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Formation of Factor 390 by Cell Extracts of *Methanosarcina barkeri*

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Cell extracts of *Methanosarcina barkeri* converted coenzyme F_{420} in an ATP-dependent reaction to the adenylylated derivative factor 390. Although it was reported previously (L. M. Gloss and R. P. Hausinger, BioFactors 1:237–240, 1988) that whole cells were unable to perform this conversion, we observed the conversion in 7 of 11 extracts, all of which were prepared from different batches of cells.

Coenzyme F_{420}, an 8-hydroxy-5-deazaflavin, plays a role as electron carrier in several redox reactions in methanogens (4). It was shown that coenzyme F_{420} was converted to factor 390 (F_{390}) when cells of *Methanobacterium thermoautotrophicum* and *Methanobacterium formicicum* (2, 3) were exposed to oxygen; AMP or GMP was coupled via a phosphodiester bond to the 8-OH group of the deazaflavin (3) to form derivatives which showed an absorbance maximum at 390 nm and which were called F_{390} (F_{390}-A or F_{390}-G, respectively). Conversion of coenzyme F_{420} to F_{390} was recently shown to occur in cell extracts of *M. thermoautotrophicum* (5).

In this study we present results which show that F_{390} formation also occurs in cell extracts of *Methanosarcina barkeri*, although such a formation was not observed earlier with whole cells (2).

M. barkeri MS (DSM 800) was grown on H_2-CO_2 (80:20, vol/vol)–25 mM methanol or 30 mM acetate, and *M. barkeri* Fusaro (DSM 804) was grown on 30 mM acetate, as previously described (6). Cells were harvested anaerobically by continuous centrifugation. Cells were washed twice anaerobically in 10 mM TES buffer [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] (pH 7.2) containing 15 mM $MgCl_2$ and were suspended in an equal volume of this buffer. Cells were disrupted by anaerobic passage through a French pressure cell (Aminco, Silver Spring, Md.) at 138 MPa. Cell debris was removed by centrifugation at 40,000 × *g* for 30 min. The supernatant was stored at −70°C under an H_2 atmosphere. Cell extracts of *M. thermoautotrophicum* ΔH (DSM 1053) were prepared as described before (5).

Formation of F_{390} was assayed spectrophotometrically at 37°C by recording a 300- to 500-nm spectrum every 2.5 min on a Hitachi U 3200 spectrophotometer. Enzyme activity was expressed as nanomoles of coenzyme F_{420} converted per minute per milligram of protein, using the molar extinction coefficients of coenzyme F_{420} and F_{390} (1, 3, 5). The assay mixture contained 1.5 ml of 50 mM TES buffer (pH 7) with 15 mM $MgCl_2$, 100 μl of 0.4 mM coenzyme F_{420}, and 100 μl of extract (about 1.5 mg of protein). After 3 min of incubation at 37°C in an open cuvette, 100 μl of 100 mM ATP was added to start the reaction.

Coenzyme F_{420} was purified from boiled extracts of *M. thermoautotrophicum* essentially as described by Eirich et al. (1). F_{390} was isolated from the incubation mixtures when no further absorbance changes occurred (5). The mixture was heated for 2 min at 100°C. After removal of precipitated proteins by centrifugation, the mixture was loaded on three in-series-connected Sep-Pak C_{18} cartridges (Waters Associates, Milford, Mass.), which were activated before use by rinsing with methanol and deionized water (Milli-Q). The cartridges were then rinsed with 2 M NaCl and deionized water. F_{390} was eluted with methanol-water (10:90, vol/vol). The F_{390} solution was freeze-dried and dissolved in deionized water.

The formation of F_{390} from coenzyme F_{420} in an extract of methanol-grown *M. barkeri* cells is shown in Fig. 1. The reaction was completely dependent on the addition of ATP. With heat-treated (5 min, 100°C) extract, no coenzyme F_{420} conversion was observed. The conversion of coenzyme F_{420} to F_{390} proceeded at a rate of about 35 nmol/min/mg of protein, whereas under the same conditions an extract of *M. thermoautotrophicum* ΔH showed an activity of 72 nmol/min/mg of protein.

After the reaction had proceeded towards completion, the product formed during the incubation was purified. The product was identified as F_{390} on the basis of its characteristic UV-visible absorption spectrum (3, 5).

The conversion of coenzyme F_{420} to F_{390} could be demonstrated in extracts from *M. barkeri* MS cells grown on methanol (four of seven) as well as in extracts from cells grown on acetate (two of two). F_{390} formation was also

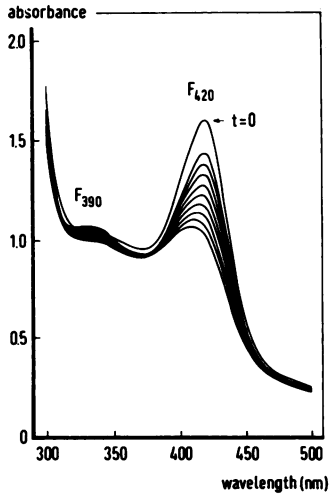

FIG. 1. Formation of F_{390} by an extract of methanol-grown *M. barkeri* MS.

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observed in an extract from acetate-grown *M. barkeri* Fusaro cells. For reasons not understood, some extracts prepared from methanol-grown cells and the only extract tested prepared from H_2-CO_2-grown cells failed to perform this conversion. Our data suggest that it is worthwhile to reinvestigate formation of F_{390} from coenzyme F_{420} in methanogens earlier reported to be negative in this respect (2).

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REFERENCES

1. **Eirich, L. D., G. D. Vogels, and R. S. Wolfe.** 1978. Proposed structure for coenzyme F_{420} from *Methanobacterium*. Biochemistry **17**:4583–4593.
2. **Gloss, L. M., and R. P. Hausinger.** 1988. Methanogen factor 390: species distribution, reversibility and effects of non-oxidative cellular stress. BioFactors **1**:237–240.
3. **Hausinger, R. P., W. H. Orme-Johnson, and C. Walsh.** 1985. Factor 390 chromophores: phosphodiester between AMP and GMP and methanogen Factor 420. Biochemistry **24**:1629–1633.
4. **Keltjens, J. T., and C. van der Drift.** 1986. Electron transfer reactions in methanogens. FEMS Microbiol. Rev. **39**:259–303.
5. **Kengen, S. W. M., J. T. Keltjens, and G. D. Vogels.** 1989. The ATP-dependent synthesis of factor 390 by cell-free extracts of *Methanobacterium thermoautotrophicum* (strain ΔH). FEMS Microbiol. Lett. **60**:5–10.
6. **Van de Wijngaard, W. M. H., C. van der Drift, and G. D. Vogels.** 1988. Involvement of a corrinoid enzyme in methanogenesis from acetate in *Methanosarcina barkeri*. FEMS Microbiol. Lett. **52**: 165–172.