The role of sulfoacetaldehyde sulfo-lyase in the mineralization of isethionate by an environmental Acinetobacter isolate

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An environmental Acinetobacter isolate, strain ICD, utilized isethionate at concentrations up to at least 20 mM as carbon and energy source, with essentially quantitative sulfate accumulation. The initial step in isethionate metabolism is likely to be its oxidation to sulfoacetaldehyde since inducible sulfoacetaldehyde sulfo-lyase activity was demonstrated in isethionate-grown cells by in vitro assay and gel zymography; sulfoacetaldehyde itself did not induce the enzyme. Isethionate-grown cells of Acinetobacter sp. ICD, unlike those of most other C-S bond-cleaving strains described, also contained an inducible sulfite-oxidizing activity. The results provide further evidence that sulfoacetaldehyde sulfo-lyase plays a central role in the mineralization of biogenic sulfonates.

Keywords: isethionate, sulfoacetaldehyde sulfo-lyase, sulfite oxidase, sulfonate, Acinetobacter sp.

INTRODUCTION

Sulfonates are compounds which contain the R-CH\(_2\)-SO\(_2\)H moiety. They are distributed widely in the biosphere, both as a result of the commercial introduction of xenobiotic arylsulfonates as detergents, dyestuffs, etc., and also through the widespread occurrence of biogenic sulfonates, which are almost exclusively aliphatic. The environmental significance of the latter group is indicated by the fact that more than 40% of organic sulfur in the O1 and O2 horizons of 14 out of 18 forest soils examined by Autry & Fitzgerald (1990) was found in the C-S linkage. Similarly, 20–40% of organic sulfur in the uppermost layers of three marine sediments consisted of sulfonate-S (Vairavamurthy et al., 1994). Sulfonates, at an oxidation state of +4, are thus likely to be significant participants in biogeochemical sulfur cycling, intermediate between sulfate (oxidation state +6) and sulfide (oxidation state –2) (Seitz et al., 1993).

Extensive research on the biodegradation of xenobiotic sulfonates has been carried out. However, there is comparatively little information on the microbial metabolism of biogenic molecules containing the C-S bond (Urià-Nickelsen et al., 1993; Kertesz, 1996), and in particular on their utilization as carbon sources. The major naturally occurring sulfonates include sulfoquinovose (6-sulfo-6-deoxyglucose), the sulfur-containing moiety of plant sulfolipid; the vertebrate amino acid taurine (2-aminoethanesulfonate); coenzyme M (2-mercaptoethanesulfonate), which is found in methanogenic Archaea (White, 1985); and methanesulfonate, a major atmospheric photooxidation product of gaseous dimethyl sulfide derived from marine organic matter (Thompson et al., 1995).

The biogenic sulfonates also include isethionate (2-hydroxyethanesulfonate), originally identified as the major anion of the squid axoplasm (Hoskin, 1971) and subsequently shown to be present at up to millimolar concentrations in the tissues of higher animals, probably as a consequence of its formation from taurine by gut micro-organisms (Ikaheimo et al., 1982). More recently, it has been found at levels of up to 1-7% by weight in some marine red algae (Holst et al., 1994); its physiological function in plants is uncertain. We now report the mineralization of isethionate by an environmental Acinetobacter isolate, and an investigation of the degradative pathway involved.

METHODS

Growth of organism. Isolate ICD was obtained from the sediment of a polluted woodland pond (Laundromat Pond, approximately 0.5 km north of Summit Lake, WI, USA) in the course of a survey of the environmental distribution of sulfonate-metabolizing bacteria (King & Quinn, 1997), using
elective culture on medium containing 10 mM isethionate as sole carbon and energy source. The strain has subsequently been identified at the National Collections of Industrial and Marine Bacteria (Aberdeen, Scotland) as a species of *Acinetobacter*.

The isolate was maintained on a medium containing [g (1 tap water)⁻¹]: NH₄Cl, 1.0; KCl, 0.2; MgSO₄.7H₂O, 0.2; CaCl₂, 2H₂O, 0.001; ferric ammonium citrate, 0.001; yeast extract, 0.05; plus vitamin solution, 1.0 ml l⁻¹ (Difco) and trace element solution, 1.0 ml l⁻¹ (Krieg, 1981). The medium was sterilized by filtration. Filter-sterilized isethionate was added as a concentration of 50 mM, and sterile 100 mM potassium phosphate buffer (pH 7.2) to a final concentration of 5 mM. Flasks (500 ml) containing 60 ml inoculated medium were incubated at 27 °C on a rotary shaker (100 r.p.m.). For bulk culture of cells, a benchtop fermenter (working volume 6.5 l) was used. Growth of cultures was followed by measuring their optical density at 650 nm, and supernatants were assayed for sulfate (Sorbo, 1987) and sulfate (Johnston et al., 1975). Each growth experiment reported was carried out on at least three occasions.

