The Coenzymic and Chemical Properties of a Carbocyclic Analogue of Vitamin B_{12} Coenzyme*

(Received for publication, October 16, 1969)

S. S. Kerwar,† T. A. Smith, and Robert H. Abeles

From the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154

SUMMARY

An analogue of vitamin B_{12} coenzyme in which the ribosyl oxygen has been replaced by —CH,— has been synthesized. This compound has spectral properties very similar to vitamin B_{12} coenzyme. The chemical properties of the analogue differ from those of vitamin B_{12} coenzyme in that reaction with CN⁻ and BH₄⁻ does not result in cleavage of the carbon-cobalt bond. The analogue functions as coenzyme in the reaction catalyzed by dioldehydrase. The reaction in the presence of analogue is one-third as fast as with the coenzyme. Other properties of the enzyme reaction in the presence of the analogue are similar to those observed with the coenzyme: spectral changes in the presence of substrate and glycolaldehyde, hydrogen exchange between substrate and coenzyme, and over-all deuterium isotope effect. It was concluded that the ribosyl oxygen of the coenzyme cannot play an essential role in the catalytic process.

Investigations of the mechanism of action of B_{12} coenzyme have led to the hypothesis that the carbon-cobalt bond of the coenzyme undergoes chemical modification, most likely dissociation, during the course of the reaction. Several proposals have been made concerning the mechanism and nature of this modification (1-5). One possibility, which we have considered, is suggested by nonenzymic reaction of cobalamin and cobaloximes containing oxygen β to the carbon-cobalt bond. In these compounds, cleavage of the carbon-cobalt bond can occur under relatively mild acid conditions (6, 7). For instance, hydroxyethyl cobalamin is converted to ethylene at 25° at pH 3 with f₂ of 2 hours.1 These reactions have led us to consider the possibility that interaction of the enzyme and coenzyme leads to cleavage of the carbon-cobalt bond through protonation of the ribosyl oxygen of the coenzyme by an acidic group on the enzyme. This results in the formation of a complex, consisting of an electron deficient cobalt, which interacts, possibly through a π complex, with the double bond of the deoxyribosyl moiety. This complex could function as an intermediate hydride acceptor in the conversion of propanediol to propionaldehyde. The formation of the activated complex is shown in Fig. 1. A similar proposal has been made for the reaction catalyzed by methylmalonyl coenzyme A isomerase (1). The validity of this type of mechanism was tested by preparing a coenzyme analogue in which the oxygen atom of the ribosyl moiety of 5,6-dimethylbenzimidazolylcobamide 5'-deoxyadenosine is replaced by —CN__. Partial structural changes of the coenzyme analogue, which will be called carbocyclic DBCC,² are shown in Fig. 2. If this analogue has coenzyme activity, the mechanism cannot be valid. The method of synthesis, coenzymic, and chemical properties of carbocyclic DBCC are reported here.

MATERIALS AND METHODS

Partial Synthesis of Carbocyclic DBCC—The carbocyclic analogue of adenosine was a gift from Dr. Y. F. Shealy, Southern Research Institute, Birmingham, Alabama (8). 2',3'-Isopropylidene carbocyclic adenosine was prepared from 10 mg of carbocyclic adenosine by the method of Hampton (9). The crude 2',3'-isopropylidene derivative was brought to dryness under reduced pressure and dried over P₂O₅ for 12 hours. To the flask containing the dried isopropylidene derivative, 0.4 ml of anhydrous pyridine (dried and distilled over KOH) was added. After all the solid material had dissolved, 10 mg of p-toluenesulfonfoly chloride was added and the solution was incubated at room temperature for 6 hours with the exclusion of moisture. The resulting 2',3'-isopropylidene 5-p-toluenesulfonyl carbocyclic adenosine was retracted with B₁₂₅ by procedures previously used for the synthesis of DBCC (10, 11). The reaction product was isolated by phenol extraction and purified by chromatography on Dowex 50 (10). 2',3'-Isopropylidene carbocyclic DBCC was extracted into phenol and back extracted into water. The aqueous solution was concentrated to 80 ml in a rotary evaporator and 10 ml of 2 N HCl were added. The solution was incubated at room temperature for 24 hours and carbocyclic DBCC was isolated by chromatography on Dowex 50 (10). The coenzyme analogue was then subjected to paper electrophoresis on Whatman No. 3MM paper with 0.5 N NH₄OH for 3 hours at 10 volts per cm. Under these conditions, the analogue migrated.

