COENZYME ACTIVITY OF 5'-DEOXYADENOSYL-10-CHLOROCOBALAMIN (10-Cl-DBCC*) IN PROPANEDIOL DEHYDRATASE SYSTEM

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Received July 17,1967

Recent studies from several laboratories have indicated that the coenzyme action of 5'-deoxyadenosylcobalamin would be initiated by the heterolytic cleavage of the linkage between the cobalt atom and 5'-deoxyadenosyl moiety. On the other hand it has been also known that the electronegativity of the group attached to the cobalt atom influences the reactivity of the hydrogen on C-10 or C-8 of the corrin nucleus and the substituent at C-10 exerts a profound effect on the cobalt atom vice versa (Hogenkamp et al., 1965). Although the 10-chloro derivative of DBCC (10-Cl-DBCC) was inactive in methylaspartate mutase reaction (Barker), it seems interesting to study the coenzyme action in the other cobamide-dependent reactions.

This paper deals with the comparison of the coenzyme activity between 10-Cl-DBCC and DBCC in the propanediol dehydratase system of Lee and Abeles (1963) and discussions concerning the possible effect of 10-Cl on the cleavage of the carbon-cobalt bond of the coenzyme-apoenzyme complex.

MATERIALS AND METHODS

Materials CN-B_{12} and DBCC were purchased from Roussel Uclaf Co., France. CH_3-B_{12} was prepared according to Smith et al. (1962). 10-Cl-CH_3-B_{12} was obtained from CH_3-B_{12} by the method of Dolphin et al. (1964), then converted

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* Abbreviations: OH-B_{12}, aquo (or hydroxo) cobalamin; CN-B_{12}, cyanocobalamin; 10-Cl-B_{12}, cobalamin chlorinated at C-10 of its corrin nucleus; DBCC, 5,6-dimethylbenzimidazolylcobamide coenzyme; 10-Cl-DBCC, 10-chloro derivative of DBCC; CH_3-B_{12} and 10-Cl-CH_3-B_{12}, methylcobalamin and its 10-chloro derivative.

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to 10-Cl-OH-B_{12} by irradiation. 10-Cl-DBCC was prepared from 10-Cl-OH-B_{12} enzymatically using a partially purified enzyme from Propionibact. shermanii (Brady et al., 1962) or by reacting its 2-electrons reduced state with 5'-iodo-adenosine according to Murakami et al. (1967). Spectrophotometric assay of 10-Cl-DBCC was performed after converted to its dicyano form. The molar extinction coefficient of the dicyano form, 26×10^3 at 370 mμ, was calculated based on that of 10-Cl-CH_3-B_{12} at 347 mμ, 12×10^3 (Dolphin et al. 1964). Propanediol dehydratase (EC 4.2.1.28) was prepared from Aerobacter aerogenes (ATCC 2784) according to Lee and Abeles (1963).

Assay of the coenzyme activity The assay of the coenzyme activity was carried out in a similar manner to that reported by Lee and Abeles (1963). In order to examine the possibility that 10-Cl-DBCC would be converted to DBCC during the enzyme reaction, the reaction mixture was photolyzed by irradiation with 500-W tungsten lamp at a distance of 15 cm for 10 min. A cobalamin separated from the apoprotein was extracted with phenol, transferred back to aqueous phase by shaking with a small amount of a mixture of ether and water, then examined by paper chromatography, paper electrophoresis and spectroscopy. In this case 150 ml of the enzyme reaction mixture containing 2 mg of 10-Cl-DBCC, 15 units of the apoenzyme, 15 m moles of the substrate and 0.05 M phosphate buffer (pH 8.0) was incubated for 10 min. at 37°.

