The Mechanism of Action of Ethanolamine Ammonia-Lyase, a B₁₂-dependent Enzyme

EVIDENCE FOR TWO INTERMEDIATES IN THE CATALYTIC PROCESS*

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SUMMARY

Ethanolamine ammonia-lyase, an enzyme catalyzing the adenosylcobalamin-dependent deamination of ethanolamine, also catalyzes the conversion of L-2-aminopropanol to propionaldehyde and ammonia. In this reaction, tritium is transferred from enzyme [5′-aH]Coenzyme B₁₂ to the C-1 position of L-2-aminopropanol, as well as to the α carbon of the product aldehyde. The labeling pattern is consistent with the mechanism of hydrogen transfer deduced with other substrates. Tryptophan transfer also occurs between enzyme [5′-aH]adenosylcobalamin and propionaldehyde in the presence of NH₄⁺. Unlike the deamination of ethanolamine, the conversion of 2-aminopropanol to propionaldehyde and NH₄⁺ is reversible, since tritiated 2-aminopropanol was isolated from reaction mixtures originally containing only propionaldehyde, ammonia, and enzyme [5′-aH]adenosyl cobalamin.

The partitioning of tritium derived from enzyme [5′-aH]adenosylcobalamin between L-2-aminopropanol and propionaldehyde was determined in reactions begun with L-2-aminopropanol as well as in reactions begun with propionaldehyde and ammonia. In the first case, the ratio [3H]propanol-2 to [3H]propionaldehyde is 1.8:1; in the second case, the ratio is 0.3:1. This difference in the partitioning of tritium from the trinitiated enzyme complex is consistent with the notion that there are at least two intermediates in the catalytic process, each exchanging tritium with coenzyme, which interconvert slowly with respect to the rate of tritium exchange.

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Ethanolamine ammonia-lyase (EC 4.1.3) from Clostridium sp., an adenosylcobalamin-requiring enzyme, catalyzes the conversion of ethanolamine to acetaldehyde and NH₄⁺ (2). It has been reported that L-2-aminopropanol is a competitive inhibitor for that reaction (3). In the course of our studies on the mechanism of action of ethanolamine ammonia-lyase, we have confirmed this fact, but found, in addition, that L-2-aminopropanol is also a substrate for that enzyme. This substrate displays some unique properties which enabled us to obtain further evidence in support of the mechanism previously proposed for adenosylcobalamin-dependent rearrangements (4–7). The results obtained with L-2-aminopropanol are reported in this and a subsequent communication.

MATERIALS AND METHODS

Enzyme, Coenzyme, and Substrate—Ethanolamine ammonia-lyase from Clostridium sp. (3) was purified and resolved of bound cobamides as previously described (8, 9). Enzyme concentration was calculated on the basis of a molecular weight of 520,000 (8) and the highest specific activity reported of 45 units per mg (10). The enzyme possesses two active sites per molecule (11–13). The enzyme was made substrate-free by dialysis against 0.01 M potassium phosphate buffer, pH 7.4.

AdoCbl was obtained from Glaxo Laboratories. Ethanolamine, L-2-aminopropanol, and propionaldehyde were obtained from commercial sources and were redistilled before use. Both ethanolamine and L-2-aminopropanol were converted to their respective hydrochlorides. All other reagents were of the highest purity obtained commercially.

Isotopically Labeled Compounds—Adenosylcobalamin containing tritium on the 5′ carbon of the adenosyl moiety ([3H]AdoCbl) was prepared from unlabeled adenosylcobalamin, DL-1,2-[1-14C]propanediol, and dioldehydrase (4, 6). L-2-Amino[U-14C]propanol was synthesized by reducing the trifluoroacetate salt of L-[U-14C]alanine with diborane (14). The trifluoroacetate was prepared by adsorbing 171 µmoles (7.7 × 10⁵ dpm per µmole) of L-[U-14C]alanine (New England Nuclear) onto a column (5 × 40 mm) of Dowex 50-X8 resin (H⁺ form). After washing the column with 20 ml of water, L-[14C]alanine

