The Mechanism of Action of Ethanolamine Ammonia-lyase, an Adenosylcobalamin-dependent Enzyme

THE SOURCE OF THE THIRD METHYL HYDROGEN IN THE 5'-DEOXYADENOSINE GENERATED FROM THE COFACTOR DURING CATALYSIS*

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Ethanolamine ammonia-lyase is an adenosylcobalamin-dependent enzyme which catalyzes the conversion of ethanolamine and propanolamine to ammonia and the corresponding aldehydes. A mechanism has been proposed for this and other adenosylcobalamin-dependent reactions which involves cleavage of the carbon-cobalt bond of the cofactor followed by abstraction of a substrate hydrogen atom by the adenosyl fragment to form 5'-deoxyadenosine. In support of this proposal, a previous study demonstrated that the deamination of propanolamine by ethanolamine ammonia-lyase is accompanied by the reversible cleavage of the carbon-cobalt bond of the cofactor, with the production of 5'-deoxyadenosine (Babior, B. M., Carty, T. J., and Abeles, R. H. (1974) J. Biol. Chem. 249, 1689-1695). The present study is concerned with the origin of the third hydrogen atom on the methyl group of the 5'-deoxyadenosine produced in that reaction. The 5'-deoxyadenosine isolated from an incubation mixture initially containing enzyme, [5',5'-D,]adenosylcobalamin, and [1,1-D,]propanolamine was chemically degraded so that the 4' and 5' carbon atoms were, respectively, converted to the carbonyl and methyl carbons of acetaldehyde. Analysis of the p-nitrophenylhydrazone of the acetaldehyde by gas-liquid chromatography-mass spectroscopy revealed 3 deuterium atoms/molecule, indicating that two of the methyl hydrogens originated from adenosylcobalamin and the third was donated by substrate. This observation provides further support for the participation of 5'-deoxyadenosine in the mechanism of adenosylcobalamin-dependent reactions.

Ethanolamine ammonia-lyase (EC 4.3.1.7) catalyzes the adenosylcobalamin-dependent conversion of 2-aminoethanol and 2-aminopropanol to ammonia and the respective aldehydes (2, 3). In the course of this reaction, a hydrogen originally attached to the carbinol carbon of substrate is transferred to the adjacent carbon atom, appearing in the methyl carbon of acetaldehyde or the methylene carbon of propionaldehyde, depending on the starting material (3, 4).

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\begin{align*}
\text{H} & \quad \text{O} \\
\text{C} & \quad \text{OH} \\
\text{H} & \quad \text{H}
\end{align*}
\]

In this process the cofactor participates as an intermediate hydrogen carrier, accepting the migrating hydrogen from the substrate and conveying it in a subsequent step to the product (5). The position on the cofactor to which the migrating hydrogen atom is transferred is the adenosine carbon atom that is bonded to the cobalt of the corrin ring (hereinafter referred to as the C-5' carbon). Much evidence has suggested that the mechanism of hydrogen transfer to the cofactor involves homolysis of the carbon-cobalt bond followed by a free radical hydrogen abstraction from the substrate (6-9). In the first step, cob(II)alamin and the 5'-deoxyadenosyl-5'-yl radical are formed, and in the second step the radical abstracts a hydrogen atom from the substrate to form 5'-deoxyadenosine and substrate radical. 5'-Deoxyadenosine is a crucial intermediate in this

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mechanism, and its formation and participation in the catalytic process is supported by numerous experiments. It has been demonstrated that ethanolamine ammonia-lyase is able to form 5'-deoxyadenosine from the adenosyl moiety of the coenzyme during catalysis (8, 9) and that several other adenosylcobalamin-dependent enzymes, including diol dehydrase (10), methylmalonyl-CoA mutase (11), and β-lysin mutase (12), generate the nucleoside under other conditions. The reversibility of the formation of 5'-deoxyadenosine from adenosylcobalamin has also been shown (9). With diol dehydrase, arguments have been made, based on stereochemical and kinetic results (13, 14), which suggest that a compound with three equivalent hydrogens (two derived from the coenzyme and one from the substrate) must be an intermediate in the catalytic process; 5'-deoxyadenosine is a logical choice for this intermediate. Kinetic evidence for the intermediate involvement of 5'-deoxyadenosine has also been presented for glutamate mutase (15) and methylmalonyl-CoA mutase (16).

