SHORT COMMUNICATIONS

BBA 63344

Coenzyme B_{12} as hydrogen carrier in the ethanolamine deaminase reaction

In all the coenzyme B_{12}-dependent reactions so far discovered, a substituent on a carbon atom is replaced by a hydrogen[1]. FREY *et al.*[2] have proposed that the coenzyme serves as an intermediate hydrogen carrier in these reactions. In support of this hypothesis, they have shown that during the course of the diol dehydrase reaction, tritium can be transferred from tritium-labeled ethylene glycol and propylene glycol to 5'-deoxyadenosyl cobalamin, as well as from 5'-deoxyadenosyl cobalamin to acetaldehyde and propionaldehyde. More recently it has been found that the B_{12}-dependent ribonucleotide reductase[3], methylmalonyl-CoA isomerase[4] and glutamate mutase[1] are also capable of catalyzing the transfer of tritium between 5'-deoxyadenosyl cobalamin and the other constituents of the reactions. We wish to report the results of similar experiments with ethanolamine deaminase, a B_{12}-dependent enzyme which catalyzes the conversion of ethanolamine to acetaldehyde and ammonia.

Ethanolamine deaminase was purified and resolved of bound cobamide by the method of KAPLAN AND STADTMAN[5]. 2-Amino[1-^{3}H]ethanol was prepared by reducing glycine ethyl ester (95 mg) with $LiAl^3HH_3$ as described by WEISSBACH AND SPRINSON[6], and purified by chromatography on Dowex 50-X8 (H^+), using 1.5 M HCl as eluant. The specific activity was 40 μC/μmole. The purified compound, when chromatographed on Whatman 3 MM paper with *n*-butanol–acetic acid–water (4:1:5, by vol.) as the developing solvent, gave a single radioactive spot which cochromatographed with authentic ethanolamine. The compound was 88% radiochemically pure as judged by the extent of its conversion to radioactive acetaldehyde in the ethanolamine deaminase reaction. 5'-Deoxyadenosyl cobalamin was the generous gift of Professor H. HOGENKAMP. Tritiated 5'-deoxyadenosyl cobalamin was kindly provided by Professor R. H. ABELES.

The ability of ethanolamine deaminase to catalyze the transfer of hydrogen from substrate to cofactor was demonstrated by the observation that in the presence of enzyme and 5'-deoxyadenosyl cobalamin, label from tritiated ethanolamine is incorporated into a photolabile compound. Substrate levels of ethanolamine deaminase were incubated for 2 min at room temperature with tritiated ethanolamine and 5'-deoxyadenosyl cobalamin as described in the legend of Fig. 1. The reaction was stopped by heating in boiling water for 15 sec. Then 0.17 ml of the reaction mixture was placed on a 20-ml column of Biogel P-2. The column was eluted with water, and 1-ml fractions were collected directly into liquid scintillation vials. Up to this point, the experiment was carried out in dim light. BRAY's[7] solution was then added to the scintillation vials, and the fractions were counted in a Nuclear Chicago liquid scintillation counter. The remainder of the reaction mixture was exposed for 10 min to a 100-W tungsten lamp at a distance of 5 cm, after which another 0.17-ml aliquot was chromatographed on the Biogel P-2 column as described above.

As chromatographic markers, cyanocobalamin and adenosine were selected (see Fig. 1b). This choice was based on the considerations that in the diol dehydrase reaction, the tritium was found to be transferred to the 5' position of the cobalt-linked