**Chemicals.** Sulfoacetaldehyde was synthesized, as the bisulfite addition complex, in the Custom Synthesis and Process Development Research Centre, School of Chemistry, Queen's University Belfast, by the method of Kondo et al. (1971). Sulfoacetaldehyde was prepared from the bisulfite adduct as described by White (1988). A fresh sample was prepared daily. All other chemicals, of highest available purity, were obtained from Sigma/Aldrich.

**In vitro enzyme assays.** Cells grown on 50 mM isethionate as source of carbon and energy were harvested in the mid-exponential phase by centrifugation (12000 g for 15 min at 4 °C), washed twice in 50 mM phosphate buffer and disrupted ultrasonically at 16 kHz on ice for 4 min in the presence of 0.01% DTT (30 s sonication followed by 2 min cooling). The homogenate was centrifuged (25000 g for 30 min at 4 °C) and the supernatant retained as the crude cell-free extract. Protein concentrations were measured by the method of Bradford (1976). C–S bond cleavage activity was assayed at 30 °C in duplicate reaction mixtures containing 50 mM phosphate buffer (pH 7.3), 10 mM substrate (isethionate or sulfoacetaldehyde), 0.01% DTT and 5 mg cell-extract protein ml⁻¹. Corresponding 'no substrate' and 'no extract' controls were prepared, and reactions initiated by the addition of cell extract. After termination by addition of 0.1 vol. 50% (w/v) trichloroacetic acid and removal of precipitate by centrifugation, supernatants were assayed for sulfate (Sorbo, 1987), and for acetate using an enzyme-based Acetate Test Kit (Boehringer Mannheim) with a lower detection limit of 10 μg ml⁻¹. Sulfitic oxidase (EC 1.8.3.1) activity in cell extracts was assayed similarly, but with freshly prepared sodium sulfite (final concentration 10 mM) as substrate in the presence of 0.2% DTT; sulfite levels were determined in assay supernatants and in control assays lacking cell extract as a measure of abiotic sulfite oxidation. Cytochrome c reductase activity was determined in triplicate assays containing 50 mM Tris/HCl buffer (pH 7.1), 0.15 mM cytochrome c (horse heart), 2 mM freshly prepared sodium sulfite and 2 mg cell-extract protein ml⁻¹. Assays were started by the addition of sulfite and the absorbance at 550 nm recorded at intervals of 15 s for 5 min.

**Gel zymography.** Sulfoacetaldehyde sulfo-lyase (EC 4.4.1.12) activity was demonstrated in extracts of *Acinetobacter* sp. ICD by a modification of the method of Kondo & Ishimoto (1975). Aliquots (12.5 μl) of crude extract (5 mg protein ml⁻¹) from cells grown on isethionate as carbon and energy source were loaded into the wells of duplicate precast 8–16% Tris/glycine gels (Novex). Following electrophoresis (125 V for 3 h) one gel was stained with Coomassie blue; the duplicate gel was stained for sulfoacetaldehyde sulfo-lyase activity by bathing for 1 h at 30 °C in darkness in an assay mixture containing 10 mM sulfooacetaldehyde, 2 mM MgCl₂, 50 mM phosphate buffer (pH 7.2) and 0.1 mM thiamin pyrophosphate. The gel was then rinsed twice with distilled water, immersed in a 0.3% (w/v) solution of malachite green and incubated as above for 2 h. It was photographed immediately after staining.

**Resting cell assays.** The regulation of C–S bond cleavage and sulfite-oxidizing activities in *Acinetobacter* sp. ICD was determined by resting cell assays. Cells pre-grown on 50 mM acetate as carbon and energy source were harvested, washed and resuspended in basal medium containing either isethionate, sulfooacetaldehyde or sodium sulfite (all 10 mM). After incubation on a rotary shaker at 27 °C for 7 h (4 h for suspensions containing sodium sulfite), cells were harvested, washed and resuspended in assay mixtures (7–0 ml) containing 10 mM substrate (isethionate, sulfooacetaldehyde or sodium sulfite), 140 mg whole cells, 50 mM phosphate buffer (pH 7.2) and 0.02% DTT (in the case of assays containing sodium sulfite). Assays were carried out in 30 ml vials incubated with gentle shaking at 27 °C, and were initiated by the addition of substrate to preincubated assay mixtures; aliquots were removed hourly and the reactions stopped by centrifugation prior to the determination of sulfite in supernatants. Control assays, from which either substrate or cell suspension had been omitted, were carried out in parallel. All substrates were stable under the assay conditions used.