1 C. P. Dunne and R. H. Abeles, unpublished results.

2 The abbreviations used are: carbocyclic DBCC, analogue of DBCC in which the ribosyl oxygen of the adenosyl group is replaced by —CH,—; DBCC, 5,6-dimethylbenzimidazolylcobamide 5'-deoxyadenosine; B₁₂₅, vitamin B₁₂ containing cobalt in the +1 oxidation state.
with the same mobility as DBCC. Carbocyclic DBCC was eluted from the paper and subjected to descending paper chromatography on Whatman No. 1 paper with water-saturated 2-butanol. Two major pink bands were detected: Fraction A, \( R_F 0.11 \); Fraction B, \( R_F 0.15 \); DBCC, \( R_F 0.12 \). Total yield (Fractions A and B), 14 mg. The homogeneity of these fractions was examined by ascending paper chromatography on Whatman No. 1 paper with the following solvents: isopropanol-HAc-H\(_2\)O-butanol-1 (70:1:99:100) and butanol-2-NH\(_2\)OH-H\(_2\)O (100:36:14). The following \( R_F \) values were obtained: Fraction A, 0.24; Fraction B, 0.32; DBCC, 0.30, and Fraction A, 0.16; Fraction B, 0.22; DBCC, 0.17. In each case a single spot was observed.

Treatment of Carbocyclic DBCC with Cyanide—To remove possibly contaminating DBCC the analogue was treated with cyanide under conditions which would convert DBCC to dicyanocobalamin. Carbocyclic DBCC (44 pg) (Fraction B) was dissolved in 1 ml of 0.1 M sodium bicarbonate buffer, pH 9.9, containing 0.16 M KCN. The solution was incubated at 37°. After 30 min, 2 ml of 2 N HCl were added and the solution passed through a Dowex 50-H\(^+\) column (0.5 × 4 cm). The column was washed with 10 ml of water and carbocyclic DBCC eluted with 10 ml of 1 N NH\(_2\)OH. The spectrum of the recovered product was identical with that prior to cyanide treatment. Of the material, 82% was recovered.

**Analytical Procedures**—The following millimolar extinction coefficients were used: DBCC or carbocyclic DBCC, 340 m\( \mu \) at pH 7.0, 12.0; B\(_{12}\)s, 352 m\( \mu \) at pH 7.0, 22, and dicyano B\(_{12}\), 367 m\( \mu \) at pH 10.0, 30.4. Spectral measurements were conducted using a Unicam SP-800 recording spectrophotometer.

Dioldehydrase was prepared and assayed as described (12). Protein determinations were by the method of Lowry et al. (13). Radiochemical assays were performed by liquid scintillation counting in an Anstron scintillation counter by the method of Bray (14).

**Substrates**—DL-1,2-Propanediol-1\(^3\)H (specific activity 1.5 × 10\(^6\) cpn per \( \mu \)mole) and DL-1,2-propanediol-1\(^3\)H were prepared as described previously (11). All other substrates were obtained from commercial sources.

