pubs.acs.org/acschemicalbiology

Articles

In Vitro Reconstitution of a Five-Step Pathway for Bacterial Ergothioneine Catabolism

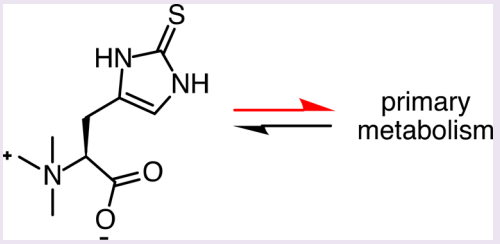
Mariia A. Beliaeva, Florian Leisinger, and Florian P. Seebeck*

Cite This: *ACS Chem. Biol.* 2021, 16, 397–403

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Ergothioneine is a histidine-derived sulfur metabolite that is biosynthesized by bacteria and fungi. Plants and animals absorb ergothioneine as a micronutrient from their environment or nutrition. Several different mechanisms of microbial ergothioneine production have been described in the past ten years. Much less is known about the genetic and structural basis for ergothioneine catabolism. In this report, we describe the *in vitro* reconstitution of a five-step pathway that degrades ergothioneine to L-glutamate, trimethylamine, hydrogen sulfide, carbon dioxide, and ammonia. The first two steps are catalyzed by the two enzymes ergothionase and thiourocanate hydratase. These enzymes are closely related to the first two enzymes in histidine catabolism. However, the crystal structure of thiourocanate hydratase from the firmicute *Paenibacillus sp.* reveals specific structural features that strictly differentiate the activity of this enzyme from that of urocanate hydratases. The final two steps are catalyzed by metal-dependent hydrolases that share most homology with the last two enzymes in uracil catabolism. The early and late part of this pathway are connected by an entirely new enzyme type that catalyzes desulfurization of a thiohydantoin intermediate. Homologous enzymes are encoded in many soil-dwelling firmicutes and proteobacteria, suggesting that bacterial activity may have a significant impact on the environmental availability of ergothioneine.



INTRODUCTION

Ergothioneine is a ubiquitous sulfur metabolite that occurs in a broad range of different organisms, including pathogenic, photosynthetic, or halophilic bacteria, fungi, plants, and animals.[1−4] Ergothioneine derives from bacterial and fungal production and migrates up the food chain either directly or via plants and animals into humans where this micronutrient is believed to participate in the cellular protection against oxidative stress.[5] Indeed, studies with genetically modified model organisms rendered unable to import ergothioneine were shown to suffer from increased susceptibility to oxidative damage.[6−8] A growing body of evidence suggests that limited dietary uptake of ergothioneine may cause long-term health problems or even reduce life expectancy.[5,9−12] Hence ergothioneine may be considered as a food additive to overcome reduced resorption in certain patients or to fortify food that does not contain sufficient amounts of this micronutrient. Indeed, concerns have been raised that modern agricultural methods that limit the exposure of plants to fungi and soil bacteria may produce food crops with reduced ergothioneine content.[13] This new connection between long-term human health and the composition of arable soil also raises renewed interest into the microbial processes that modulate ergothioneine availability.

It is now clear based on genetic evidence that ergothioneine biosynthesis is a fairly common trait. Almost all fungi and bacteria from many different phyla, including actinobacteria, cyanobacteria, firmicutes, and proteobacteria, encode ergothioneine biosynthetic enzymes.[14,15] Biochemical and structural characterization of exemplary proteins showed that the ability to convert histidine to ergothioneine has emerged at least three times through independent evolutionary processes.[4,14,16,17] Although catabolism of ergothioneine may be equally widespread and diverse, much less is known about how microorganisms compete with plants and animals for this nutrient.

Early observations documented ergothioneine degrading activity in several enterobacterial strains.[18−20] More recently, the enzyme ergothionase which converts ergothioneine to thiourocanic acid and the enzyme thiourocanate hydratase from *Burkholderia sp.* HME 13 have been identified, cloned, and characterized.[21,22] The crystal structure of ergothionase from *Treponema denticola* provided first insights into the catalytic mechanism of this enzyme.[23] The conservation of critical active site residues in hundreds of bacterial homologues suggests that ergothioneine degradation may be a common activity in gastrointestinal and soil microbiomes.

In this report, we describe the *in vitro* reconstitution of the complete five-step pathway that degrades ergothioneine to trimethylamine, glutamate, hydrogen sulfide, carbon dioxide,

Received: December 15, 2020
Accepted: January 27, 2021
Published: February 5, 2021

and ammonia (Figure 1). The third enzyme in this pathway—a thiohydantoin desulfurase—shares no discernible homology to

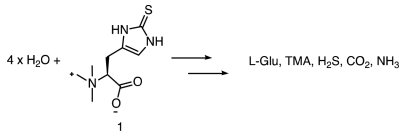


Figure 1. Five enzymes from *Paenibacillus sp.* catalyze the degradation of ergothioneine (**1**) to glutamate, trimethylamine (TMA), hydrogen sulfide (H_2S), carbon dioxide (CO_2), and ammonia (NH_3).

any protein with known function and thus represents a new type of C−S bond cleaving enzyme.[24] The last two steps are catalyzed by two metal-dependent hydrolases that share similarity with enzymes that participate in uracil degradation. The crystal structure of thiourocanate hydratase in complex with urocanate and the cofactor NAD^+ identifies key structural determinants that differentiate the catalytic activity of this enzyme from that of the closely related homologue urocanate hydratase.[25]

