Arylsulfatase Activity in Salt Marsh Soils†

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Received for publication 15 May 1979

The presence of arylsulfatase(s) was confirmed in salt marsh soils. The temperatures of maximum activity and inactivation, the pH range over which the enzyme was active, and the \( K_m \) values were similar to those of soil enzymes. Unlike soil arylsulfatases, however, the salt marsh enzymes do not appear to be repressed by sulfate. It is postulated that these enzymes may be necessary for the initiation of arylsulfate ester metabolism.

In aerated terrestrial soils ester sulfates constitute 25 to 93% of the available sulfur in such forms as choline sulfate, phenolic sulfate, and sulfated polysaccharides (4). Microorganisms, plants, mammals, and probably other animals can produce enzymes (sulfohydrolases) that hydrolyze these esters. Only a few sulfohydrolases have been studied, and of these arylsulfatase (arylsulfate sulfohydrolase, EC 3.1.6.1) has been the one most extensively studied because of the abundance of arylsulfate in soil. It has been suggested that the function of this enzyme is to provide free sulfate for organism growth in sulfate-deficient environments containing arylsulfate esters (4, 5). It has been shown that arylsulfatase production can be repressed in sulfate-rich environments.

Marine environments have not been considered likely sites for arylsulfatase activity, due to the high concentration of sulfate found in seawater. However, Dodgson et al. (3) isolated an \textit{Alcaligenes metalcaligenes} strain from intertidal mud, which possessed arylsulfatase activity in culture, despite the rich sulfur content of the growth medium. More recently, Chandramohan et al. (1) demonstrated that mangrove and subtidal marine sediments possessed arylsulfatase activity. Fitzgerald (4) suggested that the function of the enzyme in marine environments might be to enable organisms to metabolize the arylsulfate, not to provide free sulfate. Thus, the function of the enzyme in marine and terrestrial environments could differ significantly.

During a study of sulfate reduction in salt marsh sediments at Sapelo Island, GA., we found that sulfate was depleted up to 40% in some sediments (Oshrain and Wiebe, unpublished data); probably in local microzones free sulfate was in very low concentration. Thus, even in a marine system sulfate could be limiting. In this paper we examine arylsulfatase activity, its distribution within the intertidal salt marsh sediments at Sapelo Island, and some of its physical properties.

The salt marshes bordering Sapelo Island have been described by a series of authors (2, 6, 7, 12-14, 20). The marsh grass \textit{Spartina alterniflora} is the major primary producer. Two extremes in growth form are found. Along the creek banks plants are >2 m in height and roots penetrate to more than 1 m; this is the tall \textit{Spartina} (TS) zone. At higher elevations the plants are <1 m in height and roots form a compact mat 15 to 20 cm deep; this is the short \textit{Spartina} (SS) zone. Beneath-ground biomass is several times higher here than in the TS zone.

The two regions differ in many properties. Interstitial water flow is rapid in the TS zone but very restricted in the SS zone; salinity in TS soil is about that of the overlying water, whereas it can climb to 40 to 50% in SS soils (12). Christian et al. (2) showed that the microbial content, as measured by adenosine 5'-triphosphate, followed the general distribution of roots in the two zones.

Sampling sites were chosen from both TS and SS zones (see R. Oshrain, M.S. thesis, University of Georgia, Athens, Ga., 1977, for details of location). Cores 7.5 cm in diameter and at least 30 cm in length were taken from each site and subsampled within 4 h, using plastic 10-ml syringes with the ends cut off. In some experiments the soil was screened through a 1-mm nylon mesh to remove macroorganic matter.

Arylsulfatase activity was determined by the method of Tabatabai and Brenner (18, 19); the only modification from their procedure was that the samples were not air dried before assay. Samples were incubated for 1 h at 37°C except

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where noted. The p-nitrophenol content in the filtrate was calculated by reference to a calibration graph plotted from the results obtained with standards containing 0, 10, 20, 30, 40, and 50 μg of p-nitrophenol. If the color intensity of the filtrate exceeded that of the 50 μg of p-nitrophenol standard, an aliquot of the filtrate was diluted with distilled water until the colorimeter reading fell within the limits of the calibration graph. This occurred infrequently; excess p-nitrophenyl sulfate was in the reaction medium (see time course results below).

Controls were run with each analysis to allow for color not derived from p-nitrophenol released by arylsulfatase activity. The procedure used was identical to the experimental technique, except that substrate was added after the 0.5 M CaCl₂ and 0.5 M NaOH (i.e., immediately before filtration of the soil suspension).

It is necessary to inhibit microbial growth and assimilation of enzymatic reaction products in any assay of soil enzyme activity. Toluene has been found to be an effective agent for inhibiting microbial activities during the determination of extracellular enzyme activities (9, 11, 17). Several workers have found that toluene increases the activity of some enzymes in soils (17, 18, 19). According to Skujins (17), toluene is used to unmask enzymes in assays of enzymatic activities; enzymes are not released by the toluene (i.e., cell walls are not ruptured), but rather, the microbial cell membranes become permeable to substrates and enzyme reaction products. Addition of toluene to the reaction mixture increased the enzyme activity by 20%; addition of 0.25, 0.50, or 1.0 ml gave identical results.

