ENZYME KINETICS OF DddQ OF *Ruegeria pomeroyi* DSS-3: AN Fe(II)- AND Mn(II)-DEPENDENT DMSP LYASE

by

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(Under the Direction of Barny Whitman)

ABSTRACT

Dimethyl sulfide (DMS) is a climate-active gas produced from dimethylsulfoniopropionate (DMSP) by DMSP lyases, one of which is DddQ. The relative importance of DddQ compared to other lyases is not yet fully understood, and its low activity was one reason for the claim that it might not be an authentic DMSP lyase [1]. Here we examined the kinetic parameters, metal ions, and structure of DddQ of the marine bacterium *Ruegeria pomeroyi* DSS-3. We determined that the relevant metal ions for this DddQ are Fe(II) and Mn(II). The specific activity of Fe(II)-DddQ was 17.8 ± 1.6 µmol mg^{-1} min^{-1} and that of Mn(II)-DddQ was 3.3 ± 0.6 µmol mg^{-1} min^{-1}. Its activity level and relevant metal ions are comparable to those of DddW [2]. The crystal structure of this DddQ indicates a cupin-fold enzyme and high structural similarity to the DddQ of *R. lacuscaerulensis*. Based our findings, we conclude that DddQ is a bona fide DMSP lyase.

Index words: Dimethylsulfide, Dimethylsulfoniopropionate, DMSP lyase, DMSP lyase-like, DddQ, Cleavage, *Ruegeria*, DMS, Sulfur metabolism

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CHAPTER 1

INTRODUCTION

The purpose of this project was to study the enzyme kinetics and structure of dimethylsulfoniopropionate (DMSP) lyase DddQ from the marine bacteria *Ruegeria pomeroyi* DSS-3, an alphaproteobacterium that lives at the ocean surface. DMSP is ubiquitous in marine surface waters at nanomolar to micromolar concentrations, where it functions as an osmolyte, antioxidant and predator deterrent [3]–[5]. DMSP is synthesized by marine phytoplankton, algae, halophytic plants and bacterioplankton, in which the intracellular concentration of DMSP can reach up to 650 mM [6]. It can account for up to 10% of carbon fixation by marine phytoplankton and is also a source of sulfur and carbon for marine organisms [7], [8]. Phytoplankton can break down DMSP, but the most common fate of DMSP is release into the ocean. Upon assimilation by marine bacteria, DMSP is metabolized by two major pathways: the demethylation pathway and the cleavage pathway [9]–[11]. The demethylation pathway results in the formation of methanethiol and its eventual oxidation to sulfate and CO_2. The cleavage pathway results in the production of the volatile gas dimethyl sulfide or DMS. DMS has a significant environmental impact through its contribution to global sulfur cycling. Marine DMS has been identified as the most abundant source of biogenic sulfur in the atmosphere, or about 13-37 teragrams (10^9 kg) per year, resulting in the transfer of a substantial amount of sulfur from marine to terrestrial environments [12]–[14]. Although DMS is a key sulfur compound, the study of the DMS-producing Ddd enzymes in still in the beginning phase.

The key enzymes of the cleavage pathway are the Ddd enzymes, where Ddd stands for DMSP-dependent DMS. Seven Ddd enzymes have been identified: DddD, DddK, DddL, DddP, DddQ, DddW, DddY [15]–[23]. All Ddd enzymes break down DMSP to produce DMS and acrylate except for DddD,

which produces acetate and 3-hydroxypropionyl-CoA (Figure 1). Four of these enzymes possess the cupin sequence motif and the cupin fold: DddK, DddL, DddQ and DddW. Cupin-fold enzymes typically consist of a metallocenter buried in a β-barrel with 4 to 8 β-sheets. In some cupin proteins the β-barrels are surrounded by α-helices. The metallocenter is coordinated by the conserved cupin motifs $Y(X)_2H(X)H(X)_4E(X)Y$ and $G(X)_{10}H$ (Figure 2)[24], [25]. In the majority of enzymatic cupin proteins, ferrous ions are the active site metal. However, for DddQ, the active site metal has been proposed to be Zn(II), which supports a very low specific activity of 0.002 to 0.027 μmol mg^{-1} min^{-1} [26], [27]. In contrast, DddW, which has similar active site motifs, possesses either Fe(II) and Mn (II) at its active site and much higher specific activity, approximately 61 μmol mg^{-1} min^{-1} [2].