RESULTS AND DISCUSSION

Identification of Ergothioneine Degradation Enzymes in *Paenibacillus sp.* The crystal structure of ergothionase from *T. denticola* (*Td*ETL) highlighted four active site residues (Tyr54, His84, Glu412, and Lys384) that are essential for the specific recognition and turnover of ergothioneine. In the genome of *Paenibacillus sp.* Soil724D2, we found an open reading frame (ORF, KRE36541) coding for an ergothionase homologue with 49% sequence identity to *Td*ETL (seq id.) with all four critical active site residues conserved (Figure S3). The neighboring ORF (KRE36542) codes for a protein of unknown function (DUF917). The next ORF (KRE36543) codes for a protein sharing 63% seq id. with the recently reported thiourocanate hydratase (TUC) from *Burkholderia sp.* HME13, suggesting that the homologue from *Paenibacillus* may have the same activity.[22] The fourth and fifth ORFs (KRE36544 and KRE36545) code for putative metal-dependent hydrolases. Cursory inspection of the genome neighborhood of the putative thiourocanate hydratase (KRE36543) using the Genome Neighborhood Tool showed that clusters of these five genes in variable arrangements are a recurring motif in genomes of numerous firmicutes and proteobacteria.[26] To test whether this locus codes for a catabolic pathway, we produced the putative thiourocanate hydratase and the three enzymes of unknown function in *E. coli* and characterized their activity *in vitro*.

***In Vitro* Reconstitution of Complete Ergothioneine Degradation.** Thiourocanate (**2**, Figure 2) was generated by incubation of 1 mM ergothioneine in phosphate buffer (pH 8.0) with ergothionase (5 μM) from *Bacillus sp.* at 25 °C for 10 h (reaction B, Figure 3).[23] The product thiourocanate and all

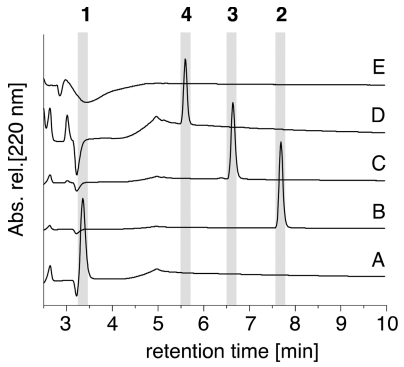


Figure 3. Reversed-phase HPLC analysis of reactions containing A: ergothioneine; B: A + ergothionase; C: B + thiourocanate hydratase; D: C + thiohydantoin desulfurase; and E: D + hydantoinase. The peaks highlighted in gray were identified as compounds **1**−**4** by ^1H NMR and HR-ESI-MS (Table S3). The product of E + glutamate carbamoylase was identified as glutamate after derivatization with *o*-phthalaldehyde and 3-mercaptopropionic acid (Figure S13).

subsequent products were identified by high resolution electrospray mass spectrometry (HR-ESI-MS, Table S3) and

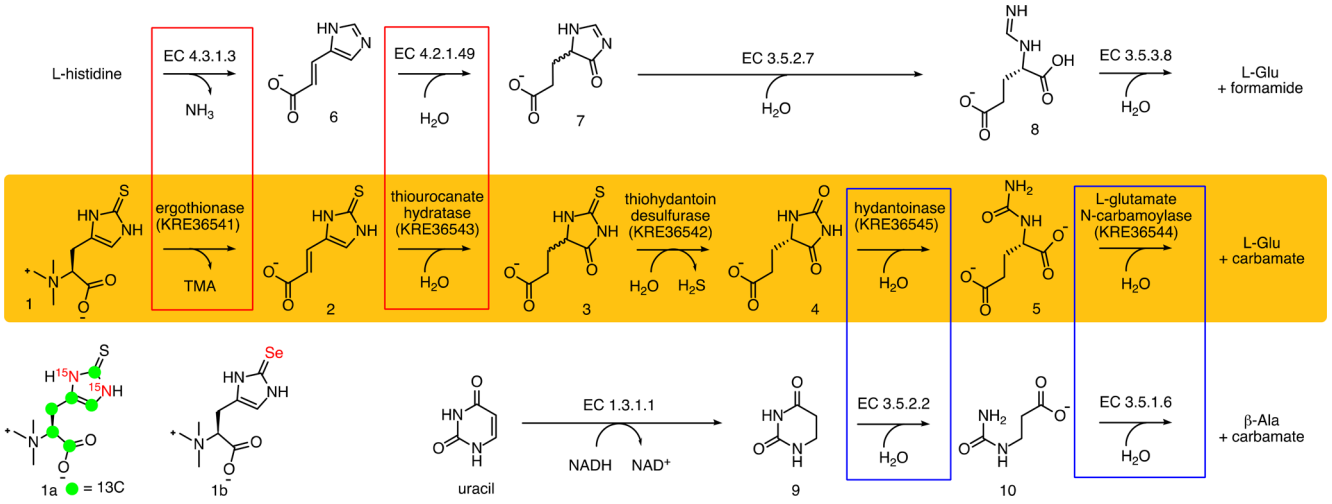


Figure 2. Yellow box: The *in vitro* reconstituted enzymes from *Paenibacillus sp.* mediate degradation of ergothioneine (**1**) and isotope labeled ergothioneine (**1a**) via the intermediates thiourocanic acid (**2, 2a**), 3-(5-oxo-2-thioxoimidazolidin-4-yl)propanoic acid (**3, 3a**, thiohydantoin), 3-(2,5,-dioxoimidazolidin-4-yl)propanoic acid (**4, 4a**, hydantoin), and *N*-carbamoyl glutamate (**5**). Selenoneine (**1b**) is degraded by the same enzymes via the equivalent intermediates **2b, 3b**, and **4b**. Red box: The first two steps are similar to the first steps in histidine degradation via the intermediates urocanate (**6**), 4,5-dihydro-4-oxo-5-imidazolepropanoate (**7**), and *N*-formimino-L-glutamate (**8**). Blue box: The last two steps share more similarities with the last steps in uracil degradation via the intermediates 5,6-dihydrouracil (**9**) and 3-ureidopropanoate (**10**).