RESULTS

The coenzyme activity of 10-Cl-DBCC in propanediol dehydratase system

It was observed that 10-Cl-DBCC exhibited the coenzyme activity in the propanediol dehydratase system. As shown in Fig. 1, its Km value was almost identical with that of DBCC, ca. 6×10^{-7}M, whereas their maximal velocities (Vmax) were markedly different. These results seem to indicate that the coenzyme analogue is able to combine with the apoprotein as efficiently as DBCC, but the catalytic activity of the analogue-apoenzyme complex is much lower than that of the normal enzyme. We observed that 10-Cl-OH-B_{12} exerted a competitive inhibitory action against DBCC in the dehydratase system. Its 50 % inhibition index was about 1.7 which was similar to that of OH-B_{12}.

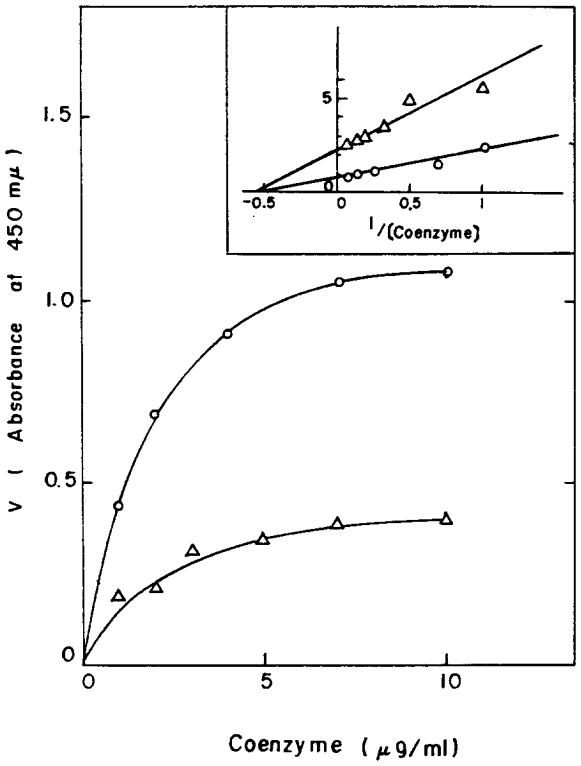


Fig. 1 Comparison of the coenzyme activities of DBCC O and 10-Cl-DBCC Δ in propanediol dehydratase reaction.
The reaction mixture contained the following in a final volume of 1 ml: apoenzyme, 0.10 unit; 1,2-propanediol, 0.1 m mole; potassium phosphate buffer (pH 8.0), 0.05 M; coenzyme, as indicated. Incubation was carried out for 10 min. at 37°. The propionaldehyde formed was assayed colorimetrically as its 2,4-dinitrophenylhydrazone at 450 mμ.

This fact suggests that the chlorine at C-10 doest not influence on the affinity of 10-Cl-OH-B_{12} or 10-Cl-DBCC for the protein. On the other hand both the compounds are known to be inactive in the methylaspartate mutase system (EC 5.4.99.1) (Barker). The discrepancy would be attributed to the difference in the affinity between the corrinoids and the apoproteins in these two systems. The only cobalamin recovered from the reaction mixture including 10-Cl-DBCC showed the same behaviors as 10-Cl-OH-B_{12} on paper chromatography and paper electrophoresis using several kinds of solvents. The occurence of OH-B_{12} could not be detected. The absorption spectrum of

the cobalamin recovered was identical with that of authentic 10-Cl-OH-B_{12}. These results would confirm the maintenance of the chlorine in 10-Cl-DBCC during the enzyme reaction. A time-course study of the propionaldehyde formation revealed that the reaction catalyzed by 10-Cl-DBCC proceeded with a constant lower rate than that catalyzed by DBCC. This suggested that the lower catalytic activity of 10-Cl-DBCC would be due to the resistance of its cobalt-carbon bond to the cleavage in the enzyme reaction caused by the change of the polarization of the bond.

