Isolation and characterization of the Co-methyl and Co-aquo derivative of 5-hydroxybenzimidazolylcobamide (factor III) from *Methanosarcina barkeri* grown on methanol

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The Co-methyl and the Co-aquo derivative of 5-hydroxybenzimidazolylcobamide (factor III) were isolated as the major natural corrinoids from *Methanosarcina barkeri* 'Fusaro'. They were characterized by their UV/VIS-spectra, their chromatographic behaviour, and their electrophoretic properties. The portion of Co-methyl-factor III was found to be 70–80% whereas Co-aquo-factor III contributed 20–30% to the total amount of corrinoids.

Co-methyl-5-hydroxybenzimidazolylcobamide *Vitamin B_{12}-factor III* Methanosarcina barkeri
Methane biosynthesis *Methanol* *Archaebacterium*

1. INTRODUCTION

Factor III was first isolated from sewage sludge [1,2]. Later it was identified as one of the several corrinoids produced by *Methanobacillus omelianskii* [3], a syntrophic association of two organisms [4]. Factor III was also isolated from pure cultures of the archaebacterium *Methanosarcina barkeri* [5–8], a methanogen being present in sewage sludge [9]. Addition of 5,6-dimethylbenzimidazolylcob(I)amide and methanol to *M. barkeri* extracts lead to the formation of Co-methyl-cobalamin [10]. A factor III-containing protein from *M. barkeri* can be chemically methylated by methyl iodide. The resulting protein is then operative in the biological methane formation [11]. But direct evidence that Co-methyl-factor III occurs in *M. barkeri* has been lacking. Since the occurrence of this corrinoid could have important implications on further metabolic studies with *M. barkeri,* we investigated which natural forms of factor III are present in this organism. This study describes the isolation of Co-methyl-factor III from *M. barkeri.*

Abbreviation: 5-hydroxybenzimidazolylcobamide, factor III

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2. MATERIALS AND METHODS

The reference compounds Co-aquo-, Co-methyl- and Co-5′-deoxyadenosyl-factor III were synthesized from Co-monocyano-factor III as described for the corresponding cobalamin derivatives [12]. *Methanosarcina barkeri* strain 'Fusaro' (DSM 804) was grown on methanol as in [6,13]. The bacteria were harvested in the early stationary phase, when they were still producing methane.

2.1. *Isolation of Co-methyl-factor III and Co-aquo-factor III*

All operations were performed under dim light. Wet *M. barkeri*-cells (88 g) obtained from 8-l fermentation, were suspended in 400 ml metha-

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nol/water (8/2), and kept at 90°C for 15 min. The suspension was centrifuged, and the sediment re-extracted with 100 ml methanol/water (8/2) as before. The methanol was removed from the combined supernatants under reduced pressure. The resulting aqueous solution was passed through a column (2 × 10 cm) of Amberline XAD-2 (100–200 mesh).

The column was washed with 2 l water. The corrinoids were eluted with methanol/water (8/2), and the solvent was removed in vacuo. The residue was dissolved in water, and extracted with chloroform in order to remove lipid contaminants. The aqueous solution was evaporated in vacuo, the residue dissolved in ethanol/water (7/3), and chromatographed on a column (1 × 10 cm) of neutral alumina 90 (Merck, Darmstadt, no. 1077) with the same solvent. The solvent was evaporated. The corrinoids (0.45–0.7 μmol/8 l fermentation) were dissolved in water, and applied to a column (2 × 20 cm) of CM-cellulose (H^+-form). Co-methyl-factor III (70–80% of the total corrinoids) was eluted with water, further purified by descending paper chromatography on Whatman 3 MM-paper with butan-2-ol/water/acetic acid (70/30/1, by vol.). The band migrating like a Co-methyl-factor III reference was cut out, and eluted with water. The solution was evaporated, the residue dissolved in ethanol/water (7/3), and chromatographed on neutral alumina as above. The spectrum (fig.1A) was taken from the preparation thus obtained.

The corrinoid remaining on the CM-cellulose column after elution with water (20–30% of the total corrinoids) was eluted with 2% aqueous acetic acid. The eluate was evaporated in vacuo. To remove the acetic acid quantitatively, the residue was dissolved in water, evaporated, and this procedure repeated once more. The resulting Co-aquo-factor III was further purified on neutral alumina as above (spectrum, fig.1B).

The corrinoids were further characterized by paper electrophoresis and paper chromatography (table 1).

