The catalytic mechanism of rhomboid protease GlpG probed by 3,4-dichloroisocoumarin and diisopropyl fluorophosphonate

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Supplemental Material

Supplemental figure legends

Fig. S1. Identification of the hydrolytic products of DCI by LC-MS. **A-C**, The positive-ion mass spectra for the intact inhibitor **1**, acid chloride **2** and carboxylic acid **3**. The molecular species (M), plus either proton or sodium ion(s), are shown in the brackets. The theoretical molecular mass for each compound is listed below the spectra. **D**, Under alkaline conditions (100mM NaOH), DCI is quickly hydrolyzed to yield a single HPLC peak, which has similar elution volume and appearance as the peak (**4**) produced by GlpG (Fig. 1A). In this experiment, 20 times more inhibitor was used to enhance the signal of mass spectrometry. **E** and **F**, The positive-ion and negative-ion mass spectra for the peak generated by alkaline treatment. Unlike **1**, **2** and **3**, only fragments of the compound are observable in the positive-ion spectrum. In the negative-ion spectrum, a clear (but not the strongest) peak at 195.1 is observed, which corresponds to the mass of the α-hydroxy acid (minus a proton; Fig. 1B). All other major peaks in the positive-ion and negative-ion spectra can be explained by the α-hydroxy acid, as they correspond in mass to fragments of the compound that have first lost a H_2O, and then a CO_2. This fragmentation pattern is characteristic for *cis*-oriented di-carboxylic acids (38). **G** and **H**, The positive-ion and negative-ion mass spectra for the peak (**4**) generated by GlpG. Although the negative-ion peak at 195.1 is not observed, probably due to lower sample amount, all other major peaks match well with those in **E** and **F**, thus confirming that **4** corresponds to the same α-hydroxy acid. Note that the α-hydroxy acid (**4**) has a much lower extinction coefficient than the intact DCI.

Fig. S2. A hypothetical mechanism for GlpG-catalyzed DCI hydrolysis. The catalytic Ser-201 attacks the carbonyl carbon (C1) of the inhibitor **1**, generating an unstable acid chloride, which spontaneously hydrolyzes to form free carboxylic acid. His-254 then functions as a base to activate a water to attack C4, resulting in the replacement of the chlorine with a hydroxyl group. The unstable acylenzyme quickly undergoes hydrolysis to release α-hydroxy acid **4**. In this mechanism, the enzyme provides a base (His-254) to facilitate the dechlorination on C4, which mimics the action of NaOH in solution.

Fig. S3. Difference Fourier analysis confirms the binding of DFP to the membrane protease. The apo structure (2ic8) was used to generate the phases for map calculation. The F_o-F_c map is contoured at +3σ (blue) and -3σ (red) levels. The concerted side chain movements are indicated by arrows.

