

Supplemental Information

Free Glycine Accelerates the Autoproteolytic

Activation of Human Asparaginase

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Inventory of Supplemental Information

Figure S1, related to Figure 1: *hASNase3 has a very slow intrinsic cleavage rate.*

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Figure S3, related to Figure 2: *Cleavage of hASNase3 is glycine dose-dependent and occurs also at physiological pH.*

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Figure S5, related to the main text section titled “Structural analysis of hASNase3”: *Effect of small metabolites on hASNase3-catalyzed asparagine hydrolysis.*

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Supplemental Figures

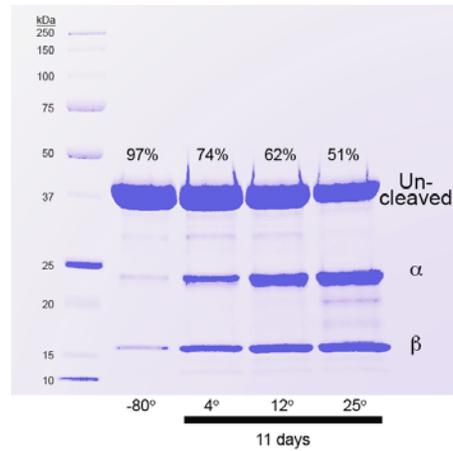


Figure S1. hASNase3 has a very slow intrinsic cleavage rate. Samples were incubated in storage buffer for 11 days, at either 4, 12, or 25 °C. These temperatures were selected since the enzyme is not stable for more than 3 days at 37° C. Percentage denotes precursor band strength relative to the sum of all three forms (precursor + α + β).

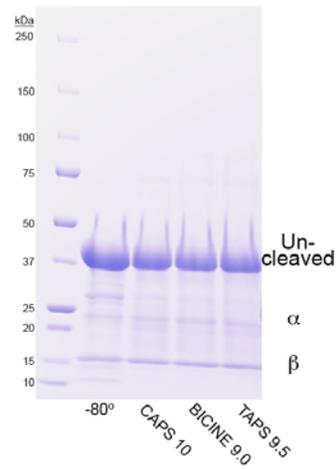


Figure S2. High pH by itself does not accelerate cleavage. hASNase3 (freezer-stored original sample labeled -80°) was incubated in 25 mM CAPS, pH 10.0, BICINE pH 9.0, or TAPS, pH 9.5 for 42 hours. Note that the enzyme remained predominantly in its uncleaved form.

