Review

Microbial desulfonation

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Abstract

Organosulfonates are widespread compounds, be they natural products of low or high molecular weight, or xenobiotics. Many commonly found compounds are subject to desulfonation, even if it is not certain whether all the corresponding enzymes are widely expressed in nature. Sulfonates require transport systems to cross the cell membrane, but few physiological data and no biochemical data on this topic are available, though the sequences of some of the appropriate genes are known. Desulfonative enzymes in aerobic bacteria are generally regulated by induction, if the sulfonate is serving as a carbon and energy source, or by a global network for sulfur scavenging (sulfate starvation induced (SSI) stimulon) if the sulfonate is serving as a source of sulfur. It is unclear whether an SSI regulation is found in anaerobes. The anaerobic bacteria examined can express the degradative enzymes constitutively, if the sulfonate is being utilized as a carbon source, but enzyme induction has also been observed. At least three general mechanisms of desulfonation are recognisable or postulated in the aerobic catabolism of sulfonates: (1) activate the carbon neighboring the C–SO₃ bond and release of sulfite assisted by a thiamine pyrophosphate cofactor; (2) destabilize the C–SO₃ bond by addition of an oxygen atom to the same carbon, usually directly by oxygenation, and loss of the good leaving group, sulfite; (3) an unidentified, formally reductive reaction. Under SSIS control, different variants of mechanism (2) can be seen. Catabolism of sulfonates by anaerobes was discovered recently, and the degradation of taurine involves mechanism (1). When anaerobes assimilate sulfonate sulfur, there is one common, unknown mechanism to desulfonate the inert aromatic compounds and another to desulfonate inert aliphatic compounds; taurine seems to be desulfonated by mechanism (1).

Keywords: Fermentation; Oxidation; Reduction; Oxygenation; Hydrolysis; Sulfonate; Novel fermentation

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1. Introduction

Sulfonated organic compounds, here those with a C SO₃ moiety, influence our picture of the world around us, whether the words are used figuratively or literally. The natural product taurine (Fig. 1) has roles in vision, neural function and the digestive tract, amongst others [1]. Many dyestuffs (Fig. 2), which add color to e.g. inks, clothes or foodstuffs, are sulfonated. As there is now little voluble complaint about sulfonated compounds in the environment, especially after the development of sensitive analytical methods (e.g. [2–4]), it seems likely that extensive degradation occurs, despite the poor biodegradability of at least some sulfonates (e.g. [3,5–8]). This review explores these degradative phenomena, which, over the years, writers with very different backgrounds have approached (e.g. [9–18]). Recent advances, however, allow a newly rationalized overview of the field.

2. Organosulfonates occurring naturally, their stability and their biosynthesis

Strictly, organosulfonates are those compounds containing the SO₃ moiety, so sulfate esters are O sulfonates, which have a very wide occurrence

naturally [19]. The O sulfonates are usually [19], but not always [20], cleaved hydrolytically by enzymes in EC 3.1.6.- (sulfuric acid hydrolases). N Sulfonates are also known (e.g. N sulfoglucosamine, representing heparin) and appear to be subject to facile hydrolysis by enzymes in EC 3.10.1.- (acting on sulfur nitrogen bonds). The C sulfonates are thermodynamically much more stable [21] and are not subject to chemical hydrolysis (taken as general knowledge by e.g. [22]). One can thus anticipate a different biochemistry in the degradation of these C sulfonates, compared with the N and O sulfonates. The sulfur atom in C sulfonates would appear to be in the 5+ oxidation state ([23,24]; cf. [18]).

Until recently, the naturally occurring sulfonates were considered to be relatively few in number, though often in important functions (e.g. taurine, coenzyme M, the plant sulfolipid, sulfolactate and methanesulfonate in Fig. 1). It is now clear that organic matter in soils (e.g. [25]) and in marine sediments [26] is sulfonated, and determinations of fractions from river samples by pyrolysis-mass spectrometry indicate this to be humic material [27]. As humus is subject to biophysical and biochemical alteration [28], including a relatively rapid flux through the sulfonate pool [29,30], one can fairly presume that the number of sulfonated compounds occurring naturally is very high. Indeed, biotransfor-

Fig. 1. Some naturally occurring organosulfonates. Taurine, discovered in 1836, indicates how long biologists have worked with sulfonates [1]. The sulfonate of methanogens, coenzyme M, is a much more recent discovery (cf. [161]), while the importance of methanesulfonate in the sulfur cycle has only now been recognized [69]. Cysteate was first observed in biological systems as a weathering product from cysteine in wool [162], but it is now better known as an intermediate in one pathway of taurine formation [1] or as a precursor [163] of the major spore component, sulfolactate [164]. Isethionate, initially found as a major anion in squid nerve [22], is now known to have a wider distribution [165]. Sulfoacetate is a degradative intermediate [166] from sulfoquinovose in the plant sulfolipid in the thylakoid membrane [40]. Bacterial and diatomal sulfolipids (not shown) are also known [167,168]. An undefined, but presumably aliphatic compound is found in high concentrations in plasma cells from heart samples of *Ascidia ceratodes* [23]. In contrast to these aliphatic compounds, only one defined aromatic sulfonate seems to be known, aeruginosin B [169]. Undefined sulfonates in soils, largely in humic materials, and in marine sediments seem to be widespread [25–27]. Humic material is characterized by its heterogeneity [28], so, as it presumably represents a major reservoir of natural organosulfonate, it is presented in the figure, but it cannot be given a structure.

mation of these numerous organosulfonates occurs naturally [30], so perhaps one should not be surprised when xenobiotic compounds are desulfonated.

There would appear to be two general routes to generate C sulfonate bonds. One route with many variations involves oxygenation of cysteine sulfur to the sulfinate (EC 1.13.11.20), decarboxylation (EC 4.1.1.29) and a subsequent oxidation (e.g. EC 1.8.1.3) to yield taurine and a large number of its derivatives [1]. Alternatively there is addition of sulfite at a C=C double bond as in the synthesis of coenzyme M [31], a variant of which is probably involved in the synthesis of the sulfoquinovose moiety of the plant sulfolipid [32].

