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Glycyl Radical Enzymes and Sulfonate Metabolism in the Microbiome

Yifeng Wei[1] and Yan Zhang[2]

[1]Singapore Institute of Food and Biotechnology Innovation, Agency for Science, Technology and Research (A*STAR), Singapore 138669

[2]Tianjin Key Laboratory for Modern Drug Delivery & High-Efficiency, Collaborative Innovation Center of Chemical Science and Engineering, School of Pharmaceutical Science and Technology; and Frontiers Science Center for Synthetic Biology (Ministry of Education), Tianjin University, Tianjin 300072, China; email: yan.zhang@tju.edu.cn

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Keywords

sulfonates, sulfate- and sulfite-reducing bacteria, bacterial microcompartment, dihydroxypropanesulfonate, terminal electron acceptor, glycyl radical enzyme, GRE, dissimilatory sulfite reductase, gut microbiome, hydrogen sulfide

Abstract

Sulfonates include diverse natural products and anthropogenic chemicals and are widespread in the environment. Many bacteria can degrade sulfonates and obtain sulfur, carbon, and energy for growth, playing important roles in the biogeochemical sulfur cycle. Cleavage of the inert sulfonate C–S bond involves a variety of enzymes, cofactors, and oxygen-dependent and oxygen-independent catalytic mechanisms. Sulfonate degradation by strictly anaerobic bacteria was recently found to involve C–S bond cleavage through O_2-sensitive free radical chemistry, catalyzed by glycyl radical enzymes (GREs). The associated discoveries of new enzymes and metabolic pathways for sulfonate metabolism in diverse anaerobic bacteria have enriched our understanding of sulfonate chemistry in the anaerobic biosphere. An anaerobic environment of particular interest is the human gut microbiome, where sulfonate degradation by sulfate- and sulfite-reducing bacteria (SSRB) produces H_2S, a process linked to certain chronic diseases and conditions.

Contents

THE DIVERSITY, UBIQUITY, AND ABUNDANCE OF SULFONATES

Sulfur is an essential element for all living organisms. The most widespread and familiar biological sulfur compounds, including cysteine, methionine, biotin, lipoic acid, thiamine, and molybdopterin, contain sulfur in the −2 oxidation state, with the electron-rich sulfur moiety often acting as nucleophiles, metal ligands, and reductants. Other biological sulfur compounds, including sulfate esters of proteins and glycans, contain sulfur in the +6 oxidation state, with the esters of the sulfur oxyanions exhibiting chemical and physical properties similar to those of phosphate esters (1).

Another class of highly abundant but underappreciated organosulfur compounds is sulfonates, which contain a C–SO_3^- moiety (2–5) with sulfur in the +4 oxidation state. Sulfonic acids are strong acids and are fully ionized at neutral physiological pH. Sulfonates are thus hydrophilic and membrane impermeable. Sulfonates are relatively inert under physiological conditions and, unlike sulfate esters, are not subject to hydrolytic cleavage. These physical and chemical properties

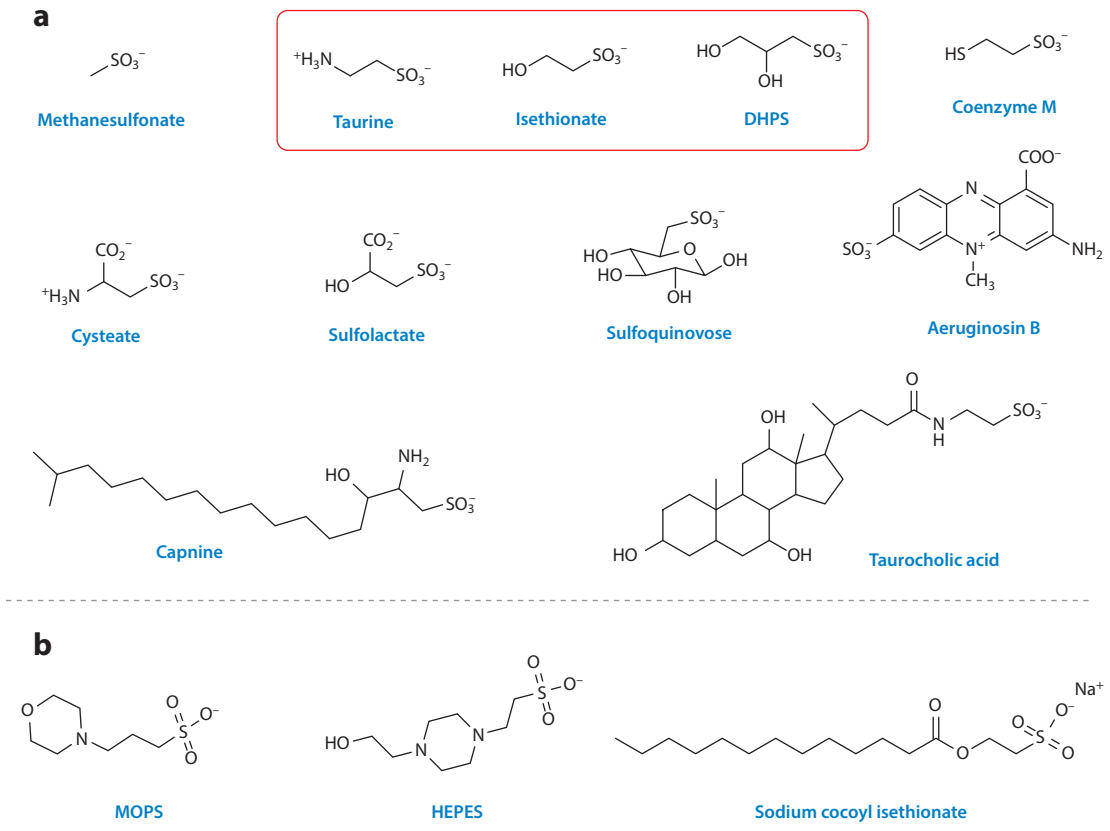


Figure 1

The chemical structures of common sulfonates. Biologically (*a*) and industrially (*b*) relevant sulfonates are displayed in order of molecular weight and are separated by a dashed line. C2 and C3 sulfonates that are the focus of this review are highlighted in the red box. Abbreviations: DHPS, dihydroxypropanesulfonate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MOPS, 3-(*N*-morpholino)propanesulfonic acid.

rationalize why nature chose sulfonates for roles such as osmolytes (e.g., taurine), surfactants (e.g., taurocholate), membrane lipids (e.g., sulfoquinovosyl diacylglycerol), and coenzymes (e.g., coenzyme M) (6, 7), and why humans utilize them in applications such as counterions, buffers, and detergents (**Figure 1**).

This review focuses on three highly abundant and biologically relevant sulfonates: the C2 sulfonates taurine and isethionate and the C3 sulfonate dihydroxypropanesulfonate (DHPS) (**Figure 1**). Taurine, first isolated from ox bile in 1827 (8), is present at high concentrations as an osmolyte in animals and certain algae (7) and is a common ingredient in food and beverages. The structurally related isethionate (**Figure 1**) is found in mammals as a by-product of taurine metabolism by gut microbiota (9); it is also a component of various detergents.

DHPS (**Figure 1**) is important because of its relationship to major primary producers on our planet: plants (10, 11) and eukaryotic phytoplankton (12–14). Marine phytoplankton use sulfate, which is present at ~28 mM in seawater, to synthesize a variety of osmolytes, including DHPS (13). DHPS is present at millimolar concentrations in diatoms, which are abundant in both marine

and freshwater environments and account for ~20% of global primary production. DHPS is also an intermediate in the microbial degradation of the sulfosugar sulfoquinovose (**Figure 1**) through a pathway termed sulfoglycolysis, which is described in the section titled Sulfoglycolysis and Other Sources of DHPS. Sulfoquinovose is the polar headgroup of the sulfonolipid sulfoquinovosyl diacylglycerol, a component of thylakoid membranes in plant chloroplasts as well as nearly all other photosynthetic organisms (11). With an annual production of 10^{10} tons, sulfoquinovose is one of the most abundant organosulfur compounds in the biosphere, rivaling cysteine and methionine.

Microbial degradation of taurine, isethionate, and DHPS, as well as other sulfonates, has long been the subject of biochemical research owing to the abundance and relevance of these sulfonates to various biological contexts.

HISTORICAL REVIEW OF MICROBIAL SULFONATE DEGRADATION

The many natural sources, combined with industrial and domestic effluents, introduce large quantities of sulfonates into the environment. Despite their chemical inertness, sulfonates do not generally persist; they are rapidly degraded by environmental bacteria, which return the sulfonate sulfur to the inorganic sulfur cycle (2). In the following sections, we outline the well-studied enzymes catalyzing C–S bond cleavage in aerobic degradation of C2 and C3 sulfonates, and then focus on the recently reported glycyl radical enzymes (GREs) involved in anaerobic degradation of these compounds. Different enzymes and pathways are generally used for assimilatory sulfonate degradation (through which the organism obtains the sulfonate sulfur) and dissimilatory sulfonate degradation (through which the organism obtains energy for growth), in keeping with their different kinetic and energetic requirements (3) (**Figure 2**). We describe the background leading to their discovery and discuss their structures, catalytic mechanisms, and metabolic functions in anaerobes, including gut bacteria. The distribution of sulfonate degradation enzymes in diverse bacteria with distinct physiology and ecological niches is also discussed and summarized (**Table 1**).

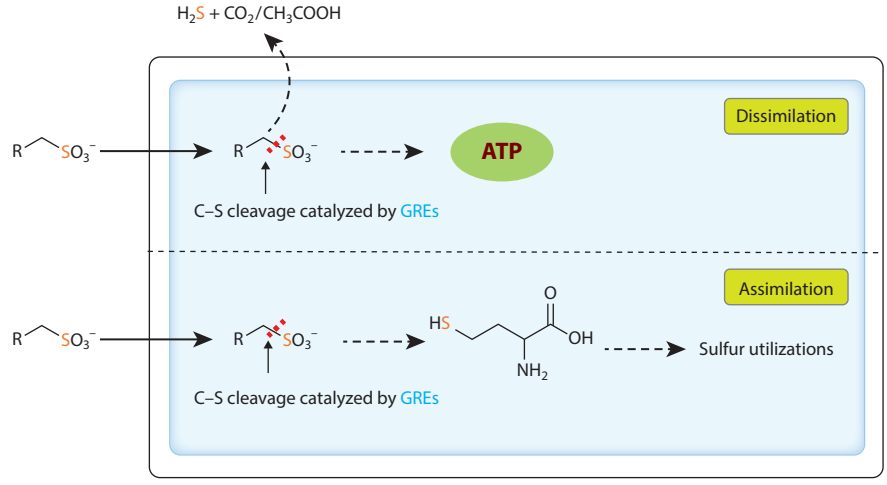


Figure 2

Physiological roles of assimilatory and dissimilatory sulfonate degradation in strict anaerobes and the involvement of GREs. Abbreviation: GRE, glycyl radical enzyme.