The abbreviations used are: AdoCbl, adenosylcobalamin (formerly known as coenzyme B₁₂); [3H]AdoCbl, [5′-aH]adenosylcobalamin.
was eluted with 1.5 N trifluoroacetic acid. The eluate was evaporated to dryness on a rotary evaporator, and the glassy oil remaining was further dried in a vacuum desiccator over P_{2}O_{5} for 2 hours. The reduction was carried out by the addition of 3 ml of 1 M diborane in tetrahydrofuran to L-^{14}C]-alanine trifluoroacetate; the reaction was allowed to proceed at room temperature for 2 hours. For isolation of the product, tetrahydrofuran was evaporated under a stream of nitrogen. The residue was dissolved in 2 ml of anhydrous methanol containing 1 drop of trifluoroacetic acid and allowed to stir for 1 hour. The resulting solution was taken to dryness on a rotary evaporator, and the residue was twice dissolved in 3 ml of methanol and immediately evaporated to dryness each time. This procedure served to remove borate as its volatile methyl ester. The residual material was dissolved in 0.2 ml of H_{2}O. L-2-Amino[^{14}C]-propanol was purified by chromatography on a column (0.9 x 36.5 cm) of Dowex 50-X8 resin using 0.2 M pyridine acetate buffer (pH 3.5) as the developing solvent (15). The aqueous solution of radioactive propanolamine was diluted to 2 ml with 0.2 M pyridine acetate buffer (pH 3.5), the pH adjusted to 2.2 with 3 N HCl, and the resulting solution applied to the Dowex column. The radioactive fractions, which were eluted between 180 and 210 ml, were pooled and lyophilized. The lyophilized material was dissolved in 1 ml of H_{2}O and passed through a column (5 x 30 mm) of Dowex 1 X8 resin (OH- form). The eluate was neutralized with 0.05 N HCl. The over-all yield was 25%, and the specific activity of the product was 7.4 x 10^{6} dpm per µmole.

**Assays**—Ethanolamine ammonia-lyase was assayed by measuring the rate of conversion of ethanolamine to acetaldehyde and ammonia. The enzyme was diluted for assay to 0.2 to 0.8 unit per ml in 0.05 M potassium phosphate buffer, pH 7.4, containing 0.05 M ethanamine hydrochloride. The assay mixtures contained 0.2 ml of diluted enzyme, 50 µmoles of potassium phosphate buffer (pH 7.4), 100 µmoles of ethanolamine hydrochloride, and 0 or 0.013 µmole of AdoCbl in a total volume of 1.0 ml. The reaction was initiated by the addition of coenzyme and was incubated at 37°C for 4 min. All reactions in which coenzyme was used were carried out in the dark. The reaction was stopped by the addition of 0.1 ml of 2 N HCl, and the amount of aldehyde produced was measured colorimetrically (16). Sodium pyruvate was used as a standard for the assay. One unit was defined as the amount of enzyme catalyzing the formation of 1 µmole of acetaldehyde per min. Protein was determined by the method of Lowry et al. (17) with appropriate correction (3).

The concentration of adenosylcobalamin was assayed spectrophotometrically at 367 nm after conversion to diecyanocobalamin with KCN, using 30.4 x 10^{4} M^{-1} cm^{-1} as the extinction coefficient (18).

L-2-Aminopropanol was measured by oxidation with periodate and subsequent colorimetric determination of the formaldehyde produced (19). Aldehyde concentrations were determined as in the enzyme assay. L-Alanine was quantitated with ninhydrin (20).

Radiochemical assays were carried out by liquid scintillation counting with a solvent system consisting of 7 g of 2, 3-diphenyloxazole, 300 mg of p-bis[2-(5-phenyloxazolyl)]benzene, and 100 g of naphthalene in 1 liter of dioxane solution. Radioactivity measurements were made using a Nuclear Chicago Mark I or Packard Tri-Carb (model 3320) liquid scintillation spectrometer. The measurements of radioactivity of the 2, 4-dinitrophenylhydrazone derivatives were corrected for quenching by internal standardization with [^{14}C]- or [^{3}H]-toluene (New England Nuclear).

