Towards a systematic understanding of structure-function relationship of dimethylsulfoniopropionate-catabolizing enzymes

Yin Chen, Hendrik Schäfer

School of Life Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom

Correspondence to Y Chen (y.chen.25@warwick.ac.uk) phone + 44 24 76528976

Abstract

Each year, several million tons of dimethylsulfoniopropionate (DMSP) are produced by marine phytoplankton and bacteria as an important osmolyte to regulate their cellular osmosis. Microbial breakdown of DMSP to the volatile gas dimethylsulfide (DMS) plays an important role in global biogeochemical cycles of the sulfur element between land and the sea. Understanding the enzymes involved in the transformation of DMSP and DMS holds the key to a better understanding of oceanic DMSP cycles. Recent work by Shao et al (2019) has resolved the crystal structure of two important enzymes, DmdB and DmdC, involved in DMSP transformation through the demethylation pathway. Their work represents an important step towards a systematic understanding of the structure-function relationships of DMSP catabolizing enzymes in marine microbes.

Dimethylsulfoniopropionate (DMSP) is arguably the best studied organosulfur compound which is produced by marine phytoplankton and bacteria. This is not least due to DMSP and dimethylsulfide (DMS), the volatile product of its degradation, being central to the CLAW hypothesis. This hypothesis states that the emission of DMS into the atmosphere and its oxidation to sulfate aerosol affect cloud nucleation leading to an increase in planetary albedo, which decreases solar irradiance and/or surface temperature, which in turn could exert a negative feedback on the growth of phytoplankton and hence the production of DMSP in the surface ocean (Charlson et al., 1987). Although the CLAW hypothesis is oversimplified as it becomes increasingly clear that the sources of oceanic CCN not only includes DMS-derived sulfate particles but also a range of organics and inorganics (Quinn & Bates, 2011), it has catalysed the collaboration of oceanographers, microbiologists and structural biologists which has led to an ever increasing understanding of the biological controls of DMSP catabolism in the oceans.

In the marine water column, DMSP can be catabolised through two major pathways, the demethylation pathway and the cleavage pathway (Figure 1). The demethylation pathway sequentially removes methyl groups from DMSP, resulting in the formation of acetaldehyde, which can be further oxidised to acetate, and methanethiol (Reisch et al 2011). This pathway is found in many ecologically important and numerically abundant marine bacteria, notably the SAR11 clade *Pelagibacter* spp. and the *Roseobacter* clade (reviewed in Curson et al., 2011).

The cleavage pathway, on the other hand, produces DMS as a product, and a diverse group of enzymes has been found to have this activity. Five of the DMSP lyase enzymes (DddK, DddQ, DddL, DddW and DddY) have a characteristic cupin motif, a metal-binding β-barrel comprised of three histidines and a glutamate residue (Lei et al., 2018). Crystal structures of three of these enzymes, DddQ, DddK and DddY (Table 1), have been resolved recently (Li et al., 2014; Brummett & Dey, 2016; Schnicker & Dey 2017; Li et al., 2017a). These cupin family

DMSP cleavage enzymes appear to be abundant in the marine environment and have been found in many marine bacteria, including *Pelagibacter* spp. and Roseobacters.

In addition to the cupin-motif DMSP lyases, at least three other DMSP cleavage enzymes have been identified to date, including DddD belonging to the CoA transferase family, DddP of the metallopeptidase family, and the algal Alma1 of the aspartate/glutamate racemase family (Alcolombri et al., 2015). Of the latter three non-cupin DMSP lyases, only the crystal structure of DddP is known (Table 1), although a model based on homology modelling for DddD has been successfully obtained (Alcolombri et al., 2014). DddP has a binuclear metal centre comprised of iron and another metal of choice (Hehemann et al., 2014; Wang et al., 2015). The *dddP* gene is widely present in many marine bacteria, notably the cosmopolitan marine SAR116 clade (Choi et al., 2015).

The first demonstration of microbial DMSP demethylation to methylmercaptopropionate (MMPA) and the suggestion of a DMSP demethylation pathway were based on analysis of DMSP degradation in anoxic marine sediments by Kiene and Taylor (1988). The gene encoding the DmdA enzyme that demethylates DMSP to methylmercaptopropionate (MMPA) was identified more than ten years ago (Howard et al., 2006). Further studies of the biochemistry and enzymology of DMSP demethylation identified the enzymes DmdB, DmdC and DmdD, catalysing the subsequent three steps of the DMSP-demethylation pathway; metagenomic data showed that these genes were widespread in marine bacterioplankton (Reisch et al., 2011). DmdA catalyses the initial step of DMSP demethylation, removing one methyl group and conjugating it to the C1 acceptor tetrahydrofolate (THF). DmdA belongs to a larger C1-conjugating glycine-cleavage T protein (GcvT) family, which includes other microbial osmolyte-catabolizing enzymes such as trimethylamine oxide demethylase (Tdm) (Zhu et al., 2014). Structural elucidation of DmdA confirms that this enzyme shares a tri-domain tertiary structure similar to that of GcvT and that the THF binding residues are highly conserved (Schuller et al., 2012).