3. RESULTS

Usually corrinoids are extracted from microorganisms in the presence of cyanide [14]. By this way the rather light-sensitive coenzyme forms are transformed into the more light-stable cyanocorrinoids. In order to isolate the undestructed corrinoids present in *Methanosarcina barkeri,* the cells were heated with aqueous methanol in the absence of light and cyanide. The corrinoids thus extracted were purified by filtration through Amberlite XAD-2, and fractionated on CM-cellulose. One

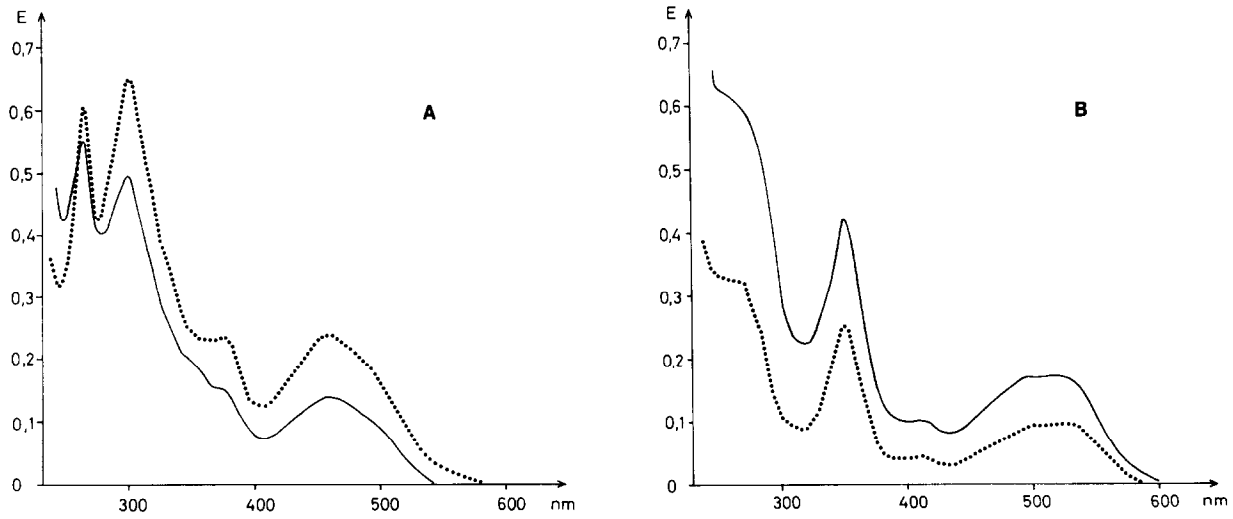

Fig.1. Spectra of corrinoids isolated from *Methanosarcina barkeri* 'Fusaro' in the absence of light and cyanide (see section 2): (A) (——) corrinoid eluted from CM-cellulose with water; (···) authentic Co-methyl-factor III; (B) (——) corrinoid eluted from CM-cellulose with 2% acetic acid; (···) authentic Co-aquo-factor III.

Table 1

Chromatographic and electrophoretic properties of the corrinoids isolated from *Methanosarcina barkeri* 'Fusaro' in the absence of light and cyanide, and of reference corrinoids

	Relative cationic mobility	Relative chromatographic mobility
Monocyano-factor III	0.00	1.00
Monocyano-Cobinamide	1.00	n.d.
Co-methyl-factor III	0.58	1.75
Co-aquo-factor III	0.91	0.82[a]
Co-5'-deoxyadenosyl-factor III	1.12	0.75
Corrinoid eluted from CM-cellulose with water	0.58	1.75
with 2% acetic acid	0.91	0.83[a]

n.d. = not determined
[a] These spots showed tailing

Electrophoresis on Schleicher a. Schüll-paper 2043a, 0.5 M acetic acid, 10 V/cm, 4 h. Descending chromatography on Whatman 3 MM paper for 24 h with butan-2-ol/water/acetic acid, 70/30/1 (by vol.)

corrinoid fraction representing about 70–80% of the total corrinoids was eluted with water, and identified as Co-methyl-factor III by its UV/VIS-spectrum (fig.1A), as well as by its chromatographic behaviour and electrophoretic properties (table 1). A second corrinoid (20–30% of the total corrinoids) was eluted from the CM-cellulose column with 2% aqueous acetic acid. This corrinoid was identified as Co-aquo-factor III (fig.1B, Table 1).

4. DISCUSSION

The procedure described here shows that Co-methyl-factor III and Co-aquo-factor III can be easily isolated from *Methanosarcina barkeri*. But in three separate experiments performed with *M. barkeri*-cells from different fermentations Co-5'-deoxyadenosyl-factor III could not be found. This is in contrast to experiments with the symbiotic culture *'Methanobacillus omelianskii'* [3] where Co-5'-deoxyadenosyl-factor III and Co-aquo-factor III were found.

Here Co-methyl-factor III was detected in the *M. barkeri*-cells whether the bacteria were extracted by heating with methanol/water or with water alone. But since the extraction with water was very incomplete, the methanol/water-procedure was preferred.

These results show that the Co-methyl-factor III is the regular and predominant constituent of *M. barkeri*. This fact suggests strongly a natural function of this corrinoid in methanogenesis [11] or in other pathways [7].

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