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Formation of D_g-1-amino-2-propanol by a highly purified enzyme from *Escherichia coli*

Specific enzymic formation of the D_g isomer* of 1-amino-2-propanol, a part of the vitamin B_{12} molecule, has not yet been reported. KRASNA, ROSENBLUM AND SPRINSON[1], using growing cultures of *Streptomyces griseus*, found that L-[^{15}N]threonine is effectively incorporated into the aminopropanol moiety of vitamin B_{12} and suggested that the amino alcohol might be formed by decarboxylase activity. A decarboxylase that converts L-threonine to D_g-1-amino-2-propanol is not known. On the other hand, the conversion of L-threonine to aminoacetone is well documented[2–6]. Furthermore, several studies[7–11] have demonstrated the presence of aminopropanol dehydrogenase (1-amino-2-propanol:DPN$^+$ oxidoreductase) activity in a number of biological sources. Such studies, however, have all been carried out with cell suspensions, homogenates, or crude extracts and the enzymic activity observed preferentially utilized the L isomer of aminopropanol. We recently obtained, in highly purified form, a dehydrogenase from *Escherichia coli* extracts that is specific for catalyzing a reversible oxidation–reduction reaction between aminoacetone and the D_g isomer of 1-amino-2-propanol; the equilibrium of this reaction is far in favor of amino ketone reduction.

Aminoacetone hydrochloride was prepared by the method of TSCHUDY *et al.*[12]; the final product was recrystallized 3 times from a mixture of ethanol–ether. DL-1-Amino-2-propanol (m.p., 0–1°) was a commercial product. The D_g and the L_g isomers of 1-amino-2-propanol were individually prepared from L-threonine and D-threonine, respectively, by the procedure of CHALELUS[13]. The products obtained were judged pure by the preparation of derivatives and the determination of optical rotation values. *E. coli* K12 was grown on a nutrient broth medium; extracts were prepared by sonic disintegration of the cells. 1-Amino-2-propanol dehydrogenase activity was measured by one of two methods: (1) colorimetric determination, by the method of MAUZERALL AND GRANICK[14], of the amount of aminoacetone formed, or (2) measurement of the rate of DPNH formation (1-amino-2-propanol oxidation) or of DPNH disappearance (aminoacetone reduction) at 340 mμ. The latter assay could be used only with purified dehydrogenase preparations; the colorimetric assay was used in purifying the enzyme. A unit of activity is defined as the amount of protein required to form 1 mμmole of aminoacetone in 30 min at 37°; specific activity refers to units of enzyme activity per mg of protein.

We succeeded in purifying 1-amino-2-propanol dehydrogenase activity over 250-fold from *E. coli* extracts by procedures including ammonium sulfate fractionation, adsorption and elution from calcium phosphate gel, and column chromatography on DEAE-cellulose; purified enzyme fractions had specific activity values in the range of 105000 to 110000. The purified dehydrogenase is optimally active between pH 8.0 and 8.6. The apparent equilibrium constant for the reaction

$$\text{Aminoacetone} + \text{DPNH} + \text{H}^+ \rightleftharpoons D_g\text{-1-amino-2-propanol} + \text{DPN}^+$$

strongly favors the formation of the amino alcohol. As shown in Table I, purified

* Configurationally related (as is the β-carbon atom of L-threonine) to D-glyceraldehyde.

TABLE I

SUBSTRATE SPECIFICITY OF PURIFIED DEHYDROGENASE

The complete reaction mixture contained 100 μmoles Tris–HCl buffer (pH 8.4), 60 μmoles of D$_g$-1-amino-2-propanol (or of the L$_g$ isomer, when tested), 5 μmoles of DPN$^+$ (or TPN$^+$, when tested), 120 μmoles of L-threonine (when tested), and 15 μg of purified dehydrogenase, specific activity 105000. Water was added to a final volume of 1 ml. Reaction was initiated by adding pyridine nucleotide; the rate of increase in absorbancy at 340 mμ was followed for 3–5 min at 37° with a recording Gilford spectrophotometer. Individual controls showed that in order to observe any enzymic reaction, D$_g$-1-amino-2-propanol, DPN$^+$, and enzyme protein were all required.

Substrate tested	*Reaction rate (absorbance change per min)*
D$_g$-1-Amino-2-propanol	0.360
L$_g$-1-Amino-2-propanol	0
L-Threonine	0
DPN$^+$	0.360
TPN$^+$	0

1-amino-2-propanol dehydrogenase is not active with L-threonine as substrate, utilizes only DPN$^+$ (not TPN$^+$), and is completely specific for the D$_g$ isomer of 1-amino-2-propanol. Of a large number of alcohols, amino alcohols, hydroxy acids, and hydroxy amino acids tested, no other compound serves as substrate for the purified enzyme.

TURNER[11] previously observed that whereas non-dialyzed cell-free extracts of *E. coli* preferentially utilized the L isomer of 1-amino-2-propanol, greater activity toward the D isomer was detected after these same extracts were dialyzed and stored at 0° for several days. Our findings with the highly purified dehydrogenase provide further support for the proposal[3,11] that a metabolic pathway may very well exist in bacteria whereby L-threonine is first converted to aminoacetone by the action of L-threonine dehydrogenase, which compound is subsequently reduced by a specific dehydrogenase yielding D$_g$-1-amino-2-propanol. A necessary component for vitamin B_{12} biosynthesis is thereby provided.

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