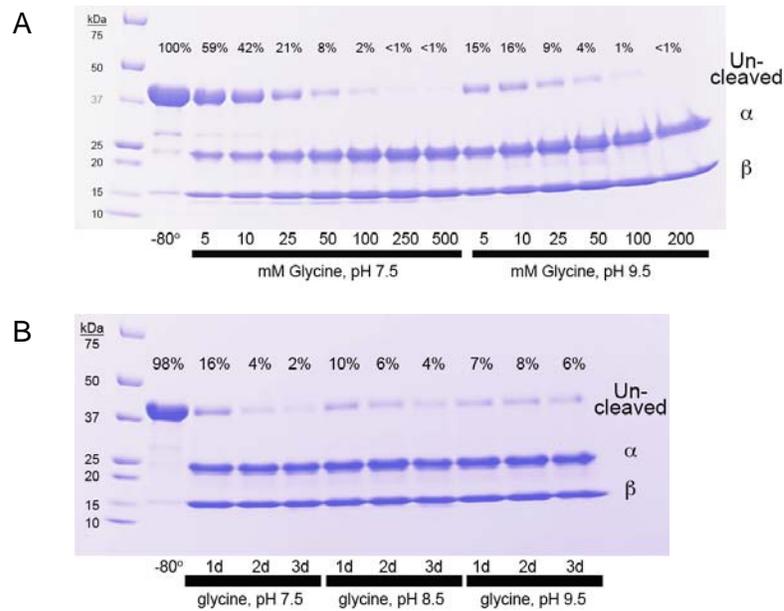


Figure S3. Cleavage of hASNase3 is glycine dose-dependent and occurs also at physiological pH. (A) Left panel; same as Figure 2a; right part, at pH 9.5. Cleavage rate is better at the higher pH, but still significant at the physiological pH of 7.5. (B) hASNase3 was incubated in storage buffer plus 100 mM glycine at pH 