

# DddD Is a CoA-Transferase/Lyase Producing Dimethyl Sulfide in the Marine Environment

Uria Alcolombri,<sup>†,‡</sup> Paola Laurino,<sup>†</sup> Pedro Lara-Astiaso,<sup>†</sup> Assaf Vardi,<sup>‡</sup> and Dan S. Tawfik<sup>\*,†</sup>

<sup>†</sup>Department of Biological Chemistry and <sup>‡</sup>Department of Plant Sciences, Weizmann Institute of Science, Rehovot 76100, Israel

## S Supporting Information

**ABSTRACT:** Dimethyl sulfide (DMS) is produced in oceans in vast amounts ( $>10^7$  tons/year) and mediates a wide range of processes from regulating marine life forms to cloud formation. Nonetheless, none of the enzymes that produce DMS from dimethylsulfoniopropionate (DMSP) has been adequately characterized. We describe the expression and purification of DddD from the marine bacterium *Marinomonas* sp. MWYL1 and its biochemical characterization. We identified DMSP and acetyl-coenzyme A to be DddD's native substrates and Asp602 as the active site residue mediating the CoA-transferase prior to lyase activity. These findings shed light on the biochemical utilization of DMSP in the marine environment.

Dimethylsulfoniopropionate (DMSP) is biosynthesized in large amounts ( $5 \times 10^{13}$  mol annually), mostly by marine phytoplankton, but also by macroalgae, corals, and some angiosperms.<sup>1–3</sup> It serves as an osmolyte and antioxidant<sup>4</sup> and is a primary source of sulfur and carbon in marine environments.<sup>1</sup> Dimethyl sulfide (DMS) is a volatile catabolite of DMSP<sup>5</sup> generated by enzymes dubbed DMSP lyases. Among other roles, DMS serves as a chemoattractant for phytoplankton, bacteria,<sup>6</sup> zooplankton,<sup>7</sup> fish, and sea birds.<sup>8,9</sup> Environmentally, DMS plays a role in cloud condensation and thus affects the atmosphere–ocean feedback loop and, possibly, the planet's climate.<sup>5</sup>

Despite its importance, the enzymes involved in DMSP biosynthesis and catabolism, and specifically in DMS production, are largely uncharacterized. Genetic studies addressed marine bacteria that possess a range of different catabolic pathways,<sup>1,2</sup> while the algal ones remain unknown. Six different marine bacterial DMSP lyases belonging mostly to abundant marine alphaproteobacteria known as the roseobacters have been reported.<sup>2</sup> However, the biochemical features of this surprisingly diverse group of enzymes remain largely unknown.<sup>1</sup> Three of these putative DMSP lyases have been purified to date, DddY, DddQ, and DddP. Only DddY exhibited high DMSP lyase activity ( $408 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ), but its molecular features remain unknown. DddP<sup>10,11</sup> and DddQ<sup>12</sup> exhibited very low activity, and their native activity might not be DMSP lyase.<sup>1,2,13</sup>

DddD is the only known DMSP lyase suggested not to generate acrylate but rather 3-hydroxypropionate (3HP),<sup>14,15</sup> although 3HP was observed in cell cultures in which other enzymes could modify the product. The sequence of DddD is similar to those of class III CoA-transferases and particularly that of CaiB, a homodimeric enzyme that transfers CoA to carnitine.<sup>14,16</sup> However, DMSP lyase activity could be detected

within living cells only upon overexpression of DddD and feeding DMSP, whereas purified DddD showed no lyase activity.<sup>14,16</sup>

Here we report the characterization of *Marinomonas* DddD identified in ref 14. We identify its substrates and products and examine its kinetic parameters and primary structural features.

The *Marinomonas* DddD gene<sup>14,15</sup> was overexpressed in *Escherichia coli*. Purification using an N-terminal His tag, followed by anion exchange, yielded the expected 90 kDa protein at  $\geq 95\%$  purity (see the experimental section of the Supporting Information). Purified DddD failed to induce the release of DMS from DMSP, as reported previously.<sup>14</sup> However, because the DddD closest sequence homologues are CoA-transferases, we tested DMS release in the presence of acetyl-CoA, the default cellular CoA donor, and found the enzyme to be active. Its specific activity,  $5 \pm 0.2 \mu\text{mol of DMS min}^{-1} (\text{mg of enzyme})^{-1}$  (at 20 mM DMSP and 0.5 mM acetyl-CoA), although low, seems to be within range for CoA-transferases (1–100, as judged by values in Brenda for EC 2.8.3.X).