**Isolation of Products**—Most of the experiments described below involved the measurement of tritium transfer from [^{3}H]-AdoCbl to 2-amino propanol and propionaldehyde. To isolate the tritiated propionaldehyde and L-2-aminopropanol produced in these experiments, the following procedure was employed. All procedures involving aldehydes were carried out at 0-4°C. After the addition of carrier propionaldehyde and L-2-aminopropanol, the pH was adjusted to pH 5.5 to 6.0 with 0.2 M K-HPO_{4}. The solution was then treated twice with 10 to 20 mg of charcoal (Daro G60, Matheson, Coleman and Bell) to remove [^{3}H]-AdoCbl. The charcoal was removed by centrifugation, and the supernatant was passed through a column of Dowex 50-X8 resin (5 x 50 mm or 10 x 100 mm, depending on the amount of carrier propionaldehyde added). Small aliquots were taken for measuring propionaldehyde, propanolamine, and radioactivity content before and after separation on Dowex. Propionaldehyde was eluted with water; and after washing the column exhaustively with H_{2}O, L-2-aminopropanol was eluted with 1.5 N HCl. The propionaldehyde was isolated as the propionaldehyde derivative. This was prepared as described previously (4) and recrystallized to constant specific activity. The melting point of the recrystallized derivative (156-157°C) was in agreement with the reported value (21). The acid eluate containing 2-aminopropanoic hydrochloride was taken to dryness on a rotary evaporator. The residue was twice dissolved in 1 ml of water and each time evaporated to dryness to remove any residual HCl. The material was further dried in vacuo over P_{2}O_{5}. The O,N-di(p-bromobenzoyl) derivative of 2-aminopropanol was prepared by a modification of the procedure of Jeger et al. (22). 2-Aminopropanol (550 to 1000 µmoles), dissolved in 1 ml of dry pyridine, was mixed with 3 ml of a suspension of 0.6 g of p-bromobenzoyl chloride in pyridine and stirred for 21 hours at room temperature. Ice water was then added to the reaction mixture. The precipitate which immediately appeared was collected and dissolved in 150 ml of benzene. This solution was extracted three times with equal volumes of a saturated solution of sodium bicarbonate to remove p-bromobenzoyl acid. After being dried over anhydrous sulfate, the organic layer was concentrated until crystals appeared. The material was recrystallized from hot benzene (m.p. 158-159°C (uncorrected); literature, 155°C (22)). The specific activity was the same after each crystallization.

The location of tritium in propionaldehyde was determined by oxidation to propionic acid, while in 2-aminopropanol its location was determined by degradation with periodate. Propionaldehyde was separated from propanolamine by ion exchange chromatography as described above. After purification by bulb-to-bulb distillation, the aldehyde was oxidized to propionic acid with KMnO_{4}. Maintaining the pH at 6.5 with additions of NaOH (23). Propionic acid was isolated by column chromatography on silica acid (24) using 4% 1-butanol as developing solvent. The fractions were titrated and assayed for radioactivity as published previously (4). The 2-aminopropanol eluted from the Dowex column (6.4 µmoles) was dissolved in 1 ml of H_{2}O and was oxidized at pH 6.4 with 1 ml of 0.075 M sodium metaperiodate. The reaction was allowed to proceed for 20 hours in the cold. Formaldehyde and acetaldehyde, formed in the reaction from the alcohol carbon (C-1) and the remainder of the molecule, respectively, were isolated by distillation. The 2, 4-dinitrophenylhydrazone derivatives of these aldehydes were then prepared by adding 3 ml of 1% 2, 4-dinitrophenylhydrazine
in 3 N HCl to the distillate. The method for synthesis of
these derivatives, their separation by paper chromatography,
and their assay have been described previously (4).