3. Xenobiotic sulfonates in the environment

The detergents used to clean clothes, dishes, or floors, usually contain sulfonated compounds, whether as surfactants [17], hydrotropic agents (*p*-toluenesulfonate, Fig. 2) or optical brighteners [8]. Some sulfonates are used in molar excess in dye-stuff processing (*m*-nitrobenzenesulfonate, Fig. 2). Yet other sulfonates (naphthalenesulfonates, Fig. 2) enter the environment in waste streams: chemical synthesis of sulfonates typically give yields of 80–90% with many sulfonates as by-products [33], and many of these compounds can be detected in e.g. rivers [3] or seepage from waste dumps [34,35]. In

addition, it is clear that some sulfonates in the environment (e.g. 'alachlor-ethanesulfonate', Fig. 2) are generated biologically from non-sulfonated xenobiotic compounds [36]. Further sulfonates or derivatives, e.g. saccharine (Fig. 2) enter the environment via foodstuffs.

An interest in desulfonation reactions involving xenobiotic compounds has been apparent since about 1950 (cf. [37]), though thorough proof of a desulfonation reaction was to wait some 15 years, till arguments about the degradation of branched-chain alkylbenzenesulfonate surfactants forced direct analyses to be done (cf. [38,39]). Indeed, the non-biodegradability of the first generation of benzene-sulfonate surfactants (e.g. [10]) led at least one author to consider the plant sulfolipid as a replacement [40]. Another 25 years went past before the first enzyme desulfonating a xenobiotic compound was purified [41], but the pace of development and the level of understanding have increased markedly in the meantime.

4. Formalism in the degradation of organosulfonates

There appear to be no reports of the accumulation of naturally occurring sulfonates under anoxic conditions, but until recently it was believed that arene-sulfonates were degraded under oxic conditions only [12,42], and desulfonation reactions in anaerobes were ignored. The rapidly growing understanding of anaerobic desulfonation reactions involving aliphatic and aromatic compounds, and their abundance, is now illustrated in Sections 9 and 10.

It is helpful to consider critically the role of the organosulfonate in the metabolism of the organism under study. In aerobes, the sulfonate can be found as a carbon and energy source only, concomitant with excretion of the sulfonate moiety. As the organisms do not grow in the strict absence of sulfate in the growth medium [37,43], it is presumed that the catabolic activities (uptake of arenesulfonate, desulfonation and excretion of the sulfonate moiety) are independent of the supply of sulfate for growth, i.e. of a sulfate transport system. In addition, the regulation involved is largely induction of a catabolic pathway (Section 6). Alternatively, the sulfonate is used as a source of sulfur for growth with concom-

itant excretion of the carbon moiety [44-48]; the regulation is a global system for scavenging sulfur (Section 8). In anaerobes, this strict formalism is perhaps better known and the different metabolic types are easily recognized; sulfonates as sulfur sources (Section 10), sulfonates as electron sinks in respiration, sulfonates as electron (and carbon) sources for e.g. nitrate respiration, and sulfonates as substrates in fermentations (Section 9). These processes will be examined in turn.

One process of almost (cf. Section 7) universal relevance for the metabolism of organosulfonates is the transport of the compound across the cell membrane. It has been largely ignored.

5. Membrane transport

Organic sulfonic acids are strong acids whose pK values, if uninfluenced by other substituents, are probably negative [49]. This means that the sulfonate group is charged at physiological pH values, with the consequence that transport is required to bring the substrate into contact with the cytoplasmic enzymes involved in its degradation (cf. Section 7 for the reverse situation).

Biedlingmaier and Schmidt detected transport systems for e.g. ethanesulfonate or taurine in *Chlorella fusca* [50,51] and in cyanobacteria [52] and reported on their kinetics. Thurnheer et al. [53] offered indirect evidence for transport processes in that the cell membrane of *Alcaligenes* sp. strain O-1 is selectively permeable to isomeric arenesulfonates. Direct transport assays were done by Locher et al. [54] with *Comamonas testosteroni* T-2 and *p*-toluenesulfonate. They presented evidence for an inducible, secondary proton symport system, which was not sufficiently stable to allow biochemical work to be done.

Genetic evidence for a transport system for taurine is available in *Escherichia coli* MC4100 [55]. The genes are located in an operon termed *tauABCD* (accession number D85613; [55]), and TauABC comprise an ABC-type transporter of unknown substrate specificity (see also Section 8). Genes for a putative transport system for methanesulfonate (cf. [56]) are under study, and manuscripts are pending (J.C. Murrell, personal communication).

Fig. 2. A few xenobiotic sulfonates. Many of these compounds are very simple, and, as in the case of the naphthalenesulfonate, representative for a large number of substituted compounds. The azo dyestuff, tartrazine, represents a widely used class of dyestuffs. 'Alachlor-ethanesulfonate' represents a class of compounds formed from chloroacetanilide herbicides, alachlor in this case [36]. More information on xenobiotic sulfonates can be found elsewhere (e.g. [170]).

6. Aerobes dissimilating sulfonates as sources of carbon and energy

Whereas a wide range of compounds has been shown to serve as a sole source of carbon and energy for the growth of aerobic bacteria (e.g. [6]), be this in pure culture or in (model) sewage, there is much less information available on the reactions involved, especially on the desulfonations. One can now be sure that there are hydrolytic desulfonations, desulfonations inherent in monooxygenations and in dioxygenations, and a formally reductive desulfonation is postulated.

6.1. Hydrolytic desulfonation of aliphatic compounds

The first desulfonation to be characterized was that of sulfoacetaldehyde, which is hydrolyzed quantitatively to acetate and sulfite by a thiamine pyrophosphate (TPP)-coupled lyase (EC 4.4.1.12) from several bacteria (cf. Fig. 6) [57,58]. It is possible to hypothesize an enol-adduct of the TPP whose formation is aided by loss of the sulfite anion, a good leaving group; addition of water across the double

bond generates the acetate and regenerates the TPP [57]. The acetate is oxidized to CO_2 and water, with anaplerotic reactions allowing channelling of intermediates for biosynthesis [59].

The first known source of sulfoacetaldehyde was taurine, but different organisms use transaminations to different amino-group acceptors (e.g. EC 2.6.1.55) (cf. [60]) or oxidation (EC 1.4.99.2) [61] for the transformation. Recent work shows that the catabolism of isethionate [62]; cf. [63]) and possibly sulfoacetate converge at sulfoacetaldehyde [64], but it is unclear how the sulfoacetate is reduced to the aldehyde. The enzymes involved in these reactions are found in a wide range of genera including *Pseudomonas* [60], *Acinetobacter* [62], *Aureobacterium* and *Comamonas* [64].

