Structural basis of substrate selectivity in urea and guanidine carboxylase enzymes.

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Background

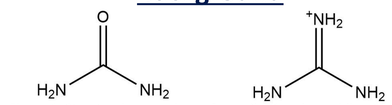
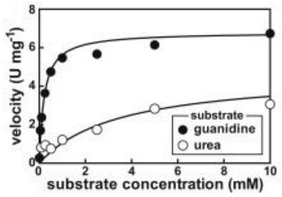


Figure 1: Structural comparison of urea (left) and guanidine (right.)

Urea is the primary nitrogenous waste product in mammalian cells. The closely related guanidine [Figure 1] is not a common metabolite, but it is a component of many compounds entering the environment. The environmental biodegradation of guanidine was previously unknown

While investigating an unidentified class of riboswitches in 2017, the Breaker lab discovered that translation of genes encoding for a urea carboxylase increased with the presence of free guanidine in the cell. They were able to show that these carboxylases react more readily with guanidine than they do with urea [Figure 2].



Substrate	V_{max} (U mg^{-1})	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M ($s^{-1}mM^{-1}$)
urea	4.9 ± 0.7	5.2 ± 1.5	5.2 ± 0.7	1.1 ± 0.4
guanidine	6.9 ± 1.2	0.19 ± 0.05	7.4 ± 1.2	40 ± 5

Figure 2: Kinetics of ATP cleavage *by Oleomonas sagarenensis* urea carboxylase in the presence of urea and guanidine (Nelson et.al, 2017).

To verify the proposed pathway, the St. Maurice Lab sought to confirm that guanidine could be decomposed into usable nitrogen. Upon failing to identify a product, bioinformatics revealed two unidentified protein sequences in the GC operon, later determined to be subunits of a heteromeric carboxyguanidine deiminase that converts carboxyguanidine to allophanate [Figure 3].

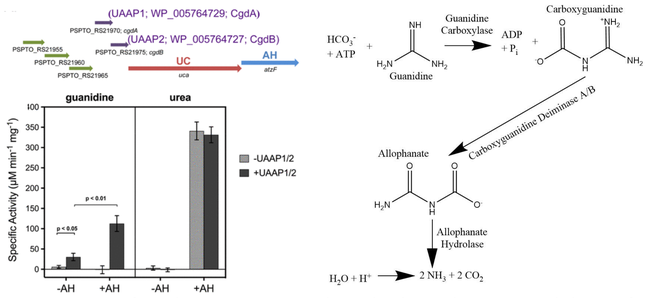
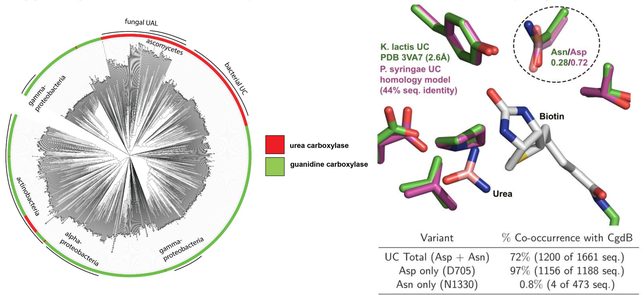


Figure 3: Kinetics of *Pseudomonas syringae* guanidine carboxylase in the presence and absence of carboxyguanidine deiminase (CgdAB, labeled UAAP1/2) and allophanate hydrolase for guanidine and urea (left.) Enzyme activity in the presence of guanidine increases drastically with the addition of CgdAB and again with the addition of allophanate hydrolase, showing that CgdAB converts carboxyguanidine into allophanate. The full pathway from guanidine to ammonia is shown on the right (Schneider et. al, 2019).

There is clear phylogenetic evidence for evolutionary divergence between UC and GC. Guanidine carboxylases always colocalize with CgdAB and almost always contain an aspartate at the equivalent to position 705 in PsGC. Urea carboxylases contain an asparagine in the corresponding location and do not colocalize with CgdAB, suggesting that this residue likely contributes to substrate selectivity [Figure 4].



Variant	% Co-occurrence with CgdB
UC Total (Asp + Asn)	72% (1200 of 1661 seq.)
Asp only (D705)	97% (1156 of 1188 seq.)
Asn only (N1330)	0.8% (4 of 473 seq.)

Figure 4: Phylogenetic tree of UC and GC enzymes (left) and co-occurrence of aspartate and asparagine residues in the D705 position with CgdAB (right). Presumably, enzymes with an aspartate are guanidine carboxylases and those with an asparagine are urea carboxylases (Schneider et al, 2019).

Future Studies

We are actively developing an assay to kinetically isolate the CT domain of urea and guanidine carboxylases, based on an assay used by the St. Maurice lab for pyruvate carboxylase [Figure 7].

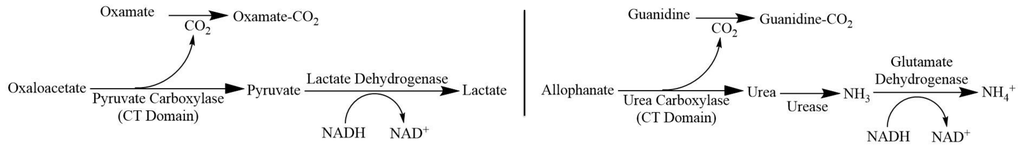


Figure 7: Assay to kinetically isolate the CT domain of pyruvate carboxylase measuring the decarboxylation of oxaloacetate to pyruvate (Lietzan et al, 2014), and the proposed corresponding assay to kinetically isolate the CT domain of urea and guanidine carboxylases by measuring the decarboxylation of allophanate to urea.