**RESULTS**

**Chemical Properties of Carbocyclic DBCC**—The spectra of Fractions A and B of carbocyclic DBCC are identical with that of DBCC (Fig. 3) (15). As in the case of DBCC, addition of acid causes a spectral shift and the appearance of a peak at 460 m\( \mu \) (not shown in Fig. 3). Exposure to light after addition of cyanide yields the spectrum of dicyanocobalamin. Addition of cyanide without light exposure causes a slight spectral change which does not undergo further changes after 1 hour. We tentatively attribute this spectral change to the formation of an adduct in which cyanide has displaced the benzimidazole base and is coordinated to the bottom position of the corrin ring. Since carbocyclic DBCC can be reisolated after cyanide treatment (see "Materials and Methods"), no covalent changes take place. Treatment of DBCC under similar conditions leads to complete conversion to dicyanocobalamin.

The relative susceptibility of DBCC and carbocyclic DBCC (Fractions A and B) to NaBH\(_4\) reduction was determined. A solution (0.4 ml) containing 0.039 M carbocyclic DBCC or DBCC, 0.1 M Tris-Cl, pH 8.0, and 0.3 M NaBH\(_4\) was incubated in the dark at room temperature for 3 min. The reaction was stopped by the addition of 0.1 ml of acetic acid and the extent of decomposition was determined from the absorbance at 352 m\( \mu \). It was found that more than 90% of DBCC was converted to hydroxyl B\(_{12}\), whereas carbocyclic DBCC (Fraction A or B) remained intact.

The rates of acid decomposition of carbocyclic DBCC and DBCC were compared. Carbocyclic DBCC (Fraction B), 5.1 × 10\(^{-4}\) M, and DBCC, 7.0 × 10\(^{-4}\) M, were heated in 0.1 N H\(_2\)SO\(_4\) at 100° in the dark. At 0, 10, 20, 30, and 50 min, 0.2-ml aliquots were withdrawn and diluted to 25 ml with 8 × 10\(^{-4}\) M NaOH. Aliquots of that solution were assayed enzymically for coenzyme activity (16). The rates of disappearance of coenzyme activity for both coenzymes followed first order kinetics. Coenzyme activity was lost in both at nearly identical rates with a \( t_\alpha \) of 10.5 to 12.0 min. It is known that DBCC under acid conditions is converted to B\(_{12}\)(a), adenine, 2,3-dihydroxy-4-penten-1-al (17). To test whether B\(_{12} (a) \) was formed from carbocyclic DBCC, the compound was heated at 100° in 0.1 N H\(_2\)SO\(_4\) and spectra were taken at 0, 15, and 50 min. A similar experiment was carried out on DBCC in 0.1 N H\(_2\)SO\(_4\).
The reaction was allowed to proceed for 10 min. Propionaldehyde 0.5 ml. The reaction mixture was previously incubated at 37° for cases, it was shown by enzymic analysis that at the end of the reaction, no coenzyme activity remained. As expected, after 50 min, the spectrum of DBCC was that of B12ca) since after light exposure, spectral change was complete after 15 min. At that point not all coenzyme activity is lost. It was concluded that carbocyclic DBCC undergoes a chemical modification in acid, which causes loss of coenzyme activity. Unlike DBCC, the product of the acid reaction may not be B12ca) and certainly is not exclusively B12ca). The significance of the spectral change observed upon heating carbocyclic DBCC in acid is not clear. Insufficient material was available to further explore this reaction.

In the reactions with CN-, NaBH4, and possibly with acid, the carbon-cobalt bond of DBCC is more susceptible to cleavage than that of carbocyclic DBCC. This difference in reactivity provides evidence for the contribution of the ribosyl oxygen in reactions in which the carbon-cobalt bond dissociates so that the electrons of that bond remain with the adenosyl moiety.