**RESULTS**

**Growth of organism**

Growth of *Acinetobacter* sp. ICD on 10 mM isethionate as carbon and energy source, and the concomitant accumulation of sulfate and transient sulfite release into

![Fig. 1. Growth of Acinetobacter sp. ICD (●), and release of sulfite (■) and accumulation of sulfate (▼) in a medium containing 10 mM isethionate as carbon and energy source.](image-url)
Isethionate metabolism by *Acinetobacter* sp.

**Fig. 2.** Cell yields of *Acinetobacter* sp. ICD (●) and supernatant sulfate levels (▼) following growth of the organism on a medium containing varying concentrations of isethionate as carbon and energy source.

**Fig. 3.** Formation of acetate from isethionate (▼) and from sulfoacetaldehyde (●) by extract of isethionate-grown cells of *Acinetobacter* sp. ICD.

**Fig. 4.** Formation of sulfate from sulfite by extract of isethionate-grown cells of *Acinetobacter* sp. ICD.

sulfonate, naphthalene-1-sulfonate, sulfanilate, *p*-toluenesulfonate.

**In vitro assay of isethionate metabolism**

Formation of acetate from both isethionate and sulfoacetaldehyde by extracts of isethionate-grown cells of *Acinetobacter* sp. ICD was observed; it was proportional to amounts of protein used in the assay (Fig. 3). Extracts also contained a sulfite-oxidizing activity (Fig. 4) with a pH optimum of 8.0 and a broad temperature optimum between 24 and 37 °C (results not shown). The values for sulfite-oxidizing activity presented in Fig. 4 have been corrected to take account of rates of abiotic sulfite oxidation observed in 'no-extract' controls – these were less than 5% of those obtained in the presence of cell extract, while in further controls lacking both extract and DTT rates of sulfite oxidation were approximately 14% of those obtained under assay conditions. Cell extract was also found to reduce cytochrome c in the presence of sulfite; control assays lacking sulfite or cell extract, or containing extract from acetate-grown cells, did not show such activity.

**Demonstration of sulfoacetaldehyde sulfo-lyase activity by gel zymography**

To confirm that the initial product of cleavage of the C–S bond of sulfoacetaldehyde was sulfite, a gel containing extract from isethionate-grown cells of *Acinetobacter* sp. ICD was stained for sulfoacetaldehyde sulfo-lyase activity. A discrete band, produced through the formation of a colourless addition product of sulfite and the dye (Kondo & Ishimoto, 1975) was observed (result not shown). It corresponded to a major protein band with a molecular mass of about 220 kDa, and

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the culture supernatant, are shown in Fig. 1. An uninoculated control experiment showed that sulfate was not spontaneously released from isethionate over the incubation period. Final cell yields were directly proportional to the concentration of isethionate supplied, up to at least 20 mM (Fig. 2), and growth of the isolate was accompanied by essentially equimolar accumulation of isethionate sulfur as sulfate. The isolate also mineralized taurine and sulfoacetate, but none of the following aliphatic and aromatic sulfonates tested: methanesulfonate, ethanesulfonate, hydroxymethylsulfonate, sulfoacetaldehyde, sulfosuccinate, benzene-
Table 1. C-S bond cleavage and sulfite-oxidizing activities in resting cells of Acinetobacter sp. ICD pregrown on acetate, following exposure to several potential inducers

<table>
<thead>
<tr>
<th>Potential inducer</th>
<th>Assay substrate</th>
<th>Activity [nmol sulfate accumulated h^-1 (mg cells)^{-1}]^*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Isethionate</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Sulfoacetaldehyde</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Sulfite</td>
<td>0.0</td>
</tr>
<tr>
<td>Isethionate</td>
<td>Isethionate</td>
<td>1020±5.6</td>
</tr>
<tr>
<td></td>
<td>Sulfoacetaldehyde</td>
<td>933±3.3</td>
</tr>
<tr>
<td></td>
<td>Sulfite</td>
<td>85.0±1.0</td>
</tr>
<tr>
<td>Sulfoacetaldehyde</td>
<td>Isethionate</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
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<td>0.0</td>
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<tr>
<td></td>
<td>Sulfite</td>
<td>0.0</td>
</tr>
<tr>
<td>Sulfite</td>
<td>Isethionate</td>
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</tr>
<tr>
<td></td>
<td>Sulfoacetaldehyde</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>Sulfite</td>
<td>54.5±2.0</td>
</tr>
</tbody>
</table>

^* Each value is the mean of triplicate assays ± standard error of the mean.

migrated in a manner similar to an active band detected in an extract of a taurine-grown environmental isolate of Pseudomonas cepacia which served as a control; the role of sulfoacetaldehyde sulfo-lyase in the bacterial metabolism of taurine is well-established (Kondo & Ishimoto, 1972; Shimamoto & Berk, 1980).