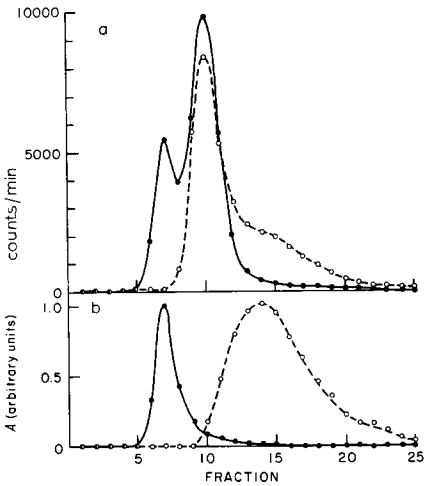

Fig. 1. Transfer of hydrogen from substrate to cofactor. The incubation mixture contained 0.5 mg ethanolamine deaminase, 5 nmoles ethanolamine-[1-^{3}H]HCl, 5 nmoles 5′-deoxyadenosyl cobalamin and 25 μmoles potassium phosphate buffer (pH 7.4) in a total volume of 0.5 ml. The experiment was carried out as described in the text. a. ●—●, before photolysis; ○- - - -○, after photolysis. b. ●—●, cyanocobalamin; ○- - - - -○, adenosine.

deoxyadenosyl moiety, and that aerobic photolysis of this triatiated compound resulted in the release of half the tritium as a proton, while the other half remained attached to adenosine 5′-aldehyde, one of the fragments of the photolytic reaction[8]. When chromatographed on Biogel P-2, cyanocobalamin would be expected to have properties similar to those of 5′-deoxyadenosyl cobalamin, while adenosine would be expected to behave like adenosine aldehyde.

The results are shown in Fig. 1. Two peaks are apparent in the chromatogram of the unphotolyzed reaction mixture. The larger peak can be attributed to the tritiated acetaldehyde produced during the incubation, while the smaller peak appears to represent radioactivity associated with coenzyme, since it cochromatographs with cyanocobalamin. The identity of the small peak is confirmed by the observation that in the chromatogram of the reaction mixture after exposure to light, this peak has been replaced by a broad, slow-moving shoulder which has the same chromatographic properties as adenosine. These results are consistent with the transfer of tritium from substrate to the 5′-deoxyadenosyl moiety of 5′-deoxyadenosyl cobalamin. Control experiments in which enzyme or 5′-deoxyadenosyl cobalamin was omitted showed only the larger peak. (In the control experiments, this peak is assumed to represent ethanolamine rather than acetaldehyde.)

To show that hydrogen from 5′-deoxyadenosyl cobalamin appears in the final product, tritiated 5′-deoxyadenosyl cobalamin was incubated with ethanolamine and enzyme, and the resulting acetaldehyde was isolated and analyzed for tritium. The reaction mixture consisted of 0.2 ml (1.5 mg) ethanolamine deaminase, 2.5 ml of an ethanol–amine buffer solution (0.111 M ethanolamine and 0.05 M potassium phosphate, pH 7.4), and 0.10 ml [^{3}H]5′-deoxyadenosyl cobalamin ($1.7 \cdot 10^{-4}$ M; 122 000 counts/min per ml). The reaction was allowed to proceed for 30 min at 25° in the dark, after which time assay by the 2,4-dinitrophenylhydrazone method[9] indicated that all of the sub-

strate had been converted to aldehyde. The solution was adjusted to pH 5.5 with 0.25 M H_2SO_4, frozen, and sublimed in the dark room. The sublimate was combined with 20 ml of a solution containing 10 mg/ml of 5,5-dimethylcyclohexane-1,3-dione and 5 mg/ml of Na_2CO_3. This was adjusted to pH 3.9 with 1 M HCl and refrigerated overnight. The resulting acetaldomethone was filtered and dried *in vacuo*. The precipitate melted at 139.5–140.5° (uncorr.) and contained 1240 counts/min per mg. After recrystallizing from ethanol–water the melting point was unchanged and the derivative contained 1220 counts/min per mg. If all of the radioactivity in the coenzyme had been transferred to acetaldehyde, the specific activity of the derivative would have been 1420 counts/min per mg. Therefore, 87% of the tritium was actually transferred to acetaldehyde.

These experiments indicate that in the coenzyme B_{12}-dependent ethanolamine deaminase reaction, the cofactor plays the role of a hydrogen carrier, and supplies further evidence for the generality of this mechanism.

The author wishes to thank Dr. P. A. FREY, who performed the experiment with tritiated 5'-deoxyadenosyl cobalamin. He is indebted to Dr. E. R. STADTMAN for helpful advice and support.

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Received June 4th, 1968

Biochim. Biophys. Acta, 167 (1968) 456–458