It seems possible that the catabolism of cysteate or sulfolactate could be channelled through taurine [65,66], but some preliminary enzyme work with cysteate leads one to suspect the presence of a novel desulfonation [67].

The only other known hydrolytic desulfonation is that involved with 4-sulfocatechol, which will be treated with the other aromatic compounds in Section 6.3.

6.2. Monooxygenases and desulfonation of aliphatic compounds

The monooxygenation of *n*-alkane-1-sulfonates, a family of surfactants, was detected many years ago [68], but it is only recently that a monooxygenation reaction, from *Methylosulfonomonas methylovora*, was characterized. The substrate is the natural product methanesulfonate [69,70] and the monooxygenase system involved [56,71,72] belongs to the mononuclear iron systems better known in the bacterial oxygenation of aromatic compounds [73]. These enzymes contain an electron transport chain, that collects electron pairs from NADH on a flavin, which delivers single electrons via [2Fe 2S] ferredoxin and/or [2Fe 2S] Rieske centers to the mononuclear iron at the reactive center (cf. [74]) where the reduced iron activates the oxygen. Activated oxygen presumably attacks the carbon atom to yield the unstable hydroxy-methanesulfonate, which spontaneously loses the good leaving group sulfite to form formaldehyde, a typical intermediate in C₁ metabolism [75]. The three-component system will oxygenate ethane- and propanesulfonates, but does not accept any further substitution on the alkane.

The destabilization of the otherwise inert C SO₃ bond by the introduction of a second heteroatom to the carbon atom, which causes spontaneous loss of sulfite, is a mechanism that will be observed many times in this review. It is presumed to be the basis of the formation of oxaloacetate from sulfosuccinate in *Pseudomonas* sp. strain B51 [76], though the nature of the monooxygenase here is still unknown. Another monooxygenation is presumed to be involved in the degradation of naphthalene 2,6-disulfonate [77].

6.3. Dioxygenases and desulfonation, including hydrolytic and putative reductive reactions

6.3.1. Setting the scene for desulfonation of the aromatic ring, largely with naphthalenesulfonates

The bulk of research on desulfonation has been done with aromatic compounds. Initial work reviewed by Cain [9] established the idea that dioxygenases destabilized the inert C sulfonate bond and that the sulfonate moiety was released as sulfite [38,78], thus correcting some earlier claims that sul-

fate was the direct product of desulfonation. Knackmuss' group gave more experimental support for this idea with a naphthalenesulfonate model [79]. Work with amino- and hydroxynaphthalene-2-sulfonates [80] led to *Sphingomonas* sp. strain BN6, but despite much work (e.g. [81]), this desulfonation has also not been examined directly because of the inactivity of the oxygenase in cell-free extracts. Indirect evidence on the nature of the oxygenase comes from an unpublished DNA sequence (accession number U65001) located adjacent to the partial DNA sequence of a putative 1,2-dihydroxynaphthalene dioxygenase (the next enzyme in the degradative pathway). A comparison of the deduced protein sequence of the ferredoxin component shows high sequence similarities with the ferredoxin components of naphthalene dioxygenase and of benzene dioxygenase.

Those working with the mononuclear iron oxygenase systems are used to characterize them into classes I, II and III, and to distinguish them from the diiron systems [82–84].

6.3.2. The benzenesulfonate/*p*-toluenesulfonate dioxygenase system

p-Toluenesulfonate (TS) has turned out to be a more rewarding substrate as regards elucidation of desulfonation reactions, and three different pathways for its degradation have been discovered or proposed (Fig. 3). The inducible dioxygenase which causes ring desulfonation has been separated in extracts of *Alcaligenes* sp. strain O-1. The reaction catalyzed is:

The enzyme is probably a three-component system that is also benzenesulfonate dioxygenase (desulfonating) [85]. This desulfonative enzyme has an incompletely explored, broad substrate range which involves deaminating *o*-aminobenzenesulfonate [85,86]. The general phenomenon of spontaneous reactions following oxygenation of the ring is large, and turns out to be widespread in nature [85,86]. In contrast to the broad substrate range of the enzyme, however, induction of the enzyme in strain O-1 is very specific [85].

Fig. 3. Three pathways for the catabolism of *p*-toluenesulfonate. The uppermost pathway was established in *Alcaligenes* sp. strain O-1 [85], the middle pathway was established in *C. testosteroni* T-2 [41,43,90] and the tentative third pathway is derived from data with a 'pseudomonad' [38,100]. The desulfonation catalyzed by PsbAC (middle pathway) is analogous to that in the uppermost pathway (see reaction in brackets), but, to simplify the figure, it is not shown.

In bacterial nutrition, the combination of utilization of TS and benzenesulfonate is widespread [37,87–89], so it is hypothesized that an enzyme system analogous to the TS-dioxygenase system in *Alcaligenes* sp. strain O-1 is widespread. Balashov et al. [88,89] find the enzyme(s) to be plasmid-encoded.

6.3.3. The degradation of *p*-toluenesulfonate via the *p*-sulfobenzoate dioxygenase system

The degradation of TS in *Comamonas testosteroni* T-2 is currently the best understood desulfonative pathway, though there are still significant gaps in our knowledge, especially in the biochemistry and genetics of the transport system (Section 5). The initial attack (Fig. 3) is monooxygenation of the methyl side chain by a two-component monooxygenase system [90,91] followed by two characterized dehydro-

genases which yield *p*-sulfobenzoate (PSB). PSB is desulfonated, as predicted above, via dioxygenation by a two-component dioxygenase involving reductase C and oxygenase A (EC 1.14.12.8; a class IA system) and spontaneous loss of the leaving group, sulfite, to yield protocatechuate [41,92]:

Reaction stoichiometry and dioxygenation have been established [41,43], and the identical oxygenase component is found in two independent isolates [92].