https://dx.doi.org/10.1021/acschembio.0c00968
ACS Chem. Biol. 2021, 16, 397−403

by $^{1}H/^{13}C$ NMR (Supporting Information). Addition of the putative thiourocanate hydratase from *Paenibacillus sp.* Soil724D2 (*Pa*TUC) converted thiourocanate to thiohydantoin (**3**, reaction C). This compound was not further modified by either of the two metal-dependent hydrolases. Instead, addition of the putative enzyme DUF917 converted **3** to hydantoine (**4**, reaction D), suggesting that this enzyme removes hydrogen sulfide from **3** by hydrolysis. Subsequently, one of the hydrolases—termed hydantoinase—converted **4** to *N*-carbamoyl glutamate (**5**, reaction E) which no longer absorbed at 220 nm. The second hydrolase—termed *N*-carbamoylase—converted **5** into glutamate. To facilitate detection, glutamate was derivatized with *o*-phthalaldehyde and 3-mercaptopropionic acid. Comparison with the same derivatives made from authentic L- or D-glutamate by chiral HPLC identified L-glutamate as the final product of ergothioneine degradation (Figure S14). Feeding the same five-step pathway with isotope labeled ergothioneine (**1a**, Figure 2) produced the same metabolites with the correspondingly higher masses (Table S3).[27] Selenoneine (**1b**), the naturally occurring selenium analog of ergothioneine, was also confirmed as a substrate of this pathway (Figure S15).[28,29] Glutamate is a central primary metabolite that is used as a building block for protein synthesis, as an amine donor for many transaminases, and—after oxidation to α-ketoglutarate—as a feedstock for the citric acid cycle. Hence, the pathway described here provides a direct channel by which ergothioneine and selenoneine can be tapped as a source of reduced carbon, nitrogen, sulfur, or selenium.

Thiourocanate Hydratase (*Pa*TUC). In the next step, we examined the individual enzymes in more detail. The second enzyme in the degradation pathway—thiourocanate hydratase—shares 51% seq ID with urocanase from *Pseudomonas putida* (PDB: 1W1U, Figure S4).[25] Urocanase, also known as imidazolone-propionate hydrolase, is a remarkable catalyst because it uses tightly bound NAD^+ as an electrophilic cofactor.[30] This enzyme catalyzes the addition of water to urocanate (**6**) as the second of three universally conserved steps in histidine degradation (Figure 2).[31] Given the close sequence homology and the similarity of the substrates, it is likely that thiourocanate hydratase also uses NAD^+ to catalyze addition of water via a similar catalytic mechanism.[22] However, despite these similarities, there must be specific differences between the two enzymes, since thiourocanate hydratase cannot turn over urocanate.[22]

In phosphate buffered solutions (pH 8.0), the recombinant *Pa*TUC catalyzes substrate turnover with slightly higher catalytic efficiency than the previously reported homologue from *Burkholderia sp.* HME13 (Table 1). No product formation was observed when *Pa*TUC was assayed with urocanate as a substrate, showing that this enzyme is at least 10^3-fold less active toward urocanate than toward thiourocanate (Figure S8). After removal of the N-terminal His-tag, thiourocanate hydratase from *Paenibacillus sp.* (*Ta*TUC) was crystallized in the presence of urocanate as an unreactive substrate analog. One of the crystals selected for analysis by X-ray crystallography diffracted to a resolution of 1.9 Å. Data collection and refinement statistics of the diffraction data are summarized in Table S4. The observed electron density map revealed a continuous polypeptide chain from Glu2 to Asn546 (numbering according to ORF KRE36543). The overall structure and most of the active site of *Pa*TUC closely resembles that of urocanase from *P. putida* (PDB: 1W1U).[25] The asymmetric unit of the crystal contains one thiourocanate hydratase molecule. Two molecules from adjacent asymmetric units form a symmetric dimer via a large monomer—monomer interface. The same dimeric structure has been observed for urocanases, suggesting that dimer formation is a conserved feature of this enzyme family.[25] The residual electron density in the putative substrate binding pocket could be modeled as urocanate bound with an occupancy of 69%. The position and orientation of this ligand is specified unambiguously by the shape of the electron density and by polar interactions to Arg352 and Tyr42 (Figure 4 and Figure S20). Residual electron density in the cofactor binding pocket could be modeled as the NAD^+ bound with full occupancy (Figure 4 and Figure S20). The nicotinamide ring adopts two orientations that emerge from a 93° rotation along the *N*-glycosidic bond. In the dominant conformer (conformer 1, Figure S21), the plane of the nicotinamide ring is positioned perpendicular to the imidazole ring of urocanate. In the minor conformer (conformer 2, Figure S21), the two aromatic rings are nearly parallel. According to the proposed mechanism of thiourocanase hydratase (see below), this minor conformation is more representative of the productive substrate/cofactor complex. Structural characterization of urocanases from *P. putida* (PDB: 1W1U),[25] *Bacillus subtilis* (2FKN), *Geobacillus stearothermophilus* (1X87), *Legionella pneumophilia* (7JFZ), and *Trypanosoma cruzi* (6UEK)[32] found NAD^+ in a number of conformers, suggesting that the nicotinamide ring retains significant rotational freedom in complex with the enzyme.

