Reactions of the Carbon-Cobalt Bond of Alkylcobalamins

A REVERSIBLE DISSOCIATION OF THE CARBON COBALT BOND*

(Rceived for publication, November 1, 1965)

R. BARNETT, H. P. C. HOGENKAMP, AND R. H. ABELES

From the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154, and the Department of Biochemistry, College of Medicine, The University of Iowa, Iowa City, Iowa

SUMMARY

The carbon-cobalt bond in cyanoethylcobalamin can be reversibly cleaved to give hydridocobalamin and acrylonitrile. Aerobic decomposition in alkali is first order in both hydroxide ion and cyanoethylcobalamin, although the kinetics is somewhat ambiguous below pH 9. This is the first demonstrated example of alkyl-cobalt cleavage in which the cobalt leaves with the bonding electrons.

The cobamide coenzymes1 are unstable in the presence of alkaline cyanide. This decomposition by cyanide has been envisaged as a nucleophilic displacement reaction which gives rise to dicyanocobalamin, adenine, and the cyanohydrins of 2,3-dihydroxy-Δ4-pentenal (1, 2). In the case of the simple alkylcobalamins, cyanide decomposition proceeds very slowly or not at all, presumably because the alkyl carbanion is a poor leaving group (2). However, Hogenkamp, Rush, and Swenson (3) recently reported that alkylcobalamins, which contain cyanoethyl and methoxy-carbonyl ethyl functions, are readily decomposed by alkaline cyanide to dicyanocobalamin. It is highly unlikely that this reaction proceeds by a displacement mechanism similar to that proposed for the cyanide decomposition of coenzyme Bi2, since the alkyl carbanion, which results from such a displacement, is extremely unstable. Both cobalamins, however, contain groups which facilitate the ionization of a C—H bond to form a carbanion. These observations suggest that these reactions may be elimination reactions, in which the electrons of the carbon-cobalt bond remain with the cobalt atom, rather than displacement reactions, in which the electrons of the organometallic bond leave with the alkyl group. Such elimination reactions should be catalyzed more effectively by hydroxide ion than by cyanide ion. Consequently, we have investigated the alkaline-catalyzed decomposition of cyanoethylcobalamin and have shown that it proceeds as shown in Equation 1 and that the reported decomposition (3) in the presence of CN− is caused by the OH− generated by the hydrolysis of potassium cyanide. The formation of dicyanocobalamin reported by Hogenkamp et al. (3) is attributed to the reaction of cyanide with the hydroxycobalamin which is formed from the oxidation of hydridocobalamin.

Hydridocobalamin is known to react with acrylonitrile in alkali to produce β-cyanoeethylcobalamin, and, therefore, the reaction shown in Equation 1 is reversible. The OH−-catalyzed decomposition of β-cyanoeethylcobalamin is the first example of a reversible cleavage of the carbon-cobalt bond of alkylcobalamins and of the dissociation of such a bond which leads to the formation of hydridocobalamin. The reaction of acylcobalamins with nucleophiles as reported by Bernhauer and Irion (4) also leads to cleavage of the carbon-cobalt bond with the formation of hydridocobalamin.

The reversible elimination reaction of alkylcobalamins provides a possible model system for those enzymatic reactions in which 5'-deoxyadenosylcobalamin is required as a coenzyme. It has been proposed that a reversible cleavage of the carbon-cobalt bond occurs upon combination of coenzyme Bi2 and diol-dehydrase. The possible formation of hydroxycobalamin as a result of this dissociation has been considered (5).

EXPERIMENTAL PROCEDURE

Materials

Hydroxycobalamin was a generous gift of E. R. Squibb and Sons. Acrylonitrile-14C was obtained from New England...
Determined in 0.1 M of decomposition in alkali over the pH range of 7.9 to 11.2 was cobalamin.