Expression of enzyme activity allowed groups of co-regulated genes to be determined [93]. The genes

encoding the enzymes for the degradation of TS via PSB in *C. testosteroni* T-2 and of PSB in *C. testosteroni* PSB-4 are located on two conjugative megaplasmids designated pTSA and pPSB, respectively [94]; cf. [13]). The genes encoding degradation of protocatechuate are chromosomal in both organisms. Both plasmids were estimated at 85 kbp, but an expanded data set indicates that pTSA is about 73 kbp [95]. pTSA belongs to the incompatibility group IncP1 β . It is readily lost under non-selective conditions, giving rise to mutant TER-1, which cannot grow with TS and was very useful for conjugation experiments to explore the catabolic genes [94].

The four genes (*tsaMBCD*) encoding the enzymes which convert TS to PSB comprise an operon, the *tsa* operon, that corresponds to regulatory unit R1 (Fig. 3) [93,94], putatively with the divergently transcribed, LysR-type regulator gene, *tsaR* (accession number U32622) [91]. The intergenic region between *tsaR* and *tsaMBCD* shows the characteristic features of LysR-type regulated systems [96] and a putative sigma⁷⁰ housekeeping promoter for *tsaMBCD* is present [91]. Many newly isolated bacteria, selected to utilize TS, contain the *tsa* operon (shown by PCR and partial DNA sequence) and show the same physiological properties as *C. testosteroni* T-2, namely the transient excretion of the intermediates *p*-sulfobenzylalcohol and PSB during growth with TS [95]. This operon, too, seems to be widespread.

A preliminary sequence [97] is available for the putative *psb* operon, which corresponds to regulatory group R3 (Fig. 3) [93]. The operon structure in strain PSB-4, *psbAXYZ*, is located between two copies of the 3.2-kbp insertion element IS1071 (Accession number M65135), and possibly represents

a class II transposon (cf. [98]), as predicted [94]. A transposition event is observed in conjugation experiments involving strain PSB-4 (donor) and a plasmid-free, *psb* recipient. The *psb* genes transpose to the chromosome with concomitant loss of plasmid pPSB [94]. Currently, the composition of operon R3 is speculative, with only *psbA* (the oxygenase component) firmly attributed and *psbC* (the reductase component) tentatively ascribed; the functions of the gene products of *psbXYZ*, open reading frames, are still unknown.

The degradation of PSB in *C. testosteroni* T-2 was difficult to study for several years, because functional reductase C was no longer found, and its activity was complemented by reductase B of the toluenesulfonate methyl-monooxygenase (TsaB in Fig. 3). It was proposed that a mutation in the putative *psb* operon had occurred [92,94]. A culture of strain T-2 has since been recovered that was stored prior to the putative mutational event, and it apparently also encodes the *psbAXYZ* structure found in strain PSB-4, whereas *psbXYZ* seem to be missing from *C. testosteroni* T-2 (DSM 6577) [97], thus confirming the hypothesis on a mutation. PSB degradation is ready and widespread [37,88,99], so one wonders whether the conjugative plasmids and the transposition observed represent the transmission of one operon, or whether other degradative pathways remain to be found. The nature and the location of the transport system, which differs from that for TS [54], is another open question.

6.3.4. The putative degradation of *p*-toluenesulfonate via a reductive reaction

The third pathway for the degradation of TS (Fig. 3) is generated from the initial identification of

Fig. 4. The degradative pathway of orthonilate (2AS) in *Alcaligenes* sp. strain O-1. The oxygenation of orthonilate to generate 3-sulfocatechol (3SC) involves an intermediate analogous to that in the upper line of Fig. 3; for simplicity, it is not shown. The product from oxygenation (and subsequent spontaneous hydrolysis) of 3-sulfocatechol is shown as the relaxed form (Z,E) of 2-hydroxymuconate (2HM). In fact all isomers can be found, but the most common (99%) is shown. This stresses the importance of spontaneous reactions in the biochemistry of degradation of aromatic compounds in general, whereas the emphasis here is on the desulfonation reaction [85,86].

3-methylcatechol [100] and the subsequent identification of sulfite as the primary fate of the sulfonate moiety [38]. Thus formulated, the pathway represents a desulfonation that is formally reductive, even if nothing about its nature is known. The validity of the hypothesis depends on the quality of the paper chromatographic methodology used to identify the organic intermediate. The hypothesis would be stronger, if another isolation of the organism were achieved.

6.3.5. The desulfonation of *o*-aminobenzenesulfonate during meta cleavage

The widespread ring dioxygenation with the accompanying spontaneous desulfonation and the reductive desulfonation both occur prior to ring cleavage. The degradative pathway for *o*-aminobenzenesulfonate (orphanilate) in *Alcaligenes* sp. strain O-1 turns out to involve a multi-component dioxygenase system which causes stoichiometric deamination to 3-sulfocatechol [85,86,101] (Fig. 4). The latter is a novel substrate for ring cleavage, and the spontaneous desulfonation is a consequence of the *meta* ring cleavage enzyme, which is co-induced with the ring-activating dioxygenase system.

The pathway thus contains a dioxygenation by a putative class IB enzyme in EC 1.14.12.-, and the reaction of this multi-component enzyme leads to the spontaneous loss of ammonia (Fig. 4); if the enzyme is supplied with TS, rapid dioxygenation and desulfonation occur [53,85]. The desulfonation of 3-sulfocatechol is caused by formation of the sulfo-aldehyde (Fig. 4), which is spontaneously hydrolyzed, probably distant from the enzyme active site [85]. So this desulfonation is brought about by an enzyme in EC 1.13.11.-, and it can equally well catalyze a standard *meta* cleavage of catechol [85]. As with the TS dioxygenase (desulfonating) in this organism (Section 6.3.2 and Fig. 3), the broad specificity of the enzymes is coupled to a very tight regulation of enzyme induction [85].

The degradation of orphanilate is encoded on a conjugative, IncP9 mega-plasmid, pSAH (172 kbp) in *Alcaligenes* sp. strain O-1 [102,103]. Intra- and interspecies matings to a plasmid-cured strain of *Alcaligenes* sp. O-1 and to *Pseudomonas putida* PaW130, respectively, are possible. The transconjugant of strain PaW130 acquires the ability to grow

Fig. 5. Convergence of catabolic pathways for arenesulfonates at 4-sulfocatechol (4SC) and the *ortho* cleavage pathway established for that compound. None of the reactions leading to 4-sulfocatechol (from 2-(4-sulfophenyl)butyrate (SPB), sulfanilate (4AS) and benzene-1,3-disulfonate (BDS)) has been characterized [106–108], but the pathway in *Hydrogenophaga palleronii* S1 and *Agrobacterium radiobacter* S2 has been thoroughly elucidated [106].

with orthanilate, indicating that all the required, pSAH-encoded genes were expressed.

