

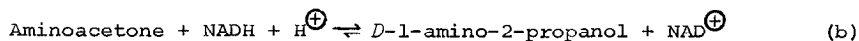
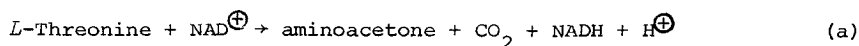
FORMATION OF *D*-1-AMINO-2-PROPANOL FROM
L-THREONINE BY ENZYMES FROM *ESCHERICHIA COLI* K-12*

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Summary: *Escherichia coli* K-12 cells contain two dehydrogenases which in sequence catalyze the net conversion of *L*-threonine to the *D*-isomer of 1-amino-2-propanol. These two enzymes are *L*-threonine dehydrogenase (*L*-threonine + NAD⁺ → aminoacetone + CO₂ + NADH + H⁺) and *D*-1-amino-2-propanol dehydrogenase (aminoacetone + NADH + H⁺ ⇌ *D*-1-amino-2-propanol + NAD⁺). Each enzyme has been obtained in purified form free of the other; the nature of the reaction catalyzed by the latter dehydrogenase alone and in a coupled system with the former enzyme has been studied. The results provide an explanation on the enzymological level for the utilization of *L*-threonine by cell suspensions of certain microorganisms for the biosynthesis of the *D*-1-amino-2-propanol moiety of Vitamin B₁₂.

The incorporation of ¹⁵N-*L*-threonine into the *D*-1-amino-2-propanol moiety of the Vitamin B₁₂ produced by growing cultures of *Streptomyces griseus* has been reported (1). Recently, Müller and co-workers (2) indicated that ¹⁴C-*L*-threonine as well as ¹⁴C-*D*-1-amino-2-propanol are utilized for Vitamin B₁₂ biosynthesis by cell suspensions of *Propionibacterium shermanii*. It was suggested by Krasna, *et al.* (1) that formation of 1-amino-2-propanol might occur by direct decarboxylation of *L*-threonine. On the other hand, Neuberger and Tait (3) proposed that this aminoalcohol might be formed by the coupled action of two enzymes, namely *L*-threonine dehydrogenase (reaction a) and a *D*-specific 1-amino-2-propanol dehydrogenase (reaction b):



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NET: *L*-Threonine \rightarrow *D*-1-amino-2-propanol + CO₂ (c)

Both of these enzymes have recently been detected in a wide variety of microorganisms (4-10). However, only the *D*-specific 1-amino-2-propanol dehydrogenase detected in extracts of *E. coli* K-12 by Dekker and Swain (11) seemed to exhibit the stereospecificity required for participation in such a pathway.

In this report, evidence on the enzymological level is provided for the sequential action of the two dehydrogenases noted [reactions (a) and (b)] whereby the net reaction (reaction c) is accomplished. Purified preparations of *L*-threonine and *D*-1-amino-2-propanol dehydrogenases have been used in coupled and individual enzyme systems. In this manner, the formation of the *D*-isomer of 1-amino-2-propanol not only from aminoacetone but also from *L*-threonine has been established.

EXPERIMENTAL AND RESULTS

Materials and Methods—*L*-threonine dehydrogenase and *D*-1-amino-2-propanol dehydrogenase were purified (approx. 50- and 500-fold, respectively) from sonic extracts of *Escherichia coli* K-12 cells. Ninhydrin-positive materials were detected and quantitated by the method of Rosen (12). The specific colorimetric procedure of Mauzerall and Granick (13) as modified by Gibson, *et al.* (14) was used to determine aminoacetone. Aminoacetone hydrochloride was chemically synthesized by the method of Tschudy, *et al.* (15) and the *D*-isomer of 1-amino-2-propanol by the procedure of Chalelus (16). Optical rotatory values were measured at ambient temperature with the use of an O. C. Rudolph and Sons polarimeter (Model 80); absorbance measurements were made with a Gilford 2000 spectrophotometer equipped with a thermostated cuvette holder.

