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Deamination of 2-Aminopropanol by Ethanolamine Ammonia-lyase, an AdoCbl-Requiring Enzyme. Kinetics and Isotope Effects for the *R* and *S* Enantiomers of the Substrate[†]

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ABSTRACT: Both (*R*)-2-aminopropanol and (*S*)-2-aminopropanol are substrates for ethanolamine ammonia-lyase, reacting to yield propionaldehyde and ammonia via a common enzyme-bound intermediate. (*S*)-Propanolamine shows a lower K_m and a higher V_{max} than the *R* enantiomer, indicating that the former is a substantially better substrate than the latter. From the isotope effects and other considerations, it appears that the rate-limiting step for (*S*)-propanolamine is the same as that for ethanolamine, namely, the transfer of hydrogen from the cofactor to the product. In contrast, isotope effects seen with (*R*)-propanolamine indicate that there are two rate-limiting steps, both located in the segment of the reaction mechanism which is unique to the *R* enantiomer. One of these is the transfer of hydrogen from the substrate to the cofactor. The other is a subsequent step whose rate is similar to that of the first hydrogen transfer step but which does not involve the rupture of a carbon–hydrogen bond. It is speculated that this second rate-limiting step may have to do with the migration of the amino group.

Ethanolamine ammonia-lyase, an AdoCbl-dependent enzyme, catalyzes the conversion of ethanolamine and (*S*)-2-aminopropanol to ammonia and the respective aldehydes (Babior, 1969; Carty et al., 1974). This conversion involves the transfer of a hydrogen initially on the carbinol carbon of the substrate to the adjacent carbon atom, the cofactor serving as intermediate hydrogen carrier. Accompanying the transfer of hydrogen is a concomitant migration of the amino group to the carbinol carbon to form the 1,1-amino alcohol, which then eliminates ammonia to yield the product aldehyde (Scheme I) (Babior, 1975; Abeles & Dolphin, 1976; Babior & Krouwer, 1979).

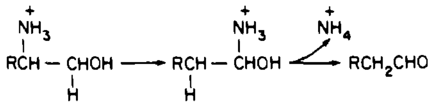
Studies with isotopically labeled ethanolamine have been conducted in the past to obtain information concerning the mechanism of this reaction. The initial studies disclosed a large isotope effect on the overall reaction rate when [1,1-$^2H_2$]ethanolamine was used as substrate (Babior, 1969). These results indicated that the reaction rate was limited by a hydrogen transfer, although the data then available did not permit a decision as to whether the limiting step was the transfer of hydrogen from the substrate to the cofactor or from the cofactor to the product. Subsequent investigations with tritiated substrate and cofactor, however, established that the latter was the rate-limiting step (Weisblat & Babior, 1971).

During the course of recent stereochemical investigations, it was found that both (*S*)-2-aminopropanol and (*R*)-2-aminopropanol were substrates for the enzyme (Diziol et al., 1980). A large deuterium isotope effect was observed with the former substrate, but a much smaller one was seen with the latter. In this paper we describe more detailed kinetic studies with the isotopically labeled propanolamines. These studies have provided further information regarding the mechanism of action of ethanolamine ammonia-lyase.

Materials and Methods

The preparation of ethanolamine ammonia-lyase from *Clostridium* sp. and its separation from bound cobamides followed the method of Kaplan & Stadtman (1968), except that 0.1 mM phenylmethanesulfonyl fluoride and 1.0 mM ethylenediaminetetraacetic acid (EDTA) were in the buffer in which the bacteria were suspended for disruption, and the ammonium sulfate precipitate was obtained by bringing the poststreptomycin supernatant to 50% saturation with $(NH_4)_2SO_4$. The protein was determined with the Bio-Rad protein assay. Calculation of the concentration of active sites was based on an equivalent weight of 260 000 (Babior & Li, 1969).

Scheme I



AdoCbl, (*R*)-alanine, and yeast alcohol dehydrogenase were purchased from Sigma Chemical Co., (*S*)-alanine methyl ester and (*S*)-2-aminopropanol were from Aldrich Chemical Co., and $LiAlD_4$ was from Alfa Chemicals. Other reagents were of the highest purity commercially available. Chemicals and reagents were used without further purification.