Two other methods of inhibiting microbial growth and product assimilation were used. Autoclaving (15 pounds [ca. 425 g] for 15 min) eliminated all activity, whereas addition of 0.01 M HgCl₂ decreased activity between 80 and 90% in both SS and TS soils.

The effect of incubation time on arylsulfatase activity was analyzed. Activity was linear with time for at least 4 h in both soil types. These results also indicate that the concentration of p-nitrophenyl sulfate used was not limiting.

Skujins (16) reported that a variety of terrestrial soil enzymes increased in activity up to about 60°C and were inactivated between 60 and 70°C. The effect of the incubation temperature on screened soil samples (0- to 15-cm pooled material) from both marsh areas is shown in Fig. 1. The maximum activity observed was at 60°C; inactivation began at 67°C.

Arylsulfatas isolated from soil bacteria have been reported to be active over a wide pH range (4), and Chandramahon et al. (1) found arylsulfatase activity from pH 4 to 10 in marine sediments. Dodgson et al. (3) showed that A. metalcaligenes arylsulfatase was active from pH 4 to 12, with maximum activity in the range pH 7.5 to 9.5. In both SS and TS soils arylsulfatase activity was suppressed almost completely at pH 4, showed slight activity at pH 4.5, and showed maximal activity from pH 5 to 9. (Activity above pH 9 was not measured.)

It was of some interest to determine the site of enzyme activity in the soil. Interstitial water, separated by centrifuging sediment for 10 min at 10,000 × g, contained no activity. This was true for TS and SS soils. Fresh active roots from the TS zone also showed no activity. SS zone roots were not examined because it was difficult to separate the growing roots from the massive mat of macroorganic material present. Although no activity was found in the interstitial water, this does not mean that the enzyme could not be extracellular. Skujins (17) noted that some extracellular enzymes require clay or soil particles for activity; they may also be bound to the particles.

In all of the physical characteristics, then, the arylsulfatase present in the salt marsh soil appears to be very similar to those described for terrestrial soils and mangrove and intertidal mud.

Depth profiles of arylsulfatase activity, from 0 to 25 cm, in three cores from the TS and SS zones are shown in Fig. 2. In the SS soil there
was a consistent, large decrease in activity with depth, whereas in TS soils the decline in activity was slight. These trends appear identical to microbial adenosine 5'-triphosphate distribution in the two zones (2) and lend some support to the idea that the enzyme is cell associated.

An attempt was made to obtain some kinetic data for the soil enzymes. The problems of such measurements have been discussed in detail by Skujins (16, 17). Substrate concentrations were varied from 0 to 30 × 10⁻³ M. Using the equation $K_m = \frac{1}{2} V_{max}$, the Michaelis constant for TS soil was 4.2 × 10⁻³ M and that for SS soil was 5.2 × 10⁻³ M. Tabatabai and Bremner (18) reported that the $K_m$ values varied from 1.37 to 5.69 × 10⁻³ M. Our values fall within their range. To our knowledge these are the only soil values in the literature.

There is some question whether the arylsulfatase was produced under anaerobic or aerobic conditions. Fitzgerald (5) noted that no authentic anaerobic arylsulfatase production has been reported. We do know that the salt marsh soils in general are highly anaerobic, based on their production of methane (10) and hydrogen sulfide (G. W. Skyring, R. L. Oshrain, and W. J. Wiebe, Geomicrobiol. J., in press). However, aerobic zones, associated with the rhizosphere of the *Spartina* plant, also occur throughout the sediment. Thus, we do not have direct evidence that the enzyme was produced under anaerobic conditions. However, the relatively high activity 20 to 25 cm deep in the TS soil suggests that anaerobic production is likely, since few roots are present at this depth (6).

The role of arylsulfatase in the salt marsh is puzzling. In bacterial pure cultures $SO_4^{2-}$, $SO_3^{-}$, or cysteine repress arylsulfatase synthesis (4, 15). In marine environments the activity persists in the presence of high concentrations of sulfate. We do not know, however, whether synthesis occurs under similar conditions or whether it occurs in sulfate-depleted microsites. The activity as measured by the $K_m$ was within the range reported for several soils (18). The presence and quantitative similarity in the abundance of arylsulfatase in a variety of environments suggest that it plays some important role in nature. Several authors (3-5, 8) have postulated that it is important for the release of inorganic sulfate. While this could be true also in the salt marsh, it seems unlikely due to high sulfate concentration in these soils. A more likely role here is the initiation of breakdown of arylsulfate esters for the microbial community.

This study was supported by National Science Foundation grant OCE 75-20842 A01.

**LITERATURE CITED**