RESULTS

The data presented in Fig. 1 show that ethanolamine ammounia-
lyase catalyzes the conversion of L-2-aminopropanol to propional-
dehyde and ammonia. In the experiment shown, 210 nmoles
of propionaldehyde and ammonia were produced for every
nanomole of active site present in the reaction mixture. The
turnover number of about 60 min⁻¹ per active site indicates
that this catalysis is considerably less efficient than that occur-
ing with ethanolamine, the natural substrate (turnover number
8000 min⁻¹ (11)). When L-2-aminopropanol was added to the
enzyme-[H]AdoCbl complex, tritium was transferred from co-
enzyme to both the product, propionaldehyde, and the starting
material, 2-aminopropanol (Table I). Essentially all of the
tritium lost from the coenzyme (i.e. not taken up by charcoal)
was found in either propionaldehyde or propanolamine. The
location of the tritium in each of the two compounds was estab-
lished by the method described above. The results, summarized
in Table II, showed that 2-aminopropanol was labeled exclusively
at C-1, while propionaldehyde was labeled in the α or β position.
Based on earlier enzymatic reactions (4, 7, 25, 26), we conclude
that the tritium was located solely on the α position. Therefore,
the action of ethanolamine ammonia-lyase on L-2-aminopropanol
is very similar to its action on ethanolamine, except that with
ethanolamine reversible hydrogen transfer between substrate
and coenzyme does not occur (26).

The results of Table III show that incubation of enzyme-
[Hz]AdoCbl complex with propionaldehyde, the reaction product,
results in the transfer of radioactivity from the coenzyme to a
compound or compounds not adsorbed by charcoal, provided
ammonia is also present. In the absence of either ammonia
or propionaldehyde, there is no loss of tritium from coenzyme.
(In the latter experiments, the tritium which remained in solution
after charcoal treatment appears to represent a contaminant of
the coenzyme, since the same fraction of tritium remained in
solution after charcoal treatment of reaction mixture in which
ethanolamine-ammonia-lyase was replaced by albumin ("con-
trol," Table I.) As expected from the results of Table I, trit-

![Fig. 1. Catalytic conversion of L-2-aminopropanol to propionaldehyde. The reaction mixture contained 10 nmoles of AdoCbl, 0.4 μmole of L-2-aminopropanol-HCl (specific activity, 7.4 x 10⁴ cpm per μmole), and 1.8 μmole of potassium phosphate buffer, pH 7.4, in a total volume of 0.2 ml; (C) 20 units (approximately 1.7 neq in "active sites") of ethanolamine ammonia-lyase, (●) no enzyme. The enzyme was allowed to react with coenzyme for 3 min before 2-aminopropanol was added to initiate the reaction. All incubations were carried out at 25°C. All groups of 0.04 ml were removed from the reaction and quenched in 0.5 ml of 0.05 N HCl. Propionaldehyde and propionaldehyde carrier (5.6 μmole) was added to every time point. After neutralization, the reaction was distilled, and propionaldehyde isolated as the 2,4-dinitrophenylhydrazone derivative as described under "Materials and Methods."](http://www.jbc.org/)

| TABLE I |
|-----------------------------------------------|
| **Enzymatic transfer of tritium to 2-aminopropanol and propionaldehyde from [H]AdoCbl** |
| The reaction mixture contained 30.5 units (approximately 2.6 neq of "active sites") of ethanolamine ammonia-lyase, 6.7 nmoles of [H]AdoCbl (specific activity, 2.95 x 10⁴ cpm per nmole), 53.2 nmoles of L-2-aminopropanol-HCl, 50 μmole of potassium phosphate buffer (pH 7.4), and 1.1 mmoles of glycerol in a total volume of 1 ml. The reaction was started by the addition of enzyme and was allowed to proceed for 5 min at 37°C. A control in which 100 μg of bovine serum albumin were substituted for enzyme was also carried out. The reaction was stopped by the addition of 0.2 ml of 0.3 N HCl, and carrier propionaldehyde (730 cpm) and L-2-aminopropanol-HCl (1125 cpm) were added. The solution was neutralized, and propionaldehyde and L-2-aminopropanol were isolated as described under "Materials and Methods." |