(*S*)-2-Amino[1,1-$^2H_2$]propanol, (*R*)-2-aminopropanol, and (*R*)-2-amino[1,1-$^2H_2$]propanol were synthesized by reducing the appropriate enantiomer of alanine methyl ester with unlabeled or fully deuterated $LiAlH_4$ as previously described (Babior et al., 1974b). The products cochromatographed with authentic (*S*)-2-aminopropanol on a Whatman 1 paper chromatogram developed with 1-butanol–acetic acid–water (4:1:1 v/v; R_f = 0.36). NMR spectra of the products were consistent with the expected structures.

The deamination of the various substrates was measured spectrophotometrically by using the previously described assay in which aldehyde production is coupled to reduced nicotinamide adenine dinucleotide (NADH) oxidation by yeast alcohol dehydrogenase (Kaplan & Stadtman, 1968). The rate of deamination of the three-carbon substrates was found to decline exponentially with time (see Results); for these substrates, initial rates were calculated as described in the Appendix.

Results

It has previously been shown that (*S*)-2-aminopropanol is a substrate for ethanolamine ammonia-lyase, reacting to yield

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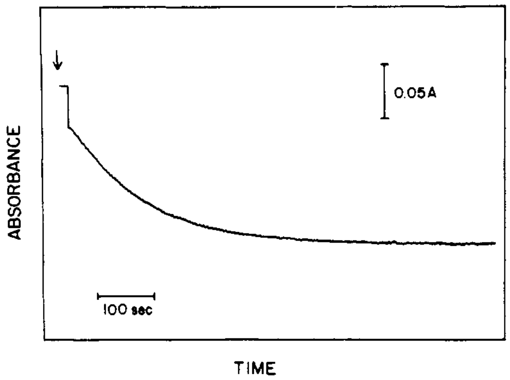


FIGURE 1: Representative reaction progress curve for the deamination of 2-aminopropanol by ethanolamine ammonia-lyase. Incubations were routinely conducted at 23 °C in 1-mL quartz cuvettes (1-cm path length), following the disappearance of NADH spectrophotometrically at 340 nm with a Cary 118C instrument. All operations were carried out under safelights to prevent photolytic destruction of the cofactor. For this particular experiment, the reaction mixture contained 0.16 nmol of ethanolamine ammonia-lyase (expressed as active sites), 0.16 μmol of (*S*)-2-aminopropanol hydrochloride (pH 7.4), 30.0 nmol of AdoCbl, 0.064 μmol of NADH, 0.1 mg of yeast alcohol dehydrogenase, and 0.1 μmol of potassium phosphate buffer (pH 7.4) in a total volume of 1.0 mL. The reaction was initiated with enzyme (arrow).

Table I: Kinetic Parameters for the Deamination of (*R*)- and (*S*)-2-Aminopropanol[a]

substrate	K_m (μM)	turnover no. (s^{-1})	V_{max}/K_m × 10^3
(*S*)-2-aminopropanol	15.5 ± 5.2	2.3 ± 0.1	148 ± 50
(*R*)-2-aminopropanol	63.6 ± 11.0	0.27 ± 0.01	4.2 ± 0.7

[a] Reaction mixtures were prepared and incubations were conducted exactly as described in Figure 2. Turnover numbers were calculated as described in the Appendix, and kinetic parameters were determined by a least-squares fitting procedure as described in the text.

propionaldehyde and ammonia. The catalytic reaction with this substrate is accompanied by a much slower reaction in which the enzyme–cofactor complex is inactivated due to the irreversible cleavage of AdoCbl to OHCbl and 5′-deoxyadenosine (Babior et al., 1974a). Both the catalytic deamination of (*S*)-2-aminopropanol and the inactivation of the enzyme–cofactor complex are represented in Figure 1, which shows the time course of aldehyde production as measured spectrophotometrically. By analyzing this curve according to a kinetic model in which the rate of the reaction is proportional to the concentration of an enzyme which is losing activity in a first-order manner (see the Appendix), it was possible to calculate the initial rate of propanolamine deamination as well as the rate constant for the inactivation of the enzyme. Initial rates were calculated in this fashion from curves obtained at a variety of substrate concentrations. These rates fell on a straight line in a Lineweaver–Burk plot (Figure 2), and, when fitted to the Michaelis–Menten equation by a nonlinear least-squares procedure (Tobenfeld et al., 1978), they gave values for the K_m and V_{max} which agreed well with previously reported figures (Table I). The agreement between the results obtained here and those obtained in previous studies (Carty et al., 1974; Holloway et al., 1978) indicates that the kinetic model used for analyzing the reaction progress curves was appropriate.