7.5, 8.5, and 9.5. Samples were taken after 1, 2, and 3 days incubation at 25 °C. The state of the starting material as taken from the freezer is shown in the lane labeled -80°. Percentage denotes precursor band strength relative to the sum of all three forms (precursor + α + β).

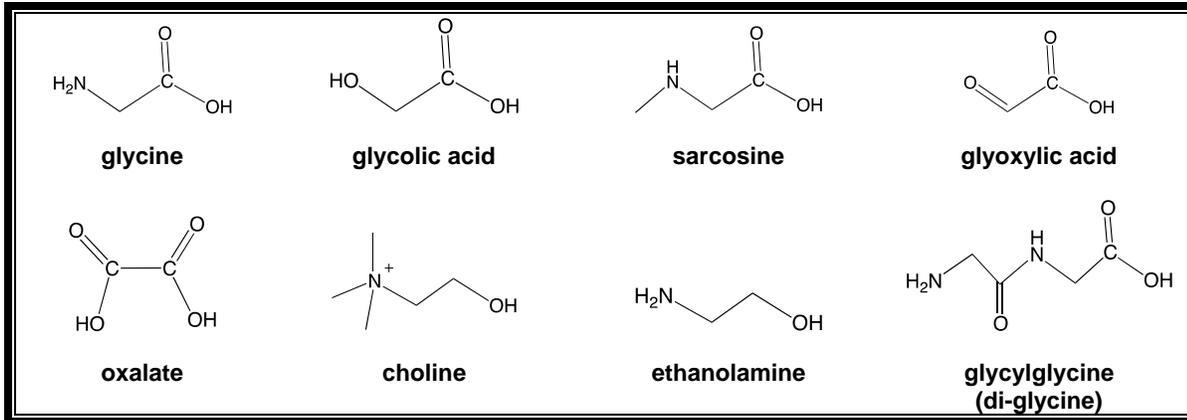
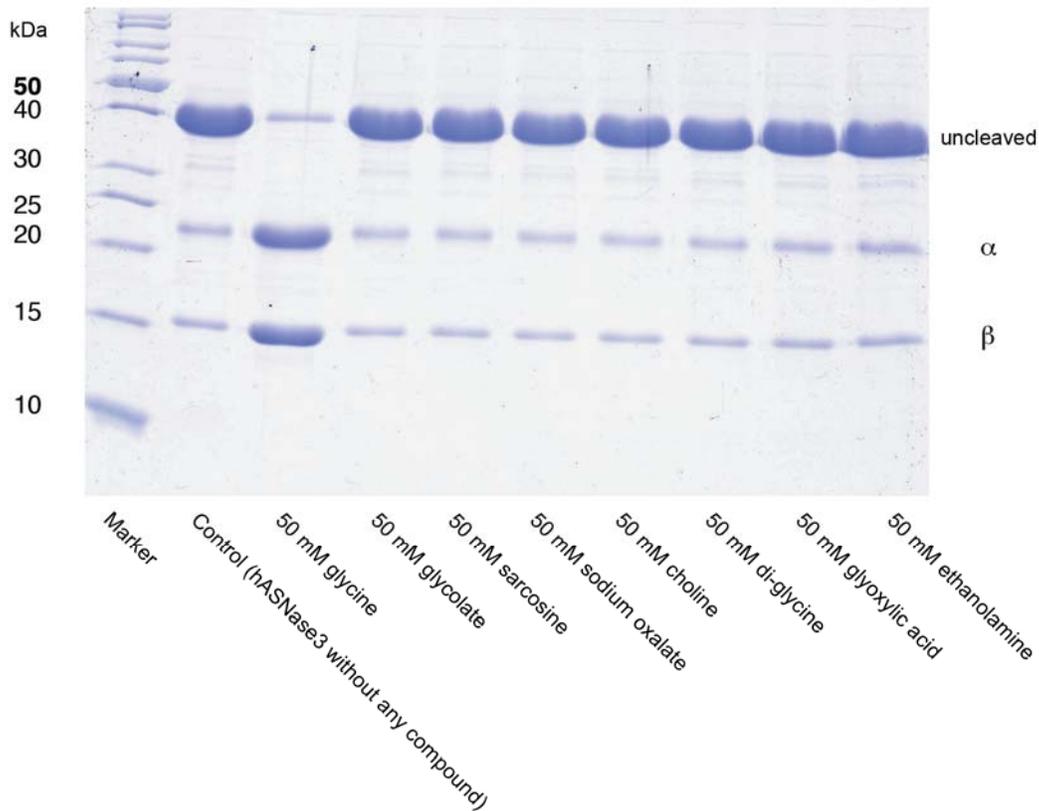