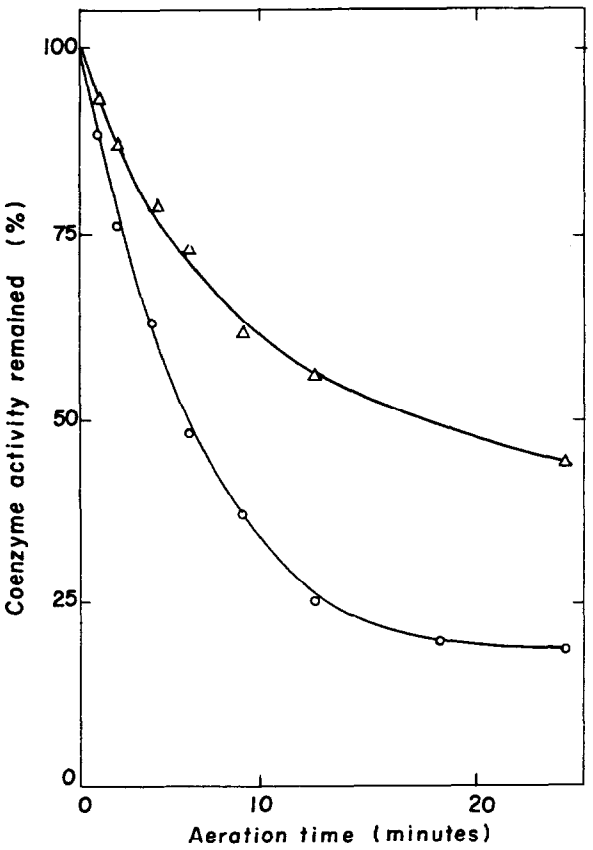


Fig. 2 Comparison of inactivation rates of DBCC-apoprotein complex O and 10-Cl-DBCC-apoprotein complex △ by oxygen.
The reaction mixture contained the following in a final volume of 1 ml: DBCC or 10-Cl-DBCC 1 μg; other components were the same as in Fig. 1 except that propanediol was omitted. Aeration was carried out at 37°. At the indicated time of aeration, 100 μ mole of propanediol was added and the activity remained was assayed.

Cleavage of the cobalt-carbon bond of 10-Cl-DBCC and the complex with the apoprotein.

Lee and Abeles (1963) and O. Wagner et al. (1966) have observed that the DBCC-apoenzyme complex was inactivated by oxygen when incubated without adding the substrate. They proposed that the nature of the cobalt-carbon bond of the coenzyme was modified by the apoprotein to react readily with oxygen. The inactivation velocity of the 10-Cl-DBCC-apoprotein complex was markedly smaller than that of the DBCC-apoprotein complex as illustrated in Fig. 2. Namely the cobalt-carbon bond of 10-Cl-DBCC is more resistant to the oxidative cleavage than that of the coenzyme.

Moreover the decomposition rate of the coenzyme analogue by cyanide was significantly lower than that of DBCC (Fig. 3). According to O. Müller and

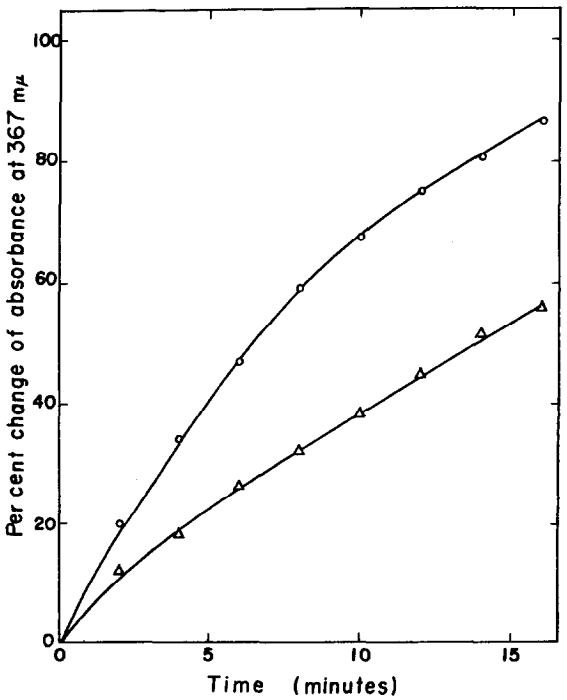
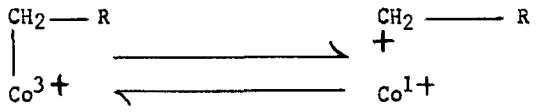