Two conspicuous differences may explain the distinct substrate specificities of urocanase and thiourocanate hydratase.[22] First, in urocanases from *P. putida* (1W1U), *B. subtilis*, *G. stearothermophilus*, and *T. cruzi*, the 2′-hydroxyl group of the nicotinamide riboside forms two hydrogen bonds. One is accepted by the backbone carbonyl of a glycine residue in a conserved GGGVG motif (Gly493 in 1W1U); the other is donated from a glutamine side chain (Gln131X in 1W1U). In *Pa*TUC, this motif is changed to GGGGY (Figure S4). The corresponding loop adopts an alternative conformation that positions the last residue of this motif (Tyr485) as the only hydrogen bonding partner of the 2′-hydroxyl group (Figure S22). The glutamine residue which interacts with the 2′-hydroxyl group in urocanases corresponds to Gly123 in *Pa*TUC. This substitution leaves the 2′-hydroxyl group of NAD^+ as a frustrated hydrogen bond acceptor. A model of this enzyme in complex with thiourocanate suggests that the thiol tautomer of this substrate would be well positioned to donate the missing hydrogen bond to the cofactor (Figure 5 and Figure S23).

The second key difference between urocanases and TUCs relates to substrate activation. *Pa*TUC interacts with the Nπ of

Table 1. Kinetic Parameters of Bacterial Thiourocanate Hydratases

	k_{cat} [s^{-1}]	k_{cat}/K_M [M^{-1} s^{-1}]
Paenibacillus sp.[a]	0.43 ± 0.01	4.2×10^4
Burkholderia sp.[b]	0.071	2.4×10^3

[a]Reactions conditions: 0.4 μM enzyme, 4–96 μM thiourocanate in 100 mM potassium phosphate buffer, pH 8.0, 23 °C. Time-dependent substrate consumption was monitored by UV spectroscopy at 311 nm (ε_{311} = 22500 M^{-1} cm^{-1}). The corresponding rate vs [S] plots are shown in Figure S7. [b]From ref 13.

https://dx.doi.org/10.1021/acschembio.0c00968
ACS Chem. Biol. 2021, 16, 397–403

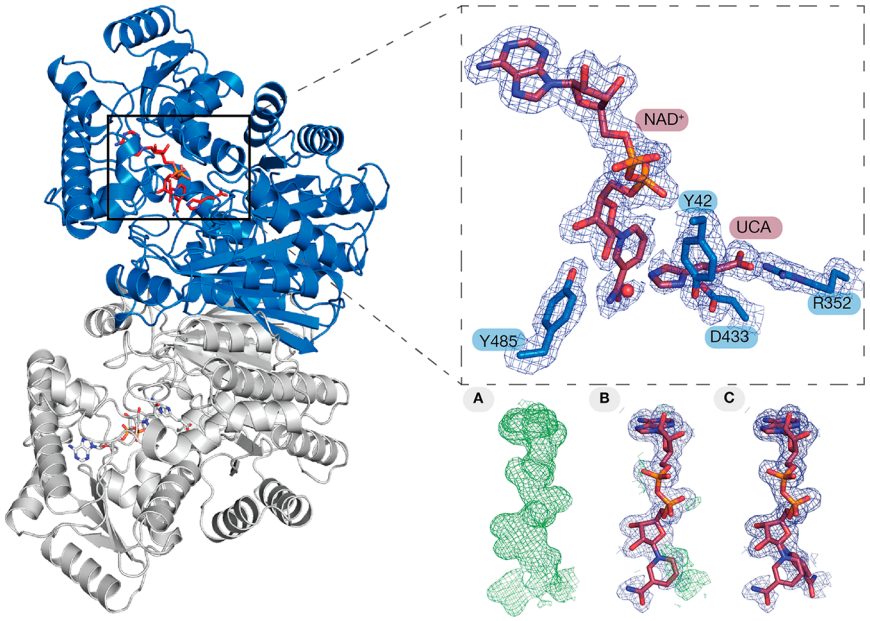


Figure 4. Left: The thiourocanate hydratase structure depicted as a physiological dimer in a ribbon representation with NAD^+ and urocanate shown as red sticks (red). **Frame:** A close-up of the most important active site residues depicted in a stick representation and H_2O as a red sphere. The $2m|F_o|-D|F_c|$ map contoured at the σ-level = 1.0 and shown as a blue mesh. NAD^+ is in a mainly populated conformation. **Bottom:** The nicotinamide ring of NAD^+ could be modeled in two distinct positions. The polder omit map of density that could be modeled by NAD^+ is depicted in **A** as a green mesh contoured at the σ-level = 3.0. **B:** The $2m|F_o|-D|F_c|$ map contoured at the σ-level = 1.0 and a stick representation of NAD^+ in the mainly populated conformation plus the $m|F_o|-D|F_c|$ map contoured at the σ-level = 3.0 (green mesh positive, red mesh negative) giving an indication for the minor NAD^+ conformation. **C:** A stick representation of the two superimposed NAD^+ species with the $2m|F_o|-D|F_c|$ map contoured at the σ-level = 1.0 as a blue mesh.