CHAPTER 2

ANALYSIS OF DDDQ'S ENZYME KINETICS AND CRYSTAL STRUCTURE

In this study, we examined the transition metal requirements of the recombinant DddQ of *Ruegeria pomeroyi* DSS-3, the kinetic parameters of DddQ under optimal conditions, as well as the crystal structure of DddQ. We found that the specific activity of DddQ is the highest in the presence of Fe(II) and Mn(II), and its specific activity was 17.8 ± 1.6 μmol mg^{-1} min^{-1} with ferrous ions. These more accurate measurements allow comparison of DddQ with other cupin lyases and has implications on its physiological importance.

CHAPTER 3

MATERIALS AND METHODS

Protein expression and purification

The gene encoding DddQ from *R. pomeroyi* DSS-3 with an N-terminal His-tag was codon optimized for expression in *Escherichia coli* and cloned in the pD444 expression plasmid ATUM/DNA2.0 under control of an isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible promoter. For expression in minimal medium, a 20 ml starter culture was inoculated into 1 liter of LB medium and then grown at 37 °C with shaking at 200 rpm. At an OD_{600} of 0.8-1.0, the cells were transferred into M9 minimal medium and 1 mM IPTG and 1mM metal ions ($MnCl_2$, $CoCl_2$, $NiCl_2$, $ZnCl_2$, $CuCl_2$, or ferric ammonium citrate) were added. The apo-DddQ culture was induced with IPTG in the absence of the addition of metal ions. Cultures were incubated at 18 °C with shaking at 200 rpm for 17 h. Cells were harvested by centrifugation (10,000 × g) for 10 minutes at 4 °C and stored at −80 °C until purification. For the expression of Fe(II)-DddQ in a highly enriched medium, the 20 ml starter culture was added to 1 liter of TB medium (1.2 % tryptone, 2.4 % yeast extract, 0.5 % glycerol, 17 mM KH_2PO_4, 72 mM K_2HPO_4) and then grown at 37 °C, with shaking at 200 rpm. At an OD_{600} of 0.8-1.0, 1 mM IPTG and 1mM ferric ammonium citrate were added. Cultures were incubated at 18 °C with shaking at 200 rpm for 17 h. Cells were harvested by centrifugation and stored as above.

For enzyme purification, cells were resuspended in TK buffer (20 mM Tris buffer at pH 8 with 200 mM KCl) containing a spatula tip of DNase, lysozyme, and SigmaFast EDTA-free protease inhibitor) and lyzed at 1200 psi with an SLM Aminco French press. The lysate was centrifuged at 60,000 × g and 4 °C for 1.5 h. The supernatant was collected and passed through a gravity flow cobalt column previously equilibrated with TK buffer. The column was then washed with three column volumes of TK buffer and

on one column volume each of TK buffer containing 15 mM and then 50 mM imidazole. Fractions were analyzed by SDS-PAGE. The protein was dialyzed to remove imidazole and stored in a pH 8 20 mM Tris buffer at −80 °C until use.

Determination of metal ion content by ICP-MS

Metal analyses of purified DddQ were performed in duplicate using an Agilent 7500ce octopole-based inductively coupled plasma mass spectrometer in FullQuant mode. For the sample dilution and container washing, trace metal grade nitric acid (70%) from Fischer Scientific and pure glass double-distilled water from a Corning MP-3A distillation apparatus (gddH_2O) were used. All containers were acid-washed by submersion in 2% (vol/vol) nitric acid for 24 h and rinsed twice by submersion in gddH_2O for 24 h. The samples were prepared by diluting DddQ to 1 mM or less by the addition of 2% (vol/vol) nitric acid in gddH_2O. The supernatants were collected by after centrifugation (2,400 × g) for 10 minutes at 25 °C. The metal ion concentrations in buffer were determined using the same volume of buffer as the sample and the same dilution with nitric acid in gddH_2O. Sample loading, instrument settings, data acquisition and processing were performed as previously described [28]–[30].

Purified DddQ was quantified with its UV absorbance at 280 nm using an extinction coefficient of 26,025 M^{-1} cm^{-1} [31]. The metal:protein ratio was calculated by dividing the moles of metal ions by the moles of DddQ monomers.

Lyase activity assays

The assay with purified DddQ enzyme was developed and optimized as follows. First, DddQ's DMSP lyase activity was assessed in various reaction buffers: MES, MOPS, HEPES, Bis-Tris and Tris at a range of reaction pH from pH 5.5 to pH 8. The DMS production was compared under a standard incubation time of 3 minutes and temperature of 30 °C, in order to minimize the non-enzymatic formation of DMS gas. Then, the linear range of concentration of DddQ in which the production of DMS

is proportional to the enzyme concentration was determined by varying the enzyme concentration in the reactions. Finally, the linear range of DddQ was adjusted after the optimization of the metal ions. After varying the assay conditions, pH 7 was determined to be the optimal pH. At pH higher than 7.5, DMS was released in detectable amounts in the absence of DddQ. In terms of reagent suitability, Bis-Tris produced the lowest spontaneous detection of DMS. The concentration of DddQ required for a linear increase of DMS was 110 µg of purified enzyme in a 1 ml reaction, equivalent to an enzyme concentration of approximately 5 µM.