| Fraction or compound assayed | Total radioactivity |
|-----------------------------|--------------------|
|                             | Reaction Control   |
|                             | cpm x 10⁻⁵         |
| Supernatant after charcoal treatment... | 11.2 2.3 |
| O-N-Di(4-bromobenzoyl)propanoiamine | 3.7 2.3 |
| Propionaldehyde             | 7.4 6.4 |
| Propanolamine               | 0.6 0.6 |

| TABLE II |
|-----------------------------------------------|
| **Location of tritium derived from [H]AdoCbl in propionaldehyde and propanolamine** |
| The reaction mixture contained 12.8 units (1.1 neq of "active sites") of ethanolamine ammonia-lyase, 0.6 nmole of [H]AdoCbl (specific activity, 1.1 x 10⁴ cpm per nmole), 0.5 μmole of L-2-aminopropanol-HCl, and 0.5 μmole of potassium phosphate buffer (pH 7.4) in a volume of 0.2 ml. The reaction was carried out for 4 min at 25°C and stopped by the addition of 0.5 ml of 0.05 N HCl. Carrier propionaldehyde (66.7 μmole) and L-2-aminopropanol-HCl (8.0 μmole) were added, and the solution was neutralized. The details for determination of the position of tritium in propionaldehyde and propanolamine are described under "Materials and Methods." |

| Compound isolated | Total radioactivity |
|-------------------|--------------------|
|                   | cpm x 10⁻⁵         |
| Propionaldehyde   | 3.7                |
| Propanolamine, derived from propionaldehyde | 3.8 |
| L-2-Aminopropanol | 10.4               |
| C-1 of L-2-aminopropanol (as formaldehyde 2,4-dinitrophenylhydrazone) | 11.2 |
| C-2 and C-3 of L-2-aminopropanol (as acetaldheyde 2,4-dinitrophenylhydrazone) | 0.0 |
Ammonia dependence for enzymatic transfer of tritium from [³H]AdoCbl in presence of propionaldehyde

Enzyme (14 units, approximately 1.19 neq of "active sites"), 0.8 nmole of [³H]AdoCbl (specific activity, 3.8 × 10⁵ cpm per nmole), and 4.5 μmoles of potassium phosphate buffer (pH 7.4) were incubated in four reaction mixtures as indicated. A control containing 2-aminopropanol was also included. The incubations were conducted in a volume of 0.3 ml in stoppered tubes at 21°. The reactions were begun by the addition of either propionaldehyde or propanolamine. At the time shown, a 0.1 ml aliquot of the reaction was withdrawn and quenched in 0.9 ml of 0.01 N HCl. Carrier propionaldehyde (400 μmoles) was added; and, in addition, 1.0 nmole of 2-aminopropanol was added to the reaction containing propanolamine. The solutions were neutralized, treated twice with charcoal, and assayed for radioactivity remaining in the supernatant.

| Addition of reaction | Total radioactivity (in supernatant after charcoal treatment) |
|---------------------|-----------------------------------------------------------|
|                     | 20 s | 5 min | 40 min |
| NH₄Cl (16 μmoles)   | 7.2  | 7.3   |
| Propionaldehyde (15.4 μmoles) | 7.1 | 7.2 |
| NH₄Cl (15.4 μmoles) and propionaldehyde (15.4 μmoles) | 15.3 | 20.1 |
| L-2-Aminopropanol (15 μmoles) | 18.0 | 39.4 |

Tritium transfer from [³H]AdoCbl to propionaldehyde and 2-aminopropanol in presence of propionaldehyde and ammonia or L-2-aminopropanol

Each reaction mixture contained 5.4 units (approximately 0.46 neq of "active sites") of ethanolamine ammonia-lyase, 0.40 nmole of [³H]AdoCbl (specific activity, 2.9 × 10⁵ cpm per nmole), and 2.3 μmoles of potassium phosphate buffer (pH 7.4) in a volume of 0.13 ml. Additional components were added as indicated. Incubations were initiated with enzyme and were carried out at 23° in stopped tubes. Thirty minutes later, the reaction was stopped with 0.9 ml of 0.01 N HCl. Carrier propionaldehyde (630 μmoles) and L-2-aminopropanol-HCl (300 μmoles) were added, and the solution neutralized. Propionaldehyde was isolated as propionaldehyde methyl and 2-aminopropanol as the O,N-di(p-bromobenzoyl) derivative, as described under "Materials and Methods."