(*R*)-2-Aminopropanol is also a substrate for the enzyme. As with (*S*)-2-aminopropanol, the deamination of this substrate was accompanied by a loss in the catalytic activity of the enzyme which was slow with respect to the turnover of the substrate. Analysis of reaction progress curves at several concentrations of (*R*)-propanolamine gave results which, while qualitatively similar to those obtained with (*S*)-propanolamine, were quantitatively very different (Table I). The differences, apparent both in V_{max} and in K_m, show that (*R*)-propanolamine is substantially worse than (*S*)-propanolamine as a substrate for ethanolamine ammonia-lyase.

Studies with deuterium- and tritium-labeled ethanolamine have shown that with this substrate, the rate-limiting step in the reaction is the second hydrogen transfer step—that is, the transfer of hydrogen from 5′-deoxyadenosine to the product radical to form enzyme-bound 1-aminoethanol (Weisblat & Babior, 1971). Experiments with (*R*)- and (*S*)-[1,1-$^2H_2$]-propanolamine were carried out in order to obtain information as to the identity of the rate-limiting step with these substrates. The results of these experiments are shown in Table II. With (*S*)-propanolamine, both V_{max} and V_{max}/K_m displayed an isotope effect of 5.5–6.0. These values are very similar to those

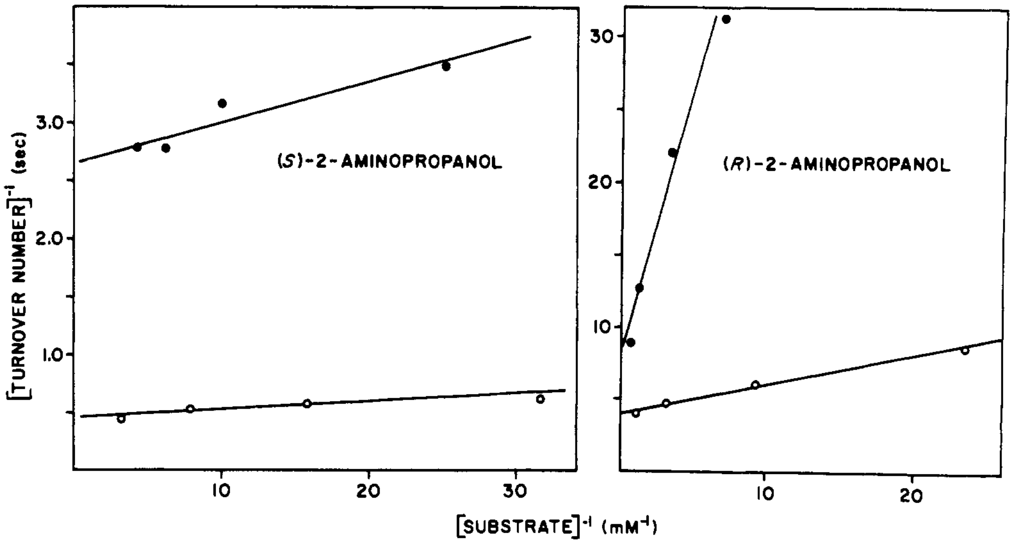
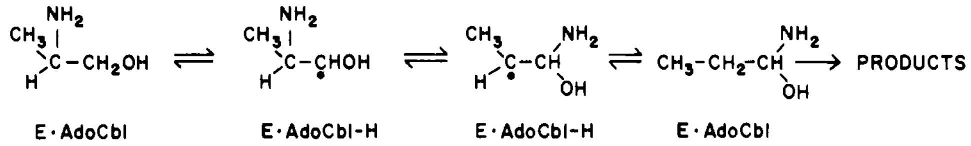


FIGURE 2: Reciprocal plots of turnover number against substrate concentrations for the deamination of the two enantiomers of undeuterated and dideuterated 2-aminopropanol. Reaction mixtures were prepared and incubations were conducted exactly as described in Figure 1, except that the amount of ethanolamine ammonia-lyase varied between 0.082 and 0.431 nmol and the configuration, isotopic substitution, and concentrations of substrates were as shown. Turnover numbers were calculated as described in the Appendix. (Left) (*S*)-2-Aminopropanol; (right) (*R*)-2-aminopropanol. (○) Undeuterated substrate; (●) 1,1-$^2H_2$-labeled substrate.