All DddQ assays were performed in triplicate in an aerobic environment with the exception of assays with ferrous or manganese ions, which were performed anaerobically. The substrate DMSP was synthesized as previously described [32]. The standard assay of 1 ml volume contained 55 µg of purified DddQ, 80 mM Bis-Tris adjusted to pH 7 with NaOH, and 40 mM of the respective metal chloride (except for the manganese reactions which contained 75 mM $MnCl_2$) in 3.7 ml sealed reaction vessels. Reactions were initiated by adding DMSP and incubated for 3 min at 30 °C. Kinetic values were determined with 1 mM to 20 mM DMSP. DMS formation in the headspace was monitored with a Supelco Chromosil 330 column and a flame photometric detector at 60°C in an SRI 8610C gas chromatograph [33]. Under these conditions, the assay was linear with time and protein concentration.

The kinetic parameters of Fe(II)-DddQ were determined with Fe(II)-DddQ enzymes obtained from highly enriched media. Prior to assay, the enzymes were pre-incubated at 30°C for 10 minutes under anaerobic conditions with equimolar concentrations of $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ and 2 mM ascorbic acid in 80 mM Bis-Tris at pH 7. The reactions were initiated by adding the pre-incubated enzymes to 1 ml of reaction mixture containing 1 mM to 20 mM DMSP, 80 mM Bis-Tris at pH 7.0, and 2 mM $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ and 2 mM ascorbic acid.

X-ray crystallization, data collection and structure determination

Recombinant DddQ expressed in rich medium was diluted from the storage concentration of 24 mg/ml to 5 mg/ml for crystallization. Initial crystallization trials for DddQ were performed at 18°C using sitting drop vapor diffusion with Hampton screens (PEG Ion 1 and 2, Index, Crystal Screen 1 and 2, Peg Rx 1 and 2) by mixing 2 µl of enzyme with 2 µl of precipitant solution. Diffraction-quality crystals of approximately 0.5 mm in diameter were obtained with 100 mM ammonium acetate, 100 mM Bis-Tris pH 5.5, 17% w/v PEG 10,000 after 20 days of incubation.

X-ray diffraction data were collected using synchrotron radiation at 100 K at the SER-CAT ID/BM-22 beamline at the Advanced Photon Source of Argonne National Laboratory. Data sets were indexed, integrated and scaled using HKL2000 [34]. The phase problem was solved by molecular replacement method using the coordinates of *R. lacuscaerulensis* DddQ (PDB ID 4LA2) as a starting model in the program Phenix, version 1.8.4_1496 [35]. Iterative refinement calculations and manual model rebuilding were performed with Phenix and COOT, version X11-2.7.7 [36], respectively. All coordinates were deposited in the RCSB databank (PDB ID 5CU1). Structural figures were made using PyMOL [37].

CHAPTER 4

RESULTS

Recombinant DddQ with an N-terminal His-tag was expressed in *E. coli* BL21 (DE3) and purified. After one chromatographic separation step with a cobalt column, DddQ was purified to 90% (Figure 3). The impurity at 40 kDa could not be removed by treating the protein samples with β-mercaptoethanol, indicating the band above the DddQ band was not formed by dimerization. Because DddQ loses all of its enzymatic activity within 4 hours after elution from the cobalt column, it was not further purified.

The crystal structure of DddQ was determined to 2.3 Å and with an average B-factor for both protein and solvent close to 22 $Å^2$. The refinement statistics are summarized in Table 1. The asymmetric unit consists of 4 monomers in a triclinic structure without a symmetry axis. The core structure contains a cupin fold with one iron center among 8 antiparallel β-sheets, which were surrounded by 4 α-helices. In the solved structure, the ferrous ion is coordinated by the tyrosine136 in proximity (4.7 Å) as well as histidine130 (2.4 Å), glutamate134 (2.3 Å) of cupin motif 1 and histidine169 (2.2 Å) of cupin motif 2.