| Addition to reaction | Total radioactivity |
|---------------------|---------------------|
|                     | Supematant (after charcoal) | Propionaldehyde | O,N-Di-(p-bromobenzyl) propionaldehyde |
| NH₄Cl (6.5 μmoles)  | 8.1 | 1.6 | 0.5 |
| NH₄Cl (6.5 μmoles) and propionaldehyde (6.8 μmoles) | 25.0 | 21.5 | 7.0 |
| L-2-Aminopropanol (0.13 μmole) | 47.6 | 18.1 | 34.2 |
| L-2-Aminopropanol (0.04 μmole) | 47.6 | 18.2 | 33.1 |

Discussion

The results reported here show that L-2-aminopropanol is a substrate for ethanolamine ammonia-lyase and is deaminated to propionaldehyde and NH₄⁺. When the reaction was carried out in the presence of [³H]AdoCbl, tritium was transferred both to propanolamine and to propionaldehyde. Tritium from the coenzyme was found in the C-1 position of propanolamine, while in propionaldehyde, the tritium was located at the carbonyl group. This labeling pattern is consistent with previously proposed mechanisms for this reaction (5, 26, 27). Therefore, we believe that the interaction of L-2-aminopropanol with enzyme-coenzyme complex results in the formation of intermediates similar to those present in the normal catalytic pathway.

The reaction with L-2-aminopropanol, however, differs from that with ethanolamine in two respects. (a) With L-2-aminopropanol, tritium from the coenzyme is transferred to the substrate. No tritium transfer from coenzyme to substrate can be detected with ethanolamine. (b) The reaction with L-2-aminopropanol is reversible. No evidence for reversibility has been detected in the reaction with ethanolamine.

The over-all reversibility of the deamination of L-2-aminopropanol was established by the appearance of tritiated propanolamine in an experiment in which only propionaldehyde and ammonia were incubated with the enzyme-[³H]AdoCbl complex. Though these results show clearly that the reaction is reversible, they give no indication as to the equilibrium constant for the
deamination of propanolamine. The results of Table IV show that 25% of the tritium lost from the cofactor appears in propanolamine, the remainder being found in propionaldehyde. It is likely, however, that the tritiated propanolamine is in equilibrium with the tritium-labeled coenzyme, since the exchange of tritium between coenzyme and propanolamine is rapid with respect to the duration of the experiment in Table IV (see Table I). Under such circumstances, it would be expected that the specific activity of the propanolamine would be the same as the specific activity of the tritiated coenzyme, except for a statistical factor of 3 (assuming that the product of the reaction between propanolamine and adenosylcobalamin is 5'-deoxyadenosine) and whatever small equilibrium isotope effect the reaction may display.

The results of Table IV show, on the other hand, that hydrogen exchange between propionaldehyde and coenzyme is far from equilibrium, since the concentration of aldehyde exceeds the concentration of adenosylcobalamin by a factor of 1.7 × 10^4, while only 18% of the tritium originally in the coenzyme has been transferred to propionaldehyde. Assuming isotopic equilibration between propanolamine and adenosylcobalamin, the quantity of propanolamine in the reaction mixture is calculated to be 0.072 nmole, compared with 6.8 µmoles of propionaldehyde. The similarity in the amounts of radioactivity can be ascribed to differences in specific activity, the propanolamine being much more highly labeled than the propionaldehyde.