Figure S1

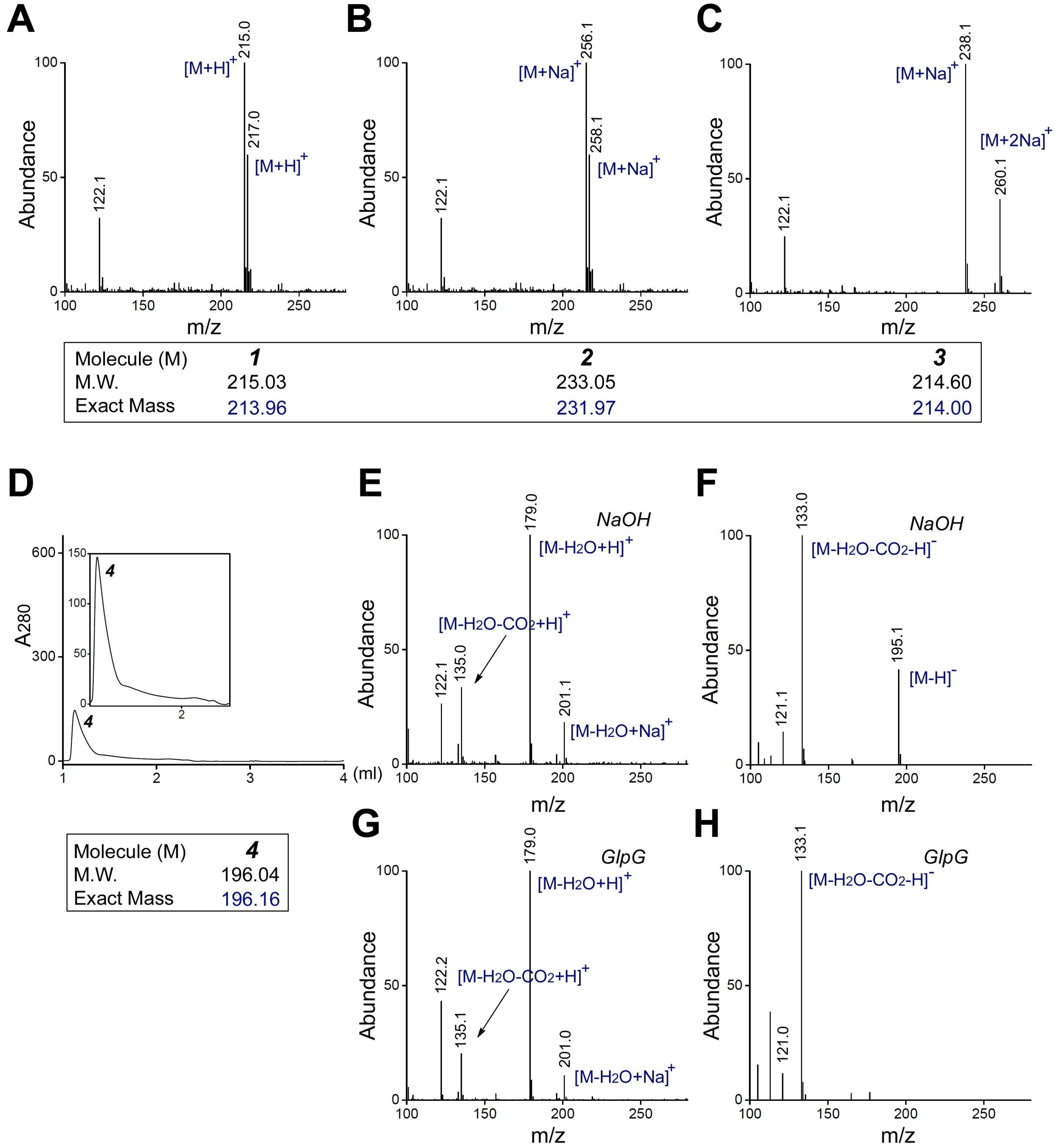

Figure S2

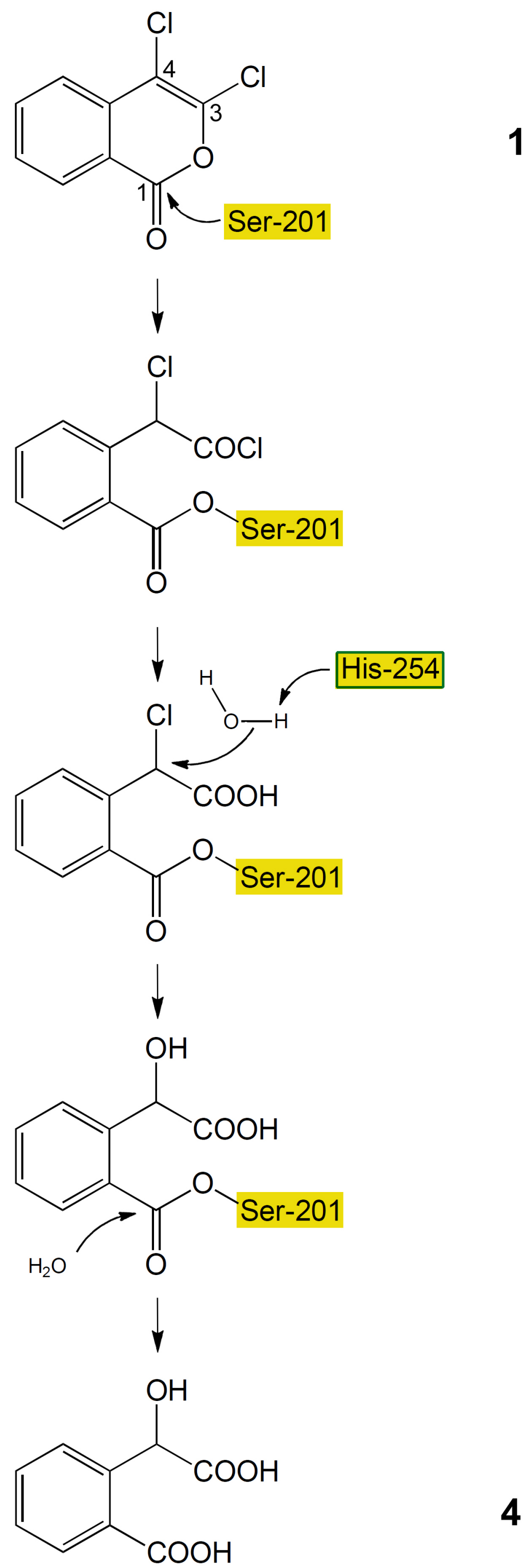

Figure S3