Based on other existing publications on DddQ, we originally planned to assay the activity of apo-DddQ by EDTA metal chelation of the purified DddQ enzyme, followed by the metal ion reconstitution of another transition metal ion. However, regardless of the EDTA concentration, the dialysis in an EDTA-containing buffer followed by the re-dialysis in an EDTA-free buffer caused DddQ to precipitate. As a consequence, the DddQ containing Fe(II), Mn(II), Ni(II), Co(II), Zn(II), or Cu(II) were produced in metal ion-supplemented M9 minimal media. According to the results from ICP-MS analysis, these enzymes did not have full metal occupancy, i.e. each enzyme molecule did not contain a metal ion in its active site (Table 2). We observed that adding Mn(II) ions to the reaction mixture with Mn(II)-DddQ increased the activity rate and made similar observations with adding Fe(II) ions to the Fe(II)-DddQ reaction, probably

due to improving the incomplete metal occupancy. However, we could not directly measure the occupancy of the metal ion in the enzyme after addition of metal ions into the reaction because of the labile nature of the metal-DddQ. In contrast to Mn(II) and Fe (II), metal additions did not increase the activity of Ni(II)-DddQ, Co(II)-DddQ, Zn(II)-DddQ, and Cu(II)-DddQ, and their activity remained below the level of detection. These findings imply that these metal ions are not binding to the site or not allowing for enzyme activity. Using the enzymes purified from supplemented minimal media, the specific activity of Mn(II)-DddQ was determined to be 8.6 µmol DMS mg^{-1} min^{-1} and Fe(II)-DddQ at 3.2 µmol DMS mg^{-1} min^{-1} (Figure 5).

After finding the suitable metal ions, the linearity of DddQ and the metal ion addition were re-optimized. The linearity study indicates that 20 µg purified enzyme in a 1 ml reaction, equivalent to an enzyme concentration of approximately 1 µM, is the minimal of the linear range where enzyme activity is directly proportional to its concentration. Therefore, the kinetics of DddQ with Fe(II) and Mn(II) were studied with 1 µM or more of DddQ. In addition, the pre-incubation procedure and expression in iron-enriched TB media further increased the activity rates of Fe(II)-DddQ by 116%. With optimizations for the metal ion occupancy, the specific activity of Fe(II)-DddQ purified from rich media was determined at 17.8 ± 1.6 µmol DMS mg^{-1} min^{-1} and the specific activity of Mn(II)-DddQ purified from supplemented minimal media was determined at 3.3 ± 0.6 µmol DMS mg^{-1} min^{-1}.

The kinetic parameters were determined through a Lineweaver-Burk plot and are summarized as follows: Fe(II)-DddQ: V_{max} = 40.1 ± 10.0 µmol/min; k_{cat} = 742 s^{-1}; K_M = 24.8 ± 9.7 mM; k_{cat}/K_M = 29.9 x 10^3 $M^{-1}s^{-1}$; Mn(II)-DddQ: V_{max} = 6.0 ± 2.4 µmol/min; k_{cat} = 55.5 s^{-1}; K_M = 16.4 ± 11.7 mM; k_{cat}/K_M = 3.4 x 10^3 $M^{-1}s^{-1}$.

CHAPTER 5

DISCUSSION

This is the first kinetic study of any DddQ that demonstrates that DddQ's activity in breaking down DMSP to DMS and acrylate and metal specificity are much closer to DddW than previously thought. Previous studies have found that DddQ's activity rate to be at 2.7×10^{-2} μmol DMS mg^{-1} min^{-1} or lower[26], [27], while in this study, its specific activity was demonstrated to be 100-fold higher at approximately 18 μmol DMS mg^{-1} min^{-1}. Although this specific activity of DddQ was not as high as DddW, reported to be 61 μmol DMS mg^{-1} min^{-1} in another publication[2], it is high enough for DddQ to be a relevant DMSP lyase in marine organisms.

Two factors could contribute to the much higher activity of DddQ in this study. First, before conducting enzyme assays we took the steps to reduce or eliminate issues related to the low stability of DddQ. In our study, the steps involving enzyme purification and buffer exchange were executed within the 2 days immediately prior to storing the purified enzyme. Second, we used ICP-MS to determine the relevant metal ions multiple times throughout this study, and the correct metal ions could render DddQ with higher activities. We determined that Fe(II) and Mn(II) are the correct metal ions and not Zn(II) or other transition metal ions such as Co(II). Although Zn(II) ions are found in all major classes of enzymes including the cupin superfamily to which DddQ belongs [25], [38], Zn(II), Fe(II) and Mn(II) can fit into a tetrahedral coordination environment, i.e. with 4 ligands. Therefore zinc ions could occupy the metal center of ferrous enzymes and make them inactive. We also considered the possible artifact of protein purification is Co(II) contamination from the $CoCl_2$ on His-tag purification column. In fact, the fluorescence scanning at the synchroton indicated that cobalt was the most abundant metal ion, followed by iron. Because the DddQ crystal contained more Co(II) than Fe(II), we investigated the

activity level of Co(II)-DddQ by assaying Co(II)-supplemented minimal media-purified DddQ with DMSP. However, despite varying Co(II) concentration, Co(II)-DddQ could not cleave DMSP. In contrast, varying Fe(II) concentrations changed the activity of DddQ. Therefore, Fe(II) was designated to be the relevant metal ion for the crystal structure of DddQ.