Enzymic Conversion of Aminoacetone to *D*-1-Amino-2-propanol—The formation of the *D*-isomer of 1-amino-2-propanol from aminoacetone, as catalyzed by *D*-1-amino-2-propanol dehydrogenase, was determined in the following manner. A mixture containing 300 μ moles of aminoacetone, 5 mmoles of sodium phosphate buffer (pH 6.0), 9 mg (12 μ moles) of NADH, and 1.2 mg of purified *D*-1-amino-

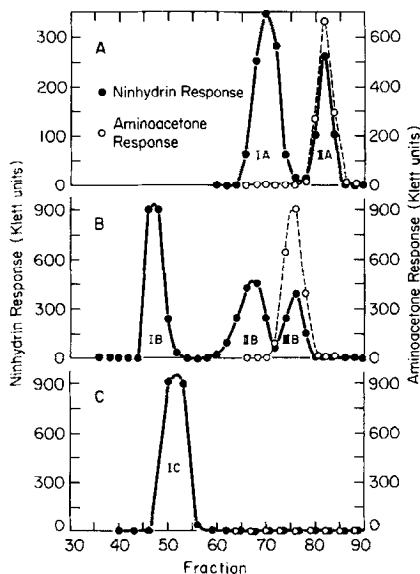


Figure 1. Elution patterns from columns of Dowex-50 (H^+) ion exchange resin of reaction mixtures in which *L*-threonine or aminoacetone was converted enzymically to 1-amino-2-propanol. The enzymic reactions and the fractionation of the cationic compounds were carried out as described in the text. The individual patterns correspond to the following experimental conditions:

A. Conversion of aminoacetone to 1-amino-2-propanol, as catalyzed by *D*-1-amino-2-propanol dehydrogenase (oxidoreductase).

B. Conversion of *L*-threonine to 1-amino-2-propanol, as catalyzed by a coupled enzyme system containing *L*-threonine dehydrogenase and *D*-1-amino-2-propanol dehydrogenase (oxidoreductase).

C. Control incubation mixture of the coupled enzyme system containing boiled *L*-threonine dehydrogenase and active *D*-1-amino-2-propanol dehydrogenase (oxidoreductase).

2-propanol dehydrogenase was prepared in a total volume of 35 ml. The main solution was incubated at 37° C while a 1-ml aliquot was concomitantly monitored at 340 nm to follow the extent of reaction as evidenced by the oxidation of NADH. Six additional aliquots, containing 12 μ moles each, of NADH were added to the main incubation mixture whenever the absorbance at 340 nm approached zero. After the mixture had been incubated for six hours, the reaction was terminated by adding 5 ml of cold 25% (w/v) trichloroacetic acid solution. The pH of the solution was then adjusted to 7.0 with 2.5 *N* sodium hydroxide. The mixture was filtered to remove any precipitated protein and the filtrate was subsequently applied to a column (2.5 x 35 cm) of Dowex-50 [H^+] ion

exchange resin. The resin column was first washed with 80 ml of water; cationic compounds were then eluted with a nonlinear gradient (0→2 *N*) of HCl prepared by passing 2 *N* HCl solution into a 400-ml reservoir of water. Fractions (10 ml) were collected and assayed for ninhydrin-positive materials as well as by the specific colorimetric procedure for aminoacetone. The elution profile obtained is shown in Figure 1A. Those fractions containing the separated compounds (substances IA and IIA) were pooled and concentrated to dryness three times *in vacuo* at 40° C. The enzymic product (peak IA), which was negative in the aminoacetone assay, was identified as L-amino-2-propanol by high voltage paper electrophoresis and paper chromatography, using a number of different buffer and solvent systems, respectively.

Conversion of L-Threonine to D-1-Amino-2-propanol; Coupled Enzyme System

The net conversion of L-threonine to D-1-amino-2-propanol was carried out by a coupled enzyme system containing both L-threonine and D-1-amino-2-propanol dehydrogenases. A reaction mixture containing 2.5 mmoles of L-threonine, 4 mmoles of sodium phosphate buffer (pH 7.0), 0.2 mmoles of NAD[⊕], 10.2 mg of L-threonine dehydrogenase, and 5 mg of purified D-1-amino-2-propanol dehydrogenase, was prepared in a final volume of 40 ml. The mixture was incubated at 37° C for 18 hours and the reaction was then terminated by adding 4 ml of cold 25% (w/v) trichloroacetic acid solution. The solution was then treated and the components fractionated and analyzed as described in the previous section. The elution profile obtained is shown in Figure 1B. Those fractions containing the separated compounds (substances IB, IIB, and IIIB) were concentrated to dryness *in vacuo* and identified as before.