Table 1 Sulfonate metabolism of bacteria described in this review[a]

Bacterial strain	Phylum	Environments	SSRB	IseG	HpsG	HpfG	Xsc	SuyAB	Aerobic/anaerobic	Notes
Bilophila wadsworthia	*Proteobacteria*	Human gut	Yes	Yes	Yes	No	No	Yes	Strictly anaerobic	C2 and C3 sulfonate degradation through IseG and HpsG
Desulfovibrio strain IC1[b]	*Proteobacteria*	Sulfidic mud	Yes	Yes	ND	ND	ND	ND	Strictly anaerobic	C2 sulfonate degradation through IseG
Desulfovibrio piger	*Proteobacteria*	Human gut	Yes	Yes	No	No	No	No	Strictly anaerobic	C2 sulfonate degradation through IseG
Desulfovibrio desulfuricans DSM642[b]	*Proteobacteria*	Tar and sand mix	Yes	Yes	ND	ND	ND	ND	Strictly anaerobic	C2 sulfonate degradation through IseG
Desulfovibrio alaskensis G20	*Proteobacteria*	Oil well	Yes	Yes	No	No	No	No	Strictly anaerobic	C2 sulfonate degradation through IseG
Desulfovibrio vulgaris Hildenborough	*Proteobacteria*	Clay	Yes	Yes	No	No	No	No	Strictly anaerobic	C2 sulfonate degradation through IseG
Anaerostipes hadrus	*Firmicutes*	Human gut	No	Yes	No	No	No	No	Strictly anaerobic	Sulfur assimilation through IseG
Brenneria goodwinii	*Proteobacteria*	Plant pathogen	No	Yes	No	No	No	No	Facultatively anaerobic	Sulfur assimilation through IseG
Clostridium butyricum str. E4 BoNT E BL5262	*Firmicutes*	Human gut	No	Yes	No	No	No	No	Strictly anaerobic	Sulfur assimilation through IseG
Clostridium cylindrosporum	*Firmicutes*	Soil	No	Yes	No	No	No	No	Strictly anaerobic	Sulfur assimilation through IseG
Clostridium chromiireducens C1	*Firmicutes*	Soil	No	Yes	No	No	No	No	Strictly anaerobic	Sulfur assimilation through IseG
Pectobacterium wasabiae	*Proteobacteria*	Plant pathogen	No	Yes	No	No	No	No	Facultatively anaerobic	Sulfur assimilation through IseG
Desulfonispora thiosulfatigenes[b]	*Firmicutes*	Sewage, lakes	No	ND	ND	ND	Yes	ND	Strictly anaerobic	Taurine fermenting, thiosulfate producing
Escherichia coli	*Proteobacteria*	Human gut	No	No	No	No	No	No	Facultatively aerobic	C2 sulfonate degradation through TauD/SsuDE

(*Continued*)

Table 1 (*Continued*)

Bacterial strain	Phylum	Environments	SSRB	IseG	HpsG	HpfG	Xsc	SuyAB	Aerobic/anaerobic	Notes
Chromohalobacter salexigens DSM 3043	*Proteobacteria*	Marine bacteria	No	No	No	No	No	Yes	Facultatively anaerobic	Taurine nitrogen assimilation (IsfD)
Klebsiella pneumoniae	*Proteobacteria*	Human gut and respiratory tracts	No	No	No	No	No	No	Facultatively anaerobic	Taurine nitrogen assimilation (IsfD)
Klebsiella oxytoca TauN1[b]	*Proteobacteria*	Unknown	No	No	No	Yes	No	No	Facultatively anaerobic	Taurine nitrogen assimilation (IsfD)
Bifidobacterium kashiwanohense	*Actinobacteria*	Gut bacteria	No	No	No	No	No	No	Anaerobic	Taurine nitrogen assimilation (TauF)
Fusobacterium nucleatum	*Fusobacteria*	Human oral bacteria	No	No	No	No	No	No	Strictly anaerobic	Fermentation of cysteine H_2S production
Cupriavidus pinatubonensis	*Proteobacteria*	Volcanic mudflow	No	No	No	No	No	Yes	Aerobic	C3 sulfonate degradation through SuyAB
Desulfovibrio sp. strain DF1[b]	*Proteobacteria*	Sewage sludge	Yes	ND	ND	ND	ND	Yes	Strictly anaerobic	C3 sulfonate degradation through SuyAB
Pseudomonas putida SQ1	*Proteobacteria*	Soil	No	No	No	No	No	No	Aerobic	Sulfo-ED
Klebsiella oxytoca strain 67[b]	*Proteobacteria*	Unknown	No	No	No	Yes	No	No	Facultatively anaerobic	C3 sulfonate degradation through HpfG
Epulopiscium (some species)	*Firmicutes*	Fish gut	No	No	No	Yes	No	No	Strictly anaerobic	C3 sulfonate degradation through HpfG
Lactobacillus (some species)	*Firmicutes*	Human gut	No	No	No	Some	No	No	Anaerobic	C3 sulfonate degradation through HpfG
Photobacterium (some species)	*Proteobacteria*	Marine bacteria	No	No	No	Yes	Some	No	Facultatively anaerobic	C3 sulfonate degradation through HpfG
Vibrio (some species)	*Proteobacteria*	Marine bacteria	No	No	No	Some	Some	No	Facultatively anaerobic	C3 sulfonate degradation through HpfG

[a]Colored shading represents different groups of bacteria. Green represents sulfonate dissimilation involving new GREs, IseG and HpsG; yellow represents sulfur assimilation from sulfonate involving IseG; blue represents nitrogen assimilation from sulfonates; white represents miscellaneous sulfonate metabolism; and orange represents HpfG-containing fermenting bacteria.
[b]No (detailed) genomic sequence information.
Abbreviations: GRE, glycyl radical enzyme; ND, no data; SSRB, sulfate- and sulfite-reducing bacteria; sulfo-ED, sulfoglycolytic Entner-Doudoroff pathway.

O_2-DEPENDENT C–S BOND CLEAVAGE IN TAURINE AND ISETHIONATE ASSIMILATORY DEGRADATION

The biochemistry of sulfonate degradation by aerobic and facultatively aerobic bacteria has been studied for decades, and as expected from the ubiquity of these substrates, the associated enzymes and biochemical pathways are widespread among bacteria (3, 4, 15, 16). Pathways for taurine and isethionate sulfur assimilation are exemplified by *Escherichia coli*, which can utilize both as sources of sulfur, but not carbon, for aerobic growth (17) (**Table 1**). Uptake of these membrane-impermeable sulfonates is catalyzed by ATP-binding cassette (ABC) transporters: TauABC for taurine and SsuABC for isethionate and other aliphatic sulfonates (**Figure 3*a***). In both cases, C–S bond cleavage releases sulfite for reduction to sulfide by the assimilatory sulfite reductase followed by incorporation into homocysteine and other sulfur utilizations (**Figure 3*a***).

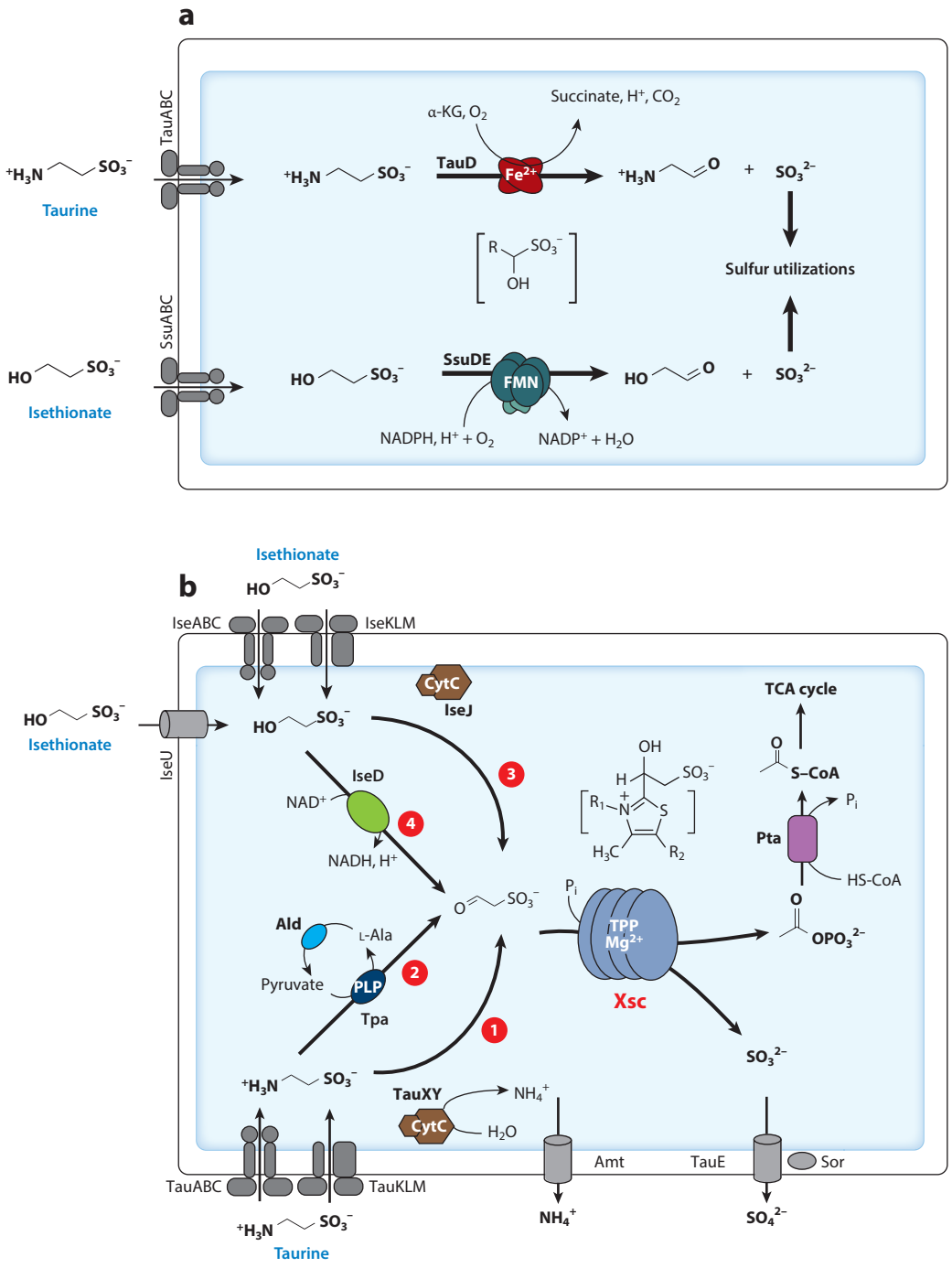
C–S bond cleavage of taurine in *E. coli* is catalyzed by an Fe(II)- and α-ketoglutarate (α-KG)-dependent taurine dioxygenase (TauD) (18–20) (**Figure 3*a***). The catalytic mechanism of TauD involves C1 hydroxylation of taurine followed by a spontaneous elimination reaction that produces 2-aminoacetaldehyde and sulfite (21) (**Figure 3*a***). A similar dioxygenase desulfonylates a variety of aliphatic sulfonates in *Saccharomyces cerevisiae* (22).

In contrast, C–S bond cleavage of isethionate and other aliphatic sulfonates in *E. coli* is catalyzed by a two-component alkanesulfonate monooxygenase, SsuDE (18, 23) (**Table 1**). The NADPH-dependent flavin reductase component SsuE catalyzes the reduction of flavin mononucleotide (FMN) to $FMNH_2$, and the $FMNH_2$-dependent monooxygenase component SsuD catalyzes the O_2-dependent desulfonylation (**Figure 3*a***). The catalytic mechanism of SsuD is still under investigation and has also been proposed to involve C1 hydroxylation (24) (**Figure 3*a***). Hydroxylation by flavoenzymes at aliphatic compounds not yet activated is rare, but a possible precedent is the two-component, long-chain alkane monooxygenase (LadA) (25). The same mechanism may also be employed by a related enzyme, SfnG, which catalyzes C–S bond cleavage of dimethylsulfone (26). Another enzyme thought to catalyze O_2-dependent desulfonylation through hydroxylation is methanesulfonate monooxygenase from the methylotrophic bacterium *Methylosulfonomonas methylovora* (27).