Given the putative identification as a carnitine CoA-transferase, we checked whether carnitine comprises a substrate or inhibitor. However, the DMSP lyase activity was unaffected by carnitine at up to 10-fold molar excess over DMSP (Figure 1 of the Supporting Information). Thus, DddD is unlikely to be a carnitine CoA-transferase that promiscuously accepts DMSP.

To identify the product of DddD's action, reactions were run with acetyl-CoA and 3-<sup>13</sup>C-labeled DMSP (experimental section of the Supporting Information). The major product is 3-hydroxypropionate, alongside 3-hydroxypropionate coupled as CoA ester, and traces of acrylic acid. Following long incubations, only 3-hydroxypropionate was observed. The transient appearance of acrylate traces may indicate that 3-hydroxypropionate is formed via elimination to acrylic acid followed by hydration.

DddD's reaction cycle was further examined. DddD's activity seems to involve a transferase step to produce DMSP-CoA, or an acylated DMSP enzyme intermediate. Free CoA is not released, as thiols were not present as indicated by the lack of reaction with dithionitrobenzoic acid (DTNB) (Figure 2 of the Supporting Information). Following a fast phase of DMS release that seems to follow the acetyl-CoA stoichiometry, DMS continues to be released at an approximately 35-fold lower rate (Figure 3 of the Supporting Information). This suggests that acetyl-CoA is DddD's preferred CoA donor. Acrylyl-CoA does not seem to react (Figure 4 of the Supporting Information), whereas the

Received: July 10, 2014

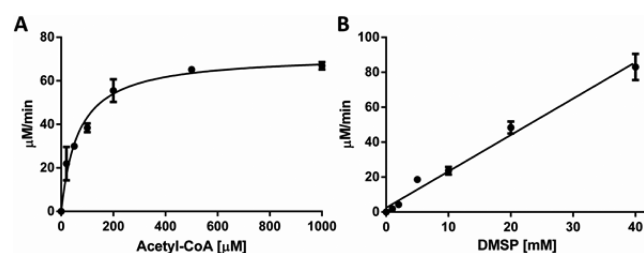
Revised: August 14, 2014

Published: August 20, 2014



assumed product, 3-hydroxy-propionate-CoA, acts as a potential CoA donor at rates much lower than that of acetyl-CoA.

Kinetic parameters were measured for DddD with respect to both DMSP and acetyl-CoA (Figure 1). At variable acetyl-CoA



**Figure 1.** DddD kinetic parameters. (A) DddD-catalyzed DMS release at varying acetyl-CoA concentrations (20 mM DMSP;  $R^2 = 0.96$ ). Kinetic parameters:  $k_{\text{cat}} = 10.8 \pm 0.2 \text{ s}^{-1}$ ,  $K_M = 67 \pm 12 \mu\text{M}$ ,  $k_{\text{cat}}/K_M = (1.6 \pm 0.3) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ . (B) DddD-catalyzed DMS release at varying DMSP concentrations (1 mM acetyl-CoA;  $R^2 = 0.97$ ). All reactions were conducted at 30 °C and pH 8.0 for 10 or 5 min, ensuring initial velocities. Kinetic parameters: apparent  $k_{\text{cat}}/K_M = 318 \pm 15 \text{ M}^{-1} \text{ s}^{-1}$ .

concentrations, DddD obeyed the Michaelis–Menten model with an apparent  $k_{\text{cat}}/K_M$  value of  $(1.6 \pm 0.3) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ ; similar  $k_{\text{cat}}/K_M$  values were measured for other type III CoA-transferases.<sup>17–20</sup> Saturation behavior was not observed with varying DMSP concentrations. The  $k_{\text{cat}}$  at the highest DMSP concentration tested was  $12.6 \pm 1.5 \text{ s}^{-1}$ , with an apparent  $k_{\text{cat}}/K_M$  of  $318 \text{ M}^{-1} \text{ s}^{-1}$ . Overall, while not indicating very high catalytic efficiency, these parameters may be reasonable given that DddD is a bifunctional enzyme that performs two subsequent reactions, transferase and lyase. For example, formyl-CoA-transferase exhibits a  $k_{\text{cat}}$  of  $5.3 \text{ s}^{-1}$  and apparent  $k_{\text{cat}}/K_M$  values of  $2.6 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  for formyl-CoA and  $1400 \text{ M}^{-1} \text{ s}^{-1}$  for oxalate.<sup>20</sup> The relatively high  $K_M$  for DMSP is also expected given its high physiological concentration ( $\leq 70 \text{ mM}$ ).<sup>21</sup> The lack of saturation with DMSP may also relate to DddD's complex reaction scheme (Scheme 1 of the Supporting Information).