**Table I**

<table>
<thead>
<tr>
<th>Coenzyme</th>
<th>Activity (umoles/alddehyde/min/unit enzyme)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbocyclic DBCC (Fraction A)</td>
<td>0.065</td>
</tr>
<tr>
<td>Carbocyclic DBCC (Fraction B)</td>
<td>0.36</td>
</tr>
<tr>
<td>Cyanide-treated and purified carbocyclic DBCC</td>
<td>0.36</td>
</tr>
<tr>
<td>DBCC (Fraction B)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Table II**

<table>
<thead>
<tr>
<th>Additions</th>
<th>Activity after prior incubation (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>Hydroxycobalamin</td>
<td>100</td>
</tr>
<tr>
<td>Carbocyclic DBCC (Fraction A)</td>
<td>12</td>
</tr>
</tbody>
</table>

![Fig. 5](image-url) Inverse plot of initial velocity against DBCC and carbocyclic DBCC concentration. The reaction mixture contained: potassium phosphate buffer, pH 8.0, 0.04 M; DL-1,2-propanediol, 0.13 M; serum albumin, 0.2 mg per ml; DBCC (○) and carbocyclic DBCC (Fraction B) (●), as indicated. Enzyme, 0.15 unit per ml with carbocyclic DBCC and 0.05 unit with DBCC. Total volume, 1.0 ml. Reaction was allowed to proceed 20 min at 37° and then assayed for propionaldehyde.

Out with DBCC. Spectra were taken at 0 and 50 min. In both cases, it was shown by enzymic analysis that at the end of the reaction, no coenzyme activity remained. As expected, after 50 min, the spectrum of DBCC was that of B12ca) and no further spectral change occurred upon exposure to light. The spectrum of carbocyclic DBCC (Fig. 4) was also changed, but the compound was not completely converted to B12ca) since after light exposure, a further spectral change occurred. This spectrum now resembles that of B12ca). The spectrum in Fig. 4 also shows that the spectral change was complete after 15 min. At that point not all coenzyme activity is lost. It was concluded that carbocyclic DBCC undergoes a chemical modification in acid, which causes loss of coenzyme activity. Unlike DBCC, the product of the acid reaction may not be B12ca) and certainly is not exclusively B12ca). The significance of the spectral change observed upon heating carbocyclic DBCC in acid is not clear. Insufficient material was available to further explore this reaction.

In the reactions with CN-, NaBH4, and possibly with acid, the carbon-cobalt bond of DBCC is more susceptible to cleavage than that of carbocyclic DBCC. This difference in reactivity provides evidence for the contribution of the ribosyl oxygen in reactions in which the carbon-cobalt bond dissociates so that the electrons of that bond remain with the adenosyl moiety.

**Coenzyme Activity of Carbocyclic DBCC**—The coenzyme activity of the two fractions of carbocyclic DBCC was tested with dioldehydrase and compared to that of DBCC. Saturating amounts of both coenzymes were used. The results are summarized in Table I. Carbocyclic DBCC can function as a coenzyme and therefore the ribosyl oxygen of the adenosyl moiety is not essential for coenzymic activity. Exposure of carbocyclic DBCC to cyanide and subsequent purification did not alter its coenzyme activity when determined under saturating and nonsaturating conditions. This eliminates the unlikely possibility that the coenzyme activity observed with carbocyclic DBCC was caused by contamination by DBCC. Carbocyclic DBCC (Fraction B) shows appreciably more coenzyme activity than Fraction A. It is possible that the small amount of activity of Fraction A is caused by contamination by Fraction B, and that Fraction B may also contain some Fraction A so that the activity reported here is a minimal activity. Insufficient carbocyclic DBCC is available at this time to further define the conditions for separation of Fractions A and B.

Fraction A inhibits the conversion of propanediol to propionaldehyde as illustrated by the results in Table II. The results show that Fraction A is as inhibitory as hydroxy B12ca an irreversible inhibitor of dioldehydrase.

Fig. 5 shows double reciprocal plots of the initial velocities against DBCC or carbocyclic DBCC concentrations. The apparent K_m obtained for the two coenzymes is quite similar (6.1 × 10^-7 M for DBCC and 8.7 × 10^-7 M for carboxylic DBCC). It is difficult to assign a mechanistic significance to these apparent K_m values since the combination between enzyme and coenzyme is essentially irreversible.