Methods

Preparation of β-Cyanocobalamin—Hydroxocobalamin (50 mg) was dissolved in 5 ml of water, deaerated with oxygen-free nitrogen, and then reduced with 0.5 M of sodium borohydride (6). After reduction to the gray-green hydroxocobalamin was complete, 1.0 ml of a 1% aqueous solution of acrylonitrile or 3-chloropropiononitrile was injected with a hypodermic syringe. After 10 min the red solution was adjusted to pH 3 with dilute hydrochloric acid, and the desired substituted cobalamin was purified by chromatography on Dowex 50 (7). The yield was 60 to 70%. The synthesis and purification were carried out in subdued light. Because cyanocobalamin decomposes during the commonly used phenol extraction (3), the cobalamin was desalted by passing 0.5 ml of a 0.5 mM solution of cyanocobalamin into a Sephadex G-10 column (1.1 x 100 cm). The column was then washed with water to yield a salt-free product. The ultraviolet and visible absorption spectra of cyanocobalamin are typical of a cobalamin containing a carbon-cobalt bond (3). The cobalamin migrated on ascending chromatography in sec-butyl alcohol-water-acetic acid (100:50:15) with an Rf of 1.29.

14C-Cyanocobalamin was prepared by the above procedure, except that 2.4 mg of acrylonitrile-14C dissolved in 1 ml of water were used. The yield based on acrylonitrile was 20%.

Decomposition of Cyanocobalamin in Alkali—The kinetics of decomposition in alkali over the pH range of 7.9 to 11.2 was determined in 0.1 M glycine and 0.1 M Tris buffers. To 1.0 ml of buffer at 25° was added, with a syringe, 0.2 ml of a 0.05 mM solution of cyanocobalamin. The decomposition of the organometallic bond was followed spectrophotometrically at 332 mp; readings were taken every 15 min for pH 7.9 to pH 9.01, every 2 min for pH 10.0 to pH 10.5, and every 10 sec for pH 11.2. A second experiment was done at pH 9.1 with 0.25 mM cyanoethylcobalamin.

The decomposition was also studied under anaerobic conditions. In these experiments a solution of cyanocobalamin in 0.1 M sodium acetate, pH 6.4, was decomposed in a Thunberg tube modified to fit a Perkin-Elmer 202 ultraviolet-visible spectrophotometer. The anaerobic cobalamin solution was in the main compartment and the alkali in the side arm. Both solutions were kept under an atmosphere of nitrogen. The spectra from 350 mp to 750 mp were recorded before and after the addition of alkali to give a final pH of 12. The final spectrum was corrected for dilution.

Analysis of Products of Anaerobic Decomposition—1. Identification of hydroxocobalamin: Oxygen-free nitrogen was bubbled through 20 ml of 0.2 mM cyanocobalamin in 0.1 M sodium acetate, pH 6.4, in a 50-ml side arm flask for 2 hours. Without introducing air, 0.5 ml of methyl iodide was injected, followed by the injection of 2 ml of 1 M sodium hydroxide. After 15 min an additional 0.5 ml of methyl iodide was added. The reaction was allowed to proceed for 2 hours under nitrogen. The solution was then adjusted to pH 7 and desalted by phenol extraction (8). The product was cochromatographed with methylcobalamin on Whatman No. 1 paper in water-saturated sec-butyl alcohol and in sec-butyl alcohol-ammonium hydroxide-water (100:14:36).

2. Identification of acrylonitrile: In the dark 20 ml of 0.152 mM 14C-labeled cyanocobalamin was decomposed by the addition of 2 ml of 1 M sodium hydroxide. After 30 min the solution was adjusted to pH 7. A derivative of acrylonitrile was prepared by a modification of Brookway's method (9). To the cobalamin solution, 0.2 ml of acrylonitrile carrier and 0.18 ml of piperidine were added, and the reaction mixture was refluxed for 10 min. After cooling, the solution was extracted once with 10 ml and three times with 5 ml of ether. The ether extracts were combined and concentrated to about 2 ml on a steam bath. To 5 ml of 5% piperie acid in absolute ethanol, 1 ml of the extract was added. The piperidinopropiononitrile picrate was recrystallized to constant activity and melting point (162.5-163°). The reported melting point is 161-162° (9).