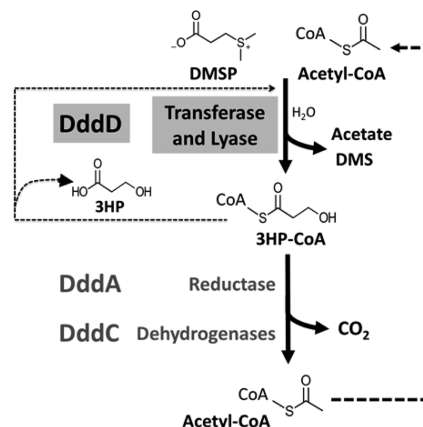
To identify DddD's active site residues, we obtained a structural homology model using Swiss-Model<sup>22</sup> (Figure 5 of the Supporting Information; other modeling programs, including Phyre2 and ModWeb, did not provide acceptable models). DddD seems to be comprised of two domains. The C-terminal domain was modeled on the basis of carnitine CoA-transferase [CaiB; Protein Data Bank (PDB) entry 1XK7]. The N-terminal domain was modeled on the basis of methylacetyl-CoA racemase (PDB entry 2YIM) for which CaiB is the closest structural homologue. CaiB is a dimer with the monomers' chains intertwined. The CoA binding site and the active site where transfer occurs are at the domain interface. Accordingly, the predicted topology of DddD comprises two CaiB-like intertwined domains fused via a long polypeptide linker (Figure 5 of the Supporting Information). The model also indicated that the C-domain contains the catalytic aspartate that mediates CoA transfer in all class III CoA-transferases. The N-domain is missing this aspartate and, instead, possesses a short insertion (LGSSY, residues 165–169). Mutating Asp602 to alanine resulted in the complete loss of DMSP lyase activity (Figure 6 of the Supporting Information), suggesting that the transferase step, i.e., formation of a DMSP ester, is a prerequisite for hydration and DMS release.

DddD's N-terminal CoA binding site is well-conserved and aligns very well with the CoA binding of CaiB. However, mutations in the predicted N-domain active site had no effect or a

relatively minor effect on activity [Ser167Ala or Tyr169Phe (Figure 6 of the Supporting Information)]. Other mutations such as Glu134Ala induced misfolding, and thus, their functional role could not be assigned. Thus, at present, whether the transferase and the hydration reactions occur within one active site (the one containing Asp602) or two remains unclear.

Summarizing our data, we propose a catalytic cycle in which, first, DMSP is transferred to the enzyme using acetyl-CoA as a donor (Scheme 1 of the Supporting Information). This step is likely executed as in other type III CoA-transferases<sup>18</sup> to give a covalent DMSP anhydride intermediate involving Asp602. The second step is hydration, directly, or via elimination and an acrylyl intermediate. Hydration may occur directly on the covalent DMSP–Asp602 intermediate, leading to a 3HP–enzyme intermediate and DMS (alternative A), or formation of DMSP-CoA first, followed by hydration (alternative B). Preliminary observations indicate that DMSP-CoA is not a substrate for DddD (Figure 4 of the Supporting Information), indicating alternative A is the more probable path. Finally, 3HP-CoA is released as the primary product, although it can serve as a donor, leading to the release of free 3HP, the main product at the completion of the reaction (experimental section of the Supporting Information). *In vivo*, 3HP-CoA probably comprises the substrate of the next enzyme in the pathway mediating DMSP catabolism (Scheme 1). Indeed, our findings confirm the

#### Scheme 1. DddD's Catalyzed Reactions and the Presumed DMSP Catabolism Pathway<sup>a</sup>



<sup>a</sup>DddD's dual action as a transferase and a lyase initiates the presumed pathway, whereby DMS and acetate are released. The third product, 3HP-CoA (3HP, 3-hydroxypropionate), is subsequently processed by two additional enzymes, DddA and DddC.<sup>15</sup> The released acetyl-CoA initiates a new reaction cycle. At a much slower rate, 3HP-CoA can also serve as a CoA donor, thereby initiating another reaction cycle and releasing free 3HP (thin dashed line).

overall proposed sequence of reactions.<sup>15</sup> However, given our discovery of DddD's dual functionality, it is likely that all steps occur via CoA esters, starting and ending with acetyl-CoA (Scheme 1).

