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

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

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,[1,2] 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.[2] However, the biochemical features of this surprisingly diverse group of enzymes remain largely unknown.[1] Three of these putative DMSP lyases have been purified to date, DddY, DddQ, and DddP. Only DddY exhibited high DMSP lyase activity (408 μmol min^{-1} mg^{-1}), but its molecular features remain unknown. DddP[10,11] and DddQ[12] exhibited very low activity, and their native activity might not be DMSP lyase.[1,2,13]

DddD is the only known DMSP lyase suggested not to generate acrylate but rather 3-hydroxypropionate (3HP),[14,15] 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.[14,16] 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.[14,16]

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[14,15] was overexpressed in *Escherichia coli*. Purification using an N-terminal His tag, followed by anion exchange, yielded the expected 90 kDa protein at ≥95% purity (see the experimental section of the Supporting Information). Purified DddD failed to induce the release of DMS from DMSP, as reported previously.[14] 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 ± 0.2 μmol of DMS min^{-1} (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-^{13}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

dx.doi.org/10.1021/bi500853s | *Biochemistry* XXXX, XXX, XXX−XXX

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

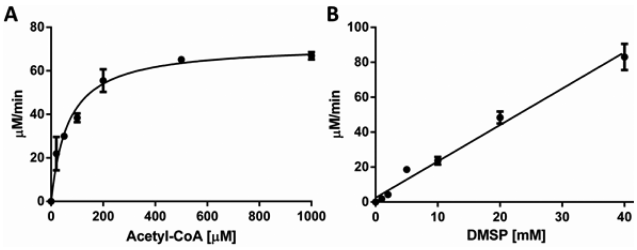


Figure 1. DddD kinetic parameters. (A) DddD-catalyzed DMS release at varying acetyl-CoA concentrations (20 mM DMSP; R^2 = 0.96). Kinetic parameters: k_{cat} = 10.8 ± 0.2 s^{-1}, K_M = 67 ± 12 μM, k_{cat}/K_M = (1.6 ± 0.3) × 10^5 M^{-1} s^{-1}. (B) DddD-catalyzed DMS release at varying DMSP concentrations (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_{cat}/K_M = 318 ± 15 M^{-1} s^{-1}.

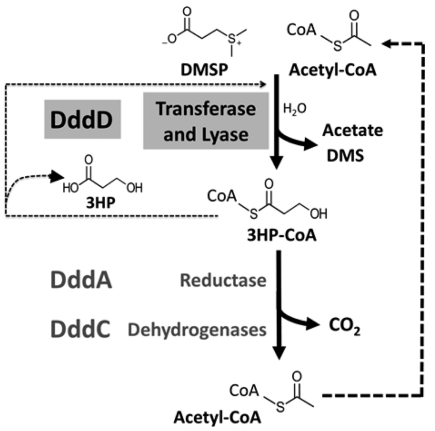
concentrations, DddD obeyed the Michaelis–Menten model with an apparent k_{cat}/K_M value of (1.6 ± 0.3) × 10^5 M^{-1} s^{-1}; similar k_{cat}/K_M values were measured for other type III CoA-transferases.[17−20] Saturation behavior was not observed with varying DMSP concentrations. The k_{cat} at the highest DMSP concentration tested was 12.6 ± 1.5 s^{-1}, with an apparent k_{cat}/K_M of 318 M^{-1} 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_{cat} of 5.3 s^{-1} and apparent k_{cat}/K_M values of 2.6 × 10^6 M^{-1} s^{-1} for formyl-CoA and 1400 M^{-1} s^{-1} for oxalate.[20] The relatively high K_M for DMSP is also expected given its high physiological concentration (≤70 mM).[21] 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[22] (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 methylacyl-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[18] 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[a]



[a]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.[15] 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.[15] 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).[1,23] 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

dx.doi.org/10.1021/bi500853s | *Biochemistry* XXXX, XXX, XXX−XXX

recycling of acetyl-CoA. CoA-transferases, including type III transferase,[24] are involved in numerous catabolic pathways.[25] 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.

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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 Benoziyo 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.

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