6.3.6. The hydrolytic desulfonation of *p*-aminobenzenesulfonate after ring cleavage

The degradative pathway for *p*-aminobenzenesulfonate illustrates yet another novel substrate for ring cleavage, 4-sulfocatechol, that is subject to *ortho* cleavage during which the sulfonate is retained [104] (Fig. 5). Formation of the lactone generates a relatively stable compound which includes a carbon atom carrying both an oxygen and a sulfono substituent; the previously stable C sulfonate bond is now subject to hydrolysis to sulfite and maleylacetate. The pathway originally found in *Hydrogenophaga palleronii* S1 and *Agrobacterium radiobacter* S2 [105,106], has also been found in a mixed culture which degrades benzene-1,3-disulfonate [107] and seems to be involved in the degradation of 2-(4-sulfophenyl)butyrate [108]. This novel substrate for ring cleavage thus fulfils the role one would predict for it; acting as a focal point for converging degradative pathways. It will be interesting to see whether all intermediates of LAS degradation, and not just this sulfophenylbutyrate, follow this pathway (Fig. 5), which has not been postulated in any review (cited in [17]). Other sulfonates could also be channelled through 4-sulfocatechol, e.g. *p*-chlorosulfobenzoate [109], whereas *m*-amino- and *m*-nitrobenzenesulfonates [110,111] could theoretically be channelled through 3- or 4-sulfocatechol. The unidentified organism, strain S1, which Locher et al. [111] isolated to utilize sulfanilate obviously also used the pathway in Fig. 5, because 4-sulfocatechol was excreted transiently.

7. Fungal peroxidases and arenesulfonates

Desulfonations of aromatic sulfonates in fungi have also been implied, because CO₂ from the ring carrying the sulfonate was released [112], or observed directly [113]. These reactions have been attributed to (extracellular) peroxidases [113,114], which sometimes lead to extensive destruction of the compounds involved, essentially as in the enzymic combustion of lignin [115]. In these cases, no transport of the sul-

fonate is required, because the fungus has already exported the non-specific peroxidase.

8. Aerobes assimilating sulfonate-sulfur

The bacterial utilization (assimilation) of organosulfonates as sources of sulfur for growth differs from the utilization of the same compounds as carbon sources in the enzymology involved and in the genetic regulation of the enzymes. Whereas bacteria usually organize catabolic enzymes in inducible operons (see above), the utilization of sulfur is regulated at the global level of the stimulon [116]. The initial observation involved two dimensional gel-electrophoresis and showed that, under sulfate-starvation conditions, *E. coli* and many other Gram-positive and -negative bacteria each produces a set of proteins which is absent in the presence of sulfate. These proteins were termed sulfate-starvation induced (SSI) proteins [117] and some of them have been identified in *E. coli* (e.g. cysteine synthase CysK, sulfate-binding protein Ssp) [118] and in *P. aeruginosa* (e.g. a sulfate-binding protein) [119]. The genes regulated by the SSI stimulon are expressed when sulfate (and/or cysteine and perhaps another, species-specific compound) has been exhausted. The stimulon also seems to regulate the distribution of sulfur within the cell [116].

The natural products taurine, cysteate and isethionate are used as sole sources of sulfur by a range of bacteria and yeasts [120-124]. Where this has been examined at the molecular level, as yet only in *E. coli*, it turns out that the enzymology is totally different from the corresponding reaction when the carbon moiety is utilized. Taurine is no longer subject to transamination and hydrolysis (Section 6.1. and Fig. 6) but to a 2-oxoglutarate-dependent dioxygenation (EC 1.14.11.-) that presumably yields the unstable 1-hydroxy-2-aminoethanesulfonate which spontaneously loses sulfite with concomitant formation of aminoacetaldehyde. Taurine is the preferred substrate, but pentanesulfonate, hexanesulfonate, 3-(*N*-morpholino)propanesulfonate (MOPS) and 1,3-dioxo-2-isoindolineethanesulfonate were also desulfonated at significant rates; the first three sulfonates also serve as sulfur sources for *E. coli* [55,125].

Fig. 6. Possibilities of taurine degradation by anaerobic bacteria. Enzyme I (taurine-pyruvate aminotransferase) has been detected in three anaerobic bacteria [139,149,157], enzymes II (alanine dehydrogenase) and III (sulfoacetaldehyde sulfo-lyase) in two organisms [139,149]. The pathway was thus presumed to be widespread. Taurine can participate as an electron acceptor (1), in combination with e.g. formate as an electron donor, in the anaerobic respiration of *B. wadsworthia* RZATAU [139]. Taurine can be utilized in anaerobic respiration (2) as an electron donor for *Alcaligenes* sp. strain NKNTAU concomitant with reduction of nitrate, nitrite or nitrous oxide, presumably as indicated [147], and (3) as putative electron donor for the reduction of iron(III) [148]. Fermentation of taurine (4) is the energy-conserving pathway of a newly isolated syntrophomonad, strain GKNTAU [149]. Assimilation of the sulfonate sulfur of taurine (5) can be catalyzed by many anaerobes, e.g. *C. pasteurianum* and *C. beijerinckii* EV4 [156,158], and as this reaction also involves a taurine transaminase in *C. pasteurianum* [157] a sulfoacetaldehyde sulfo-lyase (III) is surely also involved; the R-SH represents, e.g. a sulfur amino acid.

Taurine dioxygenase is encoded by *tauD* [55]. The gene products, CysB and Cbl, were found to be crucial for the regulation of the *tauABCD* operon, and the binding of these LysR-type transcriptional regulators to the *tauABCD* promoter has recently been shown. CysB, a regulator of the cysteine regulon, and Cbl, a protein with 41% sequence identity with CysB [126] are both positive regulators. CysB also functions as a positive regulator in *cbl* expression. Furthermore, thiosulfate and sulfide act

as anti-inducers of CysB-dependent transcription. In addition to taurine, the *cbl* gene product is essential for the utilization of several other alkane-sulfonates (*n*-alkanesulfonates (C_2 – C_6), isethionate, sulfoacetate, MOPS and piperazine-1,4-bis-2-ethanesulfonate), but their degradative genes are as yet unknown. In contrast, the absence of Cbl leads to the disappearance of the SSI proteins normally seen under sulfate starvation conditions. Thus the *cbl* and *cysB* gene products play a key role in the

degradation of alkanesulfonates under SSI conditions.