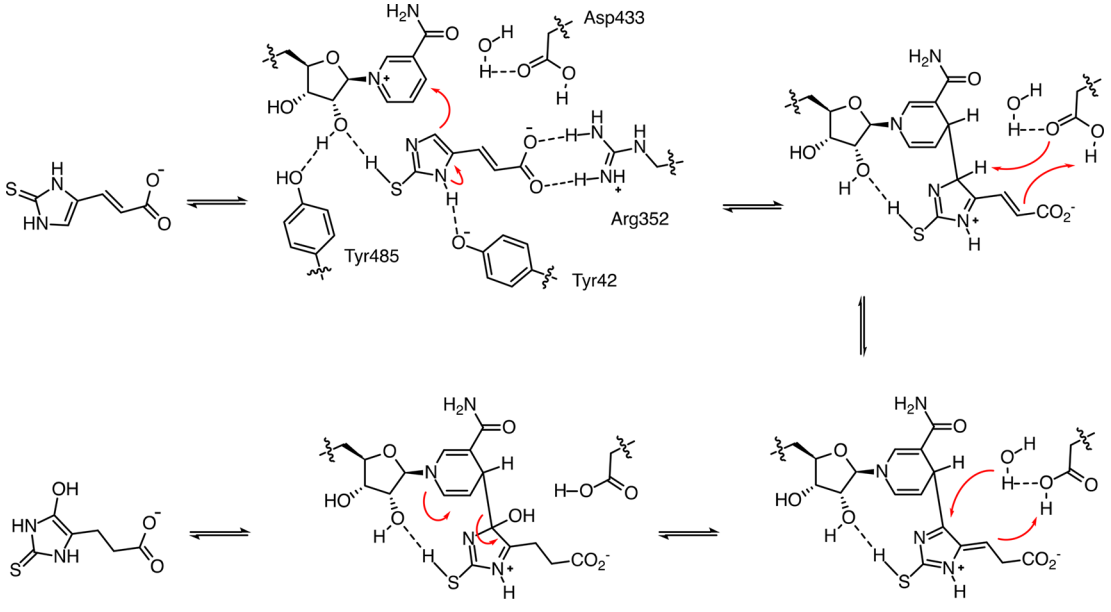


Figure 5. Proposed mechanism of thiourocanate hydratase by analogy to the mechanism of urocanase.[25]

the imidazole ring of the substrate via a hydrogen bond with Tyr42 (Figure 4). Additional hydrogen bonds to the backbone amides of Leu122 and Thr123 likely stabilize Tyr42 in an anionic form. Nτ makes no contact with any hydrogen bonding partner. Urocanases interact the same way with the urocanate Nπ (Tyr52 in 1W1U). In addition, Nτ forms a hydrogen bond with a conserved arginine residue (Arg455 in 1W1U). This second interaction is critical for catalysis because it stabilizes the imidazole of the substrate (pK_a 6.5) in neutral and therefore nucleophilic form.[25] The Arg455 to Ala mutant of urocanase from *P. putida* is completely inactive.[25] The authors of the same study noticed the existence of urocanase homologues that lack these arginine residues and correctly predicted that these enzymes may have different activities. Indeed, the structurally equivalent residue in *Pa*TUC is Ile445. Since mercaptoimidazoles are neutral at physiological pH (pK_a = 10−12), a cationic charge is not needed to bind the substrate in neutral form. Despite these two active site differences, it is safe to conclude that TUCs and urocanases catalyze water addition to their corresponding substrates via essentially the

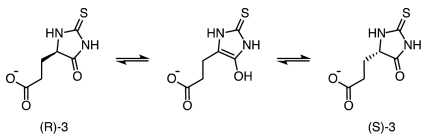
https://dx.doi.org/10.1021/acschembio.0c00968
ACS Chem. Biol. 2021, 16, 397−403

same mechanism (Figure 5).[25] The substrate specificity profiles do not overlap because of the different ways that the two enzymes solvate their substrates in activated form.

A sequence similarity network of the urocanase protein family based on sequences from the UniProtKB database shows that homologues containing the GGGGY motif form a distinct cluster that is separated from two clusters of bacterial and eukaryotic urocanases (Figure S24).[26] Species that encode thiourocanases are predominantly terrestrial firmicutes and proteobacteria that have been isolated from soil samples and plant roots (Table S5). Interestingly, *Streptococcus thermophilus* also contains a TUC homologue coencoded with an ergothionase homologue, suggesting that this lactic acid bacterium that is often used for yogurt production has the ability to degrade ergothioneine as well.

Thiohydantoin Desulfurase (THD). The third enzyme in the pathway eliminates sulfur from thiohydantoin (**3**). This hydrolase belongs to an uncharacterized protein family (PF06032) that contains homologues from bacterial, archaeal, plant, and animal species. Interestingly, many members of this enzyme family are encoded either in the same gene cluster or fused in the same ORF as hydantoinase-like enzymes. Hence, hydrolysis of heterocyclic thiones—such as sulfur-modified nucleobases[33] or small secondary metabolites[34−36] —may be a common function of this enzyme type. It is important to note that thiohydantoin **3** is not turned over by the hydantoinase at an appreciable rate, prior to removal of sulfur. Hence, desulfurization may also be an essential step in the hydrolytic breakdown of other heterocyclic thiones. In a phosphate buffered solution (pH 8.0) at 23 °C, thiohydantoin desulfurase from *Paenibacillus sp.* (*Pa*THD) catalyzes hydrolysis of thiohydantoin with a turnover frequency (k_{cat}) of 0.16 s^{-1} and a catalytic efficiency (k_{cat}/K_M) of 2300 M^{-1} s^{-1} (Figure S9). Characterization of the substrate by ^{1}H NMR in deuterium oxide showed that the C_5−H bond in the thiohydantoin ring of **3** exchanges rapidly with solvent protons, suggesting that the substrate used to assay *Pa*THD was likely racemic. Therefore, the catalytic efficiency for the preferred enantiomer may be 2-fold higher than the given value. In contrast, the hydantoin ring of **4** did not fully epimerize even after 1 day. Since intermediate **5** is even more stable with regard to stereochemistry, and the final product is the *S*-amino acid L-glutamate, we deduce that the S-isomer of **3** is the preferred substrate of *Pa*THD (Scheme 1). The simpler

