Inhibitory effects of 2-bromoethanesulfonate and protection by addition of coenzyme M in hydrogen-oxidizing marine enrichment cultures

Jordan Konisky

Department of Microbiology, University of Illinois, Urbana, IL, U.S.A.

Received 24 August 1989
Revision received and accepted 13 November 1989

Key words: HS-CoM uptake; Bromoethanesulfonate; Salt marsh sediment

1. SUMMARY

Since bromoethanesulfonate (BES) is an inhibitor of methane production (competitive with methyl-coenzyme M), cells able to accumulate large internal pools of methyl-coenzyme M via uptake of its precursor, HS-CoM, should be protected from BES by addition of HS-CoM to the growth medium. Hydrogen-oxidizing marine methanogen enrichments were prepared from anaerobic sediment samples collected at Sippewisset Salt Marsh and Oyster Bay Inlet near Woods Hole, MA. The three enrichments studied were a mixture of cell types with at least 50% of the culture comprised of methanogens. Methane production was found to be sensitive to BES with half maximal inhibition occurring at 5-20 μM BES depending on the enrichment. For each, half maximal protection against 40 μM BES occurred at a HS-CoM: BES molar ratio of 20:1 to 40:1. Since the protected enrichments exhibited normal sensitivity toward BES after removal of HS-CoM, it was concluded that methane production in the presence of both BES and HS-CoM resulted from true protection and not growth of BES-resistant mutants. These results suggest that uptake of HS-CoM may be a general property of methanogens occupying anaerobic marine sediments. It is possible that uptake of this coenzyme is an important nutritional feature of methanogens in their natural habitat.

2. INTRODUCTION

We have recently described the presence of an active transport system in the marine methanogen Methanococcus valliei which mediates uptake of 2-mercaptoethanesulfonic acid (coenzyme M) [1]. This coenzyme, which is also synthesized by this strain, is the methyl-donating substrate for the terminal biochemical reactions leading to the formation of methane [2,3]. While all examined methanogens contain coenzyme M [4], reports of significant uptake of this substrate into other methanogens has been limited to the coenzyme M requiring rumen methanogen Methanobrevibacter
ruminantium while somewhat lower levels of uptake have been observed in *Methanospirillum hungatii* and *Methanobacterium mobile*, both of which produce coenzyme M [5].

In the case of *Methanococcus voltae*, the presence of a coenzyme M uptake system was first suggested by the observation that cells could be protected from the toxic action of 2-bromoethanesulfonic acid by the exogenous addition of either coenzyme M or its methylated derivative, methyl-S-CoM [6]. In a more direct demonstration using radioactive coenzyme M it was shown that uptake occurs against a concentration gradient and with kinetic parameters characterized by a $V_{\text{max}}$ of 960 pmol/min/mg protein and an apparent $K_m$ of 61 $\mu$M [1].

The question arises as to the physiological significance of a coenzyme M uptake system in an organism which produces sufficient substrate to support growth. It is also important to establish the generality of such a system in other marine methanogens. In this paper evidence is presented which suggests the presence of coenzyme M uptake systems in methanogens comprising enrichments obtained from marine sediments.

3. MATERIALS AND METHODS

3.1 Growth medium

General salts medium contained (g/l) NaCl, 9; NaHCO$_3$, 5; Na-acetate, 1; KCl, 0.34; MgCl$_2$·2H$_2$O, 2.7; MgSO$_4$, 3.5; CaCl$_2$·2H$_2$O, 0.14; NH$_4$Cl, 0.25; $K_2$HPO$_4$, 0.14; and trypticase (Difco, U.S.A.), 2; (mg/l) NiCl$_2$, 5; (ml/l) 0.2% Fe(NH$_4$)$_2$SO$_4$, 1; 0.1% resazurin, 1; vitamin solution [7], 10; trace mineral solution [7], 10; 1.25% Na$_2$S, 40; and 1.25% cysteine, 40. Medium for isolation contained (g/l) NaCl, 9; NaHCO$_3$, 5; Na-acetate, 0.5; KCl, 0.34; MgCl$_2$·2H$_2$O, 2.7; MgSO$_4$, 0.2; CaCl$_2$·2H$_2$O, 0.14; NH$_4$Cl, 0.25; $K_2$HPO$_4$, 0.14; (mg/l) NiCl$_2$, 5; (ml/l) 0.2% Fe(NH$_4$)$_2$SO$_4$, 1; 0.1% resazurin, 1; vitamin solution [7], 10; trace mineral solution [7], 10; and 2.5% Na$_2$S, 40.

3.2 Methanogenic enrichments

Anaerobic sediment samples (0.2 ml) collected at Oyster Pond Inlet (enrichments BP1 and BP2) and the Sippewisset Salt Marsh (enrichment SW1) near Woods Hole, MA, were inoculated into 18 × 150 mm Balch tubes [7] containing 5 ml of isolation medium and a pressurized atmosphere (30 psi) of H$_2$ : CO$_2$ (80 : 20). The tubes were shaken at 30°C and assayed periodically for methane production. After 96 h substantial methane had been produced and growth was apparent as evidenced by increased turbidity of the cultures. These cultures were subcultured (0.2 ml) into fresh media (5 ml) containing both penicillin and kanamycin (each at 50 $\mu$g/ml, final concentration) to both continue the enrichment and limit growth of eubacteria. After 3 days of growth, these cultures were subcultured in an identical manner after which time they were subcultured into general salts medium lacking antibiotics. The latter medium was used for subsequent experiments.

