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Identity of *Escherichia coli* D-1-Amino-2-Propanol:NAD$^+$ Oxidoreductase with *E. coli* Glycerol Dehydrogenase but Not with *Neisseria gonorrhoeae* 1,2-Propanediol:NAD$^+$ Oxidoreductase

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The properties of D-1-amino-2-propanol oxidoreductase from wild-type *Escherichia coli* have been compared with those of a glycerol dehydrogenase from mutant *E. coli* 424 and of a 1,2-propanediol oxidoreductase from *Neisseria gonorrhoeae*. Several independent lines of evidence indicate that the former two enzymes are identical. (i) Both enzymatic activities purified to virtual homogeneity in an identical manner, and the ratio of specific activities (glycerol/aminopropanol) remained constant at all stages. (ii) When electrophoresed, both purified enzymes showed a major as well as a minor band of protein coincident with activity, and these two bands from each enzyme had the same mobility. (iii) The subunit molecular weights and isoelectric points were identical for each enzyme, and (iv) kinetic constants (K_m and V_{max} values) determined wtih three different substrates were the same. The somewhat greater stability of the glycerol dehydrogenase to controlled heat denaturation at 74°C was the only difference observed between these two enzymes. In contrast, D-1-amino-2-propanol oxidoreductase was found to be immunochemically and kinetically distinct from the 1,2-propanediol oxidoreductase from *N. gonorrhoeae*.

We have been attempting to uncover and characterize the enzymes that participate in the conversion of L-threonine to the D-1-amino-2-propanol moiety of vitamin B_{12} (1–5, 14). In earlier studies (3, 5), we demonstrated that enzyme preparations from wild-type *Escherichia coli* K-12 catalyzed the stepwise conversion of L-threonine → aminoacetone → D-1-amino-2-propanol. Subsequently, we succeeded in purifying L-threonine dehydrogenase (the first required enzyme) to homogeneity from extracts of a mutant of *E. coli* (1, 2) and, thereafter, also obtained one molecular form of a D-1-amino-2-propanol:NAD$^+$ oxidoreductase (the second required enzyme) in pure form (14).

This aminopropanol oxidoreductase catalyzed the oxidation of several *vic*-diols (such as glycerol, 1,2-propanediol, and 2,3-butanediol) besides D-1-amino-2-propanol (4, 14). Since other microbial enzymes are known which catalyze the oxidation of D-1-amino-2-propanol (16, 27), glycerol (15, 22, 24), 1,2-propanediol (11, 18), and 2,3-butanediol (26), the question regarding the similarity or differences of these proteins remained unanswered. Glycerol dehydrogenase of *E. coli* (strain 424) (24) and 1,2-propanediol:NAD$^+$ oxidoreductase of *Neisseria gonorrhoeae* (18) seemed especially suited for study since both have been purified to homogeneity and several of their properties established. This paper presents evidence, both enzymatic and physical, for the identity of the D-1-amino-2-propanol:NAD$^+$ oxidoreductase we previously purified and characterized from wild-type *E. coli* with the glycerol dehydrogenase of *E. coli* (mutant strain 424) isolated and studied by E. C. C. Lin. In contrast, the 1,2-propanediol:NAD$^+$ oxidoreductase of *N. gonorrhoeae* is a different enzyme.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. All cells were grown at 37°C. To purify D-1-amino-2-propanol oxidoreductase, wild-type *E. coli* K-12 (strain W-1485) obtained from D. L. Oxender was grown on modified Fraser and Jerrel medium (6) as previously described (14). For isolating glycerol dehydrogenase, E. C. C. Lin (Harvard Medical School) graciously provided *E. coli* K-12 (strain 424). This strain was grown for 30 h with shaking (150 r.p.m.) in four 2.8-liter Fernbach flasks, each containing 1 liter of medium consisting of buffer, salts, and casein hydrolysate (20 g/liter) as the sole carbon source (24). Cells were harvested by centrifugation, washed twice with prep solution (50 mM NaCl, 1 mM 2-mercaptoethanol, 0.02% sodium azide), and stored frozen; yield = 19.2 g (wet weight)/liter of medium.