Scheme 1



substrate derivative 2-thioxo-4-imidazolidinone (2-thiohydantoin) is not turned over at measurable rates (Figure S12), suggesting that the carboxylate side chain of **3** is important for substrate recognition. The enzyme is fully active in the presence of 0.5 mM EDTA, suggesting that the catalytic mechanism does not depend on a reversibly bound metal. The purified protein is devoid of any spectroscopic features above 280 nm (Figure S2) and does not contain any covalent modifications as inferred by HR-ESI-MS analysis (Table S1). Based on these observations we conclude that *Pa*THD is a cofactor-independent enzyme.

The Metal-Dependent Hydrolases. Hydantoin **4** is hydrolyzed to *N*-carbamoyl glutamate **5**. The corresponding hydantoinase is encoded by the ORF KRE36545 and belongs to a family of cyclic amidohydrolases (PF01979). This family includes dihydropyrimidinases (EC 3.5.2.2, Figure 2) and allantoinases. The closest homologue with known structure and function is allantoinase from *Escherichia coli* (31% seq. ID, PDB: 3E74).[37] This enzyme binds two bivalent metal ions in the active site by coordination through the side chains of four histidines, one aspartate, and a *N*-carboxylated lysine. Sequence alignment suggests that the hydantoinase from *Paenibacillus sp.* contains the same set of metal-coordinating residues (Figure S5), suggesting that this enzyme also binds two metals in the active site. The recombinant protein is active without addition of metal salts, but treatment with EDTA eliminates activity. Reconstitution of the metal-depleted enzyme with different bivalent metal salts (1 mM) induced activity with Mn^{II} and Co^{II} (100%), some activity with Zn^{II} (75%), and no measurable activity with Fe^{II}, Mg^{II}, or Ca^{II} (<5%, Table S2).

The final step removes the ureido group from **5**. This reaction is catalyzed by an *N*-carbamoylase (KRE36544). Sequence homology places this protein in the peptidase M20 enzyme family (PF01546). One of the closest homologues with known structure and function is *β*-alanine synthase (EC 3.5.1.6, Figure 2) from *Saccharomyces kluyveri* (32%, PDB: 1R3N).[38,39] *β*-Alanine synthase requires two zinc ions and catalyzes the last step in the breakdown of pyrimidine bases (EC 3.5.1.6, Figure 2). The homologous enzyme from *Paenibacillus sp.* contains the same set of metal-binding residues (Figure S6), suggesting that this enzyme also hosts a dinuclear metal center. However, in contrast to *β*-alanine synthase, the activity of the *N*-carbamoylase depends on Co^{II} instead of Zn^{II} (Table S2).

CONCLUSIONS

In this report, we delineate a complete enzymatic pathway through which select bacteria can catabolize ergothioneine. The sequence homology of the involved enzymes with catalysts from other metabolic functions sheds light onto the evolutionary origin of this pathway. Ergothionase and thiourocanate hydratase are closely related to the histidine degrading enzymes histidase and urocanase. The last two steps are catalyzed by the metal-dependent hydantoinase and *N*-carbamoylase that are related to the uracil-degrading enzymes dihydropyrimidinase and *β*-alanine synthases. These similarities suggest that this ergothioneine degradation pathway has emerged by repurposing parts of existing pathways of the primary metabolism. A detailed structural comparison of thiourocanate hydratase with urocanase showcases how few strategic modifications in the active site completely changed the substrate specificity from urocanate to thiourocanate. The high sequence identity between *Pa*TCU and urocanase from *P. pudida* (51% seq. id.) indicates that this particular ergothioneine degradation pathway may not be very old. The evolutionary origin of the thiohydantoin desulfurase is less clear, because this C−S bond cleaving enzyme is the first member of this protein family with a demonstrated function. However, the observation that desulfurization is essential for ring-opening hydrolysis by the metal-dependent hydantoinase and the frequent occurrence of genes coding for desulfurase-like enzymes in a broad range of organisms suggest that this enzyme type may participate in numerous pathways that

https://dx.doi.org/10.1021/acschembio.0c00968
ACS Chem. Biol. 2021, 16, 397−403

require hydrolysis of heterocyclic thiones. Finally, the occurrence of genes coding for ergothionases and thiourocanate hydratases in the genomes of a large number of soil-dwelling bacteria supports the idea that this bacterial activity could play a significant role in limiting the availability of ergothioneine for plants and potentially reduce the concentration of this valuable micronutrient in our nutrition. As this manuscript was under review, the discovery of thiohydantoin desulfurase from *Burkholderia sp.* HME13 was published.[40] The description of this enzyme is consistent with our characterization of the homologue from *Paenibacillus sp.*

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschembio.0c00968.