The work presented in this issue by Shao et al., (2019) significantly advances our understanding of how MMPA is further catabolized by two enzymes in this pathway: DmdB, an ATP-dependent CoA ligase, and DmdC, a flavin-containing CoA dehydrogenase. DmdB shows high similarity to bacterial short-chain fatty acid CoA ligases—and, remarkably, the two homologous DmdB proteins of *Ruegeria pomeroyi* DSS-3 can accept a range of fatty acids as substrates (Bullock et al., 2014). The crystal structure of DmdB was obtained in the presence of ADP, a competitive inhibitor of this enzyme, and the structure of an inactive Lys523Ala mutant was obtained in complex with AMP and MMPA (Shao et al., 2019). DmdB forms an asymmetric dimer with ADP bound to one chain while leaving the binding pocket of the other chain empty. The lysine residue (Lys523) is strictly conserved in DmdB of both marine and terrestrial origins, and it is crucial for enzyme activity. Lys523 plays a dual role in DmdB catalysis by forming a hydrogen bond with the substrate MMPA while its side chain interacts directly with the phosphate moiety of ADP. The structure of the DmdB/AMP/MMPA complex also points to the role of several other residues in substrate coordination—His231, Trp235, Gly302 and Pro333—mutants of which all exhibit significantly diminished DmdB activity. DmdB appears to adopt a two-stage sequential binding of its substrates. Binding of ATP leads to a conformational change from the open conformation, leading to the subsequent interaction with MMPA, which acts a nucleophile to attack the P-O bond between the alpha- and beta-phosphate in ATP. This intermediate is then ready to accept the coenzyme, and a further rotation in the C-terminal domain with the aid of several residues by stabilizing CoA resulted in the formation of MMPA-CoA. Such a conformational change and C-terminal domain rotation during catalysis appears to be common in other enzymes of this family.

Shao *et al.*, (2019) also resolved the structure of the next enzyme in this pathway, DmdC. This enzyme belongs to the CoA dehydrogenase family (Reisch et al., 2011); members of this family are best known for their role in β-oxidation of fatty acids. DmdC appears as a homodimer, and each monomer is composed of 4 recognized functional domains. Structural

alignment of DmdC with the nematode fatty acid CoA dehydrogenase allowed the identification of key residues involved in cofactor (FAD) binding and substrate co-ordination. In particular, Shao *et al.* have identified two key residues, Phe195 and Glu435, that are vital for DmdC activity. These residues are responsible for FAD-binding and MMPA-CoA catalysis, respectively. Unsurprisingly, these two residues are strictly conserved in DmdC homologues in other bacteria.

Another interesting finding from this study by Shao *et al.* (2019) is that both DmdB and DmdC have significantly higher affinity for their substrates (lower K_M values, several mM), compared to those of DmdA and DMSP lyases, both of which are responsible for the first step of DMSP degradation (Lei et al., 2018). Interestingly, they have previously shown that enzymes responsible for downstream metabolism of DMSP degradation, particularly those involved in acrylate detoxification, had at least 100-fold higher affinity for their substrates (K_M values in tens of μM) (Wang et al., 2017). However, one needs to be cautious when comparing these K_M values since the assays were not performed at the same physiological conditions. It has also been argued that such a high mM values of Ddd enzymes may suggest that DMSP may not be the *bona fide* primary substrate for these enzymes at physiological conditions and the "true" substrates are yet to be identified. Nevertheless, this work by Shao *et al*., (2019) further strengthens the idea that at least some marine bacteria are capable of regulating DMSP catabolism by kinetically regulating enzyme affinity to the substrates in the metabolic pathway.

Although the work by Shao *et al.* (2019) adds important pieces to the jigsaw of DMSP catabolism, the structure-function studies of DMSP-catabolizing enzymes are far from complete. Firstly, a high affinity DMSP transporter from these cosmopolitan marine bacteria remains to be identified. As the authors have hypothesized previously, a high affinity transporter is necessary to uptake and concentrate DMSP from the dilute seawater (usually in nM range). It is envisaged that such DMSP transporters, if they exist, would require superior affinity to DMSP so that intracellular DMSP concentrations can reach the mM range for DMSP

catabolism by either the demethylation pathway or the cleavage pathway (Figure 1). Secondly, many of the DMSP cleavage enzymes (DddD, Alma1, DddL, DddW) are yet to be structurally resolved. For example, Alma1 is the only known DMSP lyase from eukaryotic plankton, and its catalytic mechanism remains to be established. Last but not least, enzymes responsible for further oxidation of DMSP degradation products, particularly DMS and methanethiol, are yet to be characterized—including the methanethiol oxidase, which produces hydrogen sulfide (Eyice et al., 2018), and Tmm, the flavin-containing trimethylamine monooxygenase, which co-oxidises DMS to DMSO in marine Roseobacters (Lidbury et al., 2015). Understanding the catalytic mechanisms through structural biology, chemical biology and biochemistry would help us better understand the physiology of DMSP metabolism in these important marine microbes.