Despite the evidence described above, which strongly supports a catalytic role for 5'-deoxyadenosine in adenosylcobalamin-dependent rearrangements, it has not yet been directly shown that the hydrogens on the methyl group of 5'-deoxyadenosine isolated when the catalytic process is interrupted are derived solely from coenzyme and substrate, as required by the proposed mechanism. Alternative origins for 5'-deoxyadenosine can be envisioned, as for example its production if an adenosyl radical were formed which abstracts a hydrogen from the environment (protein or solvent) during the process of isolation. We have therefore investigated the origin of the methyl hydrogen atoms of the 5'-deoxyadenosine generated during the ethanolamine ammonia-lyase-catalyzed deamination of propanolamine.

**MATERIALS AND METHODS**

Ethanolamine ammonia-lyase from *Clostridium sp.* was prepared and resolved of bound cobamides by the method of Kaplan and Stadtman (2). Enzyme concentration was calculated on the basis of a molecular weight of 520,000 (17). The enzyme had previously been shown to possess two active sites per molecule (18). Adenosylcobalamin was purchased from Calbiochem. Ribonucleotide reductase was prepared by the method of Hogenkamp et al. (19). [U-14C]Adenosylcobalamin (8), L-2-amino-[1,1-D2]propanol (7), and 5'-deoxyadenosine (10) were prepared as previously described. [U-15N,1-14C]Adenosylcobalamin (4.8 x 10^5 cpm/μmol) was prepared by incubating [14C]adenosylcobalamin in D2O with ribonucleotide reductase in the presence of a reducing agent (19, 20). When this procedure was used previously, 1.6 atoms of deuterium were incorporated into the coenzyme. We therefore assume that the coenzyme obtained contained that amount of deuterium. Since only limited amounts of coenzyme were available, no deuterium analysis was carried out. Other reagents were the best grade commercially available, and were used without further purification.

**Isolation and Degradation of 5'-Deoxyadenosine**—The following two reaction mixtures were prepared. Reaction mixture A contained 40 nmol of ethanolamine ammonia-lyase, 78 nmol of [5',5'-D2,U-14C]adenosylcobalamin (3.8 x 10^5 cpm), 10 μmol of L-2-amino-[1,1-D2]propanol, and 10 μmol of potassium phosphate buffer (pH 7.4) in a total volume of 1.3 ml. Reaction mixture B was identical in composition except that it contained only 67 nmol of [U-15N,1-14C]adenosylcobalamin (3.9 x 10^5 cpm), and neither the coenzyme nor L-2-amino-

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1 Hydrogen present in the coenzyme may reduce the deuterium content of the 5'-deoxyadenosine which we subsequently isolate. As the reaction proceeds, the hydrogen originally present in the coenzyme will gradually be replaced by deuterium from the substrate. Nothing is known about the kinetics of this reaction exchange with 2-amino-propanol as substrate; therefore, no estimate can be made as to the exact deuterium content of the coenzyme when the reaction is terminated.
cobalamin was terminated with trichloroacetic acid while the enzyme was engaged in catalyzing the deamination of 2-amino-[1,1-D2]propanol. The 5'-deoxyadenosine that has been shown to be formed reversibly at the active site under these conditions was isolated, and the deuterium content of its methyl group was analyzed by gas-liquid chromatography-mass spectrometry after suitable derivatization. The presence of 3 deuterium atoms in the methyl group would indicate that the hydrogens in this position were derived solely from coenzyme and substrate as predicted by the proposed mechanism (8, 9). The incubation with undeuterated material served as control. Under the conditions of chromatography, authentic acetaldehyde p-nitrophenylhydrazone emerged from the column as a single peak with a retention time of 9 min (Fig. 1). Mass spectroscopy of this peak revealed a prominent molecular ion at m/e 179. This was the ion used for determination of the deuterium content of the experimental samples. The relative heights of the mass spectrometry peaks in the vicinity of the molecular ion are given in Table I. Data from the gas chromatograph detector showed that the experimental samples were heavily contaminated with extraneous material. With both experimental samples, however, the only compound emerging from the column in the first 18 min that gave a mass spectrometry peak at m/e 179 displayed chromatographic properties virtually identical with those of authentic acetaldehyde p-nitrophenylhydrazone (Fig. 2). From these findings we conclude that this mass spectrometry peak corresponds to the undeuterated hydrazone in the experimental material.