Comparing the activity of DddQ and DddW is interesting, because both of the genes *dddQ* and *dddW* exist in *R. pomeroyi* DSS-3. The co-existence of these genes is also found in other marine bacterial species such as *Ruegeria lacuscaerulensis* ITI_1157 and *Sulfitobacter* sp. EE36. The previous study of DddW by Brummet *et al.* identified a specific activity of approximately 61 µmol mg^{-1} min^{-1} of DddW at pH 8.0 in the presence of Fe(II)[39]. Due to lower stability of DMSP above pH 7.5, this activity rate of DddW could have been overestimated. On the other hand, the specific activity of Fe(II)-DddQ at pH 7.0 in this study is at 18 µmol mg^{-1} min^{-1}. As a result, DddQ and DddW could share quite similar activity rates under physiological conditions.

Our structural study of DddQ from *R. pomeroyi* DSS-3 indicates a similar overall and active site structure with other published DddQs [26], [39], so our activity rate of DddQ could represent all structurally similar enzymes. *Rp*DddQ's overall structure is a β barrel surrounded by α-helices, similar to that of crystallized *Rl*DddQ. In our structure, tyrosine136, the last residue of the cupin motif 1 $Y(X)_2H(X)H(X)_4E(X)$**Y**, is the closest tyrosine residue to the metal ion ((4.7 Å). Based on the distance, tyrosine136 is likely to coordinate the ferrous ion rather than to catalyze the reaction with the substrate. Tyrosine125, the first residue of the cupin motif I, is at a more distal position (5.4 Å) to the metal ion. We agree with the previous study of Brummet *et al.* on *Rl*DddQ that the first tyrosine residue of the cupin motif, i.e. tyrosine125 of *Rp*DddQ, is responsible for proton abstraction from DMSP during catalysis [39]. Similar to tyrosine120 and tyrosine77 of *Rl*DddQ, *Rp*DddQ's active site contains tyrosine125 and a proximal residue tyrosine82 to create a hydrophobic pocket (UGENE/ClustalW alignment). In *R. lacuscaerulensis* ITI_1157's DddQ, tyrosine77 promotes the anionic state of tyrosine120

during catalysis in preparation for proton abstraction from the substrate DMSP [39]. Not only the overall structure, but the active site is in close agreement with previous reports on the crystal structure of *R*lDddQ. Due to structural similarities, we believe that using our protocol, the actual activity rates of *R*lDddQ or other DddQs will be quite similar to our DddQ.

The findings of this study support the concept that DddQ and DddQ-clade enzymes are bona fide DMSP lyases, and that the DddQ-clade of enzymes could compete with DddW to break down DMSP. The DddQ-clade of enzymes was identified in the phylogenetic tree of the cupin-DLL (DMSP lyase and lyase-like) family by Lei *et al.* [1]. It consists of about one third of all cupin-DLL (DMSP lyase and lyase-like) family members, and more enzymes are categorized under this family than the DddW family. Further investigations are certainly needed, but our findings suggest that enzymes of the DddQ-clade are of known functions. It would be insightful to apply the assay methods presented here to enzymes in other DLL-cupin family clades to screen for DMSP lyase activity.

Based on the likelihood that DddQ is a true DMSP lyase as indicated by this study, an intriguing point of further investigation is the physiological roles of the multiple DMSP lyase genes in some marine bacteria. One speculation is that bacterial species possessing *dddQ* and *dddW* could both be expressed to take advantage of a temporal or seasonal difference of Fe(II) [40], [41]. Another possibility could be that DddQ and DddW possess different metal-retaining abilities in their respective protein structures. The previously published DddW activity assays from Brummet *et al.* [2] did not seem to require DddW to be stabilized or pre-incubated with metal ions to reach optimal activity rates. In our structural prediction of DddW with the local meta-threading server (LOMETS) [42], DddW possesses a simple β-sheet structure without α-helices, and this might contribute to metal-retaining differences between DddQ and DddW.