Acknowledgement

The work carried out in our groups was supported by the Natural Environment Research Council, U.K., through grants NE/I027061/1, NE/M002233/1, NE/R010404/1 and NE/L006448/1. We would like to dedicate this highlight to Professor Ron Kiene who sadly passed away recently. Ron made many seminal contributions to the field of DMSP cycling. His research on microbial degradation of DMSP and related organic sulfur compounds remains fundamental to this field and has inspired generations of researchers.

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Table 1 Structural determination of enzymes involved in DMSP catabolism

Protein	PDB code	Bacterium	References
DMSP demethylase pathway			
DmdA	3TFH	*Pelagibacter ubique*	Schuller et al., 2012
DmdB	6IHK/6IJB	*Ruegeria lacuscaerulensis*	Shao et al., 2019
DmdC	6IJC	*Roseovarius nubinhibens*	Shao et al., 2019
DmdD	4IZB	*Ruegeria pomeroyi*	Tan et al 2013
DMSP cleavage pathway			
DddQ	4LA2	*Ruegeria lacuscaerulensis*	Li et al., 2014
	5JSO	*Ruegeria lacuscaerulensis*	Brummett & Dey 2016
DddP	4B28	*Roseobacter denitrificans*	Hehemann et al., 2014
	4RZY	*Ruegeria lacuscaerulensis*	Wang et al., 2015
DddK	5TFZ	*Pelagibacter ubique*	Schnicker & Dey 2017
DddY	5XKX	*Acinetobacter bereziniae*	Li et al., 2017a
Acrylate degradation			
PrpE	5GXD	*Dinoroseobacter shibae*	Wang et al., 2017
AcuI	5GXE/ 5GXF	*Ruegeria pomeroyi*	Wang et al., 2017
AcuH	5XZD	*Roseovarius nubinhibens*	Cao et al., 2017
DMS oxidation			
Tmm	5IPY	*Roseovarius nubinhibens*	Li et al., 2017b
DmoA	6AK1	*Hyphomicrobium sulfonivorans*	Cao et al., 2018

- The structures of four other known DMSP lyases, DddD, DddL, DddW and Alma1 are yet to be characterised.

Figure legend

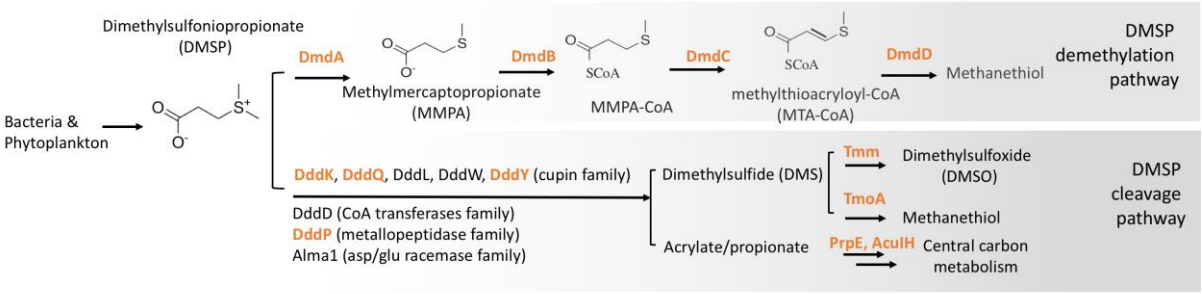

Figure 1 Key enzymes involved in degradation of dimethylsulfoniopropionate (DMSP). DMSP can be synthesized by marine microorganisms and upon release, it can be degraded by two pathways. The demethylation pathway involves DmdABCD and leads to the formation of methanethiol, while the cleavage pathway by Ddd enzymes results in the release of climate-active gaseous dimethylsulfide (DMS) and acrylate (or propionate). DMS can be further metabolised by marine microbes. The structure of two enzymes (Tmm, DmoA) leading to further catabolism of DMS have been resolved recently. Because acrylate is toxic to bacteria, further detoxification is required, and the structures of several of these enzymes (PrpE, AcuI and AcuH) have been resolved recently. Tmm is a trimethylamine monooxygenase which also oxidises DMS (Lidbury et al., 2016; Li et al., 2017b), whereas DmoA comprises the large subunit of a DMS monooxygenase (Boden et al., 2011; Cao et al., 2018). Those enzymes highlighted in orange indicate that their structures have been resolved.