Scheme II



Scheme III

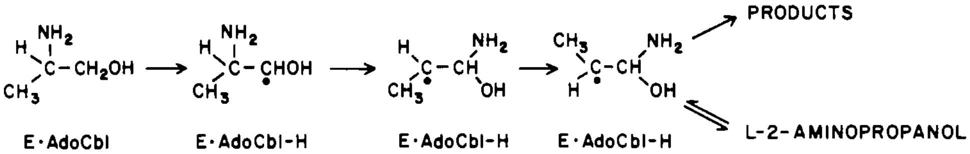


Table II: Isotope Effects for the Deamination of (*R*)- and (*S*)-2-Aminopropanol[a]

substrate	isotope effects V_{max}	isotope effects V_{max}/K_m
(*S*)-2-aminopropanol	5.9 ± 0.4	5.4 ± 2.5
(*R*)-2-aminopropanol	1.6 ± 0.2	20.0 ± 5.8

[a] Kinetic parameters for (*R*)- and (*S*)-2-amino[1,1-$^2H_2$]propanol were determined as described in Table I. From these values and those obtained for the unlabeled substrates, isotope effects were calculated for V_{max} (the turnover number) and V_{max}/K_m.

observed with ethanolamine. Interesting differences, however, were observed between (*S*)-propanolamine and (*R*)-propanolamine. The isotope effect on V_{max} was considerably smaller with the *R* than with the *S* enantiomer, while the isotope effect on V_{max}/K_m was somewhat larger. These results indicate that hydrogen transfer is at least partially rate limiting for both enantiomers, but that the hydrogen transfer step which limits the reaction rate is different in the two cases.

Despite the pronounced differences in the kinetic behavior of the two enantiomeric substrates, the rates of inactivation of the enzyme during the course of the reaction were similar. Rate constants for inactivation were 0.018 and 0.013 s^{-1} for the *S* and *R* enantiomers, respectively. Moreover, inactivation rates seen with the deuterated substrates were the same as those observed with the unlabeled compounds. These findings suggest that the rate of formation of the catalytic intermediate(s) whose aberrant reaction is responsible for the inactivation event is not limited by hydrogen transfer.

Discussion

We have previously shown that ethanolamine ammonia-lyase is able to catalyze the deamination of two enantiomeric substrates (Diziol et al., 1980). This is an unusual property for an enzyme, since most (apart from racemases) are highly specific with respect to the stereochemical configuration of the substrates they act on. There is, however, a precedent for this situation among enzymes which catalyze AdoCbl-dependent rearrangements, inasmuch as diol dehydrase, an unusually well-studied AdoCbl-requiring enzyme which catalyzes the dehydration of several vicinal diols to oxo compounds, has been shown to act on both (*R*)- and (*S*)-propylene glycol (Zagalak et al, 1966). Ethanolamine ammonia-lyase thus represents the second example of an AdoCbl-requiring enzyme which is able to act on enantiomeric substrates.

The results in Table II show that with (*S*)-propanolamine as the substrate, a primary isotope effect is expressed on both V_{max} and V_{max}/K_m. The magnitudes of both these effects are similar to those obtained with ethanolamine. These results indicate that, as with ethanolamine, a hydrogen-transfer step is rate limiting with this substrate.

The two hydrogen transfer steps in the AdoCbl-dependent deamination of amino alcohols are the abstraction of hydrogen from the substrate by the cofactor and the return of hydrogen from the cofactor to the product. With ethanolamine, the rate-limiting step has been shown to be the second hydrogen transfer step. The same seems to be true with (*S*)-propanolamine, as indicated by the similarity in isotope effects with the two substrates, by the prior finding that the migrating hydrogen in (*S*)-propanolamine exchanges rapidly with AdoCbl (Carty et al., 1974), and by the observation that the form of the substrate which accumulates at the active site during the deamination of (*S*)-propanolamine is the free radical produced in the first hydrogen transfer step (Babior et al., 1974b). A reaction mechanism applicable to this substrate is shown in Scheme II.