The sulfur sources mentioned in the previous paragraph are not carbon and energy sources for *E. coli*. TauD thus does not enable *E. coli* to utilize taurine as a source of carbon. To emphasize the different metabolic regulation involved in sulfonate catabolism and assimilation of sulfonate sulfur, TS and orthanilate do not serve as sole sources of sulfur for the growth of *C. testosteroni* T-2 or *Alcaligenes* sp. strain O-1 (cf. Sections 6.3.2, 6.3.3, 6.3.4 and 6.3.5). Indeed, the cells catabolizing a sulfonate require a constant counterflux of organosulfonate for sulfite or sulfate, whereas sulfonate-assimilating cells are surely designed to capture and never to lose sulfur.

The alkanesulfonates utilized as sulfur sources by *Pseudomonas aeruginosa* are monooxygenated by an unknown mechanism [47], but where a comparison with catabolism is possible, as with the desulfonation of methanesulfonate, a different monooxygenation is involved ([127], cf. [56]). Algae and cyanobacteria also utilize alkanesulfonates as sulfur sources [52,128].

The bacterial utilization of arenesulfonates as carbon sources is characterized by narrow substrate ranges [37,88,129], one major exception being *Sphingomonas* sp. strain BN6 [130,131]. In contrast, the assimilation of sulfur from these compounds is characterized by the wide substrate ranges of the organisms involved; *Pseudomonas putida* S-313 desulfonates several hundred compounds [44,45,132], and other bacteria have similar properties [46,129]. Indeed, many apparently unusual compounds are subject to desulfonation [48,132,133] and even to more complex transformations [134] under sulfate-limited conditions; desulfonation of a wide range of azo dyestuffs and other complex arenesulfonates has also been observed [45,97,135].

In contrast to the wide range of dioxygenations involved, sometimes indirectly, in the catabolic desulfonations of aromatic compounds (Section 6.3), only one reaction-type has been detected in the SSI stimulon, apparent monooxygenation to yield the corresponding phenol [44–46]. A similar product is observed in algae which utilize naphthalenesulfonates, though further biotransformation of the naphthol can occur [136,137].

9. Anaerobes dissimilating sulfonates as sources of carbon and energy

Anaerobic bacteria have three possible strategies to dissimilate sulfonates: (1) as the electron acceptor; (2) as the electron donor for anaerobic respirations; or (3) as the substrate for disproportionation in fermentations. An overview of known and likely pathways of taurine utilization is shown in Fig. 6.

9.1. Sulfonates as electron acceptors in anaerobic respiration

Sulfonates are utilized as electron acceptors in anaerobic respirations of sulfate-reducing bacteria [18,138,139] and of non-sulfate-reducing bacteria [139].

Reduction of sulfonates as terminal electron acceptor was first discovered in *Desulfovibrio desulfuricans* IC1, which was isolated from marine sediment [138]. The organism reduces the organosulfonates isethionate or cysteate in combination with lactate as electron donor. The carbon moiety of isethionate is oxidized to acetate, and electrons from the latter reaction and from lactate are used to reduce the sulfonate sulfur to sulfide. Thus, in the formal dissimilatory reaction, 1 mol isethionate with 1 mol lactate yield 1 mol sulfide, 2 mol acetate and 1 mol CO₂ [138]. The enzymes in the desulfonation pathway have not yet been measured, but cells of strain IC1 grown with isethionate, cysteate, fumarate and sulfite contain lower specific activities of key enzymes of sulfate reduction—ATP sulfurylase and APS reductase—than sulfate-grown cells [18]. Another *Desulfovibrio* sp., strain RZACYSA, has a somewhat broader substrate range than strain IC1 (Table 1) including taurine as an electron acceptor with lactate as electron donor [139]. A wide range of sulfate-reducing bacteria is able to reduce sulfonates [18,138].

Participation of taurine as electron acceptor in an anaerobic respiration was thoroughly studied in the non-sulfate-reducing bacterium *Bilophila wadsworthia* RZATAU. The obligately anaerobic, Gram-negative rod, that was isolated from the anaerobic digester of a communal sewage works, utilizes taurine as the electron acceptor in the presence of e.g. formate as electron source [139]. The carbon moiety of taurine is oxidized to acetate, and electrons from the

Table 1

Anaerobic bacteria and their dissimilation of organosulfonates as electron acceptor, electron donor or as fermentative substrate

	<i>Bilophila wadsworthia</i> ^a	<i>D-vibrio desulfuricans</i> ^b	<i>D-vibrio desulfuricans</i> ^c	<i>D-microbium baculatus</i> ^d	<i>D-bacterium autotrophicans</i> ^e	<i>D-vibrio</i> sp. ^f	Syn-trophomonad ^g	<i>D-vibrio</i> sp. ^h	<i>Alcaligenes</i> sp. ⁱ
Electron donor:	Formate	Lactate	Lactate	Lactate	Lactate	Lactate	Sulfonate	Sulfonate	Sulfonate
Electron acceptor:	Sulfonate	Sulfonate	Sulfonate	Sulfonate	Sulfonate	Sulfonate	Sulfonate	Sulfonate	Nitrate
Taurine	+	— ^k	—	—	—	+	+	—	+
Isethionate	+	+	+	+	—	+	—	+	—
Cysteate	+	+	—	+	+	+	—	+	—
Aminomethane-sulfonate	—	ND ^l	ND	ND	ND	+	—	+	—
Ethanesulfonate	—	—	ND	ND	ND	—	—	—	—
<i>p</i> -Toluene-sulfonate	—	—	ND	ND	ND	—	—	—	—