One of the characteristics of the reaction catalyzed by dioldehydrase is that tritium from the C-1 position of the substrate is incorporated, during the course of the catalytic process, into the C-5' position of the coenzyme (4). To establish whether this tritium exchange also occurs with carbocyclic DBCC, 60 μg of carbocyclic DBCC, Fraction B, were incubated at 10° with 242 units of dioldehydrase, 20 μmoles of potassium phosphate buffer,
**TABLE III**

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity in presence of DBCC (4.8 \times 10^{-7} M)</th>
<th>Activity in presence of Carboxyclic DBCC (2.4 \times 10^{-9} M)</th>
<th>Activity in presence of DBCC (4.8 \times 10^{-7} M) + Carboxyclic DBCC (2.4 \times 10^{-9} M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>100</td>
<td>13</td>
<td>100</td>
</tr>
</tbody>
</table>

In incubation mixture contained: potassium phosphate buffer, pH 8.0, 0.04 M; dioldehydrase, 0.48 unit; serum albumin, 0.4 mg per ml; DBCC or carboxyclic DBCC (Fraction B), or both, as indicated, in a total volume of 5.0 ml at 37°C. At times indicated, 0.1-ml aliquot of reaction mixture was removed and assayed for enzymatic activity under standard assay conditions (12).

Fig. 6. Isolation of dioldehydrase-carboxyclic DBCC complex. In a total volume of 0.845 ml, 70 units of dioldehydrase, 7.1 \times 10^{-6} M carboxyclic DBCC (tritium-labeled, specific activity = 1.16 \times 10^4 cpm per pmole), 5.9 \times 10^{-3} M K_2HPO_4 were incubated at 37°C for 10 min. The reaction mixture was layered onto a Sephadex G-75 column, 30 x 2.5 cm, equilibrated with 1 \times 10^{-3} M K_2HPO_4. At a flow rate of 2 ml per min, 3-ml fractions were collected. Each fraction was analyzed for protein concentration (X), radioactivity (O), and enzyme activity (I). Tritium-labeled carboxyclic DBCC was prepared with dioldehydrase and DL-1,2-propanediol-1-3H as described in the text.

Fig. 7. Kinetics of aldehyde formation from DL-1,2-propanediol (I) and ethylene glycol (X) with carboxyclic DBCC (Fraction B). The reaction mixture contained: potassium phosphate buffer, pH 8.0, 0.01 M; DL-1,2-propanediol or ethylene glycol, 0.2 M; serum albumin, 1 mg per ml; carboxyclic DBCC, 1.2 \times 10^{-4} M; and dioldehydrase, 0.3 unit per ml. Total volume, 1.0 ml, 37°C. Aliquots were withdrawn and assayed for aldehyde as in the assay procedure (12). Inset shows the kinetics of aldehyde formation when DBCC and 0.1 unit per ml of dioldehydrase is used (12).

with C-1-deutero-DL-propanediol in the presence of carboxyclic DBCC was determined. Under standard assay conditions the nondeuterated substrate reacted 8- to 10-fold faster than the deuterated substrate. It has been previously shown (18) that with DBCC an isotope effect of 10 to 12 is obtained under these conditions. It is therefore highly probable that the same step, the breaking of the substrate C-H bond, is rate-limiting with both coenzymes.

