THE COBALAMIN PRODUCT OF THE CONVERSION OF METHYLCOBALAMIN TO CH_4 BY EXTRACTS OF METHANOBACILLUS OMELIANSKII

M. J. Wolin, E. A. Wolin and R. S. Wolfe

Departments of Microbiology and Dairy Science
University of Illinois, Urbana, Illinois

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The formation of CH_4 from methylcobalamin (CH_3-B_{12}) by bacterial extracts was originally observed by Blaylock and Stadtman (1963). Wolin *et al*. (1963a) showed a requirement for ATP for the same reaction using extracts of *Methanobacillus omelianskii*. Although the role of ATP in this reaction has not been elucidated, this report presents evidence that the cobalamin product formed in extracts of *Mb. omelianskii* has the spectral properties of vitamin $B_{12}r$ and can be alkylated with methyl iodide. The product appears to be identical with the brown compound, recently described by Dolphin and Johnson (1963), formed by the addition of thiols to $B_{12}r$.

METHODS

The preparation of extracts and methods of assay of CH_4 formation were as previously described (Wolin *et al*., 1963b). Methylcobalamin was prepared by the method of Müller and Müller (1962).

RESULTS

During the formation of CH_4 from CH_3-B_{12}, the color of reaction mixtures turned from the deep red of CH_3-B_{12} to a deep brown. Samples were withdrawn from a typical reaction mixture after CH_4 formation from CH_3-B_{12} ceased, and the spectrum was determined. An equivalent amount of sample from a reaction mixture that did not contain CH_3-B_{12} was used as a blank. Sampling and spectral measurements were performed in the absence of oxygen. Fig. 1 shows the spectrum of the brown product formed (solid line) which is identical with that of $B_{12}r$. Aeration of the product in the absence of light changed the spectrum as shown in Fig. 1 (broken line). The oxidized compound has the spectrum of hydroxocobalamin, the expected product of $B_{12}r$ oxidation.

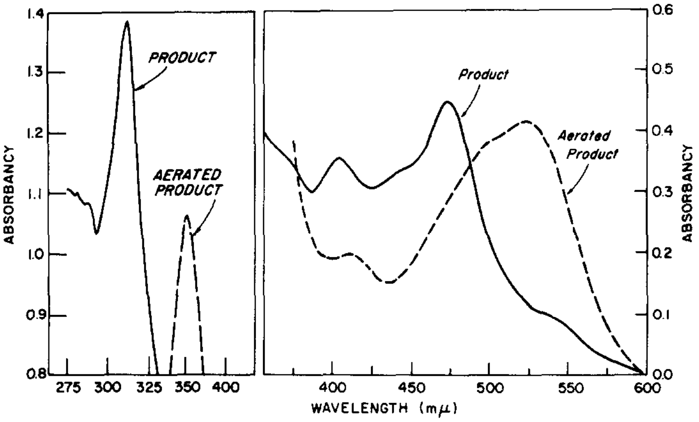

Fig. 1. Spectra of the cobalamin product and aerated product. The reaction mixtures, at 37° C, contained 1.5 ml of extract (72 mg protein); 0.06 μmole of CoASH; 10.0 μmoles of ATP; and 9.0 μmoles of methylcobalamin in a total volume of 1.86 ml with H_2 as the gas phase. After CH_4 formation ceased, a sample of the reaction mixture was removed with a syringe and transferred to an anaerobic cuvette, which had been sealed with a serum bottle-cap and contained deaerated H_2O under H_2. The product spectrum was recorded using as a blank an identically treated sample from a reaction mixture without methylcobalamin. The anaerobic cuvettes were then aerated in the dark and the aerated product spectrum was recorded. A Cary Model 14 spectrophotometer was used to record the spectra.

Recently, Dolphin and Johnson (1963) have shown that hydroxocobalamin and $B_{12}r$ can react with thiols such as NaHS to form a brown compound which has a spectrum similar to that of $B_{12}r$. The brown compound which is presumed to be a ligand formed from the thiol and $B_{12}r$, reacts with methyl iodide to form CH_3-B_{12}. Since the *Mb. omelianskii* extracts contain sulfide, it seemed possible that the HS^- ligand could actually be the product of CH_3-B_{12} cleavage. Addition of CH_3I to the reaction mixture, after CH_4 formation from CH_3-B_{12} had terminated, resulted in the conversion of the brown product with the $B_{12}r$ spectrum to a red product with the spectrum of CH_3-B_{12}.