^a*Bilophila wadsworthia* RZATAU DSM 11045 [139].^b*Desulfovibrio desulfuricans* IC1 [138].^c*Desulfovibrio desulfuricans* ATCC 29577 [138].^d*Desulfomicrobium baculatus* DSM 1741 [138].^e*Desulfobacterium autotrophicans* DSM 3382 [138].^f*Desulfovibrio* sp. strain RZACYSA [151].^gSyntrophomonad strain GRZTAU DSM 11270 [149].^h*Desulfovibrio* sp. strain GRZCYSA DSM 11493 [139].ⁱ*Alcaligenes* sp. NKNTAU DSM 11046 [147].^j+, growth.^k—, no growth.^lND, not determined.

latter reaction and from formate are used to reduce the sulfonate sulfur to sulfide. Thus, in the formal dissimilatory reaction, 1 mol taurine with 2 mol formate yield 1 mol each of ammonia, acetate and sulfide, and presumably 2 mol CO₂ (Fig. 6 (1)). Desulfovibridin, a class of sulfite reductases, was detected in *B. wadsworthia* RZATAU [139]. It is believed that the activity of this sulfite reductase, reducing the liberated sulfite to sulfide, is involved in energy conservation, so sulfite reductase and other enzymes were purified to explore the overall metabolic pathway [140]. Laue et al. find enzymes of taurine transformation to be constitutive in *B. wadsworthia* [140]; Lie et al. observe inducible proteins (bands in SDS-PAGE gels) in extracts of *D. desulfuricans* grown with different sulfonates [18].

The lower intestinal tract is the natural habitat of *B. wadsworthia* [141], and it is a suspected pathogen which is recovered from several different clinical specimens, especially from appendicitis [142,143]. Clinical isolates of *B. wadsworthia* utilize taurine and taurine-conjugated cholic acid derivatives [144] and it is now clear that the clinical isolates and strain RZATAU have indistinguishable growth with taurine as an electron acceptor and formate as electron donor [145]. Taurine is widespread in mammals [1,

so one suspects that its degradation (Fig. 6 (1)) is a major pathway of energy conservation of this organism in its natural habitat(s). In addition to taurine and e.g. taurocholate, *B. wadsworthia* utilizes cysteate and isethionate (Fig. 1), and sulfite and thio-sulfate as electron acceptors; sulfate, non-activated alkanesulfonates and arenesulfonates are not utilized [139]. The energetic advantage of reducing a sulfonate moiety in these respirations, rather than sulfate, might be that the former need not be activated in an ATP sulfurylase reaction [146].

9.2. Sulfonates as electron donors in anaerobic respiration

Enrichments for sulfonate-oxidizing, nitrate-reducing bacteria readily yield cultures that are able to utilize the naturally occurring, activated compounds taurine, isethionate and cysteate [147]. The facultatively anaerobic bacterium *Alcaligenes* sp. strain NKNTAU was examined in detail. It has a very narrow substrate range concerning sulfonates. Of 18 tested organosulfonates, the organism utilizes only taurine as a carbon and energy source for nitrate respiration. Taurine is converted quantitatively in cell material and CO₂, the sulfonate moiety being

oxidized to sulfate and the amino group released as ammonia (Fig. 6 (2)). Nitrate (nitrite or nitrous oxide) is reduced to nitrogen gas [147]. In addition to the strictly anaerobic oxidation of taurine under nitrate-reducing conditions, taurine can also be degraded by strain NKNTAU under oxic conditions. Further studies are needed to confirm that the pathway in Fig. 6 is correct and to establish whether the same enzymes are involved both in the anaerobic, nitrate-reducing and in the aerobic pathways. Another pure culture, strain KKNIS, also has a limited range of substrates as electron donor for nitrate reduction, namely isethionate and taurine. Release of sulfite in millimolar concentrations during growth of this organism indicates formation of sulfite as an intermediate during the oxidation of the sulfonate moiety [147]. A third isolate, *Paracoccus denitrificans* NKNCYSA, has a wider substrate range for sulfonates, and cell-free extracts transaminate cysteate to putative sulfoypyruvate [67,148]. These preliminary data imply a degradative pathway that does not proceed via sulfoacetaldehyde [67]. Cysteate metabolism is constitutive in this organism, whereas taurine metabolism is inducible [67].

The general concept of respiration with a sulfonate as electron donor led Cook et al. to explore alternatives to nitrate as electron acceptor (Fig. 6 (3)). Cook and associates chose to work with sulfate and iron(III). Whereas they were dissatisfied with their sulfate cultures, they believe that they obtained positive enrichments with cysteate as electron donor and iron(III) as electron acceptor [67]. It is suspected that more work in this area would yield either novel organisms or novel substrates for established respirations.

9.3. Sulfonates in fermentation

The third strategy used by bacteria which dissimilate organosulfonates is fermentation. The first organism to be isolated, strain GKNTAU, ferments taurine. It is a strictly anaerobic, spore-forming rod which has been attributed to the *Synthrophomonas* assemblage within the *Clostridium* subdivision of Gram-positive bacteria as a presumed novel genus [149]. The organism has a narrow substrate range; of 17 tested organosulfonates and 20 non-sulfonated, fermentable compounds or autotrophic regimes, only

Table 2
Selected compounds examined as sulfur sources for growth of anaerobic bacteria

	<i>Clostridium</i> sp. ^a	<i>Clostridium</i> spp. ^b	<i>Clostridium pasteurianum</i> ^c	RZLAS ^d	<i>Klebsiella</i> sp. ^e
Without sulfur	— ^f	—	—	—	—
Sulfate	+ ^g	+	+	+	+
Methanesulfonate	—	—	—	—	—
Ethanesulfonate	—	+	ND ^h	+	ND
1-Heptanesulfonate	—	+	ND	+	ND
Taurine	+	+	+	ND	—
Isethionate	ND	ND	+	ND	—
Cysteate	ND	ND	—	ND	+
Benzenesulfonate	+	+	ND	ND	ND
<i>p</i> -Sulfo benzoate	+	+	ND	ND	ND
<i>p</i> -Toluenesulfonate	+	+	+	—	—
2-(4-Sulfo phenyl)butyrate	+	+	ND	+	ND
4-(Phenyl)butane-1-sulfonate	—	+	ND	+	ND
1-(4-Sulfo phenyl)octane	+	+	ND	+	ND
LAS	—	—	ND	+	ND
Naphthalene-2,6-disulfonate	+	+	ND	ND	ND
4-Nitrocatecholsulfate	+	+	ND	ND	ND

^a*Clostridium beijerinckii* EV4 [158].