Xsc-DEPENDENT C–S BOND CLEAVAGE IN TAURINE AND ISETHIONATE DISSIMILATORY DEGRADATION

Pathways for aerobic C2 sulfonate dissimilation generally do not use the energetically inefficient O_2-dependent mechanisms for C–S bond cleavage but instead rely on the thiamine pyrophosphate (TPP)-dependent sulfoacetaldehyde acetyltransferase Xsc (28). This enzyme, isolated in 1975, was initially thought to catalyze a hydrolytic desulfonylation to generate acetate and sulfite (29). Subsequent reexamination showed that the reaction catalyzed by Xsc occurred only in phosphate buffer and produced the hydrolytically labile acetyl phosphate, similar to the TPP-dependent phosphoketolase. The *Xsc* gene commonly occurs adjacent to phosphate acetyltransferase, which converts acetyl phosphate to acetyl-CoA for oxidation via the tricarboxylic acid cycle (28) (**Figure 3*b***). Xsc serves as a hub for dissimilation of C2 sulfonates. Apart from isethionate (30) and taurine (19), these C2 sulfonates include ethanedisulfonate (31) and sulfoacetate (32), which are first converted to sulfoacetaldehyde.

For taurine dissimilation, taurine is converted to sulfoacetaldehyde either by oxidation, catalyzed by the membrane-bound cytochrome C (CytC)-dependent taurine dehydrogenase TauXY (33) (**Figure 3*b***), or by transamination, catalyzed by taurine:pyruvate aminotransferase (Tpa), coupled to alanine oxidation by NAD^+-dependent alanine dehydrogenase (Ald) (34). For isethionate



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Pathways for taurine and isethionate degradation in aerobic and facultatively aerobic bacteria. (*a*) Assimilatory pathways. (*b*) Dissimilatory pathways (❶–❹, *red*) with Xsc as a hub. Reaction intermediates of the C–S bond cleavage are presented in brackets. Abbreviations: α-KG, α-ketoglutarate; Ald, alanine dehydrogenase; CytC, cytochrome C; FMN, flavin mononucleotide; IseD, NAD^+-dependent isethionate dehydrogenase; IseJ, CytC-dependent isethionate dehydrogenase; PLP, pyridoxal phosphate; Pta, phosphate acetyltransferase; SsuDE, two-component alkanesulfonate monooxygenase; TauD, α-KG-dependent taurine dioxygenase; TauXY, CytC-dependent taurine dehydrogenase; TCA, tricarboxylic acid; Tpa, taurine:pyruvate aminotransferase; TPP, thiamine pyrophosphate; Xsc, TPP-dependent sulfoacetaldehyde acetyltransferase.

dissimilation, isethionate is oxidized to sulfoacetaldehyde either by the membrane-bound CytC-dependent isethionate dehydrogenase IseJ (30) or by the NAD^+-dependent alcohol dehydrogenase IseD (35), which was recently discovered by our laboratory.

THE PUZZLE OF C2 SULFONATE DEGRADATION IN STRICTLY ANAEROBIC BACTERIA

The first enzymatic desulfonylation reactions studied were O_2 dependent, and even facultative anaerobes such as *E. coli* assimilate sulfonate sulfur only during aerobic growth. Thus, it was once thought that the ability to degrade sulfonates may be restricted to aerobic organisms. However, subsequent studies demonstrated that taurine and isethionate could be degraded by *Clostridia*, which are a class of strictly anaerobic fermenting bacteria (36, 37), and by sulfate- and sulfite-reducing bacteria (SSRB), which are strictly anaerobic bacteria that use sulfate or sulfite as terminal electron acceptors (TEAs) for anaerobic respiration, reducing them to H_2S (38–40). For *Clostridia*, the sulfonates are used as sulfur sources for growth, whereas for SSRB, they are used as TEAs, with the sulfonate sulfur reduced to H_2S (**Figure 2**).

SSRB play an important role in the inorganic sulfur cycle in anaerobic environments, and their ability to degrade sulfonates suggests involvement in the organic sulfur cycle as well. Anaerobic environments include important ecosystems such as marine and limnic sediments and deep oil reservoirs, where sulfonate turnover is not well explored. Of particular interest is the intestinal tract of humans and other animals, where the dissimilatory degradation of taurine and related sulfonates by SSRB results in the conversion of sulfonate sulfur to toxic H_2S, a process associated with several chronic diseases and conditions (41, 42).

Taurine and isethionate degradation by both *Clostridia* and SSRB would require mechanisms for anaerobic sulfonate C–S bond cleavage (**Figure 2**). Xsc does not require oxygen for catalysis and, in theory, could provide a viable pathway for taurine and isethionate degradation by these anaerobic bacteria. However, Xsc activity was not detected in these organisms, nor were Xsc homologs identified in their genomes.

Several *Clostridium* species utilize taurine and isethionate as sulfur sources for growth (36, 43). In *C. chromiireducens* C1 (formerly *C. pasteurianum* C1), isolated from soil (36–38), Tpa activity was detected in cells grown with taurine as the sole sulfur source (37), suggesting that sulfoacetaldehyde is an intermediate, although the mechanism of desulfonylation was not resolved.

The ability to utilize isethionate as a TEA was first reported for the sulfate-reducing bacterium *Desulfovibrio* strain IC1, with lactate as the electron donor and with H_2S and acetate as products (38). The cells could be adapted to grow on isethionate, suggesting the involvement of inducible enzymes. It was later found that many SSRB could use isethionate as a TEA, although the mechanism was not further investigated.

The first strict anaerobe for which a complete pathway for taurine dissimilatory degradation was reported is *Desulfonispora thiosulfatigenes* (44), a specialist taurine degrader that grows on no other substrate and belongs neither to *Clostridia* nor to SSRB. In *D. thiosulfatigenes*, taurine is

degraded through a fermentation (disproportionation) pathway involving Tpa and Xsc, producing ammonium, acetate, and thiosulfate (**Table 1**).

The ability to use taurine as a TEA was reported for the sulfite-reducing bacterium *Bilophila wadsworthia* RZATAU (40), which was isolated from a sewage plant. In *B. wadsworthia*, use of taurine as a TEA requires the presence of an electron donor such as lactate or formate, producing acetate, ammonium, and H_2S. Growth necessitated supplementation with naphthoquinone, suggesting the requirement of a respiratory chain, and was accompanied by the induction of Tpa and Ald. Dissimilatory sulfite reductase (DsrAB) was also detected in extracts of taurine-grown cells (45). DsrAB, present in all SSRB, is an iron-sulfur hemoprotein that catalyzes the two-electron reduction of sulfite and transfers the sulfur atom to DsrC, forming a protein-based trisulfide (46), for further reduction to sulfide by the membrane-bound DsrMKJOP (46, 47) complex. Like *C. chromiireducens* C1, sulfoacetaldehyde was suggested as an intermediate, although the mechanism of desulfonylation was not resolved at that time.

All SSRB can in principle use sulfite as a TEA through the Dsr enzymes. However, sulfite-reducing bacteria such as *B. wadsworthia* are unable to use sulfate as a TEA because they lack ATP sulfurylase and adenosine 5′-phosphosulfate (APS) reductase, which catalyze ATP-dependent formation of APS from SO_4^{2-} and the subsequent reduction of APS to sulfite. Use of sulfonates as TEAs circumvents the need for ATP-dependent sulfate activation (38) but requires C–S bond cleavage to generate sulfite.

Because of difficulties in detecting C–S bond cleavage activity in anaerobic bacterial extracts, it was hypothesized that the desulfonylation reaction is catalyzed by an unstable enzyme (19). The missing enzyme catalyzing the C–S bond cleavage in anaerobic taurine and isethionate dissimilation by *B. wadsworthia* and other SSRB was recently identified independently by our group (48) and by Schleheck and colleagues (49) (**Table 1**). Our group also demonstrated that this enzyme, the isethionate lyase IseG (48, 49), is present in fermenting bacteria capable of sulfonate sulfur assimilation (**Table 1**). IseG (48, 49) is a member of the GRE family. Common characteristics of GREs, including their sensitivity to O_2 and complexity in cofactor formation, are consistent with the early prediction of IseG's instability and had hindered its discovery.

GENERAL INTRODUCTION TO GREs

GREs (InterPro family IPR004184) are a functionally diverse family of enzymes that catalyze radical-mediated reactions and play important roles in the metabolism of many obligate anaerobes (50). The radical chemistry of GREs was based on pyruvate formate lyase (PFL) and was established by the Knappe laboratory (51, 52) and subsequently by other laboratories (53). GREs contain a stable posttranslationally introduced glycyl radical (G•) cofactor generated by a separate activating enzyme that is often located adjacent to the GRE in the gene cluster. GRE-activating enzymes are members of the radical SAM (*S*-adenosyl-L-methionine) family and contain at least one [4Fe-4S] cluster ligated by three Cys residues in a conserved CX_nCXXC motif, with the fourth coordination site occupied by SAM (54) (**Figure 4*a***). The catalytic mechanism of these enzymes involves reductive cleavage of SAM by an electron from the $[4Fe\text{-}4S]^{1+}$ cluster, generating methionine and a 5′-deoxyadenosyl radical (5′-dA•), which was recently shown to be stabilized through the reversible formation of a Fe–C5′ bond with the [4Fe-4S] cluster (55). The 5′-dA• abstracts the *pro*-(*S*) hydrogen atom from a conserved Gly residue on the C-terminal segment of the GRE, forming 5′-deoxyadenosine and G• (53) (**Figure 4*a***). Once generated, G• is stable for many catalytic cycles. However, when exposed to O_2, it reacts rapidly and stoichiometrically, leading to the cleavage of the peptide at the G• position and enzyme inactivation (51).