Purification or source of enzymes. Crude extracts of the above two strains of *E. coli* K-12 were individually fractionated by a three-step procedure slightly modified from that outlined in detail earlier (14). Briefly, ca. 20 g (wet weight) of cell paste was suspended in 80 ml of prep solution and disrupted by sonic oscillation for 20 min at 0°C. After this suspension was centrifuged at 21,000 × *g* for 40 min, the supernatant fluid was heated with constant stirring to 78°C in a 90°C water bath (total time of heating, ca. 5 min) and then cooled quickly to 40°C; the denatured protein was removed by centrifuging as before. The supernatant fluid so obtained was concentrated to 20 ml by ultrafiltration. This concentrated solution was applied to a column (2.5 by 93 cm) of Bio-Gel A-0.5m that had been previously equilibrated with prep solution. The column was subsequently washed with prep solution, and the eluate was collected in 5-ml fractions. The eluted protein peak containing D-1-amino-2-propanol oxidoreductase (or glycerol dehydrogenase) activity (fractions 38 to 60) was concentrated to ca. 20 ml as before and then dialyzed for 18 h against 5 liters of prep solution. Finally, the enzyme sample was applied to a column (1.6 by

TABLE 1. Composition of enzyme assay systems

Assay[a] no. and buffer	Amt (μmol) of:			Temp (°C)	Reference
	NAD^+	Substrate	NH_4Cl		
1. 100 μmol of Tris-HCl (pH 8.4)	5	100	0	37	4
2. 100 μmol of glycylglycine (pH 8.4)	5	0.5–500	0	37	4
3. 125 μmol of CAPS[b]–NaOH (pH 10.0)	5	100	10	37	24
4. 100 μmol of Tris-HCl (pH 9.0)	5	0.5–500	250	25	18

[a] Total volume of each assay mixture was 1.0 ml.
[b] CAPS, Cyclohexylaminopropanesulfonic acid.

25 cm) of blue dextran-Sepharose 4B, prepared as described earlier (14). The column was first washed with 200 ml of prep solution and then with 200 ml of prep solution containing 2 mM NAD^+. The oxidoreductase (or dehydrogenase) activity specifically eluted by NAD^+ was concentrated by ultrafiltration and dialyzed exhaustively against prep solution. Both enzyme activities were stable for at least 3 months when stored at 4°C.

Lyophilized samples of 1,2-propanediol:NAD^+ oxidoreductase, purified from *N. gonorrhoeae*, and of sheep antiserum to this enzyme were gifts of Hugh C. McDonald, Corning Glass Works, Corning, N.Y.

Assays for enzymatic activity and protein. Four assays were used to follow the time-dependent rate of NADH formation at 340 nm with a Cary 219 spectrophotometer at the temperatures specified (Table 1). Specific activity was uniformly defined as the micromoles of NADH formed per minute per milligram of protein. Protein concentrations were estimated by the method of Lowry et al. (17) with crystalline bovine serum albumin as the standard.

Oxidoreductase activity in fractions obtained during enzyme purification was measured by assay 1. Assays 2 and 4 were used to compare the kinetic properties of purified D-1-amino-2-propanol oxidoreductase with glycerol dehydrogenase and 1,2-propanediol oxidoreductase, respectively. The effect of NH_4Cl on D-1-amino-2-propanol oxidoreductase and glycerol dehydrogenase activities was determined by assay 3.

Electrophoresis. Slab gel electrophoresis in 1% sodium dodecyl sulfate was carried out on 10% acrylamide gels at 25°C (28). In this procedure, the following proteins served as subunit molecular weight standards: bovine serum albumin (M_r 68,000), bovine liver catalase (M_r 57,500), ovalbumin (M_r 43,000), rabbit muscle fructose 1,6-bisphosphate aldolase (M_r 40,000), and bovine pancreas chymotrypsinogen (M_r 25,000).

Isoelectric focusing. One-dimensional isoelectric focusing on slab gels was done as described by O'Farrell (20) with these modifications: (i) 0.008% Triton X-100 replaced Nonidet P-40; (ii) the final concentration of ampholytes was 0.2 g/10 ml (0.16 g of pH 4 to 6 ampholytes plus 0.04 g of pH 2 to 10 ampholytes). The gels were prefocused for 2 h at 10 W constant power until the current stabilized. Protein samples were mixed with diluting solution (containing 9.5 M urea, 0.64% pH 4 to 6 ampholytes, 0.16% pH 2 to 10 ampholytes, 2% Triton X-100, 5% 2-mercaptoethanol) so that the final urea and protein concentrations were 7.6 M and 10 μg/100 μl, respectively. The samples were focused with the power initially held constant at 10 W until the voltage reached 1,000 V (ca. 2 h) and then with the voltage held constant at 1,000 V for an additional 4 h.

