Uncoupling Intramolecular Processing and Substrate Hydrolysis in the N-terminal Hydrolase hASRGL-1 by Circular Permutation

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RUNNING TITLE: Structure of Human Isoaspartyl Dipeptidase.

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SUPPORTING INFORMATION

SUPPORTING FIGURES

Figure S1. A) The pH dependence of k_{cat} for cp-hASRGL1 hydrolysis of AHA with an apparent pK_a of 6.0 ± 0.1. B) The pH dependence of cp-hASRGL1 hydrolysis of AHA upon k_{cat}/K_M with corrected values of pK_{a1} = 7.3 ± 0.2 (ascending) and pK_{a2} = 8.0 ± 0.2 (descending) limb.

Figure S1

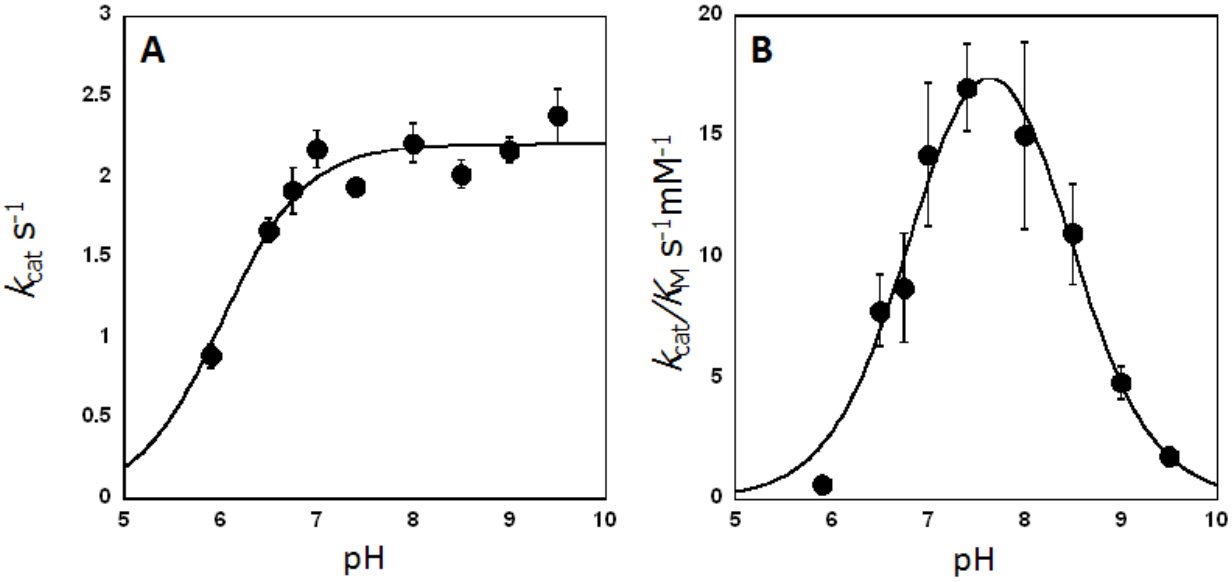


Figure S2. Density (Fo-Fc) map of sulfate ion bound in active site. The sulfate ion (shown in stick with sulfur in yellow and oxygen atom in red) is stabilized by hydrogen bonds formed by β-hydroxyl group of Ser200, amide group of Ser 200, γ-carboxyl group of Asp199 and a water molecule (red sphere), and by salt bridges formed by Arg196. The C atoms of hASRGL1 are shown in light blue and the dashed green lines indicate polar interactions. All distances are in the units of Å.

