Structural and Mechanistic Insights Into Dimethylsulfoxide Formation Through Dimethylsulfide Oxidation

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Dimethylsulfide (DMS) and dimethylsulfoxide (DMSO) are widespread in marine environment, and are important participants in the global sulfur cycle. Microbiol oxidation of DMS to DMSO represents a major sink of DMS in marine surface waters. The SAR11 clade and the marine Roseobacter clade (MRC) are the most abundant heterotrophic bacteria in the ocean surface seawater. It has been reported that trimethylamine monooxygenase (Tmm, EC 1.14.13.148) from both MRC and SAR11 bacteria likely oxidizes DMS to generate DMSO. However, the structural basis of DMS oxidation has not been explained. Here, we characterized a Tmm homolog from the SAR11 bacterium *Pelagibacter* sp. HTCC7211 (Tmm7211). Tmm7211 exhibits DMS oxidation activity in vitro. We further solved the crystal structures of Tmm7211 and Tmm7211 soaked with DMS, and proposed the catalytic mechanism of Tmm7211, which comprises a reductive half-reaction and an oxidative half-reaction. FAD and NADPH molecules are essential for the catalysis of Tmm7211. In the reductive half-reaction, FAD is reduced by NADPH. In the oxidative half-reaction, the reduced FAD reacts with O2 to form the C4a-(hydro)peroxyflavin. The binding of DMS may repel the nicotinamide ring of NADP+, and make NADP+ generate a conformational change, shutting off the substrate entrance and exposing the active C4a-(hydro)peroxyflavin to DMS to complete the oxidation of DMS. The proposed catalytic mechanism of Tmm7211 may be widely adopted by MRC and SAR11 bacteria. This study provides important insight into the conversion of DMS into DMSO in marine bacteria, leading to a better understanding of the global sulfur cycle.

Keywords: DMS, DMSO, flavin-containing monooxygenase, SAR11, catalytic mechanism

INTRODUCTION

Dimethylsulfide (DMS), one of the major biogenic sulfur species emitted into the atmosphere from oceans, is an important participant in the global sulfur cycle (Andreae, 1990; Simo, 2001; Zhang et al., 2019). Approximately 300 Tg of DMS is produced annually mainly by dimethylsulfoniopropionate (DMSP) cleavage through various DMSP lyases (Curson et al., 2011;
Among which 13–37 Tg is transferred into the atmosphere through ocean-atmosphere sulfur flux (Ksionzek et al., 2016). In the air, DMS may contribute to the formation of the cloud condensation nuclei and thus act as a global coolant (Charlson et al., 1987; Lidbury et al., 2016). DMS loss in marine surface waters is mediated by different processes, including photochemical oxidation and biological consumption, with the latter being a major component of the global sink for DMS (Brimblecombe and Shooter, 1986; Kiene and Bates, 1990; Lidbury et al., 2016). Microorganisms can transform DMS into different compounds, such as dimethylsulfide (DMSO), methanethiol, sulfate, thiosulfate and tetrathionate (deZwart et al., 1996; Vila-Costa et al., 2006; del Valle et al., 2007; Boden et al., 2010, 2011; Lidbury et al., 2016). In surface seawater, microbial oxidation to DMSO is a major fate of DMS (Lidbury et al., 2016), which accounts for approximately 70% of the total oxidized DMS in the Sargasso Sea (del Valle et al., 2007). DMSO is ubiquitous in aquatic environments, and is likely to function as cryoprotectant, free-radical scavenger or intracellular electrolyte modifier in marine organisms (Lee and De Mora, 1999; Asher et al., 2017; Speeckart et al., 2018).

The SAR11 clade and the marine Roseobacter clade (MRC) are the most abundant heterotrophic bacteria in the ocean surface seawater, and are active participants in marine carbon, nitrogen, sulfur, and phosphorus cycles (Morrison et al., 2002; Buchanan et al., 2005; Rusch et al., 2007; Chen, 2012; Carini et al., 2015; Sebastián et al., 2016; Tesmentzi et al., 2016). Previous studies have shown that trimethylamine monoxygenase (Tmm, EC 1.14.13.148) from both MRC and SAR11 bacteria likely oxidizes DMS to generate DMSO (Chen et al., 2011; Lidbury et al., 2016). It is estimated that ∼20% of the bacteria in the surface ocean contain tmm homologs (Chen et al., 2011). Physiological experiments demonstrated that MRC can oxidize DMS to DMSO using Tmm (Lidbury et al., 2016), and it is deduced that SAR11 bacteria may also play a vital role in the conversion of DMS to DMSO in marine environment (Chen et al., 2011; Lidbury et al., 2016). However, the catalytic mechanism underpinning DMS oxidation to DMSO by Tmm remains understudied.