All GREs discovered to date contain a Cys residue next to the G•, and the proposed catalytic mechanism involves the formation of a cysteine thiyl radical (Cys•) to initiate the reaction

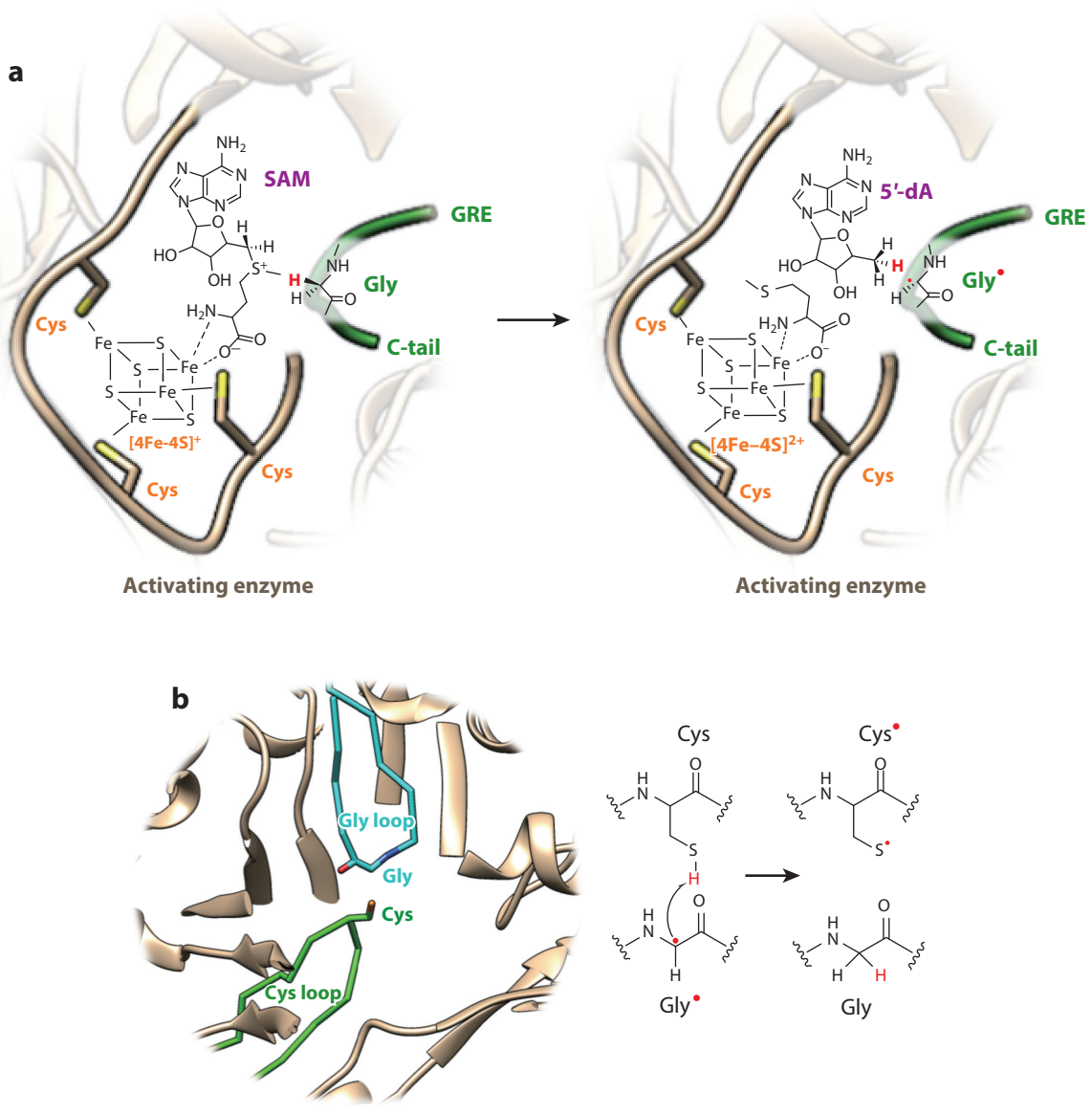


Figure 4

Mechanistic model for G• cofactor formation. (*a*) Reductive cleavage of SAM generates 5′-dA•, which abstracts a hydrogen atom (shown in *red*) from a conserved Gly residue on the C-terminal tail of GRE. The model is based on the structure of PDB ID 3CB8 (66). (*b*) The large active-site cavity and the positioning of the Gly and Cys loops, with their radical chemistry initiating the catalytic cycle of GRE. The model is based on the structure of PDB ID 5FAU (69). Abbreviations: 5′-dA•, 5′-deoxyadenosyl radical; C-tail, C-terminal tail; G•, glycyl radical; GRE, glycyl radical enzyme; PDB ID, Protein Data Bank identifier; SAM, *S*-adenosyl-L-methionine.

(**Figure 4*b***). The tertiary structure of GREs consists of a 10-stranded β/α barrel, with the N-terminal five strands oriented in one direction and the C-terminal five strands oriented in the opposite direction, enclosing a large active-site cavity that accommodates the diverse substrates of this family of enzymes (50). The glycyl radical loop bearing the G• cofactor projects into the

active site and is adjacent to a thiyl radical loop containing a critical catalytic Cys residue that can be oxidized to form a Cys• to initiate the catalysis (**Figure 4*b***).

GREs catalyze diverse radical chemistry. In addition to pyruvate formate lyase and the closely related ketobutyrate formate lyase (56), GREs include the anaerobic ribonucleotide reductase (57); the benzylsuccinate synthase (BSS) (58) and the closely related X-succinate synthase (XSS) (59); and three arylacetate decarboxylases, hydroxyphenylacetate decarboxylase (60, 61), indoleacetate decarboxylase (62), and phenylacetate decarboxylase (63). The GREs discussed in this article are involved in the desulfonylation or dehydration of C2 or C3 sulfonates and are most similar to a subclass of GREs, the 1,2-eliminases such as glycerol dehydratase (GDH) (64), propanediol dehydratase (PDH) (65), and choline trimethylamine-lyase (CutC) (66).

Public sequence databases contain many more diverse GRE sequences that have yet to be investigated (67, 68). Most of the GREs characterized to date participate in pathways for the fermentation of diverse substrates and as such are found largely in fermenting bacteria. Three of the aldehyde-forming GREs, PDH, CutC, and hydroxyproline dehydratase (67), are also found in several SSRB, where their reaction products could provide electrons for sulfate and sulfite reduction. The hydrocarbon-activating GREs, BSS and XSS, are found only in respiratory and syntrophic bacteria, because their hydrocarbon substrates are nonfermentable.

DISCOVERY OF THE GRE ISETHIONATE SULFOLYASE IseG

Two independent reports in 2019, one from our group (48) and the other from Schleheck and colleagues (49), described the discovery of a GRE isethionate lyase (IseG) and its activating enzyme (IseH) catalyzing the C–S bond cleavage of isethionate to generate sulfite and acetaldehyde. In the study by Schleheck and colleagues, IseG (which they term IslA) was identified through differential proteomics. The GRE, activating enzyme, and other proteins in its gene cluster were strongly induced in taurine- and isethionate-grown *B. wadsworthia* 3.1.6 and in isethionate-grown *Desulfovibrio desulfuricans* DSM642 and *D. alaskensis* G20. O_2-sensitive isethionate lyase activity was detected in cell-free extracts and confirmed through anaerobic assays of recombinantly produced and reconstituted GRE and activating enzyme from *B. wadsworthia* (*Bw*IseG; k_{cat} = 108 s^{-1} per G•, K_m = 8.1 mM, $k_{cat}/K_m = 1.4 \times 10^4$ M^{-1} s^{-1} per G•). The GRE from *D. desulfuricans* was also tested; results were similar. Genetic knockouts in *D. alaskensis* showed that the GRE is essential for growth on isethionate.

In our laboratory, IseG was encountered as part of a bioinformatics study of the GRE family using tools created and made publicly available by the Enzyme Function Initiative (70). A similar analysis of the GRE family was described by Balskus and colleagues (67) and Gerlt and colleagues (68). In these analyses, protein sequences were organized as a sequence similarity network, in which each node corresponds to a unique protein sequence, and closely related sequences are connected with an edge, such that sequences clustered corresponding to putative isozymes. We noticed that, although most of the GRE clusters contain sequences predominantly from fermenting bacteria, one of the clusters contains sequences predominantly from SSRB, suggesting a link to their unique metabolism.

Members of this GRE cluster were previously highlighted in a bioinformatics study by Zarzycki et al. (71), who investigated the association of GREs with bacterial microcompartments (BMCs), proteinaceous organelles that encapsulate enzymes of certain metabolic pathways. The aldehyde-generating GREs PDH and CutC, together with aldehyde and alcohol dehydrogenases downstream in the catabolic pathways, are often associated with BMCs, which are thought to sequester their volatile and toxic products. Apart from PDH and CutC, Zarzycki et al. reported a third BMC-associated GRE of unknown function that is closely related to CutC, GDH, and PDH. In its genome neighborhood, we observed the presence of aldehyde dehydrogenase, suggesting an

aldehyde product, and a TRAP (tripartite ATP-independent periplasmic) transporter, which is generally involved in the import of carboxylates and sulfonates. We thus hypothesized that this unknown GRE is an isethionate sulfolyase, which was experimentally confirmed with recombinant IseGH from the model sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough (*Dv*IseG; $k_{cat} = 92\ s^{-1}$ per G•, $K_m = 45$ mM, $k_{cat}/K_m = 2.0 \times 10^3\ M^{-1}\ s^{-1}$ per G•).

STRUCTURE AND CATALYTIC MECHANISM OF IseG

The *Dv*IseG active site is most similar to that of GDH (64), PDH (65), and CutC (66) (**Figure 5*a*,*b***), suggesting similarities between their reaction mechanisms (48) (**Figure 5*c***). The catalytic cycles of GDH and PDH have been studied both experimentally and computationally (72, 73). The C1–OH is coordinated with a conserved Glu residue, and the C2–OH leaving group is coordinated with two His residues and one Asp residue (**Figure 5*a***). In the mechanistic model, Cys• carries out a reversible abstraction of the C1–OH atom of glycerol, generating the substrate radical. Dehydration of the substrate radical is catalyzed by deprotonation of the C1–OH by the conserved Glu general base residue and by protonation of the C2–OH leaving group by a conserved His residue, generating a ketyl radical intermediate. This then abstracts the Cys hydrogen atom to form the product propionaldehyde and to regenerate Cys• (**Figure 5*c***). The mechanism of GDH is distinct from that of the coenzyme B_{12}-dependent diol dehydratase (DhaB), which involves a 1,2-migration of the C2–OH group instead of direct elimination. The difference between the two mechanisms was elegantly demonstrated with C1–^{18}OH–labeled propanediol, in which the label was retained in the GDH product but lost with 50% efficiency in the DhaB product (73).

The isethionate-bound active site of IseG contains the conserved G• and Cys• residues and the Glu general base residue, and their positions relative to isethionate suggest a reaction mechanism involving C1–H abstraction and C1–OH deprotonation, analogous to GDH, PDH, and CutC (48, 64–67) (**Figure 5*a*,*b***). However, the orientation of isethionate was markedly different from the orientation of the PDH and CutC substrates, with a 98.5° rotation between the C1–C2 axes of isethionate versus choline. The altered position of the leaving group is accommodated by shifts in two substrate-interacting loops in IseG relative to CutC, with the isethionate sulfonate group stabilized by an extensive H-bond network involving Arg187, Gln191, Arg676, and a water molecule (**Figure 5*b***). As a result of the different substrate orientation, the *pro*-(*R*) hydrogen atom of isethionate is positioned for abstraction by Cys•. A hydrogen atom of the opposite stereochemistry is abstracted in GDH, PDH, and CutC (**Figure 5*b***). Like the substrates of CutC, GDH, and PDH, isethionate is bound in a gauche conformation thought to promote leaving group elimination. A desulfonylation mechanism analogous to the reaction mechanisms of GDH, PDH, and CutC was proposed (**Figure 5*c***). Further evidence to support this model or an alternative model involving 1,2-migration of the SO_3^- group remains to be explored.

IseG IN TAURINE AND ISETHIONATE DISSIMILATORY DEGRADATION BY SSRB

Growths of gram-negative *Desulfovibrio desulfuricans*, *D. alaskensis*, *D. piger* DSM 749, and *B. wadsworthia*, with isethionate as the TEA, were accompanied by strong induction of IseG (48, 49). In earlier studies, Leadbetter and colleagues (39, 74) observed that growths of diverse gram-negative and gram-positive SSRB on isethionate induced an unidentified 97-kDa protein, which we now believe corresponds to IseG, suggesting the involvement of IseG in isethionate dissimilation in these bacteria. The SSRB converted the isethionate sulfur to H_2S and carbon to acetate (39, 74, 75). None of these SSRB is capable of further oxidizing acetate to CO_2.