Figure S4. Promotion of hASNase3 cleavage is specific to glycine. The enzyme hASNase3 at a concentration of 3.5 mg/ml was incubated with the indicated molecules at 37 °C in 50 mM sodium phosphate, pH 8, and 0.5 M sodium chloride for 24 hours. The control lane displays the very limited intrinsic cleavage of hASNase3. Addition of glycine, but not of the other molecules tested, promoted the cleavage reaction. This demonstrates the selectivity of glycine for triggering hASNase3 cleavage.

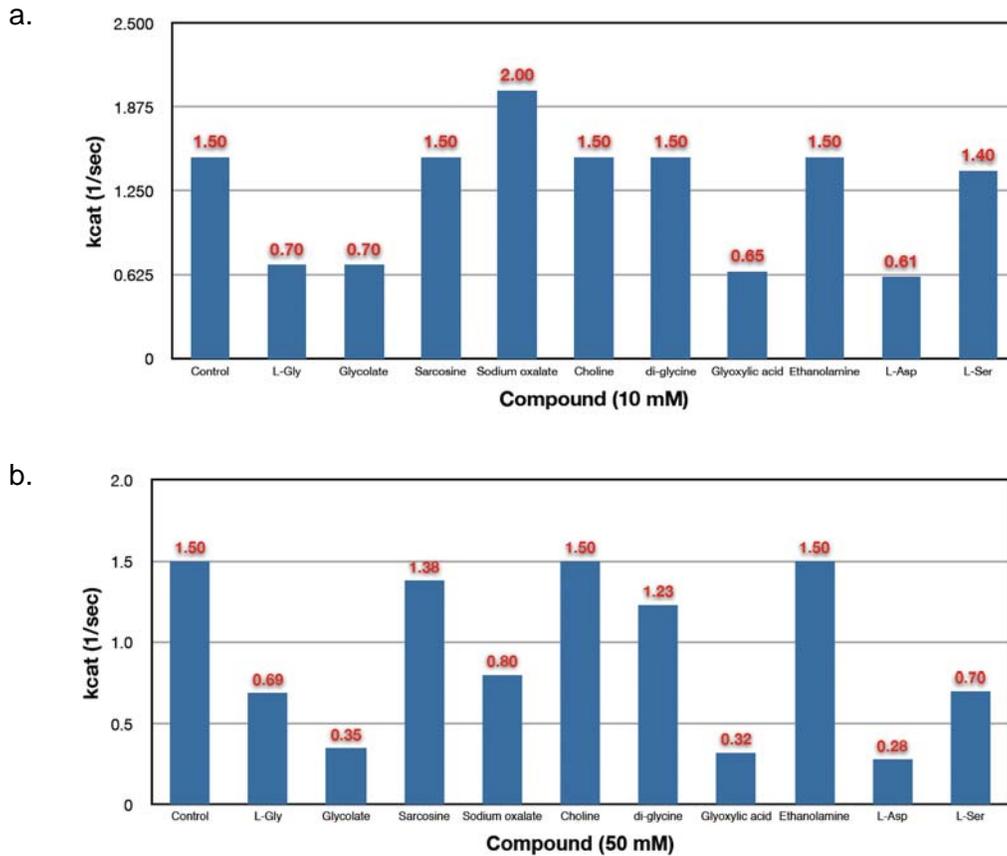


Figure S5: Effect of small metabolites on hASNase3-catalyzed asparagine hydrolysis. The enzyme was fully activated during production in *E. coli* using 200 mM glycine. After purification, cleaved hASNase3 was dialyzed against 50 mM sodium phosphate, pH 8, 0.5 M sodium chloride. The asparaginase activity was measured applying the conventional NADH dependent assay with a L-Asn concentration of 3 mM.