The data in Table IV show that the ratio between the amount of tritium transferred from the coenzyme to substrate and that transferred to product depends upon whether the reaction is started with L-2-aminopropanol or with propionaldehyde and NH₄⁺. When started with propanolamine, the ratio of tritium in propanolamine to that in propionaldehyde is 1.8, while when started with propionaldehyde and NH₄⁺, it is 0.3. Since the tritium distribution depends upon whether the reaction is started from the product or substrate side, it can be concluded that there must be more than one intermediate species which can exchange tritium with product or starting material. If there were only one such species (Scheme 1), tritium distribution should be the same whether the reaction is started with propanolamine or propionaldehyde.

\[ \text{SH} + \text{EH} \xrightarrow{k_1} \text{I-EH} \xrightarrow{k_2} \text{PH} + \text{EH} \]

**Scheme 1**

In Scheme 1, SH, PH, EH, and I·EH represent substrate, product, enzyme-AdoCbl, and intermediate-enzyme-AdoCbl, respectively. Tritiated species are denoted by superscript 3. According to this scheme, the partitioning of tritium between starting material and product, represented by the ratio \( \text{SH}:\text{PH} \), will depend only upon the fate of \( \text{I-EH} \); i.e., it will be determined by the relative rates of Steps 3 and 4. In such a scheme, \( \text{SH}:\text{PH} \) will be the same whether the reaction is started with SH or PH. Since the observed ratios depend on the nature of the starting material, the single intermediate mechanism is not applicable to the reaction investigated here.

A mechanism consistent with the results is the one shown in Scheme 2, involving two intermediates each of which can exchange tritium with the coenzyme.

\[ \text{SH} + \text{EH} \xrightarrow{k_1} \text{I-EH} \xrightarrow{k_2} \text{PH} + \text{EH} \]

**Scheme 2**

In such a mechanism there are conditions under which the \( \text{SH}:\text{PH} \) ratio would depend upon the nature of the starting material (i.e., upon whether the reaction is started with SH or with PH). If the rate of interconversion of the two intermediates is slower than the rate of transfer of tritium to starting material (i.e., if \( k_2 < k_4 \) and \( k_3 < k_3 \)), then when SH is the substrate, \( \text{SH}:\text{PH} \) will depend upon the value \( k_3/k_4 \), while \( \text{SH}:\text{PH} \) obtained with PH as substrate will depend upon \( k_2/k_3 \). If \( k_2/k_3 \neq k_4/k_4 \), then the partitioning of tritium will be different for each of the two starting materials.

Mechanisms of this type have, in fact, been proposed for reactions involving adenosylcobalamin (5, 6, 25, 27). In these mechanisms, \( (\text{I-EH})_1 \) and \( (\text{I-EH})_2 \) correspond to enzyme-bound complexes consisting of a substrate-cobalamin adduct and 5'-deoxyadenosine and a product-cobalamin adduct and 5'-deoxyadenosine, respectively. The results reported here, therefore, provide further evidence for the previously proposed mechanism (5, 6, 25, 27).

As already mentioned, no transfer of tritium from coenzyme to amino alcohol accompanies the conversion of ethanolamine to acetaldehyde catalyzed by ethanolamine ammonia-lyase. We tentatively attribute the different properties of the reaction with L-2-aminopropanol to the following. It is probable that in the deamination of ethanolamine, interconversion of the two intermediates is rapid and essentially irreversible. Even though the transfer of hydrogen from substrate to AdoCbl might be reversible in principle, the rapid removal of the intermediate formed by guest on March 24, 2020http://www.jbc.org/Downloaded from
in this hydrogen transfer \((I \cdot \text{EH})_1\) (Scheme 2) by the subsequent irreversible step would ensure that the reversibility of hydrogen transfer would be undetectable experimentally. We propose that when L-2-aminopropanol is the substrate, the interconversion of the cobalamin adducts \((I \cdot \text{EH})_1 \equiv (I \cdot \text{EH})_2\) in Scheme 2) becomes rate-determining, so that the reversibility of formation of the first complex can now be observed. Tritium transfer to both substrate and product can, therefore, occur. A consequence of the proposed change in rate-determining step is that when the substrate is propanolamine the amount of the first intermediate \((I \cdot \text{EH})_1\), which accumulates under steady state conditions, should be larger than when it is ethanolamine. In a subsequent communication, we will present evidence that this is, in fact, the case.

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