The dioldehydrase-DBCC complex reacts with oxygen in the absence of substrate to give a catalytically inactive complex (19). This oxygen inactivation involves breaking of the carbon-cobalt bond. The stability of the dioldehydrase-carboxyclic DBCC complex was examined. The results are summarized in Table III. The enzyme-carboxyclic DBCC complex is completely stable under conditions where the corresponding DBCC complex is largely inactivated. Furthermore, carboxyclic DBCC retards inactivation by DBCC. The failure to observe loss of catalytic activity in the presence of carboxyclic DBCC might be attributed to the stability of the carboxyclic DBCC-enzyme complex or to the inability of the analogue to form a stable complex with the apoenzyme in the absence of substrate. This latter interpretation is made unlikely by the demonstration that the carboxyclic analogue protects against inactivation by DBCC. To test more directly whether a stable complex is formed, dioldehydrase was allowed to react with tritiated carboxyclic DBCC in the absence of substrate and then passed through a Sephadex column. The experimental conditions and the elution pattern from the Sephadex column are shown in Fig. 6. Fractions 14, 15, and 16 contained, respectively, 6.6, 9.0, and 6.7 units of enzyme per \( \mu \)g of carboxyclic DBCC. It is in this respect similar to DBCC. It
Table IV
Effect of glycolaldehyde on activity of enzyme carbocyclic DBCC complex

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>No addition</th>
<th>Glycolaldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>104</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>1</td>
</tr>
</tbody>
</table>

has been shown that 7 to 10 units of enzyme bind 1 μg of DBCC (11). The enzyme in the column eluent was completely saturated with coenzyme, since further addition of carbocyclic DBCC or DBCC did not increase the catalytic activity. This experiment establishes that the apoenzyme has high affinity for carbocyclic DBCC in the absence of substrate and further illustrates the stability of the carbocyclic DBCC-enzyme complex. It is not possible to isolate an enzymatically active enzyme-DBCC complex under the conditions employed for the isolation of the enzyme-carbocyclic DBCC complex.

There are two additional processes which lead to the inactivation of the enzyme-DBCC complex. (a) When ethylene glycol is converted to acetaldehyde, the enzyme-coenzyme complex becomes catalytically inactive (12). This inactivation does not occur when D,L-1,2-propanediol is the substrate. (b) Addition of glycolaldehyde to dioldehydrase and DBCC leads to the formation of a catalytically inactive enzyme-DBCC-glycolaldehyde complex. Formation of this complex is accompanied by a spectral change identical with that observed in the presence of substrate (12). Carbocyclic DBCC was examined with respect to both these points. The time course of the reaction in the presence of ethylene glycol and D,L-1,2-propanediol is shown in Fig. 7. When ethylene glycol is the substrate, inactivation occurs with both coenzymes. With D,L-1,2-propanediol inactivation is observed only with carbocyclic DBCC. Inhibition of dioldehydrase by glycolaldehyde in the presence of carbocyclic DBCC is summarized in Table IV. These results are similar to those obtained with DBCC. The spectral changes observed when glycolaldehyde or D,L-1,2-propanediol are added to carbocyclic DBCC-dioldehydrase complex are identical with that previously observed in the presence of DBCC (19).

Discussion

DBCC and carbocyclic DBCC have similar spectra, light sensitivity, and chromatographic properties. An important chemical difference between DBCC and carbocyclic DBCC becomes apparent in the reactions with CN− and NaBH₄. Carbocyclic DBCC, unlike DBCC, is unreactive toward these reagents. Both CN− and NaBH₄ are known to bring about heterolytic cleavage of the carbon—cobalt bond of cobalamin in which the electrons of the bond remain with the leaving group. Presumably, this reaction is facilitated by the electron-releasing oxygen of DBCC which can stabilize the incipient negative charge at C-5'. No corresponding stabilization can occur with carbocyclic DBCC where the oxygen is replaced by —CH₂—. The acid-catalyzed cleavage of the carbon—cobalt bond of cobalamin resembles the reaction with CN− and BH₄⁻ in that it also is a heterolytic cleavage in which the electrons remain with the leaving group. It differs from the reaction with CN− and BH₄⁻ in that it probably does not involve attack of nucleophile on the carbon—cobalt bond. It was therefore of interest to compare the susceptibility of the carbon—cobalt bond of carbocyclic DBCC and DBCC to acid. Our results, pertaining to this point, are ambiguous, since we have not identified the products of the acid reaction. Although, upon exposure of carbocyclic DBCC to acid, coenzyme activity was lost, a light sensitive compound was still present. This suggests that loss of coenzyme activity may not be caused by cleavage of the carbon—cobalt bond, but by modification of another part of the molecule, and it is possible that carbon—cobalt bond of DBCC is less susceptible to acid cleavage than that of DBCC.