Control Incubation Mixture Containing L-Threonine and the Coupled Enzyme System—A control incubation mixture for examining the enzymic conversion of L-threonine to D-1-amino-2-propanol by the coupled enzyme system was prepared in the following manner. L-Threonine (2.50 mmoles), 4.0 mmoles of sodium phosphate buffer (pH 7.0), 0.2 mmoles of NAD[⊕], 1.5 mg of D-1-amino-2-propanol dehydrogenase, and boiled L-threonine dehydrogenase were mixed in a final

TABLE I

Optical Rotation Measurements on Samples of Enzymically-formed and Chemically-Synthesized l-Amino-2-propanol

l-Amino-2-propanol Sample	Concentration in Aqueous Solution	Calculated Specific Rotation Value
	(gm/100 ml)	$[\alpha]_D^{25}$
Isolated from the single enzyme system; component IA in Figure 1.	0.91%, as the HCl salt	-14°
Isolated from the coupled enzyme system, component IIB in figure 1.	2.2%, as the HCl salt	-18°
Chemically synthesized <i>D</i> -l-amino-2-propanol.	2.2%, as the HCl salt	-23°
Hexachloroplatinate salt of chemically synthesized <i>D</i> -l-amino-2-propanol. [†]	2.5%	-14°

l-Amino-2-propanol concentrations were determined by the quantitative ninhydrin method of Rosen (12); reagent grade *DL*-l-amino-2-propanol served as a standard. [†]Literature value for hexachloroplatinate salt = -12.2° (17).

volume of 36 ml. The incubation of this reaction mixture and the subsequent fractionation of the cationic compounds present was carried out as described before. The elution profile obtained in this instance is shown in Figure 1C. Only one peak of ninhydrin-positive material corresponding to *L*-threonine was observed. When all of the eluted fractions were also assayed for aminoacetone, no response was detected.

Determination of the Stereochemical Configuration of the Enzymically-Formed l-Amino-2-propanol — Paper chromatographic and high voltage paper electrophoretic data indicated that the terminal product of both the individual and the coupled enzyme systems was l-amino-2-propanol. In order to establish the stereochemical identity of the enzymic product, the optical activity of each sample was determined and the corresponding specific rotation value was calculated. The results obtained are listed in Table I.

DISCUSSION

These results provide clear support for the presence of two dehydrogenases in extracts of *E. coli* K-12 which can function in sequence and specifically catalyze the net conversion of *L*-threonine to *D*(-)-1-amino-2-propanol. The first of these enzymes is *L*-threonine dehydrogenase and the second is *D*-1-amino-2-propanol dehydrogenase(oxidoreductase). Since the reaction catalyzed by the former enzyme is essentially irreversible and the one catalyzed by the latter is far in the direction of NAD^{\oplus} regeneration, net formation of the aminoalcohol is strongly favored. Our findings, therefore, provide an enzymological basis for explaining the results of other investigators who observed that intact cells of certain microorganisms utilize *L*-threonine for the biosynthesis of the *D*-1-amino-2-propanol moiety of Vitamin B_{12} .

Although it is known that *E. coli* cannot form Vitamin B_{12} *de novo*, it is possible that this organism possesses some but not all of the enzymes required for complete vitamin biosynthesis. *D*-Specific 1-amino-2-propanol and *L*-threonine dehydrogenases have, indeed, also been detected in a number of Vitamin B_{12} -producing organisms including *Nocardia rugosa*, *Propionibacterium shermanii*, and *Streptomyces griseus* (18). It appears, however, that the levels of these two dehydrogenases in extracts of certain vitamin-producing organisms are not nearly as high as in those obtained from *E. coli*; purifying and studying these enzymes from the latter source has, therefore, distinct advantages.

As has already been noted (19), a unique aspect of *D*-1-amino-2-propanol dehydrogenase is that it exists in two different yet catalytically-active forms. The purification and properties of the enzymes considered in this report will be published in detail elsewhere.

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