Tmm is a bacterial flavin-containing monoxygenase (FMO), which belongs to the class B flavoprotein monoxygenases (Chen et al., 2011; Paul et al., 2021). FMOs are a widespread class of enzymes that are involved in the metabolism of xenobiotics (Cho et al., 2011). FMOs oxygenate a wide range of substrates, such as nitrogen-containing and sulfur-containing compounds (van Berkel et al., 2006). Tmm is also reported to act on various substrates, including trimethylamine (TMA), dimethylamine (DMA), DMS, indole, and methimazole (Chen et al., 2011). The catalytic process of Tmm to oxidize TMA, indole or methimazole can be divided into two half-reactions: a reductive half-reaction followed by an oxidative half-reaction (Beatty and Ballou, 1981a,b; Cho et al., 2011; Li et al., 2017). In the reductive half-reaction, the cofactor flavin adenine dinucleotide (FAD) is reduced by NADPH. In the oxidative half-reaction, the reduced FAD reacts with an oxygen molecule, generating the C4a-(hydro)peroxylavin, which is relatively stable in vitro (Alfieri et al., 2008). An oxygen atom from the C4a-(hydro)peroxylavin is transferred to the substrate to complete the oxidation cycle (Alfieri et al., 2008; Orru et al., 2010). However, the detailed structural basis for DMS oxidation is still lacking. Considering the important roles of DMS and DMSO in the global sulfur cycle, the structural basis of DMS oxidation to DMSO by Tmm warrants further investigation.

The SAR11 bacterium Pelagibacter sp. HTCC7211 was isolated from the oligotrophic Sargasso Sea (Sun et al., 2011). It has been reported that the recombinant Tmm from strain HTCC7211 (Tmm7211) could catalyze the oxidation of TMA to trimethylamine N-oxide (TMAO) (Chen et al., 2011). In this study, the Tmm7211 gene was synthesized and over-expressed in Escherichia coli. The recombinant Tmm7211 also exhibits DMS oxidation activity in vitro. The crystal structures of Tmm7211 and Tmm7211 soaked with DMS were solved. The catalytic mechanism of DMSO production through DMS oxidation was proposed by structural analyses and mutational assays.

**MATERIALS AND METHODS**

**Gene Cloning, Point Mutations, and Protein Expression and Purification**

The 1335-bp full-length tmm gene from Pelagibacter sp. HTCC7211 was synthesized by the Beijing Genomics Institute (China). The gene was then subcloned into the pET28a (Novagen, United States) vector with an N-terminal His tag. The point mutations in Tmm7211 were introduced using PCR-based method and verified by DNA sequencing. The Tmm7211 protein and its mutants were expressed in *E. coli* BL21 (DE3). The cells were cultured at 37°C in Lysogeny Broth medium to an OD600 of 0.8–1.0 and then induced at 20°C for 14 h with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The proteins were purified first with Ni2+-NTA resin (Qiagen, Germany) and then fractionated on a Superdex-200 column (GE Healthcare, United States). The protein concentration was determined with the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, United States), and a nine-point calibration curve of bovine serum albumin (BSA) standards was used according to the user guide.

**Gel Filtration Analysis**

A Superose 6 column was used for gel filtration analysis, because it possesses a wider fractionation range than the Superdex-200 column. The Superose 6 column was calibrated in the buffer containing 10 mM Tris-HCl (pH 8.0) and 100 mM NaCl using the following standards from GE Healthcare: thyroglobulin (669 kDa), ferritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), carbonic anhydrase (29 kDa), ribonuclease A (13.7 kDa), and aprotinin (6.5 kDa). The void volume of Superose 6 column was determined with Blue Dextran 2000 (2,000 kDa).