In vivo assay of enzyme activities in Acinetobacter sp. ICD

A study, using resting-cell suspensions, of the regulation of the enzymes believed to be involved in isethionate metabolism (Table 1) showed that cleavage of the C-S bond of both isethionate and sulfoacetaldehyde was inducible by isethionate; sulfite-oxidizing activity was induced both in the presence of isethionate and by sulfite itself. None of these activities was present in acetategrown cells, nor in cells incubated in the presence of sulfoacetaldehyde. The latter observation, and the inability of sulfoacetaldehyde to support growth of the isolate, cannot be explained by sulfoacetaldehyde toxicity since cells remained viable after exposure to this compound at the concentrations employed.

DISCUSSION

This is a report of the later steps in the bacterial mineralization of isethionate; speculation on the nature of the initial catabolic step is necessarily tentative since we did not isolate its presumed product, sulfoacetaldehyde. The enzyme responsible may, however, be analogous to the taurine dehydrogenase purified from an unidentified Gram-negative isolate by Kondo et al. (1973) which initiated a degradative pathway via sulfoacetaldehyde. In the only previous report of the microbial metabolism of isethionate, Kondo et al. (1977) demonstrated the FAD-dependent oxidation of isethionate to sulfoacetaldehyde by extracts of the same taurine-degrading strain, although a different enzyme was believed to be responsible.

Our finding of broadly similar rates of sulfate production from isethionate, sulfoacetaldehyde and sulfite in resting cell suspensions of Acinetobacter sp. ICD after exposure to isethionate (Table 1) appears to provide evidence of the physiological significance of sulfoacetaldehyde sulfo-lyase and sulfite oxidase activities in isethionate mineralization by this organism. The presence in isethionate-grown cells of an inducible sulfite-oxidizing activity is of note; although sulfite has been identified as the primary product of microbial cleavage of the C-S bond of both alkyl- and arylsulfonates, and has been shown by some workers (Kondo et al., 1971; Lee & Clark, 1993) to accumulate in both culture supernatants and active cell extracts, its oxidation to sulfate is reported in other studies (e.g. Locher et al., 1989; Feigel & Knackmuss, 1993). In the latter cases oxidation has usually been ascribed to abiotic factors, although Quick et al. (1994) have suggested the involvement of sulfite oxidase in the formation of sulfate from the sulfite produced through cleavage of the C-S bond of sulfosuccinate by resting cells of Pseudomonas sp. BS1. The sulfite-oxidizing activity in Acinetobacter sp. ICD may be necessary to minimize sulfite toxicity to the cell through, for example, interaction with thiol groups; it is similar to the majority of sulfite oxidases described from the thiobacilli, which derive energy from the oxidation of sulfur via a sulfite intermediate (Lyric & Suzuki, 1969; Southernland & Toghril, 1983), in that cytochrome c may serve as the electron acceptor.

The role of sulfoacetaldehyde sulfo-lyase in the mineralization of taurine has already been recognized (Kondo & Ishimoto, 1972; Shimamoto & Berk, 1980), while its involvement in the microbial metabolism of sulfoacetate, an intermediate in plant sulfolipid biodegradation (Martelli & Benson, 1964), has recently been demonstrated in this laboratory (King, 1996). The present report thus strengthens the suggestion that this poorly studied enzyme may play a central part in the global cycling of biogenic sulfonate-S, particularly in the light of the recent identification of isethionate as a constituent of some marine plants (Holst et al., 1994). Intriguingly, the enzyme is not induced by its substrate, sulfoacetaldehyde, in resting cell suspensions of either Acinetobacter sp. ICD (Table 1), or of two sulfoacetate-mineralizing bacterial strains isolated in this laboratory (King, 1996); the inducers are, respectively, isethionate and sulfocate. By analogy, Shimamoto & Berk (1980) have obtained evidence from a mutant strain of the taurine degrader Pseudomonas aeruginosa TAU-5 to indicate that sulfoacetaldehyde sulfo-lyase is induced (co-ordinately with taurine:pyruvate transaminase) by taurine, rather than by sulfoacetaldehyde.
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REFERENCES


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