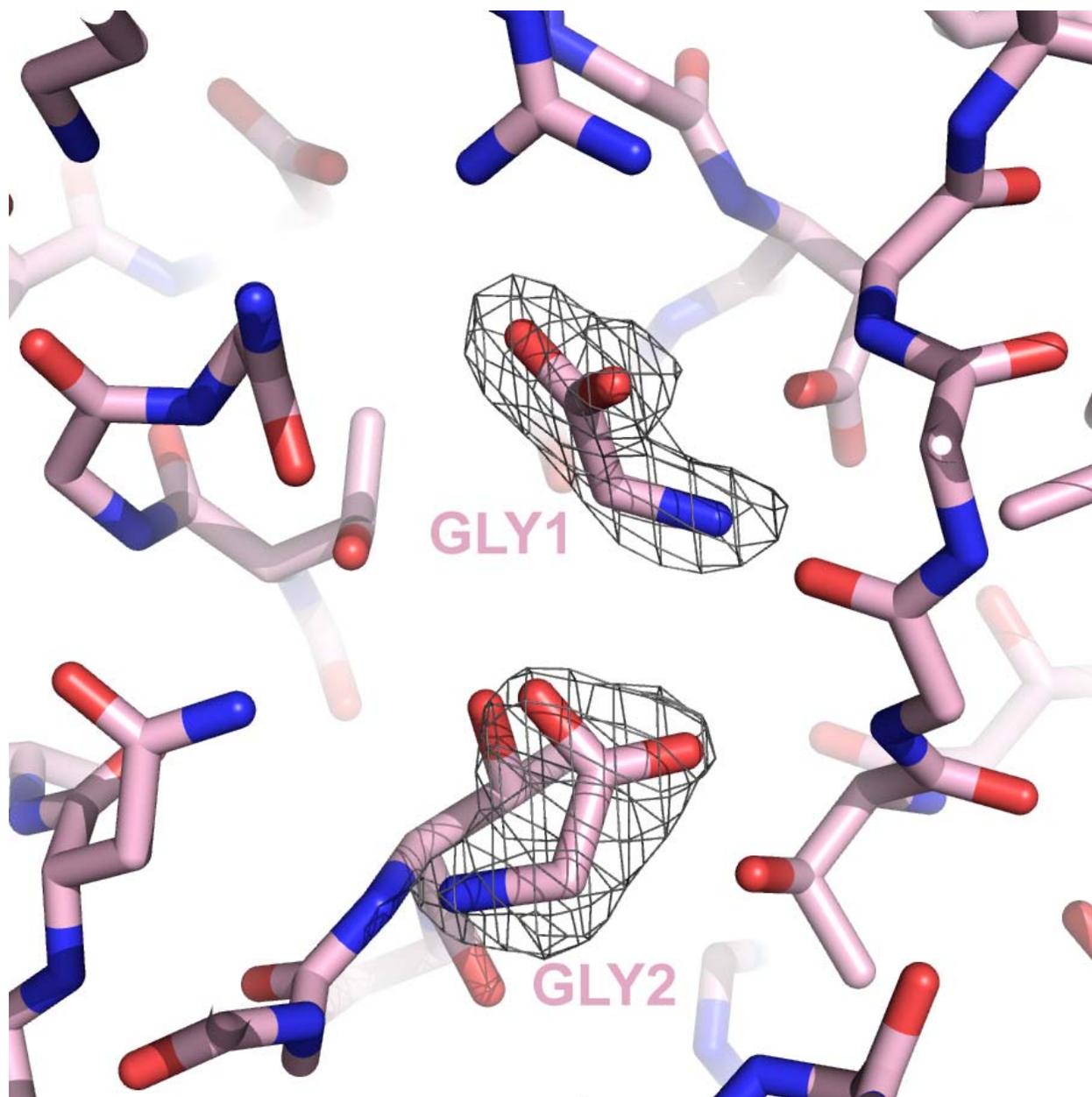


Figure S6. Fo-Fc map contoured at 3 sigma around GLY1 and GLY2. The glycine molecules were removed from the model that then underwent several rounds of refinement to eliminate model bias. The resulting Fo-Fc map clearly shows the presence of the two glycine molecules, labeled GLY1 and GLY2.

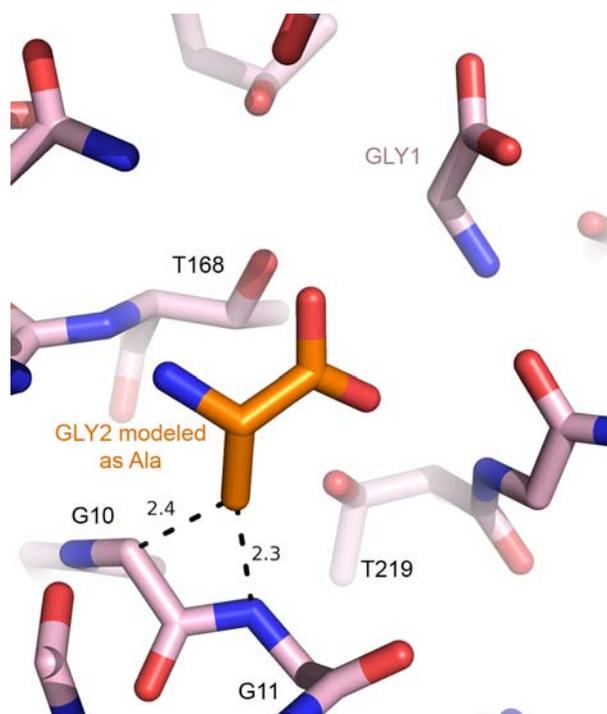


Figure S7. A model of an alanine bound in the same position as GLY2. An alanine (orange) was modeled into the same position observed for GLY2 in the pH 3.3 structure. This reveals a steric clash between the alanine side chain and the enzyme, explaining the glycine-specificity of the accelerated cleavage reaction. For orientation purposes, GLY1 is also shown.