Fig. 3 Comparison of decomposition rates of DBCC ○ and 10-Cl-DBCC △ by cyanide. The reaction mixture contained 0.2 μ mole of DBCC or 10-Cl-DBCC, 0.5 m mole of KOH and 50 μ mole of KCN. The reaction was started by the addition of KCN. The absorbance of the cyano form produced was determined at 367 mμ.

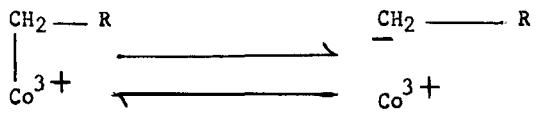
G. Müller (1962), the susceptibility to cyanide has been considered to reflect the polarization of the cobalt-carbon bond. The results mentioned above would indicate that the chlorine at C-10 of the corrin nucleus enhances the polarization of the cobalt-carbon bond in which the electrons are attracted toward the cobalt atom. Analogous effects of the substituents at C-10 have been observed on the reactivity of Co-S bond of 10-halogenocobalamin sulfonate (F. Wagner, 1965).

DISCUSSION

It has been postulated that the enzyme reaction involving DBCC would be initiated by a reversible dissociation of the cobalt-carbon bond resulting from the interaction of the coenzyme and the apoprotein (O. Wagner et al. 1966). In this case the two possible ways in which the dissociation could occur have been presented as follows.



(1)



(2)

Where $R\text{-}CH_2\text{-}Co^{3+}$ represents the cobalt-carbon bond of the coenzyme.

The result of the cyanide treatment strongly suggests that the cobalt-carbon bond of 10-Cl-DBCC should be polarized toward the cobalt atom which would be favarable to yield an electron-rich cobalt and electron-deficient 5'-deoxyadenosyl moiety (Reaction (1)). Nevertheless the coenzyme activity as well as the inactivation rate of 10-Cl-DBCC-apoenzyme complex caused by oxygen was markedly lower than those of DBCC-apoenzyme complex. This discrepancy would be explained by supposing that the polarization in 10-Cl-DBCC is inadequate and the dissociation required for the exhibition of the coenzyme action of DBCC should be as shown in Reaction (2).

Based on the supposition we postulate the action mechanism of DBCC in

propanediol dehydratase reaction as follows: The enzyme (RCH_2:Co) dissociates reversibly to protein-bound B_{12b} (Co^{3+}) and protein-bound 5'-deoxyadenosyl carbanion (RCH_2:$^-$); a hydride ion is abstracted from C-1 of the substrate to Co^{3+}; OH ion at C-2 shifts to C-1 intramolecularly in the substrate; another hydride ion (expressed as H') is transferred to C-2 of the substrate carbonium ion from C'-5 of a hydrogenated state of the coenzyme (RCH_2:--H':Co) which results in the dehydration of the substrate yielding propionaldehyde; the holoenzyme goes back to the initial state through a state consisting of Co^{1+} and 5'-deoxyadenosyl carbonium ion ($RCHH'^+$). This postulated mechanism would be able to explain the findings of Abeles·Zagalak (1966) and Frey·Abeles (1966) that a hydrogen abstracted from C-1 of a substrate was transferred to C-2 of another substrate and a hydrogen attached to 5'-C of the coenzyme was incorporated into the C-1 of the substrate.

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