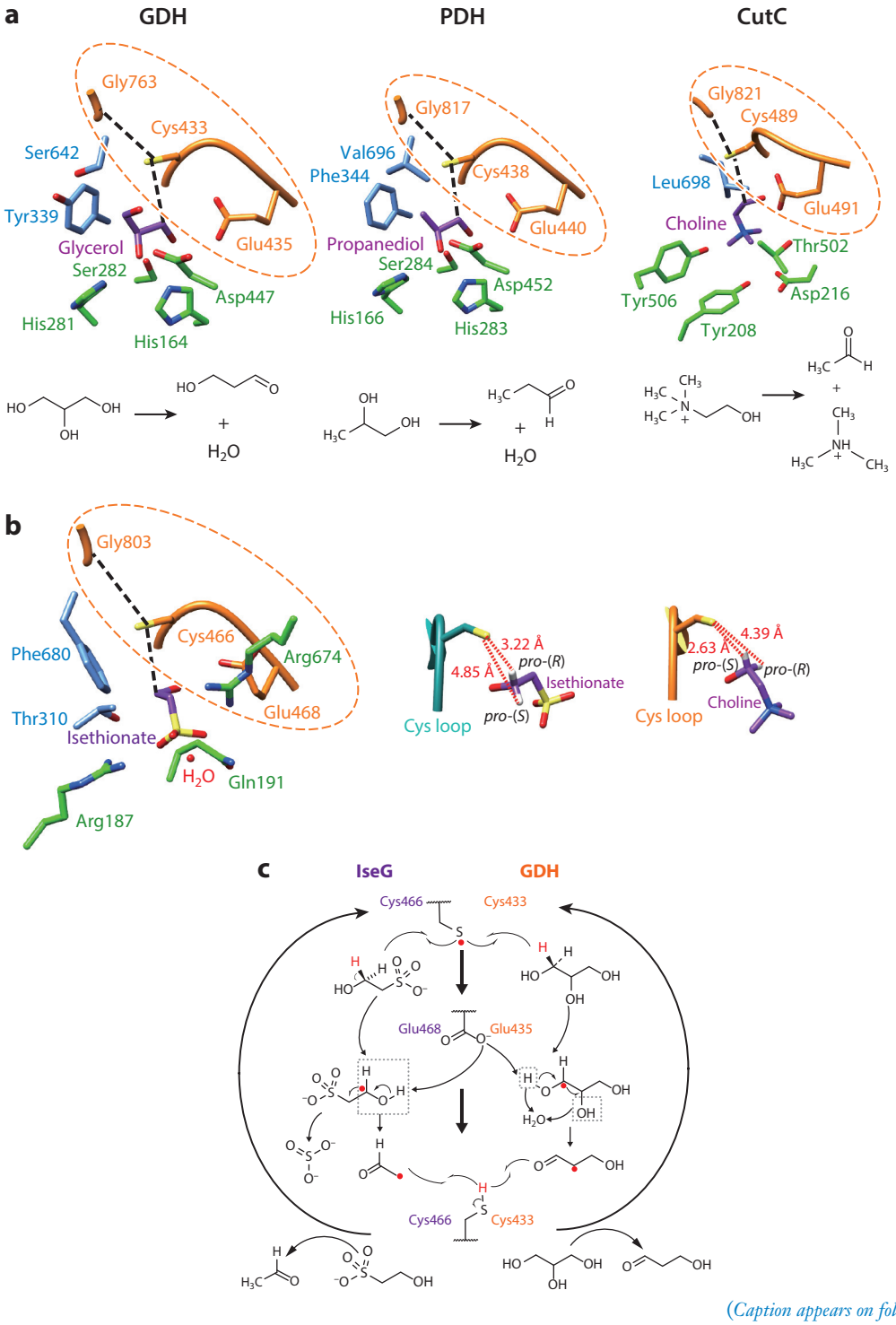


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Figure 5 (*Figure appears on preceding page*)

The active sites of known GRE 1,2-eliminases and the active site and catalytic mechanisms of IseG. (*a*) Comparisons of the active sites of GRE 1,2-eliminases: GDH, PDH, and CutC. Residues involved in conserved radical chemistry and C1–OH deprotonation are labeled in orange and circled. The proposed pathway for hydrogen atom transfer is indicated by dashed black lines. Residues interacting with the C2 leaving group and other residues contributing to the substrate specificity are labeled in green and cyan, respectively. The substrates, glycerol, propanediol, and choline, are displayed in purple. The corresponding chemical reactions catalyzed by these enzymes are shown below the structures. (*b*) The active site of IseG with the orientation of the substrate relative to the Cys thiyl radical highlighted and compared with CutC. (*c*) The proposed catalytic mechanisms of IseG and GDH. Abbreviations: CutC, choline trimethylamine-lyase; GDH, glycerol dehydratase; GRE, glycyl radical enzyme; IseG, isethionate lyase; PDH, propanediol dehydratase.

The IseG gene cluster in *B. wadsworthia* contains a NAD^+- and CoA-dependent acetaldehyde dehydrogenase (EutE), which converts the IseG product acetaldehyde to acetyl-CoA (49). Also present is phosphotransacetylase, which converts acetyl-CoA to acetyl phosphate, subsequently used for substrate-level phosphorylation by acetate kinase, generating acetate and ATP (**Figure 6*a***). IseG-containing gene clusters in SSRB are associated with a variety of putative

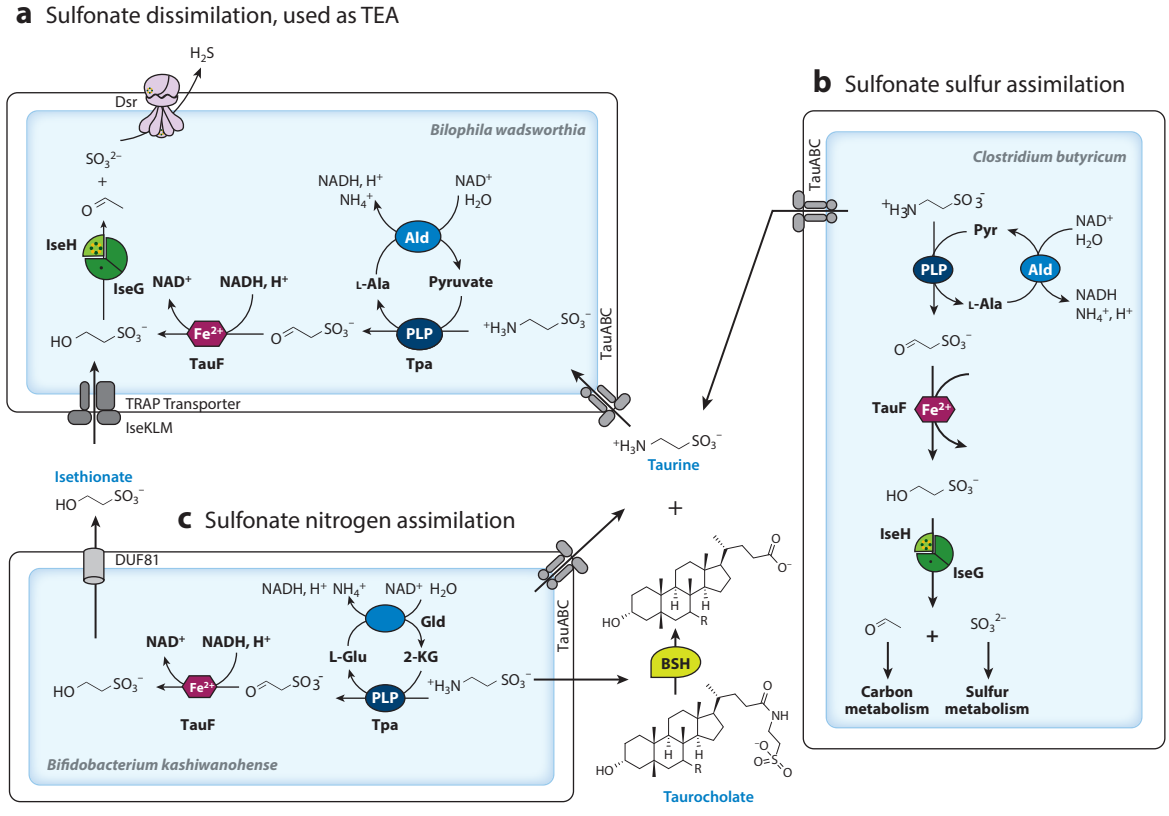


Figure 6

Interspecies metabolic web of C2 sulfonate degradation in the gut microbiome involving IseG-dependent pathways. (*a*) Sulfonate dissimilation. Use of C2 sulfonates as TEAs in SSRB, exemplified by *Bilophila wadsworthia*. (*b*) Sulfonate sulfur assimilation in anaerobic fermenting bacteria, exemplified by *Clostridium butyricum*. (*c*) Sulfonate nitrogen assimilation in fermenting bacteria, exemplified by *Bifidobacterium kashiwanohense*. Abbreviations: Ald, alanine dehydrogenase; BSH, bile salt hydrolase; Dsr, dissimilatory sulfite reductase; Gld, glutamate dehydrogenase; IseG, isethionate lyase; IseH, IseG-activating enzyme; PLP, pyridoxal phosphate; SSRB, sulfate- and sulfite-reducing bacteria; TauF, sulfoacetaldehyde reductase; TEA, terminal electron acceptor; Tpa, taurine:pyruvate aminotransferase.

isethionate transporters. Isothermal calorimetry experiments showed that the soluble periplasmic substrate-binding subunit (IseK) of a TRAP transporter binds isethionate with high affinity ($K_d = 0.5$ μM) (48).

IseG is also involved in the taurine degradation pathway in *B. wadsworthia* (48, 49). Like in many other taurine-degrading organisms, the pathway in *Bilophila* involves conversion of taurine to sulfoacetaldehyde by Tpa and Ald (76). However, instead of cleavage by Xsc, sulfoacetaldehyde is reduced to isethionate by the NADH-dependent sulfoacetaldehyde dehydrogenase TauF and then desulfonated by IseG (**Figure 6*a***). So far, IseG-dependent taurine dissimilation has been confirmed only in *B. wadsworthia* and its presence in other SSRB remains to be explored.

IseG IN TAURINE AND ISETHIONATE SULFUR ASSIMILATION BY FERMENTING BACTERIA

Several *Clostridium* species were previously reported to utilize taurine and isethionate as sulfur sources for growth (36, 43), with the mechanism of desulfonylation unknown. These species are not known to contain Xsc, and subsequent genome sequencing revealed a gene cluster containing, in addition to IseG and its activating enzyme, IseH, a putative taurine ABC transporter, an isethionate TRAP transporter, a Tpa, and a metal-dependent alcohol dehydrogenase (TauF) found to catalyze sulfoacetaldehyde reduction (77). This discovery suggested pathways for taurine and isethionate degradation similar to those in SSRB.

Predicted activities were confirmed with recombinant enzymes (*Cb*IseGH, *Cb*Tpa, and *Cb*TauF) from *C. butyricum* E4 str. BoNT E BL5262, an isolate from human feces (77) (**Figure 6*b***). *Cb*IseG shares 63% identity with *Dv*IseG, and their active-site residues are identical. However, the kinetic parameters of *Cb*IseG ($k_{cat} = 3.3\ s^{-1}$ per G•, $K_m = 6.7$ mM) differed substantially from those of *Dv*IseG. The lower turnover number and higher substrate affinity may be rationalized by the differing requirements of the assimilatory versus dissimilatory pathways. The primary sequences of *Cb*Tpa and *Cb*TauF were distantly related to those of the corresponding *Bilophila* enzymes, suggesting convergent evolution of taurine degradation in fermenting bacteria and SSRB. Other fermenting bacteria containing IseG include facultatively anaerobic gram-negative plant pathogens (e.g., *Brenneria goodwinii*, *Pectobacterium wasabiae*) and strictly anaerobic gram-positive bacteria isolated from soil (e.g., *C. chromiireducens*, *C. cylindrosporum*) and human feces (e.g., *C. butyricum*, *Anaerostipes hadrus*), suggesting the importance of sulfonates as sulfur sources in these environments. Taken together, IseG homologs can be used for sulfur assimilation from C2 sulfonates by a variety of anaerobic or facultatively anaerobic bacteria inhabiting different environments.

