ATP-DEPENDENT FORMATION OF METHANE FROM METHYLCOBALAMIN BY EXTRACTS OF METHANOBACILLUS OMELIANSKII

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The significant observation by Blaylock and Stadtman (1) that the methyl moiety of methylcobalamin serves as a precursor of methane using extracts of Methanosarcina barkeri has prompted us to extend our previous observations on cell-free methane formation (2). At the present time, it is not known whether methylcobalamin is a natural intermediate in the biological formation of methane or whether it only serves as an active methyl donor. This communication presents evidence that extracts of Methanobacillus omelianskii form methane stoichiometrically from methylcobalamin, that this formation is dependent upon ATP, and that the formation apparently does not require an added source of electrons.

METHODS

A culture of Mbac. omelianskii was kindly supplied by H. A. Barker. Cells were cultivated in 20 liter batches in a medium similar to that described by Johns and Barker (3). Extracts of washed cells were prepared daily by crushing the cells in a Hughes press (4). Methylcobalamin was prepared by the method of Müller and Müller (5) using dimethyl sulfate as the methyl donor. The reaction vessel was a Warburg flask which had been fitted with a rubber serum cap through which a gas atmosphere was added, and gas samples were taken using a hypodermic needle attached to a syringe (4). Methane was assayed

using a flame ionization apparatus attached to a silica gel column of a gas chromatograph.

RESULTS

As shown in Fig. 1 the formation of methane from methylcobalamin was dependent upon the addition of ATP, whereas the omission of CoA caused only a slight reduction in the reaction rate. Methylcobalamin was not converted to methane in the presence of boiled extract, and only negligible amounts of methane were produced in the absence of methylcobalamin.

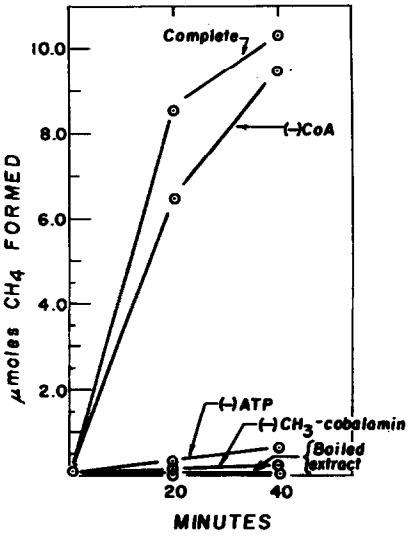

Fig. 1. Effect of ATP on the formation of methane from methylcobalamin. The complete system contained crude extract, 45 mg. protein; potassium phosphate buffer at pH 7.0, 500 μmoles; CoA, 0.05 μmole; ATP, 10 μmoles; methylcobalamin, 10 μmoles; total liquid volume 1.3 ml; hydrogen atmosphere; reaction time, 20 min. at 37°C. Values indicate total methane detected per flask.

The results of additional experiments indicated that the formation of methane was 70 to 80 percent complete in 20 minutes under the conditions used, and that incubation of the reaction mixture for periods longer than 40 minutes did not increase the amount of methane formed; the addition of 10 μmoles of methylcobalamin resulted in the formation of approximately 10 μmoles of methane.

In contrast to the pyruvate-dependent, methylcobalamin system reported by Blaylock and Stadtman (1) for extracts of Msc. barkeri, pyruvate did not stimulate the production of methane from methylcobalamin using extracts of Mbac. omelianskii

as shown in Table 1 nor did pyruvate substitute for ATP. ADP partially replaced the ATP requirement, and AMP was ineffective.

TABLE 1

Comparison of the effect of AMP, ADP, ATP and pyruvate on the formation of methane from methylcobalamin.

	Constituents Added*					Total
Exp.	AMP	ADP	ATP	Pyruvate	CH_3 cobalamin	CH_4 Formed
						μmoles
1	-	-	-	-	+	0.5
	-	-	-	+	+	0.5
	-	-	-	+	-	0.8
	-	-	+	-	+	7.4
	-	-	+	+	+	7.5
	-	-	-	-	-	0.1
2	+	-	-	-	+	0.4
	-	+	-	-	+	4.7
	-	-	+	-	+	7.9

*Each reaction vessel contained crude extract, 45 mg. protein; potassium phosphate buffer at pH 7.0, 500 μmoles; CoA, 0.05 μmole; methylcobalamin where indicated, 10 μmoles; AMP, ADP, and ATP where indicated, 10 μmoles each; sodium pyruvate where indicated, 70 μmoles; total liquid volume 1.4 ml; hydrogen atmosphere; reaction time, 20 min. at 37°C.

Previous observations (2,4) using extracts of Mbac. omelianskii have shown that methane formation from CO_2 or pyruvate is dependent upon a hydrogen atmosphere. As shown in Table 2 methane formation from methylcobalamin occurs equally well under nitrogen or argon, only a slight stimulation occurring under hydrogen.

Although pyruvate was added as an electron source in the experiments of Blaylock and Stadtman (1), it is possible that its role may have been to generate ATP.

TABLE 2

Effect of gas atmosphere on the formation of methane from methylcobalamin.

Flask	Gas Atmosphere	Methyl-cobalamin added	Total CH_4 Formed
			μmoles
1	N_2	-	0.2
2	N_2	+	9.3
3	A	-	0.2
4	A	+	9.9
5	H_2	-	0.3
6	H_2	+	10.7

Each reaction vessel contained crude extract, 45 mg. protein; CoA, 0.05 μmole; ATP, 10 μmoles; potassium phosphate buffer at pH 7.0, 500 μmoles; methylcobalamin where indicated, 10 μmoles; and gas atmosphere as indicated; total liquid volume, 1.35 ml. Reaction time was 20 min. at 37°C.

Four other possible methyl donors, L-methionine methyl sulfonium (Cal Biochem), S-adenosyl-L-methionine (Cal Biochem), 5-methyl tetrahydrofolate (kindly provided by Dr. Warwick Sakami), and 5'-methyl thioadenosine (kindly provided by Dr. F. Schlenk), were tested as precursors of methane using extracts of Mbac. omelianskii; no methane formation from these compounds was detectable. Dimethyl sulfate or methyl iodide were not precursors of methane in the reaction mixture when added in the presence of catalytic amounts of methylcobalamin or cobalamin which had been reduced with zinc dust.

Although our previous results (2,4) have demonstrated an ATP requirement for the formation of methane from hydrogen and CO_2, it was unexpected that ATP would be required at the methyl level in the formation of methane. This finding raises interesting questions as to the nature of the terminal, active intermediates.

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