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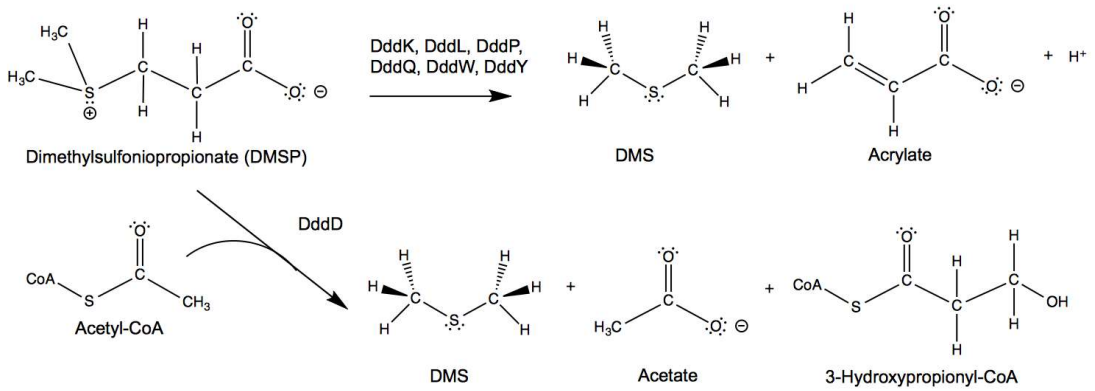
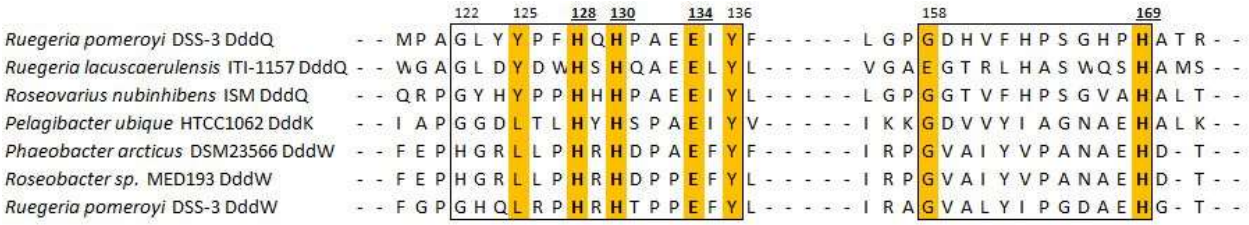
Figure 1



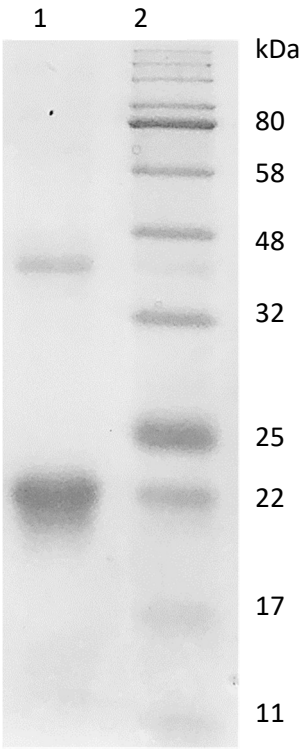
Bacterial DMSP cleavage reactions. Bacterial enzymes DddK, DddL, DddP, DddQ, DddW and DddY generate DMS and acrylate through their cleavage reaction (top). DddQ is investigated in this study to further the understanding of the required conditions to cleave DMSP. DddD is a CoA-transferase and produces DMS, acetate, and 3-hydroxy-propionyl-CoA (bottom).