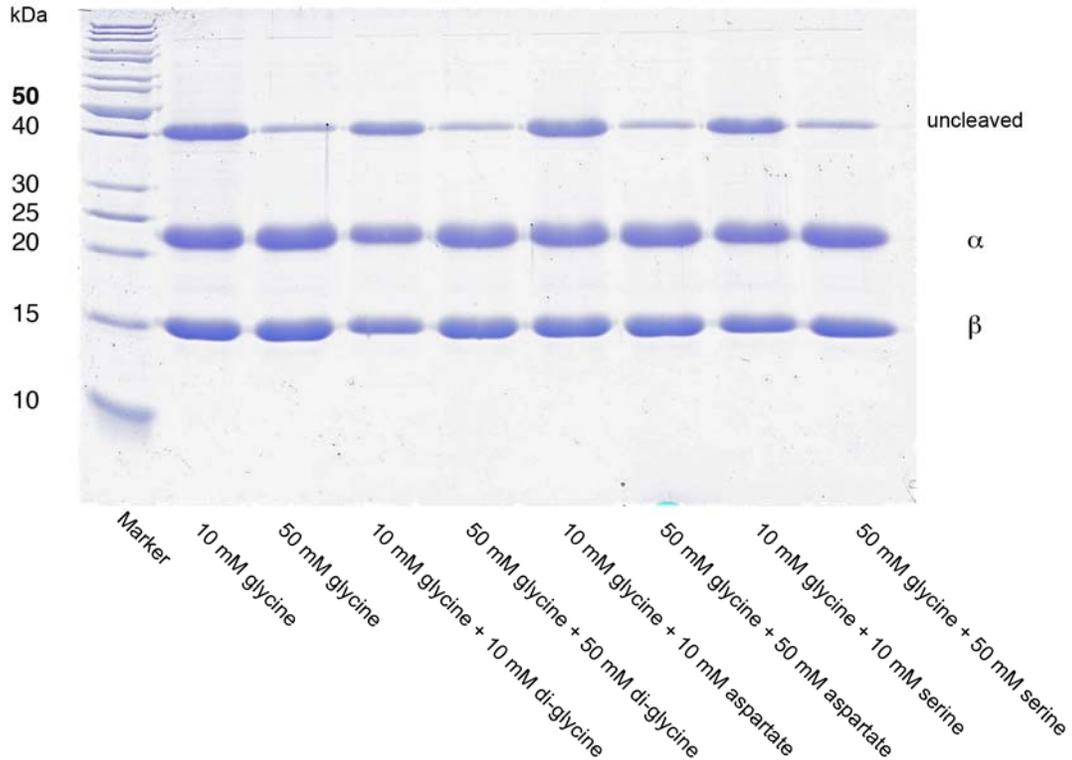


Figure S8. Promotion of hASNase3 cleavage by glycine is not inhibited by di-glycine, aspartate, or serine. The enzyme hASNase3 at a concentration of 3.5 mg/ml was incubated with the indicated molecules at 37 °C in 50 mM sodium phosphate, pH 8, and 0.5 M sodium chloride for 24 hours. Addition of di-glycine, aspartate, or serine did not reduce the amount of cleaved protein promoted by 10mM or 50 mM glycine.

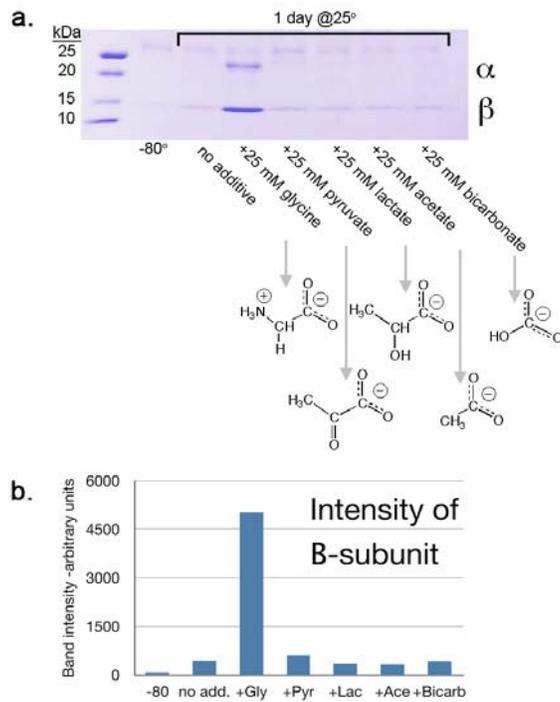


Figure S9. Small-molecule metabolites other than glycine that are expected to be present in glycolytic cells do not duplicate glycine's ability to accelerate cleavage. (A) Recombinant hASNase3 was incubated for 1 day at 25 °C in Storage Buffer with no additives, or with 25 mM of glycine, pyruvate, lactate, acetate, or bicarbonate. Only the enzyme in the presence of glycine showed a significant increase in the amount of the cleaved form, as demonstrated by the increase of the α - and β -chains. The structure of the various additives is shown below the gel, demonstrating the structural similarity of the molecules. **(B)** Quantification of the β -chain band intensity shows the very specific effect of glycine on cleavage.