Chemicals. Special chemicals were obtained from the following sources: DL-1-amino-2-propanol, DL-1,2-propanediol, DL-1,2-butanediol, DL-2,3-butanediol, DL-3-amino-1,2-propanediol, and hydroxyacetone from Aldrich Chemical Co., Inc., Milwaukee, Wis.; 3-chloro-1,2-propanediol from Matheson, Coleman and Bell; Coomassie brilliant blue-G and Sepharose 4B from Sigma Chemical Co., St. Louis, Mo.; and sodium dodecyl sulfate from Pierce Chemical Co., Rockford, Ill. Bio-Gel A-0.5m and the reagents used for polyacrylamide gel electrophoresis or isoelectric focusing were purchased from Bio-Rad Laboratories, Richmond, Calif. The individual D- and L- isomers of 1-amino-2-propanol were synthesized and characterized as before (4). All other chemicals were of the highest purity commercially available.

RESULTS

Comparative purification of 1-amino-2-propanol oxidoreductase and glycerol dehydrogenase activities. The results of purifying *E. coli* D-1-amino-2-propanol oxidoreductase (strain W-1485) and glycerol dehydrogenase (strain 424) side by side via the same fractionation steps are shown in Table 2. As can be seen, the overall results were quite similar and

TABLE 2. Comparative purification of 1-amino-2-propanol oxidoreductase and glycerol dehydrogenase activities

Enzyme source	Fraction	Vol (ml)	Amt of total protein (mg)	Sp act[a] (U/mg)	Recovery (%)	Purification (fold)
E. coli K-12 (strain W-1485)	1. Crude extract[b]	88.0	2,415	0.67	100	1.0
	2. Heated to 78°C	23.0	345	2.27	50	3.4
	3. Bio-Gel A-0.5m eluate	32.0	184	5.10	58	7.6
	4. Blue dextran-Sepharose 4B eluate	4.0	21	29.7	38	46.0
E. coli (strain 424)	1. Crude extract[c]	86.0	2,470	0.61	100	1.0
	2. Heated to 78°C	20.8	339	3.79	85	6.2
	3. Bio-Gel A-0.5m eluate	20.1	117	9.81	76	16.1
	4. Blue dextran-Sepharose 4B eluate	4.9	6	30.80	13	51.4

[a] Activity was determined by assay 1 with DL-1-amino-2-propanol as the substrate.
[b] Twenty grams of cell paste was used.
[c] Nineteen grams of cell paste was used.

the final specific activities of the two purified enzymes were nearly identical. Furthermore, the elution profiles of glycerol dehydrogenase activity from the Bio-Gel and blue dextran-Sepharose 4B columns were essentially the same as those routinely obtained in purifying D-1-amino-2-propanol oxidoreductase activity. One slight apparent difference was that the recovery of glycerol dehydrogenase activity from the affinity column was lower than usually observed for aminopropanol oxidoreductase activity; this was largely due to nonspecific elution of dehydrogenase activity during the loading and washing of the column.

In addition, the activity (assay 1) of protein samples obtained at each step in the purification of glycerol dehydrogenase (*E. coli* 424) was measured with either glycerol or DL-1-amino-2-propanol as the substrate, and the ratio of specific activities (glycerol/aminopropanol) was calculated. The ratio was 0.50 and 0.52 for fractions 1 and 2 (Table 2), respectively. A relatively constant value (range, 0.47 to 0.52) was also found for individual tubes obtained from the Bio-Gel and blue dextran-Sepharose columns. Furthermore, the most purified sample of glycerol dehydrogenase (fraction 4; Table 2) had a ratio of 0.48, a value identical to that determined for aminopropanol oxidoreductase purified to homogeneity by the same procedure.