CONVERSION OF TAURINE TO ISETHIONATE FOR NITROGEN ASSIMILATION BY FERMENTING BACTERIA

Isethionate in the human body is thought to be a by-product of taurine metabolism by intestinal bacteria (9), although the microbial species responsible has not been isolated. Studies from the Cook laboratory show that taurine nitrogen can be assimilated by environmental bacteria, accompanied by excretion of carbon and sulfur as C2 sulfonates, including isethionate (78, 79), sulfoacetaldehyde (80), or sulfoacetate (80, 81). In the marine gammaproteobacterium *Chromohalobacter salexigens* DSM 3043, taurine is imported by TauABC and converted to sulfoacetaldehyde by a taurine:oxoglutarate aminotransferase, and then sulfoacetaldehyde is reduced to isethionate by the NADPH-dependent sulfoacetaldehyde reductase (IsfD) and exported by a transporter in the TauE family (IsfE) (79). This pathway is also present in the gammaproteobacterium *Klebsiella*

pneumoniae (79), a facultative anaerobe and commensal bacterium from the human gut (**Table 1**). The key isethionate-forming enzyme IsfD is a member of the short-chain dehydrogenase/reductase (SDR) family. Structural and biochemical characterization of IsfD from *Klebsiella oxytoca* showed that it was indistinguishable from ubiquitous 3-hydroxyacid dehydrogenases (82), reflecting the structural similarities of their substrates.

Other possible sources of isethionate are strictly anaerobic fermenting bacteria, including members of *Firmicutes* and *Bifidobacteriales*. Some of these species contain a gene cluster similar to that in *C. salexigens*, but with IsfD replaced by TauF, which is a member of the metal-dependent alcohol dehydrogenase family (82, 83). Enzymatic activity was confirmed for taurine:oxoglutarate aminotransferase and TauF from *Bifidobacterium kashiwanohense*, an isolate from human feces (**Figure 6*c***; **Table 1**). However, we were unsuccessful at culturing any of the bacteria containing this cluster in defined media with taurine as the sole nitrogen source. *B. kashiwanohense* taurine:oxoglutarate aminotransferase and TauF were crystallographically characterized (82, 83), which will facilitate further bioinformatics-based prediction of isethionate-producing gut bacteria.

SULFONATE DEGRADATION AND INTESTINAL H_2S PRODUCTION

Metabolism of sulfonates and other sulfur compounds by the consortium of anaerobic bacteria in the intestinal system is of special relevance to human health (84). While carbon and nitrogen are metabolized largely to ammonia and a variety of organic acids and gases, a large proportion of sulfur is converted to H_2S, with potentially toxic consequences. Examples include fermentation of cysteine by *Fusobacterium nucleatum* (85) (**Table 1**), reduction of sulfate derived from host-derived sulfated glycans by *D. piger* (41), and degradation of taurine by *B. wadsworthia* (40, 42), all of which produce H_2S and have been linked to various diseases and conditions.

The normal physiological functions and pathological effects of H_2S in the human body are subjects of ongoing research and are just beginning to be understood. At low levels, H_2S is recognized as a gasotransmitter (86) and is involved in a myriad of physiological processes, including blood pressure regulation (87), neuronal protection (88, 89), mitochondrial biogenesis (90), antioxidative stress (91), and anti-inflammation (92). In mammals, endogenous H_2S is produced primarily by three enzymes: cystathionine-β-synthase, cystathionine-γ-lyase, and 3-mercaptopyruvate sulfurtransferase. It exists and is transmitted in different forms (e.g., sulfane sulfur, polysulfide, HS^-) (93) and modifies proteins primarily by sulfhydration of key Cys residues, modulating enzyme activities (88), ion channel gating (94, 95), protein–protein interactions (91, 96), transcriptional levels (90), and protein degradations (96). However, at high levels, H_2S is toxic (97). Neuronal toxicity caused by Down syndrome and inflammatory cytokines, associated with the overexpression of cystathionine-β-synthase and hence elevated H_2S levels, has been observed (96).

H_2S production by intestinal SSRB is thought to exert toxic effects through several mechanisms that have been investigated. H_2S is a genotoxic agent, with connections to colorectal cancer (98, 99). It cleaves disulfide linkages in the gut mucosal barrier, increasing its permeability to toxins and pathogens and thus leading to chronic inflammatory conditions (100). H_2S is a metabolic toxin to colonocytes, inhibiting their O_2-consuming butyrate oxidation (101), and the resulting decreased anaerobicity promotes dysbiosis through the expansion of facultatively aerobic gram-negative bacterial pathogens (102). In addition, H_2S is oxidized by gut epithelial tissue during the inflammatory response, generating tetrathionate as a TEA for growth of pathogenic *Salmonella* species (103). Thus, an understanding of intestinal sulfonate-derived H_2S production has great relevance to human health and disease.

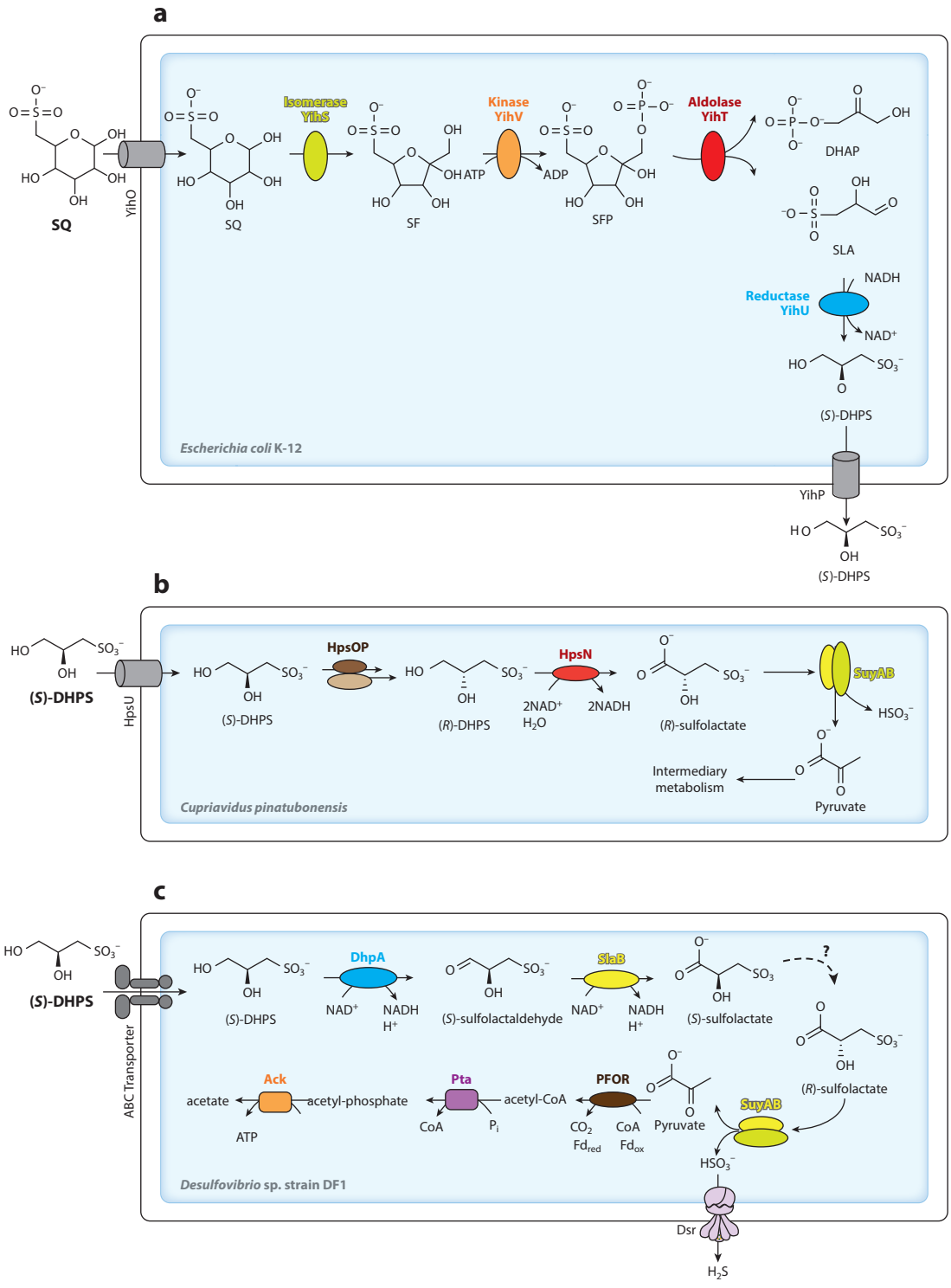
The sulfonates most relevant to gut sulfur metabolism are taurine and its derivatives. Taurine is biosynthesized from cysteine and also acquired from the diet (7). Mammals are unable to degrade taurine. Excess taurine is excreted through urine or to the gut in the form of conjugated bile salts such as taurocholate, which aids the emulsification and digestion of dietary fat. Once in the gut, taurocholate is hydrolyzed by fermenting bacteria such as lactobacilli and bifidobacteria through secreted bile salt hydrolases, making taurine available as a substrate for utilization by anaerobic gut bacteria (**Figure 6*c***). Humans produce two forms of conjugated bile salts, taurine-conjugated taurocholate and glycine-conjugated glycocholate, with the former being more abundant in a meat-rich diet (42). In addition, bile secretion is stimulated by a diet rich in saturated animal fat. Therefore, the abundance of taurocholate and taurine in the gut, and the consequent H_2S production and associated ailments, is highly correlated with diet (42).

B. wadsworthia is the sulfonate-degrading SSRB most closely associated with human health and disease. This bacterium was first isolated from gangrenous appendiceal tissue (104), and its growth requirement for bile was later traced to its dependence on taurine or taurine-conjugated bile salts (105). Its pathogenicity was linked to its ability to use taurine as a TEA and to the reduction of the sulfonate sulfur to H_2S (19, 42). *B. wadsworthia* was found at low levels in the normal fecal flora, but its outgrowth has been correlated with inflammation in the colon (104). Besides diseased gut tissue, it has also been isolated from a wide variety of other infections, for example, biliary tract infection, liver abscess, and ear infections, where growth may be supported by taurine release from necrotic tissues (40). *B. wadsworthia* lacks common virulence factors such as a capsule, but it possesses endotoxin as do most gram-negative bacteria (104). It can use electron donors available in the gut lumen, including lactate, formate, and H_2 (40, 106), and it uses as TEAs sulfite, thiosulfate, and several sulfonates, including taurine, isethionate, cysteate (40), sulfolactate (107), DHPS (108), but not sulfate. Thus, the IseG-dependent taurine reduction pathway is expected to play an important part in the metabolic niche of this bacterium in the gut. *B. wadsworthia* is a prime model organism of SSRB for the investigation of sulfonate dissimilation, and a better understanding of the metabolism of this pathogenic organism may facilitate development of prophylactics and therapeutics.