The dioldehydrase-DBCC complex and dioldehydrase-carbocyclic DBCC complex have similar properties in many respects. Vₘₐₓ with the carbocyclic coenzyme for the conversion of D,L-1,2-propanediol to propionaldehyde is one-third that with DBCC. With both coenzymes, tritium exchange between substrate and coenzyme is observed during the course of the catalytic process. Identical spectral changes are obtained when either substrate or glycolaldehyde are added to the complex. Glycolaldehyde inactivates both complexes. In addition, the Kₘ and over-all kinetic isotope effects are quite similar. The mechanisms of the reactions with the carbocyclic coenzyme and with DBCC are probably identical and it can be concluded that the ribosyl oxygen does not play an important part in the catalytic process. Therefore, any mechanism involving carbon—cobalt bond cleavage in which the stabilization of the incipient negative charge at C-5' is dependent on oxygen must be excluded for the reactions in which B₁₂ coenzymes participate. A specific example of this type of mechanism was discussed in the introduction of this paper. This conclusion does not imply that carbon—cobalt bond cleavage does not occur by other mechanisms. We believe that it is very likely that such a process occurs during the course of the reaction.

An important difference between the two coenzymes is the greater stability of the carbocyclic DBCC-dioldehydrase complex than the corresponding DBCC-enzyme complex, in the absence of substrate. Although it is known that oxygen is involved in the inactivation of DBCC-enzyme complex, the mechanism of the inactivation process is unknown. Therefore, it is difficult to propose a reason for the stability of the carbocyclic DBCC-enzyme complex.

The catalytic conversion of D,L-1,2-propanediol to propionaldehyde in the presence of carbocyclic DBCC ceases after about 30 min, whereas in the presence of DBCC, it is linear for more than an hour. With both coenzymes, the conversion of ethyleneglycol and 1,2-propanediol is shown in Fig. 7. When ethylene glycol is the substrate, inactivation occurs with both coenzymes. With D,L-1,2-propanediol inactivation is observed only with carbocyclic DBCC. Inhibition of dioldehydrase by glycolaldehyde in the presence of carbocyclic DBCC is summarized in Table IV. These results are similar to those obtained with DBCC. The spectral changes observed when glycolaldehyde or D,L-1,2-propanediol are added to carbocyclic DBCC-dioldehydrase complex are identical with that previously observed in the presence of DBCC (19).

3 The products of the reductive cleavage of the carbon—cobalt bond are B₁₂, adenosine, and a mixture of D-erythro-2,3-dihydroxy-Δ₄-pentenal and its reduction product, D-erythro-3,4-dihydroxy-Δ₄-pentanol. C. P. Dunne and R. H. Abeles, unpublished results.
glycol to acetaldehyde stops after 30 min. We have shown that in the presence of ethylene glycol, inactivation of DBCC occurs. Presumably, a similar phenomenon occurs with propanediol and carbocyclic DBCC. Why this occurs with carbocyclic DBCC and not DBCC is not known. It could be, as many phenomena in enzymology, blamed on conformational effects.

REFERENCES


The Coenzymic and Chemical Properties of a Carbocyclic Analogue of Vitamin B<sub>12</sub> Coenzyme

S. S. Kerwar, T. A. Smith and Robert H. Abeles


Access the most updated version of this article at [http://www.jbc.org/content/245/5/1169](http://www.jbc.org/content/245/5/1169)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/245/5/1169.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/245/5/1169.full.html#ref-list-1](http://www.jbc.org/content/245/5/1169.full.html#ref-list-1)