Comparative electrophoresis and isoelectric focusing of aminopropanol oxidoreductase and glycerol dehydrogenase. Samples of each purified enzyme were subjected to polyacrylamide gel electrophoresis at pH 8.0 under nondenaturing conditions. Each enzyme preparation contained a major (form L; R_m = 0.18) as well as a minor band (form S; R_m = 0.53) of protein and activity. The two bands from each preparation had the same mobility; the relative proportion of form S was slightly greater for glycerol dehydrogenase. Although the two individual enzyme preparations showed varying levels of response when stained for activity with each of four compounds as the substrate, the actual intensity of the stain was virtually identical in each corresponding case. Whereas only the two protein-stainable bands that also showed enzymatic activity could be seen with the aminopropanol oxidoreductase, two additional bands that were faintly stainable for protein but enzymatically inactive were visible with the glycerol dehydrogenase preparation. When the subunits of the two purified enzyme samples were examined by polyacrylamide gel electrophoresis in 1% sodium dodecyl sulfate, a single protein band of the same mobility and a molecular weight of 38,500 was observed in both cases.

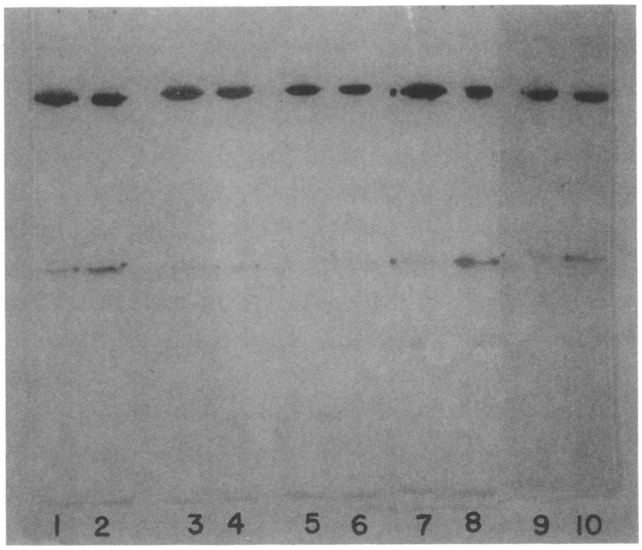

FIG. 1. Polyacrylamide gel electrophoresis of 1-amino-2-propanol oxidoreductase and glycerol dehydrogenase. Lanes: 1, 3, 5, and 7, aminopropanol oxidoreductase activity (0.1 U); 2, 4, 6, and 8, glycerol dehydrogenase activity (0.1 U); 9 and 10, oxidoreductase (10 μg) and dehydrogenase (10 μg), respectively. The separating gel consisted of 7.5% acrylamide (pH 8.0) (8); constant power (5 W) was applied at 4°C until tracking dye reached the end of the gel. Lanes 1 to 8 were subjected to a staining procedure for visualizing enzyme activity in which the formation of NADH is coupled to phenazine methosulfate and *p*-nitrobluetetrazolium (9, 14); 100 mM of the following substrates were used: DL-1-amino-2-propanol (lanes 1 and 2), DL-2,3-butanediol (lanes 3 and 4), glycerol (lanes 5 and 6), and DL-1,2-propanediol (lanes 7 and 8). Protein in lanes 9 and 10 was stained with Coomassie brilliant blue-G.

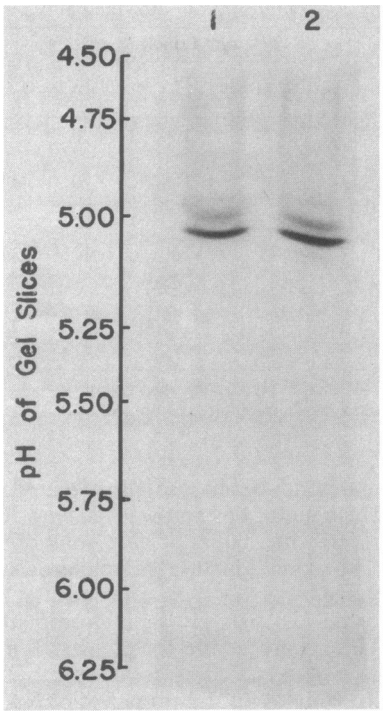

FIG. 2. Isoelectric focusing of 1-amino-2-propanol oxidoreductase and glycerol dehydrogenase. Isoelectric focusing under denaturing conditions was carried out as described in the text. Lanes: 1, purified oxidoreductase (10 μg); 2, purified dehydrogenase (10 μg). The pH gradient was estimated by measuring the pH of water extracts (1 ml) of gel slices (2.5 mm) from two lanes to which no protein was applied. After the gels were rinsed three times with fixing solvent (methanol-acetic acid-water, 4:1:5), protein was stained with 0.25% Coomassie brilliant blue-G in fixing solvent.