**Spectrophotometric Analysis**

The UV spectra of Tmm7211 (0.1 mM protein in the buffer containing 10 mM Tris-HCl (pH 8.0) and 100 mM NaCl) were measured by a V550 UV/VIS spectrophotometer (Jasco, Japan) in a cell with 1.0 cm path length (Response: Medium; Band width: 0.5 nm).
The DMSO produced by the enzymatic activity of Tmm7211 toward DMS was measured by high performance liquid chromatography (HPLC) (Dionex, America) on a SunFire C18 column (Waters, America). The detection wavelength was 210 nm because DMSO exhibited an absorbance maximum at ~210 nm. The samples were eluted in HPLC buffer (2.5% (v/v) acetonitrile, 0.2% (v/v) phosphoric acid in double-distilled H2O) over 20 min at a flow rate of 1 ml/min. The reaction system contained 6 mM DMS (Sigma-Aldrich, America), 1.5 mM NADPH (Sigma-Aldrich, America), 0.15 mM Tmm7211, 10 mM Tris-HCl (pH 7.0) and 100 mM NaCl. The reaction was performed at 25°C, pH 7.0 for 3 h, and terminated by adding 10% phosphoric acid. The reaction system was centrifuged at 15,000 g for 15 min, and then the supernatant (20 µl) was injected for HPLC analysis. The control group had the same reaction system except that Tmm7211 was not added.

**Coexistence Analysis of Enzymes Involved in Dimethylsulfide Metabolism**

Related protein sequences DddD (Pseudomonas putida, WP_062573753.1), DddK (Candidatus Pelagibacter ubique HTCC1062, WP_011281678.1), DddP (Mesorhizobium loti, WP_109668646.1), DddQ (Mesorhizobium loti, WP_109668666.1), DddW (Ruegeria pomeroyi, WP_011046214.1), DddL (Pantoea agglomerans, WP_099909581.1), DddY (Alcaligenes faecalis, WP_123051132.1), DMSOR (Rhodobacter capsulatus, Q52675.2), Tmm (Pelagibacter abyssi, APZ51459.1), DdhA (Sagittula stellata E-37, EBA07058.1).

[1]http://www.pymol.org/
MddA (*Pseudomonas deceptionensis*, WP_048359798.1), DsoB (*Acinetobacter* sp. 20B, BAA23331.1) and DmoA (*Hyphomicrobium sulfonivorans*, E9JFX9.1) were obtained from National Center for Biotechnology Information (NCBI) database as seed sequences. For multifunctional strains screening, the seed sequences were used to search against the genomes of isolated strains on the IMG/M metagenomics database (Chen et al., 2019) with parameters of similarity > 40%, E-value of < $10^{-50}$ and coverage > 70% to elevate the accuracy and precision of blast hits. Data processing was performed via scripts compiled in Python code. The biological networks of related proteins were built via software Cytoscape 3.8.0 (Kohl et al., 2011).

### Expression and Characterization of Tmm$_{7211}$

The *tmm* gene of *Pelagibacter* sp. HTCC7211 contains 1335 nucleotides and encodes a protein of 444 amino acid residues, with a calculated molecular mass of 52 kDa. Tmm$_{7211}$ shares ~53% amino acid sequence identity with RtTmm, a previously reported Tmm homolog from an MRC strain *Roseovarius nubinhibens* ISM (Li et al., 2017). Full-length *tmm* of strain HTCC7211 was synthesized and was expressed in *E. coli* BL21 (DE3) cells, and the recombinant Tmm$_{7211}$ was purified (Figure 1A) and characterized. The purified Tmm$_{7211}$ is yellow, suggesting that FAD has been already bound in the recombinant Tmm$_{7211}$ during protein expression in *E. coli*, which is further supported by spectroscopic analysis. The purified Tmm$_{7211}$ exhibited the typical absorbance maxima (around 372 and 442 nm) of fully oxidized FMOs (Figure 1B; Alfieri et al., 2008; Orru et al., 2010). Addition of equimolar amount of NADPH should lead to the formation of the enzyme-(hydro)peroxyflavin-NADP$^+$ complex exhibiting a typical absorbance maximum at around 360 nm (Alfieri et al., 2008; Orru et al., 2010).