Overall, our data suggest that DddD is an enzyme that mediates the first two steps in DMSP catabolism in *Marinomonas* and other bacteria possessing this gene, and especially in gammaproteobacteria that grow well on DMSP as a sole carbon source (representatives shown in Figure 5a of the Supporting Information).<sup>1,23</sup> Although its detailed mechanism is unclear, our data show that DddD's bifunctionality couples DMS release to 3-hydroxypropionate-CoA formation and to the downstream

recycling of acetyl-CoA. CoA-transferases, including type III transferase,<sup>24</sup> are involved in numerous catabolic pathways.<sup>25</sup> However, bifunctional ones are relatively rare (for a recent example, see ref 26), and to the best of our knowledge, DddD is the first example of a bifunctional type III CoA-transferase. Finally, DddD is probably only one representative of a diverse group of DMS-producing enzymes, whose biochemical properties are yet to be discovered.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

Figures, alignment, NMR spectra, and extended experimental section. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*E-mail: [tawfik@weizmann.ac.il](mailto:tawfik@weizmann.ac.il).

### Funding

Financial support by the Sasson & Marjorie Peress Philanthropic Fund to D.S.T. and by a European Research Council [ERC; StG (INFOTROPHIC Grant 280991)] grant to A.V. are gratefully acknowledged. D.S.T. is the Nella and Leon Benozio Professor of Biochemistry.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We thank Prof. Andrew W. B. Johnston for providing the DddD plasmid and Dr. Shira Albeck for help with protein purification. The graphic for the table of contents was adapted from Tiago Fioreze, Wikipedia.

## ■ REFERENCES

- Curson, A. R., Todd, J. D., Sullivan, M. J., and Johnston, A. W. (2011) Catabolism of dimethylsulphoniopropionate: Microorganisms, enzymes and genes. *Nat. Rev. Microbiol.* 9, 849–859.
- Reisch, C. R., Moran, M. A., and Whitman, W. B. (2011) Bacterial Catabolism of Dimethylsulphoniopropionate (DMSP). *Front. Microbiol.* 2, 172.
- Sievert, S. M., Kiene, R. P., and Schulz-Vogt, H. N. (2007) The sulfur cycle. *Oceanography* 20 (2), 117–123.
- Sunda, W., Kieber, D. J., Kiene, R. P., and Huntsman, S. (2002) An antioxidant function for DMSP and DMS in marine algae. *Nature* 418, 317–320.
- Lovelock, J. E., Maggs, R. J., and Rasmussen, R. A. (1972) Atmospheric Dimethyl Sulphide and the Natural Sulphur Cycle. *Nature* 237, 452–453.
- Seymour, J. R., Simo, R., Ahmed, T., and Stocker, R. (2010) Chemoattraction to dimethylsulphoniopropionate throughout the marine microbial food web. *Science* 329, 342–345.
- Steinke, M., Malin, G., and Liss, P. S. (2002) Trophic interactions in the sea: An ecological role for climate relevant volatiles. *J. Phycol.* 38, 630–638.
- DeBose, J. L., Lema, S. C., and Nevitt, G. A. (2008) Dimethylsulphoniopropionate as a Foraging Cue for Reef Fishes. *Science* 319, 1356.
- Nevitt, G. A. (2008) Sensory ecology on the high seas: The odor world of the procarriniform seabirds. *J. Exp. Biol.* 211, 1706–1713.
- Todd, J. D., Curson, A. R., Dupont, C. L., Nicholson, P., and Johnston, A. W. (2009) The dddP gene, encoding a novel enzyme that converts dimethylsulphoniopropionate into dimethyl sulfide, is widespread in ocean metagenomes and marine bacteria and also occurs in some Ascomycete fungi. *Environ. Microbiol.* 11, 1376–1385.