The two purified proteins also behaved the same when subjected to isoelectric focusing (Fig. 2); four protein bands were visible in each case (pI [major band] = 5.10; pI [slightly less intense band] = 5.04; pI [two faint bands] = 4.97 and 5.15).

Controlled heat denaturation of aminopropanol oxidoreductase and glycerol dehydrogenase. We subjected the two purified enzymes to heat treatment at 74°C, at which temperature activity is slowly destroyed over a period of ca. 1 h. Although glycerol dehydrogenase activity was found to be somewhat more stable under these conditions, the ratio of specific activities (glycerol/aminopropanol as substrates) re-

TABLE 3. K_m and V_{max} values for 1-amino-2-propanol oxidoreductase and glycerol dehydrogenase

Substrate[a]	K_m (mM) for:		V_{max} (U/mg) for:	
	Aminopropanol oxidoreductase	Glycerol dehydrogenase	Aminopropanol oxidoreductase	Glycerol dehydrogenase
DL-1-Amino-2-propanol	33.7	33.3	29.9	31.8
Glycerol	107.0	139.4	75.0	87.8
DL-1,2-Propanediol	1.0	1.1	102.2	110.3

[a] Activity was determined by assay 2 (at eight substrate concentrations ranging from 0.5 to 500 mM). K_m and V_{max} values were estimated by unweighted linear regression analyses of double reciprocal plots.

mained nearly constant for both enzymes throughout this procedure and was of the same magnitude as found earlier in the purification studies (i.e., 0.47 to 0.52).

Kinetic constants and substrate specificity of aminopropanol oxidoreductase and glycerol dehydrogenase. K_m and V_{max} values were determined for both purified enzymes with each of three substrates (Table 3). The K_m values with either DL-1-amino-2-propanol or DL-1,2-propanediol were found to be identical. Althouth it is not apparent why the K_m values with glycerol should differ somewhat, the relative magnitude of these values for each of the three substrates is the same. As can also be seen in Table 3, the V_{max} values determined for both enzymes with each substrate were very similar and agree with those previously published (14) for form L of D-1-amino-2-propanol oxidoreductase. Additionally, the specific activities of both enzymes were similarly increased when 10 mM NH_4Cl was added to the reaction mixture (Table 4). With NH_4Cl added, reaction rates were 3 times higher with DL-1-amino-2-propanol as the substrate and 2 times higher with either glycerol or DL-1,2-propanediol.

Comparative immunological studies with D-1-amino-2-propanol oxidoreductase and 1,2-propanediol oxidoreductase. Lyophilized samples of 1,2-propanediol:NAD^+ oxidoreductase (4 U; purified from extracts of *N. gonorrhoeae*) and of goat antiserum to this enzyme were reconstituted in sterile 50 mM sodium phosphate buffer (pH 7.4). Two immunological tests were performed with these oxidoreductases. First, a double-diffusion technique (10) was carried out which showed that, whereas precipitin bands readily formed with the gonococcal enzyme, the antiserum did not cross-react with the aminopropanol oxidoreductase. Secondly, the ability of the antiserum to inhibit the catalytic activity of the two enzymes was tested. For this purpose, samples (0.1 U) of each purified enzyme were allowed to react for 15 min at 25°C with 0.1 ml of various dilutions of the antiserum; enzyme activity with DL-1,2-propanediol as the substrate was then determined by assay 4. Under these conditions, a 1:1,250 dilution of antiserum inhibited propanediol oxidoreductase activity 50%, whereas a 1:6 dilution was required to inhibit the *E. coli* aminopropanol oxidoreductase activity to the same extent.

Comparative substrate specificity and the effect of NH_4Cl on 1-amino-2-propanol oxidoreductase and 1,2-propanediol oxidoreductase activities. When compared with *E. coli* 1-amino-2-propanol oxidoreductase, we found that the gonococcal propanediol oxidoreductase was much less effective catalytically and showed considerably narrower substrate specificity (Table 5). The rates of oxidation of several substrates, as catalyzed by propanediol oxidoreductase, were typically one order of magnitude lower than those obtained with the aminopropanol oxidoreductase. The propanediol oxidoreductase effectively utilized only short-chain, unsubstituted *vic*-diol compounds (i.e., 1,2-propanediol, 1,2-butanediol, and 2,3-butanediol) as substrates; a small amount of oxidation occurred with glycerol as well as with DL- or D-1-aminopropanol when 250 mM NH_4Cl was added to the assay mixture.