### RESULTS

**Expression and Characterization of Tmm$_{7211}$**

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However, the absorption spectrum showed that the absorbance maximum of Tmm\textsubscript{2111} with the addition of equimolar amount of NADPH was around 350 nm (Figure 1B). Because NADPH absorbs at 340 nm (Alfieri et al., 2008), this spectrum probably reflected a mixture of the enzyme-(hydro)peroxyflavin-NADP\textsuperscript{+} complex and some residual NADPH, which may due to some inactive enzymes in the purified Tmm\textsubscript{2111} solution. Incubation of recombinant Tmm\textsubscript{2111} with DMS and NADPH yielded DMSO and NADP\textsuperscript{+} (Figure 1C), demonstrating that Tmm\textsubscript{2111} has DMS oxidation activity \textit{in vitro}. The optimal temperature for Tmm\textsubscript{2111} enzymatic activity toward DMS was \~25\degree C (Figure 1D), and the optimal pH was 7.0 (Figure 1E). Furthermore, Tmm\textsubscript{2111} only retained \~40\% of its highest enzymatic activity at 30\degree C, whereas RnTmm still retained \~70\% of its highest enzymatic activity at 40\degree C (Li et al., 2017), suggesting that Tmm\textsubscript{2111} is more sensitive to high temperature than RnTmm.

The substrate specificity of Tmm\textsubscript{2111} was also analyzed. Tmm\textsubscript{2111} can oxidize DMS, TMA, DMA, and methimazole, with TMA showing the highest affinity (Table 1). In general, the apparent $K_M$ values of Tmm\textsubscript{2111} to different substrates are slightly higher than those of RnTmm, and the $k_{cat}$ values of Tmm\textsubscript{2111} are lower (Table 1; Li et al., 2017), indicating that the enzymatic activity of Tmm\textsubscript{2111} is lower than that of RnTmm \textit{in vitro}.

\textbf{Overall Structure of Tmm\textsubscript{2111}}

To gain insight into the putative active site of Tmm\textsubscript{2111}, we solved the crystal structure of Tmm\textsubscript{2111} to 1.8 Å (Table 2). The crystals of Tmm\textsubscript{2111} belong to the P2\textsubscript{1} space group, with two molecules arranged as a dimer in an asymmetric unit (Figures 2A,B). Gel filtration analysis (Figure 2C) indicated that Tmm\textsubscript{2111} functions as a dimer in solution, which is supported by the result of the PISA server prediction.\textsuperscript{4} After structural refinement, the NADP\textsuperscript{+} and FAD molecules can be clearly observed in the structure (Figure 2A). The overall structure of Tmm\textsubscript{2111} is similar to those of other reported bacterial FMOs (Alfieri et al., 2008; Cho et al., 2011; Li et al., 2017), with the root mean square deviations (RMSDs) between Tmm\textsubscript{2111} and other bacterial FMOs of no more than 0.6 Å. Tmm\textsubscript{2111} also comprises an NADPH binding domain and an FAD binding domain (Figure 2B). These two domains are connected through two hinge regions (Ser163–Pro168 and Cys268–Leu272) (Figure 2B).

\textbf{To obtain the crystal structure of Tmm\textsubscript{2111} in complex with DMS, we first tried to co-crystallize Tmm\textsubscript{2111} with DMS. However, this failed, probably due to the volatile nature of DMS that has a low boiling point (~37\degree C) in the crystallization buffer. Next, we tried the soaking method and solved two crystal structures of Tmm\textsubscript{2111} soaked with DMS for different soaking time (Table 2). For briefness, the crystal structures of Tmm\textsubscript{2111} soaked with DMS for 5 min and for 20 min were termed as Tmm\textsubscript{2111}-5-min and Tmm\textsubscript{2111}-20-min, respectively. The overall structures of Tmm\textsubscript{2111} soaked with DMS are similar to that of Tmm\textsubscript{2111}, with the RMSD between Tmm\textsubscript{2111} and Tmm\textsubscript{2111}-5-min of 0.1 Å, and the RMSD between Tmm\textsubscript{2111} and Tmm\textsubscript{2111}-20-min of 0.2 Å.}