Figure 2



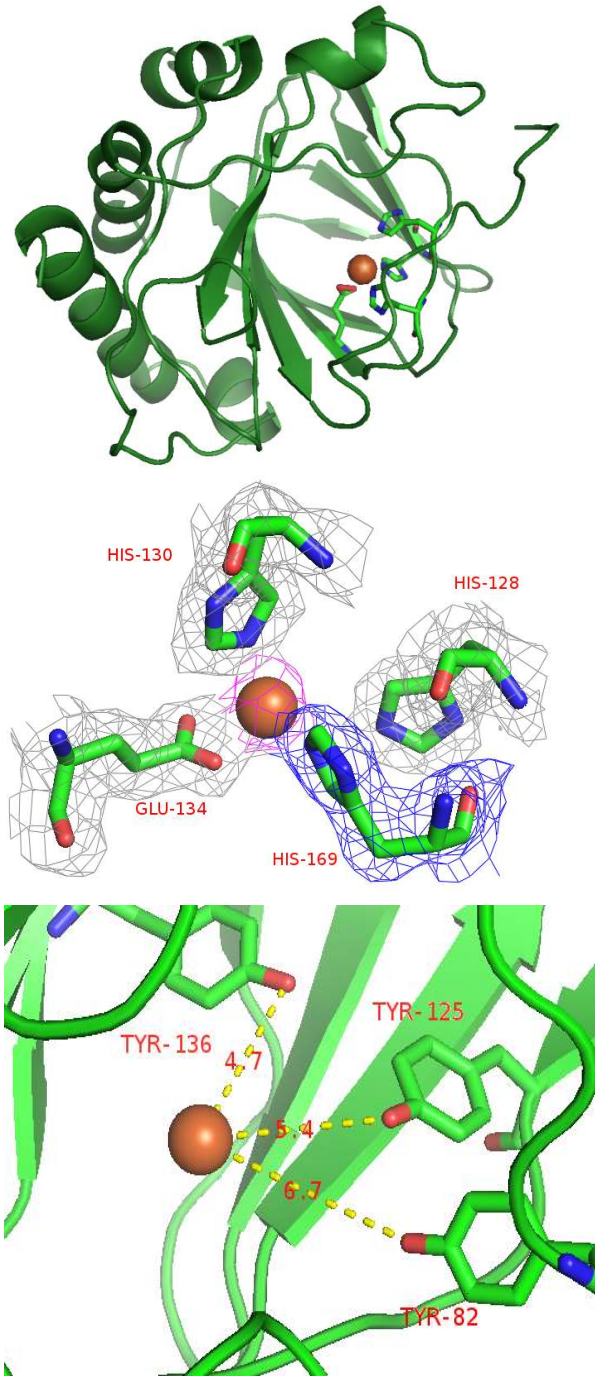
Protein sequence alignment of the cupin regions of DMSP lyases. Sequences of cupin motifs of DMSP lyases DddQ, DddK and DddW were aligned with ClustalW using sequences deposited in roseobase.org and NCBI. The first conserved cupin motif $Y(X)_2H(X)H(X)_4E(X)Y$ and the second conserved cupin motif $G(X)_{10}H$ are highlighted. The metal-binding residues are in bold. Residues were numbered using *R. pomeroyi* DSS-3 DddQ as reference.

Figure 3



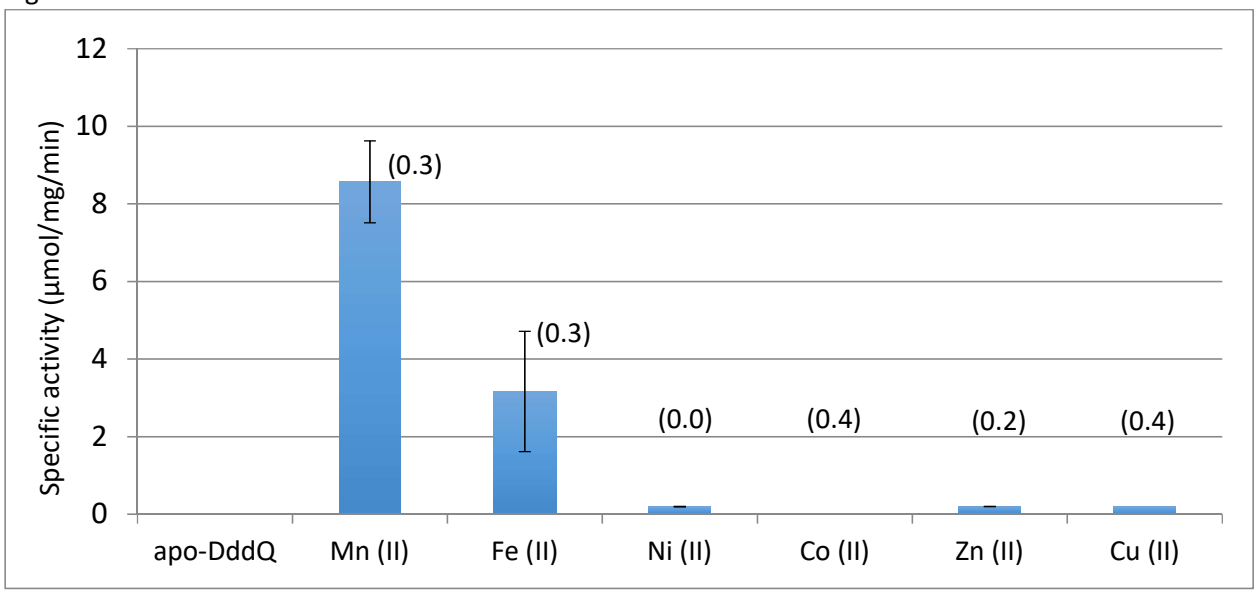
SDS-PAGE of purified recombinant *R. pomeroyi* DSS-3 DddQ. SDS-PAGE of purified recombinant *R. pomeroyi* DSS-3 DddQ obtained from rich medium. [Lane 1] The recombinant *Rp*DddQ enzyme purified using a HisTrap column. [Lane 2] Broad-range protein marker (New England Biolabs). A 15% SDS-PAGE gel was used. The single-step purification yielded DddQ with 90% purity (determined by ImageJ).