D. piger is another SSRB that is highly relevant to human biology. This bacterium is the most common sulfate-reducing bacterium in a survey of healthy US adults and has served as a model human gut SSRB (48). Its prevalence has also been associated with inflammatory bowel disease (109). IseG is present in all five SSRB genomes currently in the National Institutes of Health Human Microbiome Project (**https://www.hmpdacc.org/**), suggesting that sulfonate degradation is an important factor in the metabolic niche of gut SSRB (48). Despite the abundance of taurine in the gut, most sulfonate-reducing SSRB that have been investigated, including *D. piger*, can use isethionate but not taurine as a TEA. Thus, cross-feeding from fermenting bacteria that convert taurine to isethionate may be an important component of sulfonate sulfur metabolism in the gut, and this requires further investigation (**Figure 6*c***).

SULFOGLYCOLYSIS AND OTHER SOURCES OF DHPS

The C3 sulfonate DHPS is present in high concentrations as an osmolyte in marine phototrophs; it is also a product of microbial sulfoquinovose degradation through certain recently discovered pathways termed sulfoglycolysis (110, 111). Three sulfoglycolytic pathways have been reported; the first two are analogous to the Embden-Meyerhof-Parnas (sulfo-EMP) pathway (111) (**Figure 7*a***) and the Entner-Doudoroff (sulfo-ED) pathway, respectively (112, 113), and the third is a transaldolase-dependent (sulfo-TAL) pathway (114, 115). Despite similarities between the glycolytic and corresponding sulfoglycolytic pathways, the analogous reactions are indeed catalyzed



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(*S*)-DHPS formation and degradation. (*a*) Sulfo-EMP pathway in *Escherichia coli* K-12. (*b*) SuyAB-dependent (*S*)-DHPS degradation in hydrogen-oxidizing, facultatively chemolithotrophic bacteria *Cupriavidus pinatubonensis*. (*c*) SuyAB-dependent (*S*)-DHPS degradation in anaerobic SSRB *Desulfovibrio* sp. strain DF1. The question mark represents an unresolved intervening step that converts (*S*)-sulfolactate to (*R*)-sulfolactate. Abbreviations: Ack, acetate kinase; DhpA, NAD^+-dependent (*S*)-DHPS 3-dehydrogenase; DHPS, dihydroxypropanesulfonate; Dsr, dissimilatory sulfite reductase; Fd_{ox}, oxidized form of ferredoxin; Fd_{red}, reduced form of ferredoxin; HpsN, NAD^+-dependent (*R*)-DHPS 3-dehydrogenase; HpsO, $NADP^+$-dependent (*S*)-DHPS 2-dehydrogenase; HpsP, NAD^+-dependent (*R*)-DHPS 2-dehydrogenase; Pta, phosphate acetyltransferase; SFOR, (*S*)-sulfolactaldehdye:Fdx (ferredoxin) oxidoreductase; SlaB, NAD^+-dependent sulfolactaldehyde dehydrogenase; SQ, sulfoquinovose; SSRB, sulfate- and sulfite-reducing bacteria; sulfo-EMP, sulfoglycolytic Embden-Meyerhof-Parnas; SuyAB, (*R*)-sulfolactate sulfolyase.

by completely different enzymes belonging to separate enzyme families. In all three pathways, desulfonation does not occur and a C3 sulfonate is produced, which may be subsequently degraded by other bacteria (116).

The sulfo-EMP pathway was studied in *E. coli*; it is widespread among *Enterobacteriaceae*, which are typically facultatively anaerobic bacteria (111). In this pathway, sulfoquinovose is converted to (*S*)-DHPS, which is excreted, and dihydroxyacetone-phosphate, which is further metabolized in the cell. The pathway functions during aerobic growth and anaerobic mixed acid fermentation (107) (**Figure 7*a***). The sulfo-ED pathway was discovered in the soil isolate *Pseudomonas putida* SQ1 (**Table 1**) and is present in diverse gram-negative bacteria, including *Alpha-*, *Beta-*, and *Gammaproteobacteria*, which are typically aerobic bacteria with respiratory metabolism. In the sulfo-ED pathway, sulfoquinovose is converted to (*S*)-sulfolactate, which is excreted, and pyruvate, which is further metabolized via the tricarboxylic acid cycle (112). The occurrence of the sulfo-EMP and sulfo-ED pathways in different types of bacteria mirrors that of the classic EMP and ED pathways. The sulfo-TAL pathway is present in diverse gram-positive bacteria and may generate either (*S*)-DHPS or (*S*)-sulfolactate (114). In summary, all three sulfoglycolytic pathways produce a C3 sulfonate as a waste product in the form of either sulfolactate or DHPS.

DESULFONYLATION OF C3 SULFONATES IN AEROBIC AND FACULTATIVELY AEROBIC BACTERIA

The microbial degradation of C3 sulfonates has been studied for decades, and similar to the case for C2 sulfonates, much more is known about the processes in aerobic bacteria than in anaerobic bacteria. The diverse pathways for dissimilation of the C3 sulfonates in aerobic and facultatively aerobic bacteria have been previously reviewed (16). Several of these C3 sulfonates, including DHPS, are biosynthesized in large volumes by globally abundant eukaryotic marine phototrophs, and their release through secretion or cell lysis and subsequent rapid degradation by aerobic bacteria constitute a significant proportion of carbon flux in the aerobic surface ocean (13, 14).

Enzymes catalyzing C–S bond cleavage of C3 sulfonates include the pyridoxal phosphate (PLP)-dependent L-cysteate sulfolyase (CuyA) (117) and the Fe(II)-containing (*R*)-sulfolactate sulfolyase (SuyAB), a homolog of altronate and galactarate dehydratases (118) (**Figure 7*b***). Unlike for C2 sulfonate degradation, an important factor in the degradation of C3 sulfonates is the presence of a stereogenic center (119). For example, in the DHPS degradation pathway in *Cupriavidus pinatubonensis* (120), the stereocenter of (*S*)-DHPS is inverted via oxidation by the $NADP^+$-dependent (*S*)-DHPS 2-dehydrogenase HpsO and subsequent reduction by the NAD^+-dependent (*R*)-DHPS 2-dehydrogenase HpsP. Oxidation of the (*R*)-DHPS to (*R*)-sulfolactaldehyde and then to (*R*)-sulfolactate by the NAD^+-dependent (*R*)-DHPS 3-dehydrogenase HpsN is followed by cleavage by SuyAB (**Figure 7*b***; **Table 1**).

DESULFONYLATION OF C3 SULFONATES IN STRICTLY ANAEROBIC BACTERIA

Sulfoquinovose is found in a diet that includes vegetables and other green plant materials, and could contribute to sulfur metabolism in the anaerobic human gut. Anaerobic sulfoquinovose fermentation by bacteria such as *E. coli* is expected to generate (*S*)-DHPS, and a study by Schleheck and colleagues (107) demonstrated that the (*S*)-DHPS could be subsequently degraded by SSRB, generating H_2S. *Desulfovibrio* sp. strain DF1, isolated from sewage sludge, carried out DHPS fermentation, using it as the sole carbon and energy source with no requirement for additional electron donors or acceptors. A coculture of *E. coli* and *Desulfovibrio* carried out the complete degradation of sulfoquinovose, converting the sulfonate sulfur to H_2S.

Genome sequencing and differential proteomics analysis of *Desulfovibrio* sp. strain DF1 grown with racemic DHPS suggested that (*S*)-DHPS is imported by a DHPS ABC transporter and oxidized to (*S*)-sulfolactaldehyde by the NAD^+-dependent (*S*)-DHPS 3-dehydrogenase DhpA. Oxidation of (*S*)-sulfolactaldehyde to (*S*)-sulfolactate is catalyzed through either the NAD^+-dependent sulfolactaldehyde dehydrogenase SlaB or a putative (*S*)-sulfolactaldehyde:Fdx (ferredoxin) oxidoreductase (SFOR), a homolog of the TPP and [4Fe-4S]-containing pyruvate:Fdx oxidoreductase. The genes that encode DhpA, SlaB, and SFOR are located on the same gene cluster and are induced in DHPS-grown cells. Activity of DhpA was confirmed with the recombinant enzyme, whereas the activity of the O_2-sensitive SFOR was not detected in lysates or for the recombinant protein and requires further investigation. The last step in the pathway is desulfonylation catalyzed by SuyAB. An intervening step that has yet to be resolved is the conversion of (*S*)-sulfolactate to (*R*)-sulfolactate (see the question mark in **Figure 7*c***; **Table 1**). SuyAB is present in many SSRB, including *Bilophila*, which was reported to use sulfolactate as a TEA.

(*S*)-DHPS DEGRADATION INVOLVING THE GRE DHPS SULFOLYASE HpsG

Two alternative pathways for anaerobic DHPS degradation involving the GREs (*S*)-DHPS sulfolyase HpsG (**Figure 8**) and (*S*)-DHPS dehydratase HpfG (**Figure 9**) were recently reported. A closer examination of the IseG cluster in the GRE sequence similarity network led to the discovery of HpsG, a close homolog of IseG (108). *B. wadsworthia* contains both IseG and HpsG, which are specifically induced during growth on isethionate and DHPS, respectively (48, 108). Unlike IseG, HpsG was not associated with BMC proteins, consistent with its product being acetol, not an aldehyde. Activity of *B. wadsworthia* HpsG (*Bw*HpsG) was confirmed in assays with racemic DHPS ($k_{cat} = 26\ s^{-1}$ per G•, $K_m = 13$ mM). HpsG is associated with the TRAP transporter HpsKLM, and isothermal calorimetry experiments with the soluble substrate-binding protein and racemic DHPS demonstrated that HpsK bound DHPS ($K_d = 6.7\ \mu M$) but did not bind isethionate or 3-hydroxypropane-1-sulfonate.