\begin{table}[h]
\centering
\caption{Crystallographic data collection and refinement of Tmm\textsubscript{2111}.}
\begin{tabular}{|c|c|c|c|}
\hline
Parameters & Tmm\textsubscript{2111} & Tmm\textsubscript{2111}-5-min & Tmm\textsubscript{2111}-20-min \\
\hline
\hline
\textbf{Diffraction data} & & & \\
Space group & $P2_1$ & $P2_1$ & $P2_1$ \\
\hline
Unit cell & a, b, c (Å) & 69.4, 82.1, 97.9 & 69.0, 81.8, 97.9 & 68.7, 81.7, 97.6 \\
\hline
Resolution range (Å) & (1.83–1.80) & (1.86–1.80) & (2.0–2.00) \\
\hline
Redundancy & 3.3 (3.3) & 4.6 (6.9) & 6.6 (6.3) \\
\hline
Completeness (%) & 98.1 (98.6) & 97.7 (98.6) & 100.0 (100.0) \\
\hline
\hline
\textbf{Refinement statistics} & & & \\
R-factor & 0.22 & 0.16 & 0.16 \\
Free R-factor & 0.25 & 0.18 & 0.20 \\
RMSD from ideal geometry & & & \\
Bond lengths (Å) & 0.006 & 0.006 & 0.006 \\
Bond angles (°) & 1.1 & 1.2 & 1.1 \\
Ramachandran plot (%) & Favored & 93.8 & 94.7 & 94.7 \\
& Allowed & 6.0 & 5.1 & 5.1 \\
& Outliers & 0.2 & 0.2 & 0.2 \\
B-factors (Å$^2$) & Protein & 29.7 & 23.7 & 31.5 \\
& NADP$^+$ & 25.1 & 21.8 & 28.7 \\
& FAD & 23.2 & 18.5 & 27.3 \\
& Water & 38.5 & 33.5 & 37.4 \\
& All atoms & 30.6 & 24.9 & 32.0 \\
\hline
\end{tabular}
\begin{flushright}
*Numbers in parentheses refer to data in the highest-resolution shell.
** $R_{merge} = \sum_i |I(hkl) - <I(hkl)>|/\sum_i I(hkl)$, where $I$ is the observed intensity, $<I(hkl)>$ represents the average intensity, and $I(hkl)$, represents the observed intensity of each unique reflection.
\end{flushright}
\end{table}

\begin{table}[h]
\centering
\caption{Kinetic parameters for Tmm\textsubscript{2111} and RnTmm.}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Enzyme} & \textbf{Substrate} & \textbf{$K_M$ (µM)} & \textbf{$k_{cat}$ (min$^{-1}$)} & \textbf{$k_{cat}/K_M$ (min$^{-1}$ mM$^{-1}$)} & \textbf{References} \\
\hline
\textbf{Tmm\textsubscript{2111}} & DMS & 250.5 ± 23.0 & 4.5 ± 0.3 & 18.0 & This study \\
& TMA & 139.0 ± 10.7 & 22.4 ± 0.8 & 161.2 & This study \\
& DMA & 181.4 ± 23.7 & 17.9 ± 1.7 & 98.7 & This study \\
& Methimazole & 116.2 ± 13.8 & 5.2 ± 0.3 & 44.8 & This study \\
\hline
\textbf{RnTmm} & TMA & 110.5 ± 14.5 & 31.8 ± 2.4 & 287.8 & Li et al., 2017 \\
& DMA & 164.9 ± 36.5 & 10.2 ± 1.2 & 61.9 & Li et al., 2017 \\
& Methimazole & 123.3 ± 44.6 & 13.2 ± 1.8 & 107.1 & Li et al., 2017 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{4}http://www.ebi.ac.uk/pdbe/prot_int/pistart.html
Residues Involved in Binding NADP⁺ and Flavin Adenine Dinucleotide

From the surface view of Tmm7211, we can only observe part of the NADP⁺ molecule and the FAD molecule was not visible (Figure 3A). The nicotinamide ring of NADP⁺ is located inside Tmm7211, and the entire FAD molecule is deeply bound in the protein (Figures 3A,B). The binding of NADP⁺ and FAD mainly depends on hydrogen bonds formed between Tmm7211 residues and them (Figures 3C,D). For NADP⁺ binding, residues Trp70 and Arg409 form hydrogen bonds with the nicotinamide ring, Asn72 and Gln315 interact with the ribose ring via water-mediated hydrogen-bonds, and Tyr170, Ser202, Ser203, Ser205, Arg226, His227, and Asn288 interact with the other parts of NADP⁺ (Figure 3C). For FAD binding, residues Asn72 and Thr318 interact with the isoalloxazine ring, Glu37 forms hydrogen bonds with the ribose ring, Val125 forms a hydrogen bond with the adenine moiety, and Gly10, Leu45, Trp46, Gly160, Ser163, and Gln315 interact with the other parts of FAD (Figure 3D).