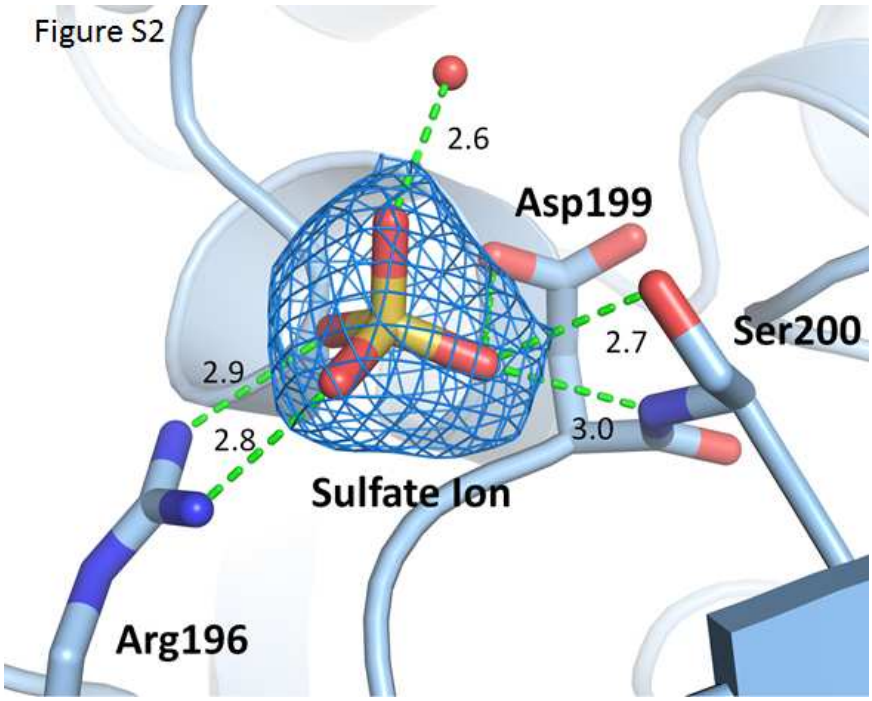


Figure S3. Sequence alignment of plant-type asparaginases from diverse phylogenies reveals a conserved HGG motif (rectangle).

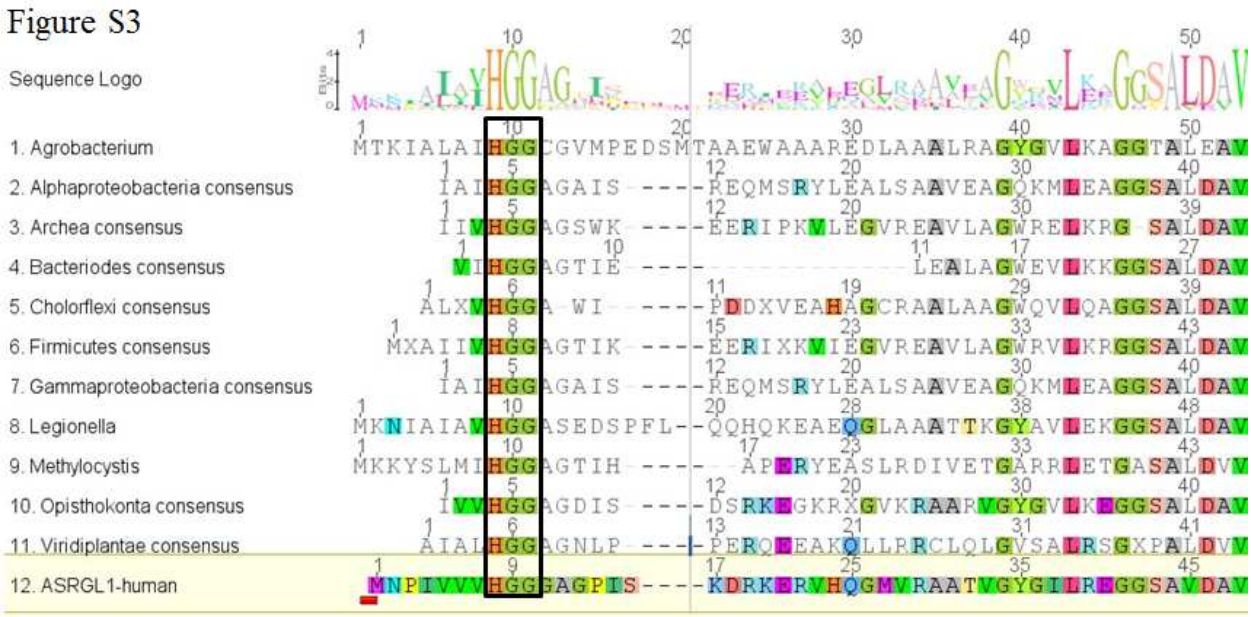


Figure S4. Hydrogen bonding around Thr168. cp-hASRGL1 active site nucleophile Thr168 forms hydrogen bonds with β-hydroxyl group Thr219, γ-amide and α-carbonyl of Asn62, and an intramolecular hydrogen bond between its own α-amino and β-hydroxyl groups. Dashed green lines indicate the hydrogen bond distances in Å.

