\( ^{14} \)C]threonine specifically into the
amino alcohol moiety of vitamin B12. The two dehydro-
genase activities required in such a pathway have also been
found in extracts of several microorganisms capable of syn-
thesizing vitamin B12 de novo (41).

A method is described in this paper for the purification of
D-1-amino-2-propanol:NAD\(^+\) oxidoreductase activity (which
catalyzes the second step in the proposed pathway) 500-fold
from E. coli K-12. It is recognized that E. coli cannot make
corrinoids from precursors simpler than cobinamide (which
already contains the D-1-amino-2-propanol moiety) (42-44),
but the possibility exists of E. coli having vestigial enzymes of
a complete pathway functional in known vitamin-producing
cells. A surprising feature uncovered in this study is that D-1-
amino-2-propanol oxidoreductase activity occurs in two dif-
ferent molecular, yet catalytically active, forms. Disruption of
cells by diverse procedures, including lysozyme treatment,
sonication, grinding with alumina, and the use of a mechanical
pressure homogenizer, uniformly gives extracts which when
passed over a column of Sephadex G-200 show only the
presence of the larger form (Form L). This suggests that Form
L exists in situ although the presence of small amounts of Form S in the cell cannot be ruled out unequivocally. A
readily reversible equilibrium between the two forms does not
appear to exist; rather, only an irreversible conversion of Form
L to Form S has so far been observed. We have found that
routine protein fractionation procedures convert some Form
L to Form S. In numerous applications of the purification
procedure outlined (Table I), no Form S is observed in the
preparation through and including Step 2 (i.e. heat treat-
ment). If, however, this preparation is fractionated by precip-
itation with either ammonium sulfate (Step 3, Table I), ace-
tone, or polyethylene glycol, or if it is adsorbed and eluted
from ion exchange columns with salt gradients, mixtures of Forms L and S are routinely observed.1 As shown in Fig. 2,
conversion of Form L to Form S also occurs at some time in
the procedure of polyacrylamide gel electrophoresis at pH 9.5
but not at pH 8.0. Possible buffer-protein interactions and/or
residual ammonium persulfate may in part explain this pH-
dependent instability of Form L in gel electrophoresis. Thus,
certain experimental conditions will induce the conversion
of Form L of the oxidoreductase to Form S. The process, how-
ever, is not spontaneous since Form S is not derived from
Form L under conditions of gel filtration (Fig. 3) and sucrose
density centrifugation (Fig. 7). Furthermore, the conversion
of Form L to Form S does not appear to be the consequence
of proteolysis since the presence of phenylmethylsulfonyl
fluoride throughout the purification and separation steps does
not prevent the formation of Form S.

When the general catalytic properties of the two forms of
the enzyme are compared, only modest differences are appar-
ent. Both forms (a) are stereospecific for the D-isomer of
D-1-amino-2-propanol and utilize only NAD\(^+\) as coenzyme, (b)
catalyze the oxidation of several diol analogs (although Form
S is somewhat more active), (c) exhibit a similar pH optimum
for Form L under conditions of gel filtration (Fig. 3) and sucrose
density centrifugation (Fig. 7). Furthermore, the conversion
of Form L to Form S does not appear to be the consequence
of proteolysis since the presence of phenylmethylsulfonyl
fluoride throughout the purification and separation steps does
not prevent the formation of Form S.

Forms L and S of the oxidoreductase are, however, signifi-
cantly different in two respects. Form S is more labile when
stored in the cold or when heated. In addition, the data show a
very marked difference in molecular weights. By molecular
sieving techniques (gel filtration and polyacrylamide gel elec-
trophoresis), a molecular weight of 380,000 and 420,000,
respectively, was found for Form L whereas corresponding val-
ues for Form S were 87,100 and 86,000, respectively. These results also substantiated a
conclusion made earlier that the two forms of the oxidoreduc-
tase are molecularly stable under certain conditions after they
are separated from each other; only one peak of enzyme
activity is seen for each of the two forms of the enzyme after
being sedimented. Moreover, the molecular weight of each of
the two forms of the oxidoreductase (Gradient B, 289,500; Gradient C, 81,200) are nearly identical with those values
obtained when both forms (L and S) of the enzyme are
centrifuged as a mixture (Gradient A; 293,000 and 84,850,
respectively).

1 Unpublished results.
erable divergence are obtained for the larger form whereas
those for Form S are in reasonably good agreement. The
conclusions reached on the basis of data in hand are that
Form L is an asymmetrically shaped tetramer of Form S and
the induced conversion of Form L → Form S is associated
with a change in molecular shape as well as in size. In spite of
such changes, however, Form S is equally if not more active
catalytically than is Form L.

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