To confirm the importance of Tmm7211 residues involved in binding NADP⁺ and FAD, we generated site-directed mutations to the related residues and quantified the enzymatic activities of the mutants. All mutants had significantly decreased activities of the mutants. All mutants had significantly decreased activities of the mutants. All mutants had significantly decreased activities of the mutants. All mutants had significantly decreased activities of the mutants. All mutants had significantly decreased activities of the mutants. All mutants had significantly decreased activities of the mutants. All mutants had significantly decreased activities of the mutants. All mutants had significantly decreased activities of the mutants. All mutants had significantly decreased activities of the mutants. All mutants had significantly decreased activities of the mutants.

Conformational Change of NADP⁺ During Soaking Dimethylsulfide

To elucidate the catalytic mechanism of Tmm7211 for DMS oxidation, it is important to ascertain the location of DMS. Despite the two structures of Tmm7211 soaked with DMS were solved, the explicit electron density of DMS in the structures could not be identified. Previous structural analyses demonstrated that the substrate of bacterial FMOs with a ring structure, such as indole and methimazole, is located in the position of the nicotinamide ring of NADP⁺, forming stacking interactions with the isoalloxazine ring of FAD (Eswaramoorthy et al., 2006; Cho et al., 2011; Li et al., 2017). There is no direct interaction between the residues of bacterial FMOs and the substrates (Eswaramoorthy et al., 2006; Cho et al., 2011; Li et al., 2017). For substrates with no ring structure, such as DMS and TMA, there may be no effective interactions to stabilize their conformations, which may be the reason why we could not find the DMS molecule in the structures of Tmm7211 soaked with DMS.

By comparing the structures of Tmm7211, Tmm7211-5-min and Tmm7211-20-min, we noticed that with the extension of soaking time, the electron densities of the nicotinamide ring and the ribose ring of NADP⁺ become increasingly weaker (Figures 4A–C), indicating that the nicotinamide ring and the ribose ring become flexible during soaking. Combined with previous studies that the indole and methimazole molecules located in the binding site of NADP⁺ (Eswaramoorthy et al., 2006; Cho et al., 2011; Li et al., 2017), this result suggests that DMS may also be bound in the position of the nicotinamide ring of NADP⁺, and that the entry of DMS may repel NADP⁺, leading to a conformational change of the nicotinamide ring and the ribose ring.

The conformational change of NADP⁺ was also observed in RnTmm when soaked with TMA, and this conformational
change was shown to be important for TMA oxidation (Li et al., 2017). After conformational change, the ribose ring of NADP⁺ in RnTmm forms a hydrogen bond with Asp317, shutting off the substrate entrance to promote a protected micro-environment for catalysis (Li et al., 2017). Because the electron densities of the ribose ring in Tmm7211-5-min and Tmm7211-20-min are rather poor (Figures 4B,C), we could not ascertain whether the ribose ring can form a hydrogen bond with Asp314 of Tmm7211, the equivalent residue to Asp317 of RnTmm. To further probe this, we generated mutants Asp314Ala and Asp314Glu, and measured their enzymatic activities. The enzymatic activity and the apparent $K_M$ of Tmm7211 toward NADPH are only slightly affected by Asp314Glu mutation (Figure 3E and Table 3), probably due to the similar properties of aspartic acid and glutamic acid. However, although the residue Asp314 is far away from the catalytic center of Tmm7211, the mutation of Asp314 to alanine decreased the activity of Tmm7211 significantly (Figure 3E), suggesting that Asp314 is involved in the catalytic reaction of Tmm7211. The CD spectrum of the mutant Asp314Ala was indistinguishable from that of WT Tmm7211, suggesting that the enzymatic activity loss in the mutant is caused by residue replacement rather than structural
TABLE 3 | Kinetic parameters for Tmm7211 and its mutants toward NADPH.

| Enzyme     | apparent $K_M$ (µM) | $k_{cat}$ (min$^{-1}$) |
|------------|---------------------|-----------------------|
| Wild type  | 12.8 ± 0.4          | 4.5 ± 0.3             |
| Asn72Ala   | 34.1 ± 1.0          | 1.9 ± 0.2             |
| Ser203Ala  | 134.8 ± 11.1        | 1.0 ± 0.1             |
| Arg226Ala  | 101.8 ± 8.6         | 0.9 ± 0.1             |
| His227Ala  | 41.2 ± 2.3          | 2.5 ± 0.2             |
| Asn288Ala  | 61.1 ± 4.0          | 1.3 ± 0.1             |
| Arg409Ala  | 44.9 ± 5.6          | 2.7 ± 0.1             |
| Asp314Ala  | 32.0 ± 0.6          | 2.1 ± 0.1             |
| Asp314Glu  | 9.7 ± 0.5           | 5.2 ± 0.2             |

