Copyright ©

Kieler Meeresforsch., Sonderh. 8, 102-106. Kiel 1991

Do sulfate-reducing bacteria respond to thymidine incorporation assays in marine sediments?

L. Piker and W. Reichardt
Institut für Meereskunde an der Universität Kiel
Düsternbrooker Weg 20, D-2300 Kiel, Germany

Abstract

In anoxic sediment from Kiel Bight short-term incorporation of tritiated thymidine (tdr) into DNA was partly reduced by molybdate, a fairly specific inhibitor of sulfate-reducing bacteria (SRB). Preliminary incorporation experiments with cultures of SRB, however, failed to provide substantial clues if and how SRB account for incorporation of tdr in anoxic sediments. One single positive response in four strains tested was by two orders of magnitude too low to compare with aerobic heterotrophic bacteria.

Introduction

Sulfate-reducing bacteria (SRB) represent the main fraction of mineralizing bacterial populations in coastal marine sediments once these have turned anoxic (JØRGENSEN 1978). In these environments, frequent changes of the redox conditions ought to have an impact on the population dynamics of SRB. As yet, however, techniques to assess population dynamics of such physiological groups of bacteria in sediments have not been developed.

On the other hand, current measures of total bacterial productivity may be specified by using discriminatory inhibitors. Inhibition by sodium molybdate of incorporation rates of tritiated thymidine (tdr) in anoxic sediments from Kiel Bight suggested a positive response by SRB. To calibrate rates of tdr incorporation in terms of DNA replication or productivity of SRB, it was attempted to measure incorporation of tdr by cultures of SRB.

Material and methods

Sediment (muddy sand, E_h = -50 to -150 mV) was obtained from Kiel Bight at 20 m water depth (54°37.3 N; 10°18.8 E) using a Reineck box core sampler.

For culture experiments, the following strains of sulfate-reducing bacteria were employed: *Desulfobacter postgatei*, DSM 2034, *Desulfovibrio vulgaris*, DSM 1744, strain LAC-C2, a lactate utilizing isolate from Kiel Bight and strain AC-C2, an acetate utilizing isolate from the same habitat. Cultures were grown under N_2/CO_2 (90:10 v/v) in a carbonate-buffered mineral base containing 10 mM of lac-

tate and 15 mM of acetate, respectively, as electron donors according to WIDDEL (1980).

Sediment slurries were prepared under N_2/CO_2 (90:10 v/v)-atmosphere by suspending 0.1 cm^3 of sediment in WIDDEL's carbonate-buffered mineral base containing Na_2S as reducing agent (-150 mV; WIDDEL 1980). To inhibit SRB, sodium molybdate was added at a final concentration of 20 mM. Short-term assays (20 min at *in situ* temperature) were started by adding 37 kBq of [*methyl-^3H*] tdr with a specific activity of 3.034 TBq $mmol^{-1}$ (MORIARTY 1986). For correcting the pool size of tdr, isotope dilution analyses were carried out according to FORSDYKE (1968).

To measure incorporation of tdr by SRB, 1 cm^3 aliquots of exponential phase culture suspensions (OD_{580nm} = 0.01) were incubated for up to six hours in WIDDEL's artificial sea water base with 37 kBq [*methyl-^3H*] tdr at 20 °C. All blanks received 6 % of formaldehyde (final concentration) which was added at least 30 min before the assay was started.

To separate DNA and proteins, the extraction procedure after FINDLAY et al. (1984) was used. Radioactivity in these fractions was determined in Lumagel (Baker) using a Packard 1900 CA Tri-Carb scintillation counter. Quench corrections were based on the external standard method.

Total cell densities of bacteria were determined using acridine orange epifluorescence microscopy (HOBBIE et al. 1977, REICHARDT 1988). During incubation of culture suspensions with tdr, cell densities were checked every second hour. Most probable numbers of SRB in sediment samples were determined using WIDDEL's (1980) sea water base supplemented with 10 mM lactate, 15 mM acetate, and 5 mM succinate (BUSSMANN 1989).