To determine the amount of the brown cobalamin product formed, a simultaneous equation was used which was based on the molar extinction coefficients of vitamin $B_{12}r$ and methylcobalamin at 312.5 mμ and 520 mμ. The spectral data for calculating the extinction coefficients were taken from Diehl and Murie (1952) for $B_{12}r$ and from Müller and Müller (1962) for CH_3-B_{12}. The calculated extinction coefficients were 8.1×10^3 (520 mμ) and 1.2×10^4 (312.5 mμ) for CH_3-B_{12} and 3.5×10^3 (520 mμ) and 2.1×10^4 (312.5 mμ) for $B_{12}r$ for molar solutions and a 1 cm light path. The calculations from the data of the experiment of Fig. 1 showed that 8.1 µmoles of cobalamin product were produced from 9.0 µmoles of CH_3-B_{12}. Results of gas analysis showed that a total of 7.8 µmoles of CH_4 were also produced. These results approximate a stoichiometric conversion of a mole of CH_3-B_{12} to a mole of cobalamin product + a mole of CH_4.

An experiment was performed to measure the time course of cobalamin product and CH_4 formation from CH_3-B_{12}. For each time interval, the gas sample for assay of CH_4 was removed immediately prior to immersion of the reaction vessel in an ice bath. Samples of the inactive reaction mixture were then removed for spectral analysis and were assayed in a Cary recording spectrophotometer. The results in Table 1 show that, except for an amount of brown product formed at zero time, a 1:1 ratio of the two products was obtained during the course of the reaction. The initial formation of 0.75 µmole of brown product may indicate that transmethylation reactions occurred as the reaction mixture was cooled to 0° C.

TABLE 1

Time Course of CH_4 and Cobalamin Product Formation From Methylcobalamin

Flask	Time	CH_4	Cobalamin Product	Cobalamin Product/CH_4
	Min	µmoles	µmoles	
1	0	0.00	0.00	----
2	10	0.60	0.71	1.18
3	20	2.15	2.25	1.04
4	30	2.99	3.30	1.0

The reaction mixtures and procedures were identical to those described for Fig. 1. Values were obtained by using identical reaction mixtures, each flask being rapidly cooled to 0° C at the indicated time interval after samples were removed for gas analysis. The cobalamin product was calculated using the extinction coefficients for B_{12r} and CH_3-B_{12} as described in the text. A zero time value of 0.75 µmole of cobalamin product was subtracted from the total product measured at each time period.

The experiments reported in Fig. 1 and Table 1 were performed in a hydrogen atmosphere. Conversion of CH_3-B_{12} to CH_4 and the cobalamin product also occurred in an argon atmosphere, and no hydrogen production accompanied the conversion as measured by gas chromatography.

According to present knowledge of the valence of cobalt in methylcobalamin (Co^3), $B_{12}r$ (Co^2) and the thiol complexes of $B_{12}r$ (Co^2), it would appear likely that an endogenous electron source is present in the extracts which would allow methane formation by the addition of an electron and a proton to CH_3-B_{12} to form CH_4 and $B_{12}r$ or its thiol complex. Direct cleavage of CH_3-B_{12} to a cobalamin-containing (Co^2) compound such as $B_{12}r$ would imply that the methyl group is released at the oxidation level of the methyl radical and would require addition of an electron and proton to form CH_4. Cleavage, also, could lead either to a methyl carbonium ion or a methyl carbanion with a correspondingly more reduced or more oxidized B_{12} moiety as a product. Further reactions of the methyl group and the B_{12} moiety could result in the net formation of CH_4 and $B_{12}r$ or its thiol complex. It will be necessary to identify the electron donor in the extracts, the point at which it participates in the reaction, and the role of ATP in order to more clearly outline the mechanism of CH_4 formation from CH_3-B_{12}.

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