DISCUSSION

DMS and DMSO are widespread in marine environment, and the oxidation of DMS to DMSO is an important biogeochemical reaction. Tmm7211 is a bacterial FMO which is able to catalyze the conversion of DMS to DMSO. Based on our results and previous studies of bacterial FMOs (Alfieri et al., 2008; Cho et al., 2011; Li et al., 2017), the structural basis of Tmm7211 for DMS oxidation to DMSO is proposed (Figure 5). The catalytic cycle of Tmm7211 consists of a reductive half-reaction and an oxidative half-reaction, similar to other bacterial FMOs (Alfieri et al., 2008; Cho et al., 2011; Li et al., 2017). In the reductive half-reaction, FAD is reduced by NADPH. In the oxidative half-reaction, the reduced FAD reacts with an oxygen molecule and the (hydro)peroxyflavin intermediate forms (Figures 1B, 5), which is a common trait for FMOs (Krueger and Williams, 2005; Eswaramoorthy et al., 2006; Alfieri et al., 2008). The nicotinamide ring of NADP$^+$ buried in Tmm7211 likely undergoes a conformational change and forms a new hydrogen bond with Asp314, which is important for the catalysis of DMS oxidation.
A proposed catalytic cycle of Tmm\textsubscript{7211} oxidizing DMS to generate DMSO. In the reductive half-reaction, FAD is reduced by NADPH. In the oxidative half-reaction, the reduced FAD reacts with O\textsubscript{2}, and a C4a-(hydro)peroxyflavin (FAD intermediate) is formed. The nicotinamide ring of NADP\textsuperscript{+} protects the FAD intermediate from solvent attack. When DMS enters the catalytic pocket, NADP\textsuperscript{+} generates a conformational change to form a hydrogen bond with Asp314, shutting off the substrate entrance and exposing the FAD intermediate to DMS. After the reaction, DMSO, NADP\textsuperscript{+} and a water molecule are released and the oxidized FAD is regenerated.

(1), NADP\textsuperscript{+} forms a hydrogen bond with Asp314, shutting off the substrate entrance and creating a protected micro-environment for catalysis; (2), the nicotinamide ring of NADP\textsuperscript{+} no longer protects the C4a-(hydro)peroxyflavin, exposing the active C4a-(hydro)peroxyflavin to DMS to complete the oxidation cycle (Figure 5). After the reaction, DMSO, NADP\textsuperscript{+} and a water molecule are released and the oxidized FAD is regenerated, enabling Tmm\textsubscript{7211} to get ready for the next catalytic cycle.

To elucidate the catalytic mechanism of Tmm\textsubscript{7211} to oxidize DMS, it is important to obtain structures of Tmm\textsubscript{7211} and Tmm\textsubscript{7211} in complex with DMS. Here, although we solved Tmm\textsubscript{7211} structures in three states, all our attempts to obtain the structure of Tmm\textsubscript{7211} in complex with DMS failed. As such, we propose the structural basis for DMS oxidation to DMSO by Tmm\textsubscript{7211} based on our structural and mutational analyses, and the previous studies of bacterial FMOs (Alfieri et al., 2008; Cho et al., 2011; Li et al., 2017). Tmm\textsubscript{7211} shares \textasciitilde 53\% sequence identity with three other reported bacterial FMOs from Nitrincola lacisaponensis (NiFMO) (Loncar et al., 2019), Methylophaga sp. strain SK1 (mFMO) (Alfieri et al., 2008) and R. nubinhibens ISM (RnTmm) (Li et al., 2017). The overall structure as well as the locations of NADP\textsuperscript{+} and FAD of Tmm\textsubscript{7211} are similar to those of mFMO (PDB code: 2VQ7), RnTmm (PDB code: 5IPY) and NiFMO (PDB code: 6HNS) (Figure 6), with the RMSDs between Tmm\textsubscript{7211} and mFMO, RnTmm and NiFMO of 0.5 Å, 0.6 Å and 0.5 Å, respectively. This suggests that Tmm\textsubscript{7211} may adopt a similar catalytic mechanism to oxidize DMS as these bacterial FMOs. Indeed, the catalytic mechanism of Tmm\textsubscript{7211} oxidizing DMS we proposed is similar to that of RnTmm oxidizing TMA (Li et al., 2017), which includes a reductive half-reaction and an oxidative half-reaction. Asp317 of RnTmm was reported to form a hydrogen bond with the ribose ring of NADP\textsuperscript{+} after its conformational change (Li et al., 2017). Here, our structural and biochemical results indicate that Asp314 of Tmm\textsubscript{7211} also likely forms a hydrogen bond with NADP\textsuperscript{+} after its conformational change, which is important for the catalysis of DMS oxidation. Moreover, sequence analysis showed that the residue Asp314 and most residues involved in binding NADP\textsuperscript{+} and FAD in Tmm\textsubscript{7211} are highly conserved in the Tmm sequences in both MRC and SAR11 bacteria (Li et al., 2017), suggesting that these residues are important residues in bacterial Tmms and that MRC and SAR11 bacteria containing Tmm may adopt a similar mechanism in oxidizing both DMS and TMA. Despite these analyses, further efforts, especially attempts to obtain the Tmm-DMS complex structure, are needed to provide more details of the catalytic mechanism of Tmm to oxidize DMS.