- Kirkwood, M., Le Brun, N. E., Todd, J. D., and Johnston, A. W. (2010) The dddP gene of *Roseovarius nubinhibens* encodes a novel lyase that cleaves dimethylsulphoniopropionate into acrylate plus dimethyl sulfide. *Microbiology* 156, 1900–1906.
- Todd, J. D., Curson, A. R., Kirkwood, M., Sullivan, M. J., Green, R. T., and Johnston, A. W. (2011) DddQ, a novel, cupin-containing, dimethylsulphoniopropionate lyase in marine roseobacters and in uncultured marine bacteria. *Environ. Microbiol.* 13, 427–438.
- Alcolombri, U., Elias, M., Vardi, A., and Tawfik, D. S. (2014) Ambiguous evidence for assigning DddQ as a dimethylsulphoniopropionate lyase and oceanic dimethylsulfide producer. *Proc. Natl. Acad. Sci. U.S.A.* 111, E2078–E2079.
- Todd, J. D., Rogers, R., Li, Y. G., Wexler, M., Bond, P. L., Sun, L., Curson, A. R., Malin, G., Steinke, M., and Johnston, A. W. (2007) Structural and regulatory genes required to make the gas dimethyl sulfide in bacteria. *Science* 315, 666–669.
- Todd, J. D., Curson, A. R., Nikolaidou-Katsaraidou, N., Brearley, C. A., Watmough, N. J., Chan, Y., Page, P. C., Sun, L., and Johnston, A. W. (2010) Molecular dissection of bacterial acrylate catabolism: Unexpected links with dimethylsulphoniopropionate catabolism and dimethyl sulfide production. *Environ. Microbiol.* 12, 327–343.
- Curson, A. R., Rogers, R., Todd, J. D., Brearley, C. A., and Johnston, A. W. (2008) Molecular genetic analysis of a dimethylsulphoniopropionate lyase that liberates the climate-changing gas dimethylsulfide in several marine alpha-proteobacteria and *Rhodobacter sphaeroides*. *Environ. Microbiol.* 10, 757–767.
- Engemann, C., Elssner, T., Pfeifer, S., Krumbholz, C., Maier, T., and Kleber, H. P. (2005) Identification and functional characterisation of genes and corresponding enzymes involved in carnitine metabolism of *Proteus* sp. *Arch. Microbiol.* 183, 176–189.
- Berthold, C. L., Toyota, C. G., Richards, N. G., and Lindqvist, Y. (2008) Reinvestigation of the catalytic mechanism of formyl-CoA transferase, a class III CoA-transferase. *J. Biol. Chem.* 283, 6519–6529.
- Ricagno, S., Jonsson, S., Richards, N., and Lindqvist, Y. (2003) Formyl-CoA transferase encloses the CoA binding site at the interface of an interlocked dimer. *EMBO J.* 22, 3210–3219.
- Toyota, C. G., Berthold, C. L., Gruez, A., Jonsson, S., Lindqvist, Y., Cambillau, C., and Richards, N. G. (2008) Differential substrate specificity and kinetic behavior of *Escherichia coli* YfdW and *Oxalobacter formigenes* formyl coenzyme A transferase. *J. Bacteriol.* 190, 2556–2564.
- Reisch, C. R., Moran, M. A., and Whitman, W. B. (2008) Dimethylsulphoniopropionate-dependent demethylase (DmdA) from *Pelagibacter ubique* and *Silicibacter pomeroyi*. *J. Bacteriol.* 190, 8018–8024.
- Arnold, K., Bordoli, L., Kopp, J., and Schwede, T. (2006) The SWISS-MODEL workspace: A web-based environment for protein structure homology modelling. *Bioinformatics* 22, 195–201.
- Ansedé, J. H., Friedman, R., and Yoch, D. C. (2001) Phylogenetic analysis of culturable dimethyl sulfide-producing bacteria from a spartina-dominated salt marsh and estuarine water. *Appl. Environ. Microbiol.* 67, 1210–1217.
- Schurmann, M., Hirsch, B., Wubbeler, J. H., Stoveken, N., and Steinbuechel, A. (2013) Succinyl-CoA:3-sulfino-propionate CoA-transferase from *Variovorax paradoxus* strain TBEA6, a novel member of the class III coenzyme A (CoA)-transferase family. *J. Bacteriol.* 195, 3761–3773.
- Muntner, P., Colantonio, L. D., Cushman, M., Goff, D. C., Jr., Howard, G., Howard, V. J., Kissela, B., Levitan, E. B., Lloyd-Jones, D. M., and Safford, M. M. (2014) Validation of the atherosclerotic cardiovascular disease Pooled Cohort risk equations. *JAMA, J. Am. Med. Assoc.* 311, 1406–1415.
- Chantigian, D. P., Thoden, J. B., and Holden, H. M. (2013) Structural and biochemical characterization of a bifunctional ketoisomerase/N-acetyltransferase from *Shewanella denitrificans*. *Biochemistry* 52, 8374–8385.