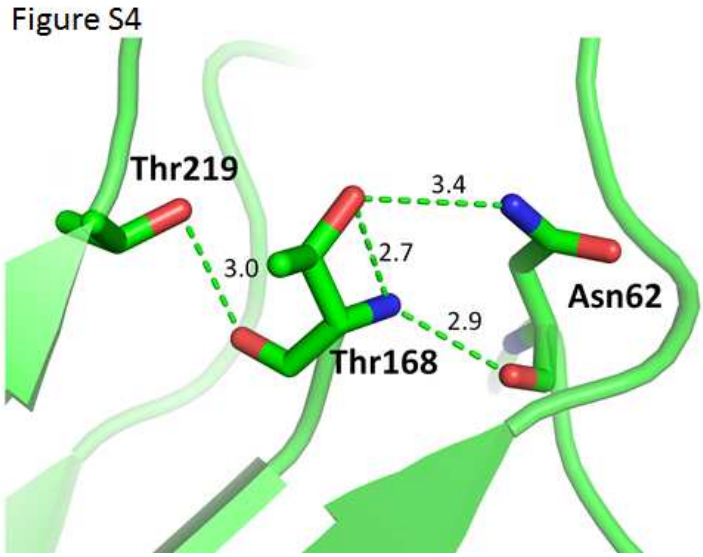
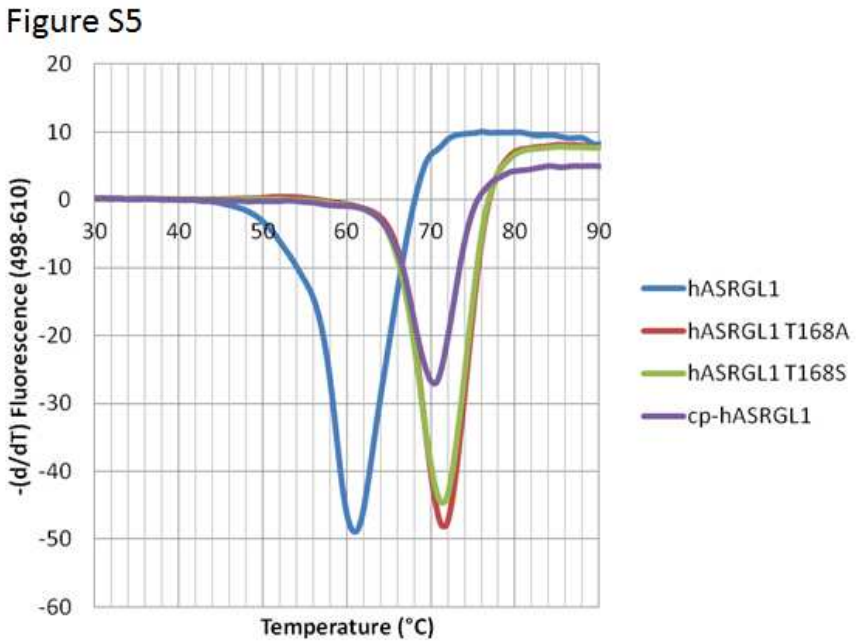


Figure S5. Derivative thermal melting curves for hASRGL1 and variants Thr168Ala, and Thr168Ser with T_m values of 61.0 ± 0.1 C°, 71.0 ± 0.1°C, and 71.0 ± 0.1°C respectively. For comparison cp-hASRGL1 was also included with a T_m value of 70 ± 0.1 C°. The hASRGL1 and hASRGL1 variants color codes are marked on the right side of the plot.



SUPPORTING TABLES

Table S1.

Table S1
ESI-MS analysis of cp-hASRGL1 and variants

Variant	Mass (calc) (-Met1) Da.	Mass (exp) Da.
cp-hASRGL1-Thr168Cys	33535	33525 ± 11
cp-hASRGL1	33533	33523 ± 11
cp-hASRGL1-Thr16Ser	33519	33513 ± 11

Table S2.