The mass spectra of the sample obtained from the experiment with deuterated substrate and cofactor are presented in Fig. 2 (right). In this figure, the size of the peaks at m/e 179, 181, and 182 (i.e. M, M + 2, and M + 3) are plotted against the retention time.2 The peak heights are corrected for 13C, assuming a molecular formula of C9H16DnN2O (n = 0, 2, 3) for the various species represented. Relative proportions of the three species were determined by experimental integration of the three curves in Fig. 2 (right), a procedure accomplished by cutting out and weighing Xerox copies of the curves. The predominant species are those corresponding to M and M + 3, comprising 91.1% and 7.2% of the total, respectively, while M + 2 only accounts for 1.7% of the total. The results obtained from mass spectrometry thus show that 8.9% of the molecules present contain deuterium. From the original reaction, 49 nmol of 5'-deoxyadenosine were isolated and diluted with 370 nmol of unlabeled 5'-deoxyadenosine. Therefore, 11.7% of the 5'-deoxyadenosine molecules should be deuterated, if one assumes that the original cofactor was completely deuterated. This number is in reasonable agreement with the percentage determined independently by gas-liquid chromatography-mass spectrometry. The data show that 81% of the deuterated acetaldehyde methyl groups contained 3 deuterium atoms and 19% contained 2 deuterium atoms. Thus, of the methyl groups of 5'-deoxyadenosine, 81% contained 3 deuterium atoms, indicating that the third hydrogen atom which is required to convert the 5'-methylene group of the coenzyme to a methyl group is primarily derived from the substrate, not from the environment (protein or solvent). The deuterated species could have arisen from a number of sources. (a) Some loss from the methyl group of acetaldehyde could have occurred during the process of isolation. (b) Dideuterated acetaldehyde could have appeared because the coenzyme used was not completely deuterated (see above). For reasons indicated elsewhere, it is difficult to estimate how much hydrogen this would contribute to the methyl group. (c) The 5'-deoxyadenosyl radical, a species which we believe participates in the catalytic process, may well be present under steady state conditions and could be converted to 5'-deoxyadenosine by abstraction of a hydrogen atom from the environment during isolation.

According to the mechanism which we have proposed, an important and sometimes disputed step in the sequence of reactions catalyzed by ethanalamine ammonia-lyase is the abstraction of a hydrogen atom from the substrate by a radical derived from the adenosyl moiety of the coenzyme to form 5'-deoxyadenosine. We believe that the evidence now available leaves little doubt concerning the involvement of 5'-deoxyadenosine in the catalytic process with ethanalamine ammonia-lyase, and (by analogy) supports its participation in other adenosylcobalamin-dependent rearrangements such as those catalyzed by diol dehydrase (10, 13, 14), glutamate mutase (15), and methylmalonyl-CoA mutase (11, 16).

*No ion was detected at m/e 180 (M + 1).
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The mechanism of action of ethanolamine ammonia-lyase, an adenosylcobalamin-dependent enzyme. The source of the third methyl hydrogen in the 5'-deoxyadenosine generated from the cofactor during catalysis.

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