

Coenzyme M, Essential for Growth of a Rumen Strain of *Methanobacterium ruminantium*

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A growth factor present in rumen fluid and essential for growth of a rumen strain of *Methanobacterium ruminantium* was shown to be coenzyme M, 2-mercaptoethanesulfonic acid.

In previous studies (1, 2) strain M1, a rumen strain of *Methanobacterium ruminantium*, was shown to require hydrogen and carbon dioxide or formate as an energy source, NH_4^+ as the major nitrogen source, and acetate and 2-methylbutyrate as carbon sources for growth. Several amino acids, none of which were individually essential, and an unidentified growth factor present in rumen fluid, anaerobic sludge, and diverse species of methanogens including a sewage strain of *M. ruminantium*, *Methanospirillum hungatii*, *Methanosarcina barkeri*, and methanobacterium strain MOH (probably an *M. formicicum* variety), but not in nonmethanogens so far tested, were also necessary. The factor present in rumen fluid was a highly polar, relatively strong acid without positive charge, and had a relatively low molecular weight. Column chromatography on silicic acid with 0.05 N H_2SO_4 as the stationary phase and with a mobile phase of 30% *tert*-butanol in chloroform, followed by ethanol or gradient elution from triethylaminoethyl-cellulose with NH_4HCO_3 , showed two peaks of activity in assays, suggesting that two forms of the factor were present. The factor was required in very small amounts since an amount of purified factor satisfactory for half-maximal growth of a 25-liter culture of M1 weighed less than 1 mg.

The factor had many features in common with coenzyme M, a compound involved in

methyl transfer reactions in methane formation (3), and somewhat purified preparations of coenzyme M were active in growth of M1 (3; unpublished data). Taylor and Wolfe (J. Biol. Chem., in press) recently identified coenzyme M as 2-mercaptoethanesulfonic acid and chemically synthesized it.

Results in Fig. 1 show that either chemically synthesized coenzyme M or its oxidized form, 2,2'-dithiodiethanesulfonic acid, replaces the rumen fluid factor requirement of strain M1. Other results indicate that about 3 to 5 ng of either form per ml is necessary for half-maximal growth and that 2,2'-dithiodipropionate does not substitute for coenzyme M.

It is thus evident that rumen fluid contains coenzyme M or a very closely related compound and that the compound has coenzyme-vitamin functions reminiscent of B vitamins. Regarding the source of the compound in rumen fluid, one can speculate that some rumen strains of *M. ruminantium* or other rumen methanogenic species biosynthesize it, as do methanogens from other ecosystems.

Whether other organisms require coenzyme M for growth is not known; however, Paynter and Hungate (4) obtained some evidence suggesting that *Methanobacterium mobilis* requires a similar factor.

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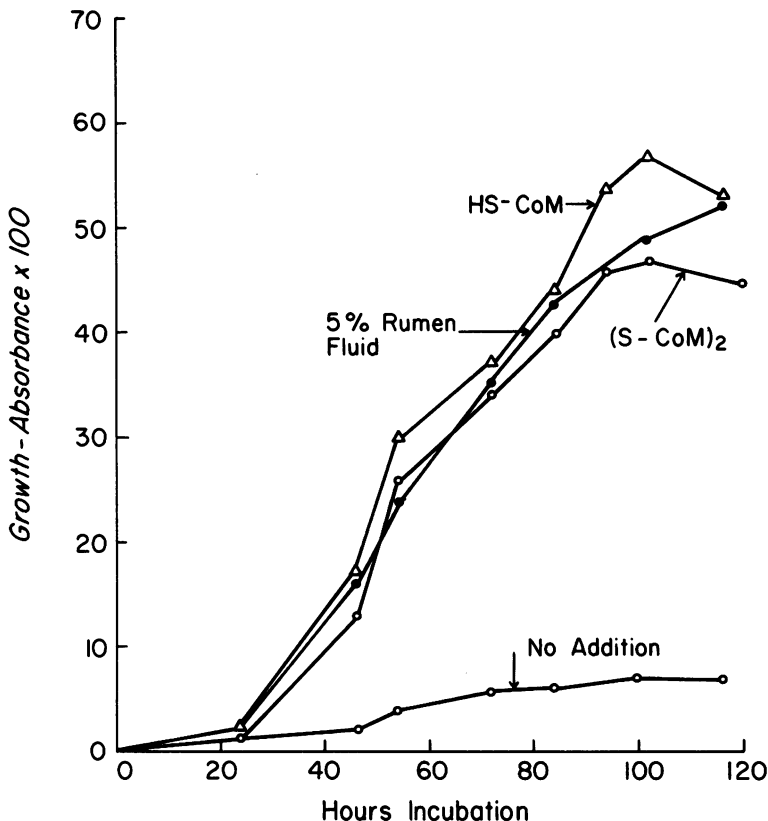


FIG. 1. Growth response of strain M1 to coenzyme M (HS-CoM; 98 ng/ml), its disulfide [(S-CoM)₂; 93 ng/ml], and rumen fluid in a complex assay medium. The assay medium contained a H₂-CO₂ (1:1) gas phase, 0.2% sodium formate, 0.2% Trypticase, 0.2% yeast extract, 0.246% sodium acetate 0.01% isobutyric, 0.01% isovaleric, 0.01% DL-2-methylbutyric and 0.01% n-valeric acids, minerals, carbonate, and cysteine-sodium sulfide reducing solution (1). The preparation of media and washed inoculum and determination of absorbance at 600 nm were as described previously (1). Each point represents the mean of four replicate tubes.

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