Results and Discussion

Under anaerobic conditions the alkaline decomposition of cyanocobalamin yields a material with absorption maxima at 410 mp and 480 mp (Fig. 1), which correspond to the absorption maxima of vitamin B12 (2). Under anaerobic conditions the spectrum of hydroxocobalamin does not change upon the addition of alkali. The absorption near 350 mp and 550 mp is probably caused by small amounts of hydroxocobalamin. This evidence indicates that the products of decomposition are vitamin B12 and hydroxocobalamin. However, it appeared possible that the initial product in the decomposition actually is hydroxocobalamin, which is immediately oxidized to vitamin B12, and hydroxocobalamin by unavoidable traces of oxygen left in the reaction solution. To test this hypothesis, the reaction was carried out in the presence of methyl iodide. Any hydroxocobalamin formed would react with methyl iodide to give methylcobalamin. This is essentially the same procedure as that used by Bernhauer and Irion to demonstrate that hydroxocobalamin is produced from the cleavage of acetylcobalamin by hydroxylamine (3). It was found by paper chromatography in two solvent systems that the main product of the decomposition in the presence of methyl iodide is identical with methylcobalamin, an indication that anaerobic, alkaline decomposition of cyanocobalamin yields hydroxocobalamin. The other product of decomposition is acrylonitrile. To ascertain this, cyanocobalamin labeled with 14C in the alkyl substituent.
was decomposed with alkali in the absence of light. After the addition of carrier acrylonitrile, the presence of acrylonitrile-1\(^{14}\)C was demonstrated by conversion to the piperidinopropiononitrile pterate. The activity of the pterate obtained was 3240 cpm per mg. The theoretical activity, based on 100\% decomposition of the cyanoethylcobalamin, was calculated to be 3500 cpm per mg. Hence cyanoethylcobalamin decomposes under alkaline anaerobic conditions to give hydridocobalamin and acrylonitrile as shown in Equation 1.

Aerobic decomposition of cyanoethylcobalamin with alkali produces hydroxocobalamin (Fig. 2) since hydridocobalamin is rapidly oxidized in the presence of air. For pH values greater than 9, the decomposition is first order in cyanoethylcobalamin. At or below pH 9, the kinetics is somewhat ambiguous. There is an initial rapid rate followed by a first order portion (Fig. 3). The initial burst may be caused by small amounts of an impurity with a different pH sensitivity. Complete spectra of the reaction mixture during the initial burst period gave no evidence for an intermediate. Additional evidence for the presence of an impurity is that the burst is not reproducible.

A study of the pH dependence of the pseudo-first order rate constants indicates a linear dependence on hydroxide ion concentration. A plot of pH with respect to \(-\log k_1\) gives a straight line with a slope of \(-1\) (Fig. 4). For pH values below 9, the first order portion of the decomposition was used. Fig. 4 shows that the rate constant is identical for the two buffer systems shown. In experiments with ammonia-ammonium sulfate buffer systems over wide ranges of buffer concentrations, no effect on the rate constant was observed. This indicates that general base catalysis does not contribute significantly to the reaction under the conditions employed. In addition, a 10-fold increase in the concentration of cyanoethylcobalamin at constant pH does not affect the pseudo-first order rate constant. Hence, over the range of pH and concentrations of cyanoethylcobalamin studied, the decomposition obeys the rate law:

\[
R = k_1 [\beta\text{-cyanoethylcobalamin}] [\text{OH}^-]
\]

where \(k_1 = 230 \text{ M}^{-1} \text{ min}^{-1}\).

The rate of ionization of propionitrile has not been determined, but it is probably of the same order of magnitude as acetonitrile, which has been estimated to be 2 to 3 \(\times 10^5\) sec\(^{-1}\) in 1 M NaOH (10). This rate is approximately five orders of magnitude slower than the rate of decomposition of cyanoethylcobalamin. This suggests that the reaction either proceeds by an \(E_1\) elimination or that, if it proceeds through a carbanion, the cobamide significantly enhances the acidity of the hydrogen \(\alpha\) to the nitrile.

Hogenkamp \textit{et al.} (3) have previously reported that cyanoethylcobalamin is cleaved by cyanide ion, the rate of cleavage being \(1.15 \times 10^{-3}\) sec\(^{-1}\) for 33 mM potassium cyanide. The pH of the solution used is approximately 10.5. The rate of basic decomp-
position at pH 10.5, obtained by interpolation on Fig. 4, is $1.4 \times 10^{-3}$ sec$^{-1}$. Hence the cyanide decomposition must actually be a basic decomposition caused by the hydroxide ion generated by the hydrolysis of potassium cyanide.

REFERENCES

Reactions of the Carbon-Cobalt Bond of Alkylcobalamins: A REVERSIBLE DISSOCIATION OF THE CARBÖN-COBALT BOND
R. Barnett, H. P. C. Hogenkamp and R. H. Abeles


Access the most updated version of this article at http://www.jbc.org/content/241/7/1483

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

Click here 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/241/7/1483.full.html#ref-list-1