3.3 Experimental conditions and methane assay

In all experiments growth was carried out with shaking at 30°C in 18 × 150 mm Balch tubes [7] containing 5 ml of general salts medium in a pressurized (30 psi) atmosphere of H$_2$ : CO$_2$ (80 : 20). For methane assays, 10 $\mu$l of the gas phase was removed with a gas tight Hamilton syringe and assayed for methane content using a Varian gas chromatograph (PID detector, porapak Q column). The instrument was standardized with pure methane. While the data presented are based on samples obtained from 48-h cultures, identical patterns of methane production were obtained for samples taken 12, 24 and 36 h. Methane production was linear during this sampling period.

4. RESULTS AND DISCUSSION

4.1 The enrichments

As determined by phase contrast microscopy, each of the enrichment cultures consisted of a mixed bacterial consortium with short rods (0.5 to 2.0 $\mu$m) and irregular cocci (0.5 to 1.0 $\mu$m in diameter) predominating. Approximately 50% of the cells exhibited the epifluorescence characteristics of methanogens [8,9].

4.2 Rationale for uptake assay

The toxicity of bromoethane sulfonic acid (BES)
toward methanogens is based on its structural relatedness to coenzyme M, and it has been shown that the cellular target for BES is the CH₃-S-CoM reductase system for which it functions as a competitive inhibitor [2]. On this basis, it was reasoned that strains able to take up coenzyme M from the medium might be able to accumulate the substrate to sufficient intracellular levels to protect cells from the action of BES. On the other hand, cells lacking the ability to accumulate coenzyme M would be equally sensitive to BES in the presence and absence of exogenously added coenzyme M. These assumptions have been borne out by our studies in M. voltae [1,6].

4.3 Sensitivity of enrichments to BES

The results in Fig. 1 represent a typical experiment in which the effect of BES on methane production was determined in cultures growing on H₂/CO₂. An identical pattern of inhibition was obtained in parallel experiments in which optical density was used to monitor cell growth. As can be seen, methanogenesis in all three enrichments was nearly totally inhibited by 40 µM BES. However, the concentration of BES needed to inhibit methanogenesis varied somewhat in different experiments and it was not established whether the different enrichments manifested a real difference in sensitivity. While the BES concentration required to inhibit methanogenesis in these enrichments is similar to that observed in pure culture, our results contrast studies carried out with sediment samples where it was observed that as much as 10–100 mM BES was required to inhibit methanogenesis [10].

4.4 Protection by HS-CoM

Various amounts of HS-CoM were added to enrichments treated with 40 µM BES. As can be seen HS-CoM protected each enrichment in a concentration-dependent manner (Fig. 2). In a control experiment, we observed that addition of 5.6 mM HS-CoM alone did not stimulate either methanogenesis or growth, indicating that these results do not merely reflect a stimulation of growth by the cofactor.

While fuller characterization of the subpopulations comprising each enrichment will be necessary to explain the observed difference in the response curves, it is possible that these data reflect differences in the capacity of the resident methanogens to take up exogenous HS-CoM from the medium. The fact that protection is afforded at a HS-CoM : BES ratio of 10 : 1 to 40 : 1, results which are similar to our studies with M. voltae [6], indicates that these populations have rather efficient systems for HS-CoM uptake.
To rule out the possibility that the presence of HS-CoM led to the accumulation of BES-resistant mutants, we determined the sensitivity of a HS-CoM-protected culture to subsequent BES treatment. Enrichment culture BP1 was grown for 24 h to mid-log phase in medium containing BES (40 μM) and HS-CoM (5.6 mM). While no growth was observed at 24 h in a parallel culture containing BES, but no HS-CoM, continued incubation over a period of 4 days led to the development of turbidity, presumably due to the growth of BES-resistant mutants. Both cultures were next subcultured (0.1 ml) into fresh medium (5 ml) lacking both BES and HS-CoM and grown to stationary phase. The cultures were then subcultured twice more in an identical fashion.

As seen in Fig. 3, the protected enrichment culture manifested a degree of sensitivity to BES which was identical to that of the untreated culture. In contrast, the culture which developed after prolonged incubation with BES was refractory to subsequent BES addition.

In conclusion, these results suggest that the ability to take up thiols present in marine sediments may be a general property of methanogens occupying marine sediments. While there is a report that intertidal Biscayne Bay (FL) sediments contained a low level of HS-CoM (< 0.5 μM) [11], it is not known if such HS-CoM has any ecological significance.

ACKNOWLEDGEMENT

These experiments were carried out at the Marine Biological Laboratory, Woods Hole, MA, where the author participated as a student in the 1988 microbiology course. This endeavor was supported by a Department of Energy Research Grant DEFG 0284 ER 132 awarded to the author.

REFERENCES