^bThree *Clostridium* spp., strain KKNDS, RZES, RZHS [159].

^c*Clostridium pasteurianum* [156].

^dStrain RZLAS [160].

^e*Klebsiella* sp. growing under fermentative conditions [156].

^f—, no growth.

^g+, growth.

^hND, not determined.

taurine serves as a carbon and energy source. In contrast to the utilization of taurine as an electron acceptor in anaerobic respiration of *B. wadsworthia* or the oxidation of taurine by the nitrate-reducing *Alcaligenes* sp. strain NKNTAU, where an additional substrate (as electron donor or electron acceptor, respectively) is necessary, taurine alone suffices for growth in salts medium. The fermentation involves a transformation of the sulfonate moiety to thiosulfate as the stoichiometric product (Fig. 6 (4)). To the authors' knowledge, this is the first metabolic pathway to yield thiosulfate as an end product, despite the fact that thiosulfate is widely used in natural ecosystems as electron donor and as electron acceptor [150]. This novel end product is simultaneously a novel pathway of energy conservation.

A different isolate ferments cysteate [151]. The fermentation involves not only a different organism, but also different products from the sulfonate moiety. Strain GRZCYSA was attributed to the genus *Desulfovibrio* because of its substrate range, especially the ability to reduce sulfate coupled with its morphology and the presence of desulfovibrin. The sulfonate moiety of cysteate or isethionate is disproportionated to equimolar amounts of sulfate and sulfide [151]. Presumably, (a) the sulfonate moiety is released as sulfite; and (b) energy conservation during the fermentation of cysteate or isethionate is analogous to the disproportionation of sulfite to sulfate and sulfide [152]. The amino group of cysteate is recovered as ammonia and the carbon skeleton is oxidized to acetate and CO₂ [151].

9.4. Widespread reactions

The dissimilation of taurine in aerobes has been described in some detail in two systems ([60,153]; cf. [154]) (Section 6.1) where the common reaction is the sulfoacetaldehyde sulfo-lyase. The anaerobes studied in some detail (*B. wadsworthia* RZATAU and strain GKNTAU) appear to have the pathway found in *P. aeruginosa*, namely a pyruvate-dependent taurine aminotransferase and a sulfo-lyase [60,139,149] (Fig. 6). The sulfo-lyase (EC 4.4.1.12) from strain GKNTAU differs in molecular properties from the only purified sulfo-lyase [57,155]. Thus, the same degradative pathway for taurine is involved in aerobes and anaerobes, the difference being the metabolic

surroundings, which give different end products from the common intermediates sulfite, from the sulfonate moiety, and acetate from the carbon skeleton.

The bacteria which dissimilate organosulfonates belong largely to the broad group of sulfate reducing bacteria (Table 1). Indeed, as some of these sulfate reducers were obtained from culture collections and not by enrichment, one can deduce how widespread the anaerobic dissimilation of sulfonates must be [138]. The same conclusion was drawn from work with the phylogenetically related sulfite-reducer, *Bilophila wadsworthia*, as a normal part of the gut flora [139]. In contrast, the taurine-oxidizing *Alcaligenes* sp. strain NKNTAU belongs to a completely different group [147], and reminds one how many genera oxidize taurine aerobically (Section 6.1). The anaerobic oxidation of cysteate by a strain of *P. denitrificans* led to the type strain being examined. The latter organism can also oxidize at least one sulfonate anaerobically [67]. Work with the fermentation of taurine by strain GKNTAU shows that similar organisms can be found in sewage works and sediments from at least two continents [155]. The anaerobic dissimilation of sulfonates is thus widespread both geographically and phylogenetically.

10. Anaerobes assimilating sulfonate-sulfur

The first desulfonative anaerobes to be isolated were bacteria which utilize sulfonate-sulfur as a sulfur source [156]. Chien et al. [156] worked largely with the naturally occurring sulfonates and isolated not only the facultative anaerobe *Klebsiella* sp. which utilizes cysteate under fermentative conditions, but also a strain of the strict anaerobe, *Clostridium pasteurianum*, which utilizes taurine with the same growth yield as sulfate and isethionate and *p*-toluenesulfonate with a lower yield (Table 2). The work with *C. pasteurianum* C1 has been expanded to show an inducible taurine-pyruvate aminotransferase activity involved in the assimilation of taurine sulfur (Fig. 6 (5)) [157]. Although one thus expects the same reactions with taurine in assimilatory and dissimilatory growth (Fig. 6), one may also expect very different enzymes or transport systems, reflecting the different affinity for a substrate (K_m) in a dissimilatory reaction (K_m values in the mM range) and

in an assimilatory reaction (K_m values in the μM range).

Denger et al. initially identified only strains of *Clostridium* in their work on arenesulfonates and alkanesulfonates [158,159], but the anaerobic desulfonation is also found in an unidentified, facultatively anaerobic bacterium [160]. Growth is concomitant with substrate utilization and molar growth yields are independent of the sulfur growth substrate. In contrast to the narrow substrate ranges of dissimilatory desulfonations in anaerobes, the bacteria assimilating the sulfonate sulfur have broad substrate ranges (Table 2) similar to those of aerobic bacteria described in Section 8. Besides the naturally occurring, activated sulfonates taurine, cysteate and isethionate [156], the non-activated compounds ethanesulfonate and 1-heptanesulfonate [159] and arenesulfonates [158-160] are used as sole source of sulfur; all organisms catalyze the same arenesulfonate reaction [160]. In contrast to *Clostridium beijerinckii* EV4, able to desulfonate only the activated alkane-sulfonate taurine and arenesulfonates [158], three *Clostridium* spp. are able to utilize in addition the non-activated alkanesulfonates [159] (Table 2). This leads to the assumption that there are at least three different sulfonate activities in these *Clostridium* spp.: an arenesulfonate, a taurine desulfonating pathway and an alkanesulfonate. The desulfonation mechanism of arenesulfonates under anaerobic conditions must be completely different from the monooxygenation in aerobic bacteria involved in assimilation of sulfur from arenesulfonates. The anaerobic mechanism has not been elucidated [159].

The substrate ranges of anaerobic bacteria able to assimilate sulfonates seem to be similar to those of aerobic bacteria. One wonders whether regulation analogous to the SSI stimulon (Section 8) will be found in anaerobes.

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