The active site in the substrate-bound crystal structure of *Bw*HpsG is highly similar to that of *Dv*IseG but contains several alterations in order to accommodate the larger substrate (108) (**Figure 8*b***). The relative positions of the conserved Cys• and Glu464 general base residues favor C2–H abstraction and perhaps C2–OH deprotonation of (*S*)-DHPS, and the sulfonate group is coordinated by Arg183, Gln187, and Arg672. The conformation of (*S*)-DHPS in HpsG was different from that of isethionate in IseG, with a 160° rotation between the C2–C3–S plane of (*S*)-DHPS relative to the C1–C2–S plane of isethionate. However, this change in conformation preserved the gauche conformation of the substrate, favoring elimination of the leaving group (**Figure 8*b***). The replacement of Phe680 and Thr310 in IseG with the less bulky Ile676 and

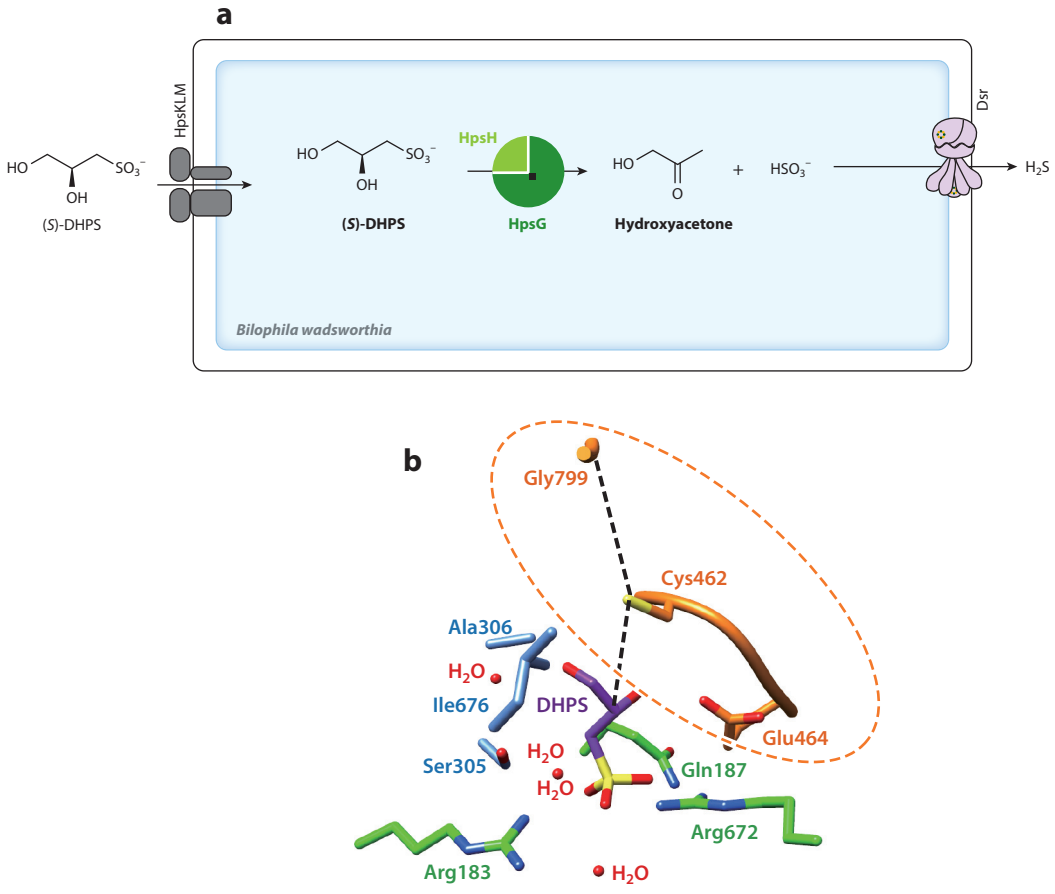

Figure 8

A GRE-dependent (*S*)-DHPS degradation pathway in SSRB and the active site of HpsG. (*a*) HpsG-dependent (*S*)-DHPS degradation pathway in *Bilophila wadsworthia*. (*b*) The active-site structure of HpsG. Residues involved in conserved radical chemistry and C2–OH deprotonation are labeled in orange and circled. The proposed pathway for hydrogen atom transfer is indicated by dashed black lines. Residues interacting with the sulfonate leaving group and other residues contributing to the substrate specificity are labeled in green and cyan, respectively. The substrate, (*S*)-DHPS, is displayed in purple. Abbreviations: DHPS, dihydroxypropanesulfonate; Dsr, dissimilatory sulfite reductase; GRE, glycyl radical enzyme; HpsG, (*S*)-DHPS sulfolyase; HpsH, HpsG-activating enzyme; SSRB, sulfate- and sulfite-reducing bacteria.

Ala306 in HpsG (compare **Figure 5*b*** with **Figure 8*b***) results in a larger active-site cavity that accommodates the C1 hydroxymethyl group and altered (*S*)-DHPS conformation, and the substrate is stabilized by an H-bond network involving three water molecules in the active site (**Figure 8*b***).

IseG and HpsG occupy two distinct branches on the phylogenetic tree. Although isethionate desulfonylation may be initiated in theory by the abstraction of either of the diastereotopic C1–H atoms, only one C2–H atom is available for abstraction in (*S*)-DHPS. A scenario in which IseG evolved from DHPS might explain the unusual stereochemistry of hydrogen atom abstraction in IseG compared with that in other mechanistically related GREs. UniProt (**https://www.uniprot.org/**) contains many more sequences for IseG than for HpsG, which, apart from the intestinal bacterium *B. wadsworthia*, are also present in environmental SSRB, including several from anaerobic marine environments, where DHPS is expected to be abundant.

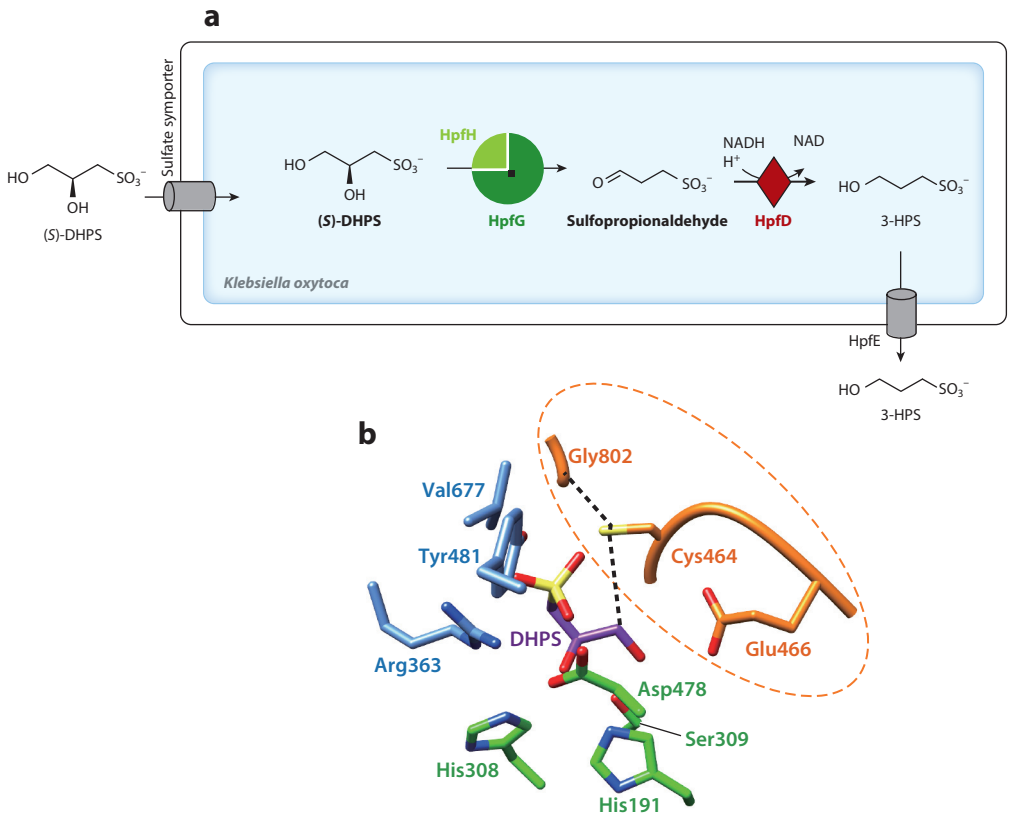

Figure 9

A GRE-dependent (*S*)-DHPS degradation pathway in fermenting bacteria and the active site of HpfG. (*a*) HpfG-dependent (*S*)-DHPS degradation pathway in *Klebsiella oxytoca*. (*b*) The active site of a homology-modeled HpfG structure. Residues involved in conserved radical chemistry and C1–OH deprotonation are labeled in orange and circled. The proposed pathway for hydrogen atom transfer is indicated by dashed black lines. Residues interacting with the C2 hydroxyl leaving group and other residues contributing to the substrate specificity are labeled in green and cyan, respectively. The substrate, (*S*)-DHPS, is displayed in purple. Abbreviations: 3-HPS, 3-hydroxypropane-1-sulfonate; DHPS, dihydroxypropanesulfonate; GRE, glycyl radical enzyme; HpfD, NADH-dependent sulfopropionaldehyde reductase; HpfG, (*S*)-DHPS dehydratase; HpfH, HpfG-activating enzyme.

(*S*)-DHPS DEGRADATION INVOLVING THE GRE DHPS DEHYDRATASE HpfG

DHPS has two C–OH groups and in theory could be a substrate of a GRE diol dehydratase catalyzing C–O cleavage. Analysis of the GRE sequences in UniProt and subsequent biochemical investigations indeed led to the identification of such an enzyme, the (*S*)-DHPS dehydratase HpfG (108). Activity was confirmed with recombinant HpfG from *K. oxytoca* HpfG (*Ko*HpfG; k_{cat} = 130 s^{-1}, K_m = 5.0 mM). Like other GRE diol dehydratases, the active site in the homology model of HpfG contains a Glu general base residue for deprotonation of the substrate C1–OH and two His residues and one Asp residue for coordination and protonation of the C2–OH, respectively (compare **Figure 5*a*** with **Figure 9*b***). The Arg363 residue, absent in GDH/PDH, may be involved in sulfonate binding. HpfG is associated with NADH-dependent sulfopropionaldehyde reductase HpfD and an exporter in the TauE family (HpfE), suggesting reduction of sulfopropionaldehyde

to sulfopropanol followed by export via HpfE. The physiological role of this pathway is believed to restore redox balance during fermentation by consuming NADH.

HpfG is present in gram-negative *Gammaproteobacteria* (e.g., *Vibrio*) and gram-positive *Clostridia* and lactobacilli, where it may be involved in different fermentation pathways (**Table 1**). Several of the HpfG sequences are associated enzymes in the sulfo-EMP pathway in *Gammaproteobacteria* and the sulfo-TAL pathway in *Firmicutes* bacteria, suggesting that they extend the pathways for mixed acid fermentation of sulfoquinovose by converting the (*S*)-DHPS product to sulfopropanol. HpfG is present in many bacteria isolated from marine environments, including facultatively anaerobic *Vibrio* and *Photobacterium* species present in seawater, in marine sediments, and as symbionts of marine animals and strictly anaerobic *Epulopiscium* species found in the gut microbiota of fish, suggesting a role in many marine anaerobic niches that exist in the largely aerobic ocean.

SUMMARY POINTS

1. Microbes, including aerobic and anaerobic bacteria, degrade sulfonates through a variety of assimilatory and dissimilatory pathways to obtain sulfur, nitrogen, carbon, and energy.
2. The glycyl radical enzymes (GREs) isethionate sulfolyase IseG and (*S*)-dihydroxypropanesulfonate (DHPS) sulfolyase HpsG catalyze sulfonate C–S bond cleavage.
3. Both IseG and HpsG are found predominantly in sulfate- and sulfite-reducing bacteria (SSRB), where they produce sulfite for use as a terminal electron acceptor.
4. IseG is also found in fermenting bacteria, where it produces sulfite for use as a sulfur source for growth.
5. Both IseG and HpsG are present in intestinal SSRB and play a role in intestinal H_2S generation.
6. Both IseG and HpsG are present in environmental bacteria and may play roles in sulfonate mineralization in anaerobic environments.
7. The GRE (*S*)-DHPS dehydratase HpfG is present in fermenting bacteria and provides an alternative and competing pathway for (*S*)-DHPS degradation.

FUTURE ISSUES

1. Intestinal bacterial species involved in sulfonate metabolism, particularly conversion of taurine to isethionate, require further investigation.
2. Several other GREs commonly present in SSRB have yet to be investigated and may reveal further radical desulfonation mechanisms.
3. The fate of SSRB sulfonate degradation products, such as 3-hydroxypropanesulfonate and 3-sulfopropionate, remains to be investigated.
4. Aromatic sulfonate sulfur assimilation in *Clostridium* species has been reported, but the mechanism of anaerobic aromatic desulfonation remains unexplored.
5. Anaerobic bacterial metabolism of other globally abundant sulfonates, such as the chemically inert methanesulfonate, remains to be established.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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