Metabolism of Low Molecular Weight Organic Compounds by Sulfate-Reducing Bacteria in a Delaware Salt Marsh

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Abstract. Oxidation of acetate, lactate, pyruvate, and ethanol to CO₂ in anaerobic salt marsh sediments was rapid, with the oxidation rate being significantly inhibited (60–90% decrease) in the presence of 2 mM sodium molybdate, an inhibitor of sulfate-reducing bacteria (SRB). 2-Bromoethanesulfonic acid (BES), an inhibitor of methanogenic bacteria, generally had no effect on the oxidation rate. Acetate was the only intermediate product detected in the oxidation of lactate and ethanol. Competition studies with lactate, acetate, and ethanol indicated that the preferred order of substrate utilization was lactate, then acetate, then ethanol. The turnover times of these three compounds in salt marsh sediments via the combined CO₂ plus acetate pool was rapid (10–13 hours) with a two- to threefold increase in the turnover time in the presence of molybdate. These results strongly suggest that SRB play a major role in the terminal metabolism of low molecular weight organic compounds in anaerobic salt marsh sediment.

Introduction

Sulfate-reducing bacteria (SRB) are commonly associated with decomposing organic matter in anaerobic marine and freshwater sediments. However, it is only in the past decade that the in situ activities of SRB have received detailed attention. Quantitative sulfate reduction rates have been measured in natural sediments with considerable study of the effects of organic amendments and metabolic inhibitors [3, 4, 19, 27, 31, 33, 39]. These investigations have made it clear that sulfate reduction is a major process in the terminal oxidation steps in a number of anaerobic systems. In addition, the metabolism of pure cultures and enrichment (mixed) cultures have shown that the diversity of electron donors usable by SRB is much wider than previously thought [18, 36, 37].

The various studies involving natural sediment have used a number of approaches: 1) the rates of oxidation of low molecular weight organic compounds and H₂ have been measured [1, 14, 17, 32]; 2) intermediate products in the metabolism of these compounds have been identified and measured [6, 7, 18]; and 3) interactions between SRB and methanogenic bacteria in relation to

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energy source (electron donor) have been investigated [2, 15, 22, 23, 30]. However, no single study has pursued all these objectives simultaneously. The present project was initiated to combine these 3 approaches along with measurement of organic turnover times [8, 11, 40, 41] in the examination of sulfate reduction activity in salt marsh sediments.

Materials and Methods

Study Site and Sample Collection

The study site was located in the Canary Creek marsh in Lewes, Delaware (USA). This marsh is dominated by Spartina alterniflora (Loisel.) vegetation with an extensive series of man-made drainage ditches dug in the 1930s. These ditches, as well as Canary Creek, are lined by dense stands of tall Spartina and form major sites of interaction between the incoming tidal water and the marsh proper. Samples were obtained along a 200-m section of one of these ditches at a site several hundred meters inland from the mouth of the ditch. The top 3–5 cm of the sediment from the central portion of the ditch was obtained using a hand trowel and placing the sediment into a sterile Whirl-Pak bag (NASCO). Further details of the study site and sample collection are described elsewhere [10].

Mineralization Studies

The mineralization of various organic compounds by SRB was measured as follows. Small sediment samples (approximately 1.0 g wet weight) were placed into pre-tared vials (17 x 60 mm). The vials were sealed with Hungate stoppers and caps (Belco) and then evacuated and refilled with O2-free nitrogen 3 times. A small amount (15 µl) of the desired 14C-labeled substrate was injected into each vial, and the vials were then incubated at room temperature for 4 hours. All substrate solutions were prepared to result in a final interstitial water concentration of 1 mM for the desired organic compound. The solutions were prepared by adding a known amount of the undiluted stock 14C-solution to an unlabeled solution of the same compound, such that each vial received 100,000–125,000 cpm. The 14C-substrates used were: Na-L-U-14C-lactate (161 mCi/mmol); Na-L-14C-acetate (59.6 mCi/mmol); L-14C-ethanol (61.6 mCi/mmol), and Na-U-14C-pyruvate (10 mCi/mmol) (Amersham). At the end of the incubation period, 1.0 ml of a pH 9.0 Tris buffer (tris [hydroxymethyl] aminomethane, 0.05 M) was injected into each vial; the vials were shaken for 30 min on a reciprocal action shaker to trap 14CO2. The vials were analyzed for 14CO2 using a modification of the sulfide distillation technique previously described [10]. A series of 4 traps was employed. The first 2 traps contained a 2% (w/v) Zn-acetate solution to remove sulfide [16], and the third and fourth traps contained 30 ml of a 10% (v/v) CarboSorb solution (Packard). After trapping the CO2 in the vial, the sediment was transferred to the reaction vessel by rinsing the vial with distilled water and immediately attaching the reaction vessel to the distillation apparatus. 14CO2 was liberated from the sediment slurry by the addition of 5 ml of 6 N HCl, and the distillation then proceeded for 30 min. Traps 1 and 2 removed all sulfide (and small amounts of ethanol; see Results), while 14CO2 was recovered in the last 2 traps. At the end of the distillation period, 0.5 ml of the CarboSorb solution from each trap (traps 3 and 4) was removed and added to 10.0 ml of formula 963 scintillation fluid (New England Nuclear) and assayed for 14CO2 activity using a Beckman LS-230 liquid scintillation counter. To determine the amount of residual label in the sediment, the slurry was centrifuged at 3,000 × g for 20 min in a Sorvall RC-5 refrigerated centrifuge (DuPont). The resulting supernatant was assayed for residual organic label by adding 0.5 ml to scintillation fluid and counting as described above. The sediment pellet was assayed for adsorbed label by resuspending the pellet in 10 ml of Tris buffer, mixing for 1 min with a vortex mixer, and counting as