In our hands, propanediol oxidoreductase activity was stimulated by added NH_4Cl to a significantly lesser degree than was originally reported (18); this could well be the consequence of the enzyme having been stored in lyophilized state for 2 years. In contrast, the reconstituted enzyme we used was inhibited by antiserum to the same extent as noted earlier.

Depending on the substrate oxidized, the addition of 250 mM NH_4Cl to assay mixtures (assay 4) for 1-amino-2-propanol oxidoreductase activity had three different kinetic effects. Whereas the V_{max} was elevated (to a greater or lesser extent) in each case, the K_m value was not significantly affected with *vic*-diols like DL-1,2-propanediol, DL-1,2-butanediol, and DL-2,3-butanediol; however, it was lowered for glycerol and dramatically increased for DL- and D-1-amino-2-propanol.

DISCUSSION

The D isomer of 1-amino-2-propanol is a constituent part of vitamin B_{12}, serving as the link between the corrin ring system and the dimethylbenzimidazole ribonucleotide moiety of the molecule. Although much information has been obtained concerning the pathways by which the corrinoid and dimethylbenzimidazole portions are biosynthesized (7), much less is known about how the aminoalcohol is made and incorporated into the vitamin. The initial proposal of Neuberger and Tait (19) that L-threonine might be converted to 1-amino-2-propanol by sequential action of a threonine and an aminopropanol dehydrogenase was experimentally established in our laboratory (3), and each enzyme has subsequently been obtained in pure form (2, 14).

The discovery and characterization of oxidoreductase activity in extracts of wild-type *E. coli* that not only stereospecifically catalyzed the reduction of aminoacetone (or oxidation of D-1-amino-2-propanol) but also effectively utilized several *vic*-diols as substrates raised questions as to its identity. Multiple lines of evidence presented here, considered collectively, confirm earlier indications in this regard (13) and firmly establish that the *E. coli* aminopropanol oxidoreductase and glycerol dehydrogenase activities which have been studied independently in our laboratories and those of E. C. C. Lin are the same enzyme. They differ

TABLE 4. Stimulation of 1-amino-2-propanol oxidoreductase and glycerol dehydrogenase activities by NH_4Cl

Substrate	Concn of NH_4Cl (mM)	Sp act (U/mg)[a] for:		Ratio of sp act
		Aminopropanol oxidoreductase	Glycerol dehydrogenase	
DL-1-Amino-2-propanol	0	16.3	18.7	0.87
	10	45.8	59.2	0.77
Glycerol	0	54.8	52.3	1.05
	10	116.9	101.8	1.15
DL-1,2-Propanediol	0	72.4	74.0	0.98
	10	143.0	147.5	0.97

[a] Enzyme activity was determined by assay 3.

TABLE 5. Comparative kinetic constants for *E. coli* D-1-amino-2-propanol oxidoreductase and 1,2-propanediol oxidoreductase of *N. gonorrhoeae* in the presence and absence of NH_4Cl

Substrate	K_m (mM)[a] for:				V_{max} (U/mg)[a] for:			
	Aminopropanol[b] oxidoreductase		Propanediol[b,c] oxidoreductase		Aminopropanol oxidoreductase		Propanediol oxidoreductase	
	$-NH_4Cl$	$+NH_4Cl$	$-NH_4Cl$	$+NH_4Cl$	$-NH_4Cl$	$+NH_4Cl$	$-NH_4Cl$	$+NH_4Cl$
DL-1,2-Propanediol	0.70	0.83	3.5	4.3	15.5	45.1	1.7	2.3
DL-1,2-Butanediol	0.76	1.30	4.1	4.4	15.5	39.8	0.9	1.5
Glycerol	46.60	25.30		2.6	10.2	24.6	0.0	0.1
DL-2,3-Butanediol	3.60	3.70	28.7	31.7	13.4	31.1	0.7	2.1
DL-1-Amino-2-propanol	12.40	267.70		10.5	8.6	12.4	0.0	0.1
D-1-Amino-2-propanol	6.40	107.80		6.5	8.2	12.3	0.0	0.1
L-1-Amino-2-propanol					0.0	0.0	0.0	0.0

[a] The activity of each enzyme was determined by assay 4 with (+) and without (−) 250 mM NH_4Cl added. Ten concentrations of each substrate, ranging from 0.5 to 500 mM, were used; the kinetic parameters were estimated by unweighted linear regression analyses of double-reciprocal plots.