Results and discussion

Rates of tdr incorporation in anoxic slurries from Kiel Bight sediment ranging between 1.0 and 4.3 pmol $cm^{-3}h^{-1}$ showed usually significant inhibition in the presence of 20 mM sodium molybdate (PIKER and REICHARDT, ms. in prep.). Up to 1.7 pmol cm^{-3} h^{-1} proved molybdate inhibitable and therefore attributable to SRB (Table 1).

Table 1. Incorporation of thymidine in anoxic sediment from Kiel Bight

Sampling Date	TdR Incorporation [pmol h^{-1} cm^{-3}] Total	Inhibitable by Mo	Mo-inhibitable Incorporation/MPN [10^{-3}fmol h^{-1} $cell^{-1}$]
November 2, 1989	4.29	1.72	n.d.
December 12, 1989	2.31	0.33	5.79
January 31, 1990	1.45	0.37	n.d.

n.d. MPN not determined

Table 2. Rates of tdr incorporation in relation to cell densities and production of sulfate reducing bacteria.

Strain	Radioactivity in DNA-Fraction [dpm]			Tdr Incorporation Rate [fmol $h^{-1}cm^{-3}$]	Cell Density [10^6 cells cm^{-3}]	Incorporation Rate/Cell [10^{-8}fmol h^{-1} $cell^{-1}$]	Cell Production [10^3 cells h^{-1} cm^{-3}]
	uncorrected	blank	corrected				
LAC C2	452 (64)	104 (55)	348	1.27*	25.7 (5.1)	4.94	704
D. vulgaris	740 (67)	189	551	2.02	45.6 (2.3)	4.43	808
AC C2	137 (107)	68 (50)	69	0.25	5.76 (1.3)	4.39	57
D. postgatei	147 (50)	87	60	0.22	7.81 (0.2)	2.82	64

* significant incorporation (p=0.05)
standard deviation in brackets (n=3)

On the other hand, only one of four sulfate-reducing bacteria examined (LAC C2) incorporated tdr into its DNA at a rate (1.27 fmol $h^{-1}cm^{-3}$) that was significantly (P= 0.05) different from formaldehyde-treated blanks (Table 2).

On a cellular basis, this weak and rarely significant response of cultured cells ranged from 2.8 to 4.9 10^{-8} fmol h^{-1} per cell. Essentially, this result seems to confirm earlier statements denying participation of SRB in tdr incorporation on the basis of yet unpublished data (MORIARTY 1986).

Nevertheless, not all the arguments in favor of the existence of an incorporation potential for tdr in SRB have been refuted yet: In our culture experiments corrections for isotope dilution have been applied only to *Desulfovibrio vulgaris*. Therefore underestimates are possible in the remaining assays. Furthermore it is not known to what degree the affinity of uptake systems for tdr is affected by different physiological states of SRB. In the diluted culture suspensions used for our assays, competition between tdr and lactate or acetate for uptake sites cannot be excluded, until these assays have been repeated with starved cells. The same argument applies to microautoradiographic assays using nutrient-starved cells in which the strains tested (*Desulfovibrio vulgaris* and Lac C2) showed erratic uptake responses for ^{3}H-tdr (unpublished results).

To solve the apparent discrepancy between positive indications of tdr incorporation by SRB in marine sediments and the rather negative conclusions drawn from preliminary culture experiments, two points seem to be crucial: 1) whether molybdate inhibits only and specifically SRB, and 2) to what extent nutrient limitation in sediments accounts for the largely positive responses.

Evidently, inhibitory effects of molybdate are mainly confined to sulfate reduction, whereas other processes and their bacterial catalysts (for example: denitrifyers) are likely to be stimulated (OREMLAND and CAPONE 1988). In fact, one of our assays with Kiel Bight sediment showed enhanced rates of tdr incorporation in the presence of molybdate (PIKER and REICHARDT, ms. submitted).