Detailed descriptions of all experiments, Figures S1−S24, and Tables S1−S5 (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Florian P. Seebeck − *Department of Chemistry, University of Basel, Basel 4002, Switzerland;* orcid.org/0000-0003-4625-1369; Email: florian.seebeck@unibas.ch

Authors

Mariia A. Beliaeva − *Department of Chemistry, University of Basel, Basel 4002, Switzerland*

Florian Leisinger − *Department of Chemistry, University of Basel, Basel 4002, Switzerland*

Complete contact information is available at: https://pubs.acs.org/10.1021/acschembio.0c00968

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank D. Lim for HR-ESI-MS measurements and J. C. Yadan (Tetrahedron) for the kind gift of ergothioneine. The authors also thank the Swiss Light Source (SLS Villigen, Switzerland) for beamtime and for the outstanding support from the beamline staff making measurements possible despite the Corona crisis. This project was supported by the Swiss National Science Foundation, the University of Basel, and the NCCR for Molecular Systems Engineering.

■ REFERENCES

(1) Genghof, D. S. (1970) Biosynthesis of Ergothioneine and Hercynine by Fungi and Actinomycetales. *J. Bacteriol. 103* (2), 475−478.

(2) Pfeiffer, C., Surek, B., Schöming, E., and Gründemann, D. (2011) Cyanobacteria produce high levels of ergothioneine. *Food Chem. 129*, 1766−1769.

(3) Sheridan, K. J., Lechner, B. E., Keeffe, G. O., Keller, M. A., Werner, E. R., Lindner, H., Jones, G. W., Haas, H., and Doyle, S. (2016) Ergothioneine Biosynthesis and Functionality in the Opportunistic Fungal Pathogen, Aspergillus fumigatus. *Sci. Rep. 6*, 35306.

(4) Burn, R., Misson, L. E., Meury, M., and Seebeck, F. P. (2017) Anaerobic Origin of Ergothioneine. *Angew. Chem., Int. Ed. 56* (41), 12508−12511.

(5) Halliwell, B., Cheah, I. K., and Tang, R. M. Y. (2018) Ergothioneine - a diet-derived antioxidant with therapeutic potential. *FEBS Lett. 592* (20), 3357−3366.

(6) Cheah, I. K., Ong, R. L., Gruber, J., Yew, T. S., Ng, L. F., Chen, C. B., and Halliwell, B. (2013) Knockout of a putative ergothioneine transporter in Caenorhabditis elegans decreases lifespan and increases susceptibility to oxidative damage. *Free Radical Res. 47* (12), 1036−1045.

(7) Paul, B. D., and Snyder, S. H. (2010) The unusual amino acid L-ergothioneine is a physiologic cytoprotectant. *Cell Death Differ. 17*, 1134.

(8) Pfeiffer, C., Bach, M., Bauer, T., da Ponte, J. C., Schöming, E., and Gründemann, D. (2015) Knockout of the ergothioneine transporter ETT in zebrafish results in increased 8-oxoguanine levels. *Free Radical Biol. Med. 83*, 178−185.

(9) Ames, B. N. (2018) Prolonging healthy aging: Longevity vitamins and proteins. *Proc. Natl. Acad. Sci. U. S. A. 115* (43), 10836−10844.

(10) Cheah, I. K., Feng, L., Tang, R. M. Y., Lim, K. H. C., and Halliwell, B. (2016) Ergothioneine levels in an elderly population decrease with age and incidence of cognitive decline; a risk factor for neurodegeneration? *Biochem. Biophys. Res. Commun. 478* (1), 162−167.

(11) Hatano, T., Saiki, S., Okuzumi, A., Mohney, R. P., and Hattori, N. (2016) Identification of novel biomarkers for Parkinson's disease by metabolomic technologies. *J. Neurol., Neurosurg. Psychiatry 87* (3), 295−301.

(12) Tang, R. M. Y., Cheah, I. K., Yew, T. S. K., and Halliwell, B. (2018) Distribution and accumulation of dietary ergothioneine and its metabolites in mouse tissues. *Sci. Rep. 8* (1), 1601.

(13) Beelman, R. B., Kalaras, M. D., Phillips, A. T., and Richie, J. P. J. (2020) Is ergothioneine a 'longevity vitamin' limited in the American diet? *J. Nutr. Sci. 9*, e52.

(14) Seebeck, F. P. (2010) In vitro reconstitution of Mycobacterial ergothioneine biosynthesis. *J. Am. Chem. Soc. 132*, 6632−6633.

(15) Jones, G. W., Doyle, S., and Fitzpatrick, D. A. (2014) The evolutionary history of the genes involved in the biosynthesis of the antioxidant ergothioneine. *Gene 549* (1), 161−170.

(16) Liao, C., and Seebeck, F. P. (2017) Convergent Evolution of Ergothioneine Biosynthesis in Cyanobacteria. *ChemBioChem 18* (21), 2115−2118.

(17) Stampfli, A. R., Blankenfeldt, W., and Seebeck, F. P. (2020) Structural basis for ergothioneine biosynthesis. *Curr. Opin. Struct. Biol. 65*, 1−8.

(18) Wolff, J. B. (1962) Ergothionase from Escherichia coli. *J. Biol. Chem. 237* (3), 874−881.

(19) Yanasugondha, D., and Appleman, M. D. (1957) Degradation of ergothioneine by Alcaligenes faecalis. *J. Bacteriol. 74* (3), 381−385.

(20) Kelly, B., and Appleman, M. D. (1961) Degradation of ergothioneine by cell-free extracts of Alcaligenes faecalis. *J. Bacteriol. 81*, 715−720.