[b] No oxidation of the following compounds was observed at any concentration: ethanol, 2-propanol, 2-amino-1-ethanol, 1-chloro-2-propanol, 3-hydroxy-2-butanone, lactic acid, and glycerol-3-phosphate.

[c] No oxidation of the following compounds was observed at any concentration: 1,2-ethanediol, 3-amino-1,2-propanediol, 3-chloro-1,2-propanediol, and 3-mercapto-1,2-propanediol.

somewhat only in relative stability to heat treatment; the glycerol dehydrogenase was found to be somewhat more stable under the experimental conditions used. The fact that the aminopropanol oxidoreductase used in this particular study was purified 1 month before the glycerol dehydrogenase may relate to this difference. Although we have found that the purified aminopropanol oxidoreductase activity is stable over several months, the possibility cannot be discounted that minor structural alterations occur during storage at 4°C which may increase its sensitivity to heat denaturation but not affect its catalytic activity. Our past studies have clearly shown that although the two molecular forms of D-1-amino-2-propanol oxidoreductase (i.e., form L and form S) are essentially the same catalytically, the latter form is significantly more heat labile (4).

In contrast to such results, both immunochemical and kinetic data establish that the 1,2-propanediol oxidoreductase of *N. gonorrhoeae* and the D-1-amino-2-propanol oxidoreductase of *E. coli* K-12 are different enzymes. Comparatively, the *E. coli* oxidoreductase has broader substrate tolerance and is, under the conditions of assay 4, a much more effective catalyst. We found that the gonococcal enzyme catalyzed the oxidation of compounds other than short-chain, unsubstituted *vic*-diols (i.e., glycerol and DL- or D-1-amino-2-propanol) only when the concentration of NH_4Cl was very high (250 mM); moreover, only the D isomer of 1-amino-2-propanol served as a substrate. Attention is again called to the fact that the sample of the gonococcal oxidoreductase used in these studies had been stored as a lyophilized powder at −20°C for 2 years; hence, although the results we obtained regarding its substrate tolerance agree with those reported before (18), its catalytic effectiveness relative to the *E. coli* oxidoreductase should probably be viewed with caution.

Although the identity of *E. coli* aminopropanol oxidoreductase (wild-type strain) and glycerol dehydrogenase (mutant strain 424) activities has now been established, its true role in the cell still remains to be determined; this question has also been considered by others (13). Several facets of the data in hand suggest that the primary function of this enzymatic activity is to catalyze the reduction (versus the oxidation) of available substrates. For example, the levels of this activity are markedly induced by oxidized substrates (i.e., aminoacetone, hydroxyacetone, and dihydroxyacetone) and also by compounds involved in the pathway postulated for threonine catabolism/D-1-amino-2-propanol formation (i.e., L-threonine and aminoacetone); except for glycerol, reduced substrates are ineffective (14). Also, the pH optimum for the oxidative reaction is sufficiently high (4) to suggest that the rate of reaction in this direction might be very low under physiological conditions. Furthermore, the equilibrium of the reaction determined in vitro is far in favor of reduced substrate formation (15). A correlate that may imply a catabolic function is the almost complete specificity of this activity for NADH as a cosubstrate (4, 24). Such considerations aside, it is apparent that the level of glycerol dehydrogenase activity in the *E. coli* mutant is under some kind of novel regulation which is neither catabolic nor respiratory repression (25). Certainly, better insight on the transcriptional and translational regulations of this enzymatic activity is required and, as already noted (13), determining its structural and regulatory genes may better define its true role.

Reports of bacterial glycerol dehydrogenases from a multitude of sources have appeared previously (12, 15, 21, 22, 24, 29, 30); for the most part, however, their physiological roles have not been elucidated, and it is not known how many are capable of catalyzing the oxidation of D-1-amino-2-propanol. We found that commercially available glycerol dehydrogenase I from *Klebsiella pneumoniae* catalyzed the oxidation of D-1-amino-2-propanol (unpublished data), but detailed studies with this enzyme have not been carried out. Since glycerol dehydrogenase II from *K. pneumoniae* has been found to be immunochemically identical with the glycerol dehydrogenase from *E. coli* 424 (23), by inference it is most likely very similar to if not identical with our *E. coli* aminopropanol oxidoreductase and would thus be specific for the D isomer of this aminoalcohol.

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