An influence of starvation for organic carbon sources on uptake and incorporation of tdr by SRB appears more likely. Comparisons of sulfate reduction rates and MPN suggest that many SRB in marine sediments are metabolically active, but not able to divide in enrichment media (JØRGENSEN 1978, GIBSON et al. 1987). Based on one MPN estimate of SRB in Kiel Bight sediment, molybdate inhibitable tdr incorporation amounted to 5.8 10^{-3} fmol h^{-1} per cell (Table 1), whereas growing cells of our lactate utilizing isolate incorporated only 4.9 10^{-8} fmol h^{-1} per cell. Even if considering an underestimation of MPN of SRB by a factor of 1000 (JØRGENSEN 1978), culture suspensions would incorporate tdr at rates being two orders of magnitude below their analogues in sediments.

Incorporation rates of tdr are usually converted into rates of bacterial biomass production (BELL 1990, RIEMANN et al. 1987). Such estimates are derived from increments of cell counts in plankton. Since these procedures are not applicable to sediments, culture suspensions serve as calibration standards (MORIARTY 1986). Based on cell production of strain Lac C2, incorporation of tdr would amount to 1.8 10^{-6} fmol per cell (Table 2). This would lead to a conversion factor of 5.55 10^{20} cells produced per mol of tdr. Yet, conversion factors published for aerobic heterotrophs are by two orders of magnitude greater (MORIARTY 1986, FUHRMAN and AZAM 1980).

References

BELL, R.T., 1990. An explanation for the variability in the conversion factor deriving bacterial cell production from incorporation of [^{3}H]thymidine. Limnol. Oceanogr. 35, 910-915.

BUSSMANN, I., 1989. Einfluß der Makrofauna der Kieler Bucht auf die Abundanz Sulfat-reduzierender Bakterien. Diploma Thesis. University of Kiel.

FINDLAY, S.E.G., J.L. MEYER and R.T. EDWARDS, 1984. Measuring bacterial production via rate of incorporation of ^{3}[H]thymidine into DNA. J. Microbiol. Meths. 2, 57-72.

FORSDYKE, D.R., 1968. Studies of the incorporation of [5-^{3}H]uridine during activation transformation of lymphocytes induced by phytohaemagglutinin. Biochem. J. 107, 197-205.

FUHRMAN, J.A. and F. AZAM, 1980. Bacterioplankton secondary production estimates for coastal waters of British Colombia, Antarctica and California. Appl. Environ. Microbiol. 39, 1085-1095.

GIBSON, G.R., R.J. PARKES and R.A. HERBERT, 1987. Evaluation of viable counting procedures for the enumeration of sulfate-reducing bacteria in estuarine sediments. J. Microbiol. Meths. 7, 201-210.

HOBBIE, J.E., R.J. DALEY and S. JASPER, 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33, 1225-1228.

JØRGENSEN, B.B., 1978. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments III. Geomicrobiol. J. 1., 49-64.

MORIARTY, D.J.W., 1986. Measurement of bacterial growth rates in aquatic systems from rates of nucleic acid synthesis. Adv. Microb. Ecol. 9, 245-292.

OREMLAND, R.S. and D.S. CAPONE, 1988. Use of "specific" inhibitors in biogeochemistry and microbial ecology. Adv. Microb. Ecol. 10, 285-383.

REICHARDT, W., 1988. Impact of bioturbation by *Arenicola marina* on microbiological parameters in intertidal sediments. Mar. Ecol. Prog. Ser. 44, 149-158.

RIEMANN, B., P.K. BJØRNSEN, S. NEWELL, and R. FALLON, 1987. Calculation of cell production of coastal marine bacteria based on measured incorporation of ^{3}H-thymidine. Limnol. Oceanogr. 32, 471-476.

WIDDEL, F., 1980. Anaerober Abbau von Fettsäuren und Benzoesäure durch neu isolierte Arten Sulfat-reduzierender Bakterien. Doctoral Thesis, University of Göttingen.