Although the aspartate and asparagine residues at position 705 are the most prominent difference between urea and guanidine carboxylases, three other residues in the active site were identified also differ between UC and GC enzymes [Figure 8].

PsGC Residue	KlUC Residue	CaUC Residue	ScUC Residue	Consurf Conservation Score
Gly 1027	Ser 1633	Ser 1616	Ser 1637	0.852 (A,G,S,P)
Val 986	Cys 1591	Cys 1577	Cys 1596	0.644 (F,I,A,L,C,V)
Asp 705	Asn 1330	Asn 1319	Asn 1335	0.945 (N,S,D)
Tyr 981	Phe 1586	Phe 1570	Phe 1591	0.821 (I,Y,W,F)

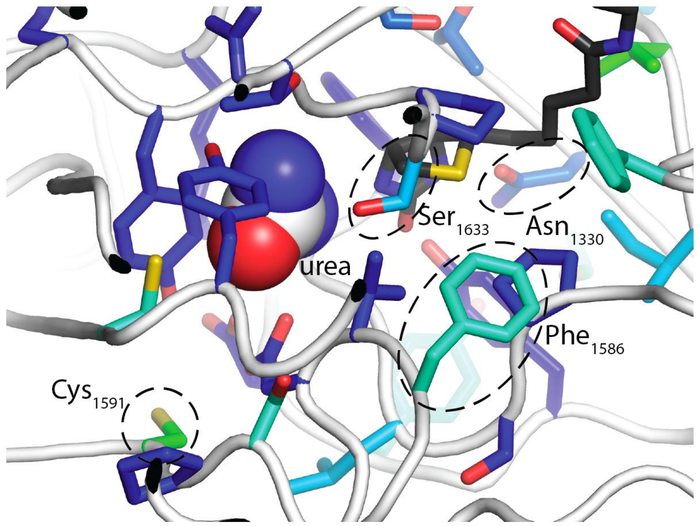


Figure 8: Structure of the active site of urea carboxylase, where darker colors indicate the most conserved residues. The four residues circled and highlighted in the table differ between GC and UC.

Mutagenesis will be performed on each of the residues highlighted in Figure 8, including different combinations of those residues. Mutant enzymes will be analyzed with the CT domain assay [Figure 7] along with other standard assays to gain insight into the structural basis of substrate selectivity in UC and GC enzymes.

Applications

1. Guanidine is a usable nitrogen source for many bacteria in nature.
2. Metformin, the fourth most prescribed drug in the world, is a biguanide that treats type-II diabetes. Currently, it pollutes the environment via wastewater and cannot be decomposed into safe derivatives. A GC reaction may be used to clean up metformin waste.
3. Nitroguanidine, a toxic derivative of guanidine released into the environment during the combustion of many military weapons, may be converted to clean nitrogen in a guanidine carboxylase centered reaction.
4. CgdAB is a member of a largely unidentified and unstudied family of enzymes called DUF1989. Further studies on CgdAB may provide valuable information about working with and studying these enzymes.

Substrate Selectivity of PsGC D705N

Hypothesis

Mutating the aspartate residue in the D705 position of *Pseudomonas syringae* guanidine carboxylase will alter substrate selectivity so that the enzyme becomes preferential to urea over guanidine.

Methods

PCR-based site-directed mutagenesis was performed on the wild type PsGC in a pTXB1 *E. coli* expression vector to change the aspartate at position 705 to an asparagine. The mutated plasmid was transformed into TOP10 competent *E. coli* cells and the DNA sequence was confirmed. The pTXB1 plasmid encoding PsGC D705N was transformed into BL21(DE3) *E. coli* cells for protein expression, and the protein was purified using a standard protocol.

The activity of wild type and D705N PsGC was assayed by measuring ATP cleavage in the presence of urea or guanidine [Figure 5].

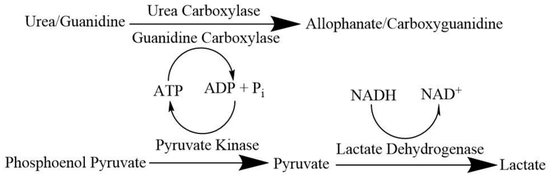


Figure 5: Schematic of an ATP cleavage assay for UC and GC using a PK/LDH enzyme coupled reaction.

Results

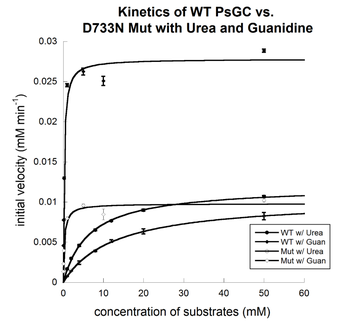


Figure 6: Kinetics of wild type and D705N *Pseudomonas syringae* guanidine carboxylases in the presence of guanidine and urea.

While the data clearly shows that an asparagine in the D705 position decreases the enzyme's preference for guanidine, it does not make the enzyme preferential toward urea [Figure 6]. The hypothesis that the residue in the D705 position is the determining factor in distinguishing guanidine and urea carboxylases must be rejected.

References

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