Table S2.
Experimental and calculated pK_a values of cp-hASRGL1 and substrate AHA

	pK_{a1} (exp)	pK_{a2}(exp)	p$K_{a\ (}$calc)*
[a]cp-hASRGL1(k_{cat})	6.0 ± 0.1		
[b]cp-hASRGL1 (k_{cat}/K_M)	7.3 ± 0.2	8.0 ± 0.2	
[c]AHA-NH_3^+ ⇔AHA-NH_2			8.05
[d]R-T168-NH_3^+⇔ R-T168-NH_2			7.62

* pK_a values were calculated using software from ChemAxon
[a] pK_a of k_{cat} dependence on pH, [b] p$K_{a,}$ values of k_{cat}/K_M dependence on pH, [c] pK_a of substrate AHA amine, [d] pK_a of the amine from an N-terminal Thr residue

Table S3.

Table S3(Related to Figure 3)
Summary of Thr168 sidechain interactions modeled into hASRGL1-Thr168Ala structure

Thr168 rotamers	% obs.	χ angle	Thr168 β-hydroxyl (H-bonds with)	Thr168 γ-methyl (clashes with)
p form	49	59°	T218 C=O	S200 SC
m form	43	-61°	S200 SC, T186 SC	S185 C=O, V169 C=O
t form	8	-171°	S185 C=O, S200 SC	T218 C=O

C=O = carbonyl, SC = side chain, γ-methyl clashes range from 2.67-2.18 Å

Table S4.

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Table S4
Oligonucleotides used in the construction of cp-hASRGL1 and point mutant variants

New N-term-Thr-F	5'-CCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGACAGTCGGCGCTGTCGCCCTG
α-β linker-R	5'-CCCAGCACCGCTACCGGCACCACTGCCAGCGCCCGGCAGATCGGTTATGGTCGTG
α-β linker-F	5'-GGCGCTGGCAGTGGTGCCGGTAGCGGTGCTGGGgggaatcccatcgttgtggttcatg
New C-Term-R	5'-TATGAATTCTCAATGGTGATGGTGATGGTGCCCAAGGTTCTTCTGGCAATCGG
New N-term-Cys-F	5'-CCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGtgcGTCGGCGCTGTCGCCCTG
New N-term-Ser-F	5'-CCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGtccGTCGGCGCTGTCGCCCTG
Gly9Ala-F	5'-ATCGTTGTGGTTCATGCAGGCGGAGC-3'
Gly9Ala-R	5'-GCTCCGCCTGCATGAACCACAACGAT-3'
Gly10Ala-F	5'-CGTTGTGGTTCATGGTGCCGGAGCCGGACCG-3'
Gly10Ala-R	5'-CGGTCCGGCTCCGGCACCATGAACCACAACG-3'
Gly11Ala-F	5'-GTGGTTCATGGTGGCGCAGCCGGACCGATTAGCAAAGATCGTAAAGAACGTGTG-3'
Gly11Ala-R	5'-CACACGTTCTTTACGATCTTTGCTAATCGGTCCGGCTGCGCCACCATGAACCAC-3'
Gly167Ala-F	5'-GATTGCCAGAAGAACCTTGCCACAGTCGGCGCTGTCGC-3'
Gly167Ala-R	5'-GCGACAGCGCCGACTGTGGCAAGGTTCTTCTGGCAATC-3'
Gly167Asp-F	5'-GATTGCCAGAAGAACCTTGACACAGTCGGCGCTGTCGC-3'
Gly167Asp-R	5'-GCGACAGCGCCGACTGTGTCAAGGTTCTTCTGGCAATC-3'

SUPPORTING METHODS

Phylogenetic analysis- Using a Linnaeus Blast algorithm (Geneious) [1] we analyzed 1000 plant-type asparaginase sequences from across all available phylogenies. Sequences from individual organisms and consensus sequences from major classes of organisms were selected for constructing Figure S2 for easy visual comparison of the conserved HGG motif within the plant-type asparaginase family.

SUPPLEMENTARY REFERENCES

1. Drummond, A.J., Ashton, B., Cheung, M., Heled, J., and Kearse, M. (2009). Geneious v4. 8, 2009. Biomatters Ltd., Auckland, New Zealand.