(21) Muramatsu, H., Matsuo, H., Okada, N., Ueda, M., Yamamoto, H., Kato, S., and Nagata, S. (2013) Characterization of ergothionase from Burkholderia sp. HME13 and its application to enzymatic quantification of ergothioneine. *Appl. Microbiol. Biotechnol. 97* (12), 5389−5400.

(22) Muramatsu, H., Miyaoku, H., Kurita, S., Matsuo, H., Kashiwagi, T., Kim, C. S., Hayashi, M., Yamamoto, H., Kato, S. I., and Nagata, S. (2020) Gene cloning and characterization of thiourocanate hydratase from Burkholderia sp. HME13. *J. Biochem. 167* (3), 333−341.

(23) Maurer, A., Leisinger, F., Lim, D., and Seebeck, F. P. (2019) Structure and Mechanism of Ergothionase from Treponema denticola. *Chem. - Eur. J. 25* (44), 10298−10303.

(24) Dunbar, K. L., Scharf, D. H., Litomska, A., and Hertweck, C. (2017) Enzymatic Carbon-Sulfur Bond Formation in Natural Product Biosynthesis. *Chem. Rev. 117* (8), 5521−5577.

(25) Kessler, D., Rétey, J., and Schulz, G. E. (2004) Structure and action of urocanase. *J. Mol. Biol. 342* (1), 183−194.

(26) Zallot, R., Oberg, N., and Gerlt, J. A. (2019) The EFI Web Resource for Genomic Enzymology Tools: Leveraging Protein, Genome, and Metagenome Databases to Discover Novel Enzymes and Metabolic Pathways. *Biochemistry 58* (41), 4169−4182.
(27) Beliaeva, M. A., Burn, R., Lim, D., and Seebeck, F. P. (2021) In Vitro Production of Ergothioneine Isotopologues. *Angew. Chem., Int. Ed.*, DOI: 10.1002/anie.202011096.
(28) Yamashita, Y., and Yamashita, M. (2010) Identification of a Novel Selenium-containing Compound, Selenoneine, as the Predominant Chemical Form of Organic Selenium in the Blood of Bluefin Tuna. *J. Biol. Chem. 285* (24), 18134−18138.
(29) Lim, D., Gründemann, D., and Seebeck, F. P. (2019) Total synthesis and functional characterization of Selenoneine. *Angew. Chem., Int. Ed. 58* (42), 15026−15030.
(30) Rétey, J. (1994) The urocanase story: a novel role of NAD+ as electrophile. *Arch. Biochem. Biophys. 314* (1), 1−16.
(31) Bender, R. A. (2012) Regulation of the histidine utilization (hut) system in bacteria. *Microbiol. Mol. Biol. Rev. 76* (3), 565−584.
(32) Boreiko, B., Silva, M., Melo, F. P., Silber, A. M., and Lulek, L. (2020) Structure of Urocanate Hydratase from the protozoan Trypanosoma cruzi. *Int. J. Biol. Macromol. 146*, 716−724.
(33) Shigi, N. (2018) Recent Advances in Our Understanding of the Biosynthesis of Sulfur Modifications in tRNAs. *Front. Microbiol. 9*, 2679.
(34) Coyne, S., Chizzali, C., Khalil, M. N., Litomska, A., Richter, K., Beerhues, L., and Hertweck, C. (2013) Biosynthesis of the antimetabolite 6-thioguanine in Erwinia amylovora plays a key role in fire blight pathogenesis. *Angew. Chem., Int. Ed. 52* (40), 10564−10568.
(35) Lee, T. H., Khan, Z., Kim, S. Y., and Lee, K. R. (2019) Thiohydantoin and Hydantoin Derivatives from the Roots of Armoracia rusticana and Their Neurotrophic and Anti-neuroinflammatory Activities. *J. Nat. Prod. 82* (11), 3020−3024.
(36) Yu, M. Y., Q, X. J., Peng, X. R., Wang, X., Tian, X. X., Li, Z. R., and Qiu, M. H. (2017) Macathiohydantoins BeK, novel thiohydantoin derivatives fromLepidium meyenii. *Tetrahedron 73*, 4392−4397.
(37) Kim, K., Kim, M. I., Chung, J., Ahn, J. H., and Rhee, S. (2009) Crystal structure of metal-dependent allantoinase from Escherichia coli. *J. Mol. Biol. 387* (5), 1067−1074.
(38) Martínez-Rodríguez, S., García-Pino, A., Las Heras-Vázquez, F. J., Clemente-Jiménez, J. M., Rodríguez-Vico, F., García-Ruiz, J. M., Loris, R., and Gavira, J. A. (2012) Mutational and structural analysis of L-N-carbamoylase reveals new insights into a peptidase M20/M25/M40 family member. *J. Bacteriol. 194* (21), 5759−5768.
(39) Lundgren, S., Gojković, Z., Piskur, J., and Dobritzsch, D. (2003) Yeast beta-alanine synthase shares a structural scaffold and origin with dizinc-dependent exopeptidases. *J. Biol. Chem. 278* (51), 51851−51862.
(40) Muramatsu, H., Maguchi, H., Harada, T., Kashiwagi, T., Kim, C. S., Kato, S., and Nagata, S. (2020) Identification of the gene encoding 3-(5-oxo-2-thioxoimidazolidin-4-yl) propionic acid desulfhydrase in Burkholderia sp. HME13. *Biosci. Biotechnol. Biochem.*, DOI: 10.1093/bbb/zbaa066.

https://dx.doi.org/10.1021/acschembio.0c00968
ACS Chem. Biol. 2021, 16, 397−403