The deamination of (*R*)-propanolamine appears to proceed by a pathway whose steps lead to an intermediate which is common for both the *R* and *S* enantiomers. This conclusion is based on the observation that when the enzyme and (*R*)-propanolamine are incubated with [5′-^{3}H]AdoCbl, tritium appears in propionaldehyde and (*S*)-propanolamine but not in (*R*)-propanolamine (Diziol et al., 1980). This result indicates that the initial abstraction of hydrogen from (*R*)-propanolamine must be irreversible under the experimental conditions, since otherwise label would have been transferred from the cofactor back to the starting material. It also indicates that the enzyme is able to invert the configuration of the asymmetric carbon of the substrate, a process most easily envisioned as proceeding by way of the rearranged product radical, a species in which the amino group has been removed from the asymmetric carbon atom and replaced by an unpaired electron. This rearranged and inverted product radical would then be the common intermediate referred to above. The mechanism proposed for the deamination of (*R*)-propanolamine is shown in Scheme III.

The isotope effects observed with (*R*)-propanolamine are very different from those observed with ethanolamine and (*S*)-propanolamine, indicating that the hydrogen transfer step which limits the rate of this reaction is in the portion of the overall reaction path which is unique to (*R*)-propanolamine. The only hydrogen transfer step in this portion of the path is the abstraction of hydrogen from the substrate by the cofactor. This step, rather than the second hydrogen transfer step, is thus rate limiting in this reaction.

This cannot be the only rate-limiting step, however. The fact that the isotope effect on V_{max} is so much smaller than the isotope effect on V_{max}/K_m means that another step is partly rate limiting as well. This step should follow the rate-limiting hydrogen transfer step, should be irreversible under the conditions of the experiment, and should not itself involve a hy-

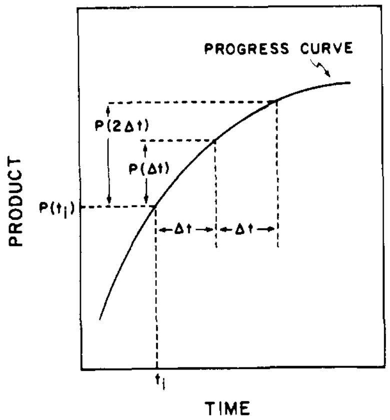


FIGURE 3: Theoretical reaction progress curve. For details see the Appendix.

drogen transfer (Northrup, 1975). It must also be restricted to the portion of the path unique to (*R*)-propanolamine, since the reactions in the other portions of the path are reversible under the experimental conditions. The two steps of the proposed mechanism which fulfill these criteria are the radical rearrangement step and the subsequent step in which the product radical inverts (formally accomplished by a rotation of the radical such that the hydrogen atom and the methyl group exchange places on the enzyme). Both are shown as irreversible in Scheme III, but the kinetics allow one of the two to be reversible. The relationship between the $V_{\max}$ and $V_{\max}/K_m$ isotope effects suggests that the step in which hydrogen is not transferred is slower than the partially rate-limiting hydrogen transfer step by a factor of 10–20.

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Appendix

The kinetic parameters were calculated from a model in which the reaction rate is proportional to the concentration of an enzyme whose activity is declining in first-order fashion. The formation of product in such a system is expressed by

$$\mathrm{d}P/\mathrm{d}t = kE_0e^{-ct}$$

where k is the zero-order rate constant for the enzyme-catalyzed reaction, E_0 is the initial concentration of active enzyme, and c is the rate constant for the decay of catalytic activity. This equation assumes that the reaction is pseudo zero order in substrate.

The progress curve for such a reaction is shown in Figure 3. The rate constant k can be determined from this progress curve in the following manner. The amount of product formed from the beginning of the reaction to time t_i is

$$P(t_i) = (k/c)E_0(1 - e^{-ct_i})$$

The amount of additional product formed from t_i to $t_i + \Delta t$ is

$$P(\Delta t) = (k/c)E_0e^{-ct_i}(1 - e^{-c\Delta t})$$

and from t_i to $t_i + 2\Delta t$ is

$$P(2\Delta t) = (k/c)E_0e^{-ct_i}(1 - e^{-c\Delta t})$$

Dividing

$$P(2\Delta t)/P(\Delta t) = (1 - e^{-2ct})/(1 - e^{-c\Delta t}) = 1 + e^{-c\Delta t}$$

From this expression it is possible to calculate c, and by substituting this value in the expression for $P(t_i)$ the value for k may be calculated.

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