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Observations on the microbial incorporation of thymidine and leucine in marine sediments

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Abstract

The simultaneous incorporation of radiolabelled thymidine and leucine was followed in intact muddy sand sediments from the North Sea. As it could be shown, incorporation activities covaried over sediment depth. Parallel analysis of the enzymatic decomposition of organic material (by means of the hydrolysis of fluoresceindiacetate) revealed that stimulations of microbial biomass production coincided with stimulations of enzymatic activities although maxima of both processes occurred at different sediment depths.

Introduction

To evaluate the role of microorganisms in mineralization processes and biomass production in marine sediments, measurements of microbial activity are essential. Of the various methods applied to estimate microbial activity, measurements of microbial biomass production seem to be most desirable since extrapolations on microbial growth rates may be derived. For the determination of microbial biomass production, the incorporation of radiolabelled thymidine into DNA and leucine into protein (FUHRMAN and AZAM 1982, KIRCHMAN et al. 1985) have been applied. Although a number of technical and conceptual problems exists (KIRCHMAN et al. 1986, McDONOUGH et al. 1986), the estimation of protein synthesis can complement estimates of DNA synthesis. As shown by CHIN-LEO and KIRCHMAN (1988) incorporation rates of leucine and thymidine covaried in marine microbial assemblages. We report here on observations of the simultaneous microbial incorporation of thymidine and leucine in marine sediments.

Material and methods

Cores were withdrawn using a multiple corer in August 1989 from a muddy sand sediment (water depth 35 m) located south of the island of Helgoland (North Sea). Intact subcores were taken in plexiglas tubes perforated at 0.5 cm intervals and sealed with silicone rubber. Through the ports 10 µl of a mixture of ^{3}H-thymidine (90 Ci/mmol; final concentration 0.4 µCi) and ^{14}C-leucine (342 mCi/mmol; final concentration 0.024 µCi) were injected (for details of the core-injec-

tion technique cf. MEYER-REIL 1986). Parallel cores were incubated at *in situ* temperature (16 °C) for 1, 2 and 4 h. The incubation was terminated by freezing in dry ice. For the analysis cores were sectioned, and the centres of the horizons were cut out with a cork drill. Macromolecules were extracted using a modification of the method of MARMUR (1961). Sediments were washed 4 times with 3 ml of formalin (5 %) and extracted with 0.8 ml of NaOH (0.3 N), EDTA (25 mM) and SDS (0.1 %) for 12 h at 25 °C. After centrifugation the sediment was finally washed with 0.3 N NaOH. The supernatants were combined, chilled at 0 °C and neutralized. Macromolecules were precipitated by adding carrier protein (2 mg) and TCA (final concentration 5 %). After washing the pellet with 5 % TCA, DNA was hydrolyzed at 95 °C for 30 min. Protein was solubilized in 0.3 ml of 0.3 N NaOH (60 °C, 15 min). Both fractions were radioassayed.

Results and discussion

In the investigation the core-injection technique applied proved to be very useful. Through the injection of radiolabelled substrates into intact sediment cores, microbial assemblages are minimally disturbed and biological and chemical gradients are minimally altered (MEYER-REIL 1986). Because of this, the core-injection technique is preferable over other methods of substrate addition (pore water replacement, sediment slurries).

To derive microbial biomass production rates from the measurement of the incorporation of thymidine and leucine, respectively, is still problematic. One of the most important problems is the assessment of isotope dilution by internal and external pools of thymidine and leucine (POLLARD and MORIARTY 1984). Therefore in the frame of this study, only substrate incorporation rates will be reported.

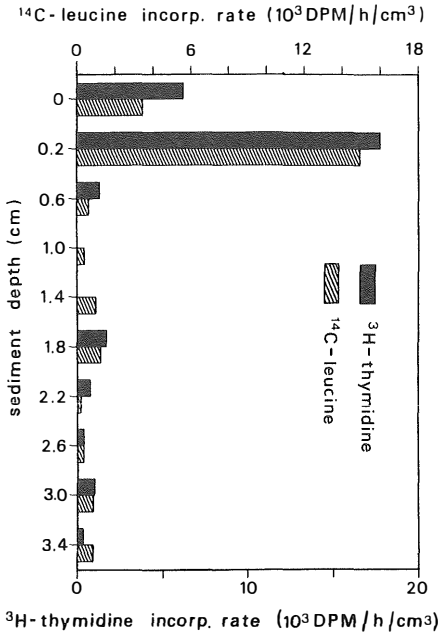

Fig. 1. Simultaneous incorporation of ^{3}H-thymidine and ^{14}C-leucine in intact sediments from the North Sea.

Incorporation rates of thymidine and leucine simultaneously injected into intact sediments covaried with depth (Fig. 1). Highest activities were measured at the sediment surface. Below the surface incorporation rates sharply decreased although some stimulations may occur in subsurface horizons. Although the covariance of thymidine and leucine incorporation was observed frequently, instances when incorporations failed to covary have been reported. The lack of covariance may be explained by specific problems with either method or by periods of unbalanced growth when bacteria shift from one growth rates to another and rates of macromolecular synthesis are uncoupled (KIRCHMAN et al. 1986).

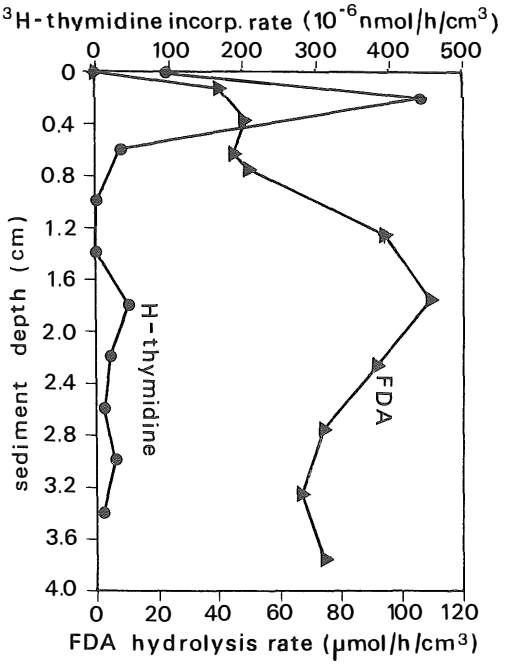

Fig. 2. Depth profiles of the incorporation of ^{3}H-thymidine and the enzymatic decomposition of organic material (hydrolysis of fluoresceindiacetate) in intact sediments from the North Sea.

Stimulations of the incorporation of thymidine and leucine seemed to coincide with stimulations of the enzymatic decomposition of organic material (as measured by the hydrolysis of fluoresceindiacetate, a model substrate to follow esterase activity; cf. MEYER-REIL 1991, MEYER-REIL and KÖSTER 1991). However, highest microbial production was measured at the sediment surface, whereas highest enzymatic activity was recorded in subsurface horizons (Fig. 2). The differences in variation patterns may reflect differences in the availability of organic material to microbial metabolism. Whereas at the sediment surface, the high microbial production may rely on available organic carbon, in subsurface horizons the enzymatic hydrolysis of higher molecular weight organic material may be the limiting step in microbial carbon utilization.

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