The volatile DMS is predominately produced from microbial degradation of DMSP through various DMSP dethiomethylases (colloquial “DMSP lyases,” EC 4.4.1.3) and the DMSP CoA-transferase/lyase DddD (EC 2.3.1.x) (Curson et al., 2011; Acolombri et al., 2014; Johnston et al., 2016). Despite this, DMS can also be generated from DMSO reduction catalyzed by the
DMSO reductase DMSOR (EC 1.8.5.3) (Bray et al., 2001), and from methanethiol (MeSH) via the methyltransferase MddA (EC 2.1.1.334, methanethiol S-methyltransferase) (Carrión et al., 2017; Boden and Hutt, 2019). In addition to Tmm, three other enzymes, namely DMS dehydrogenase DdhABC (EC 1.8.2.4, DMS:cytochrome c reductase) (McDevitt et al., 2002; Boden and Hutt, 2019), assimilatory DMS S-monoxygenase DsoABCDEF (EC 1.14.13.245) (Horinouchi et al., 1997; Boden and Hutt, 2019) and dissimilatory DMS monooxygenase DmoAB (EC 1.14.13.131) (Boden et al., 2011; Boden and Hutt, 2019), also participate in the bacterial consumption of DMS. DdhABC and DsoABCDEF convert DMS to DMSO, while DmoAB converts DMS to MeSH (Horinouchi et al., 1997; McDevitt et al., 2002; Boden et al., 2011). To investigate the prevalence and the coexistence of the enzymes involved in DMS metabolism, we searched homologs of DMS metabolism related enzymes using the IMG/M database (Chen et al., 2019). In total, 3,182 non-redundant bacterial genomes were filtered out to possess at least one type of enzymes related to DMS metabolism, among which 415 contain more than one types of DMS related genes. All these enzyme combinations yielded 22 different one-to-one enzyme configuration modes (Supplementary Figure 1). The relatively frequent co-occurrence between Tmm and DMSP dethiomethylases suggests that some bacteria may channel DMS generated from DMSP cleavage to DMS oxidation to DMSO.

CONCLUSION

The oxidation of oceanic DMS to DMSO is an important step in the global sulfur cycle, which can be catalyzed by Tmm (Lidbury et al., 2016). Tmm is present in ~20% of the bacteria in the surface ocean, and is particularly common in the cosmopolitan marine heterotrophs such as MRC and SAR11 bacteria (Chen et al., 2011). In this study, the recombinant Tmm7211 protein from the SAR11 bacterium Pelagibacter sp. HTCC7211 was purified and characterized. The crystal structures of Tmm7211 and Tmm7211 soaked with DMS were also solved. Based on structural analysis and mutational assays, the catalytic mechanism for Tmm7211 oxidizing DMS was proposed, which may be widely adopted by MRC and SAR11 bacteria. This study offers a better understanding of how marine bacteria oxidize DMS to generate DMSO.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

C-YL and Y-ZZ designed the research. X-LC and H-HF directed the research. C-YL, X-JW, NZ, and Z-JT performed the experiments. PW, W-PZ, and YC helped in data analysis. C-YL and X-LC wrote the manuscript. YC edited the manuscript.
All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.735793/full#supplementary-material

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