Figure 4



Crystal structure of *Rp*DddQ. Top: The overall structure of *Rp*DddQ with a close-up of the metal-binding residues. Middle: Iron is shown as a red-colored sphere, and the metal-coordinating residues with histidine130, glutamate134 and histidine169 (the catalytic triad) are shown in sticks: carbon in protein light green, oxygen in red, and nitrogen in blue. Histidine128 in this *Rp*DddQ is responsible for DMSP-anchoring, similar to the function of histidine130 of *Rl*DddQ [2]. Bottom: the view of the active site tyrosine residues. The hydrogen bonding distances are shown in yellow dash lines.

Figure 5



Effects of transition metal ions on the DMSP cleavage activity of DddQ. The *Rp*DddQ of this experiment were expressed in minimal media with supplemented metal ion as described in the experimental procedures. The recombinant *Rp*DddQ enzyme showed activity with Mn(II) and Fe(II), and no significant activity with Co(II), Ni(II), Co(II), or Zn(II). The error bars represent the standard deviation for three separate enzyme assays with independent measurements of the DMS gas produced. In parenthesis are the ratios of the molar quantities of the specific metal ions to recombinant *Rp*DddQ.

Table 1 Data collection and refinement statistics of DddQ crystal structure

	DddQ
	Data collection
Protein Data Bank entry	5CU1
space group	P21212
unit cell dimensions a, b, c (Å)	62.582, 69.878, 49.731
completeness (%)	99.88 (93.7)
unit cell redundancy	5.9
number of reflections	10150
I/σ	9.69 (1.95)
$CC_{1/2}$	0.990 (0.818)
R_{meas} (%)	17 (48)
R_{mrgd} (%)	14 (41)
R_{pim} (%)	9 (27)
	Refinement
resolution (Å)	2.28 (2.37-2.28)
R_{work}/R_{free}	0.23/0.29
R_{free} in highest resolution shell	0.33
number of atoms protein/ligand/water	1528/1/61
B-factors protein/ligand/water	22/35/22
	Stereochemical ideality
bond lengths (Å)	0.008
bond angles (deg)	1.119
ϕ,ψ preferred region (%)	96.41
ϕ,ψ additionally region (%)	3.59
ϕ,ψ disallowed region (%)	0

Table 2 Metal to protein ratio in DddQ expressed in supplemented minimal media

Metal: protein ratio	Mn	Fe	Ni	Co	Zn	Cu
apo-DddQ	0.016	0.188	0.038	0.072	0.078	0.019
	0.008	0.034	0.008	0.006	0.002	0
Mn (II)-DddQ	0.256	0.295	0.01	0.075	0.071	0.002
	0.066	0.06	0.001	0.002	0.001	0
Fe (II)-DddQ	0.007	0.273	0.026	0.107	0.057	0.023
	0.002	0.03	0.02	0.011	0.008	0.011
Ni (II)-DddQ	0.207	0.016	0.039	0.011	0.021	0.008
	0.498	0.002	0.039	0.195	0.01	0.015
Co (II)-DddQ	0.003	0.025	0.021	0.408	0.077	0.008
	0	0.014	0.004	0.021	0.055	0.102
Zn (II)-DddQ	0.905	0.041	0.018	0.025	0.199	0.012
	0.435	0.002	0.005	0.024	0.037	0.002
Cu (II)-DddQ	0.003	0.051	0.04	0.109	0.175	0.413
	0	0.014	0.004	0.021	0.055	0.102

The metal to protein ratios were calculated from the ICP-MS quantification of transition metal ions in DddQ expressed in metal-supplemented minimal media. The left column indicates the minimal media's metal supplements. The label above the values indicate the metal detected by the ICP-MS. The metal to protein ratios were calculated according to the procedure as described in the methods section. The two ratios under each DddQ were obtained from two protein samples, each measured three times at the ICP-MS. The metal ions supplements include manganese, iron, nickel, cobalt, zinc and cobalt. Additionally, other common enzymatic metal including molybdenum, tungsten, magnesium and calcium were tested but their quantities found in enzymes were insignificant. The results above indicate that *E. coli*'s metal ion integration into DddQ was incomplete, but in most cases the designated type of metal ion was integrated. One notable exception is expression in manganese-supplemented minimal media, where DddQ enzymes were occupied with both manganese and iron. Two other exceptions were Ni(II)-DddQ and Zn(II)-DddQ, which indicated a higher manganese ion level in two samples. These Mn(II) values appear to be incorrect, therefore, further ICP-MS experiments have been conducted to confirm the metal concentrations. At the timepoint of submission of this thesis, the data evaluation is not yet available.