

SUPPLEMENTARY ONLINE DATA

A molten globule-to-ordered structure transition of *Drosophila melanogaster* crammer is required for its ability to inhibit cathepsinTien-Sheng TSENG^{*1}, Chao-Sheng CHENG^{*1}, Dian-Jiun CHEN^{*}, Min-Fang SHIH^{*}, Yu-Nan LIU^{*}, Shang-Te Danny HSU^{*†2} and Ping-Chiang LYU^{*‡2}^{*}Institute of Bioinformatics and Structural Biology, National Tsing Hua University, Hsinchu, 30013, Taiwan, [†]Institute of Biological Chemistry, Academia Sinica, Taipei 115, Taiwan, and [‡]Graduate Institute of Molecular Systems Biomedicine, China Medical University, Taichung, 40402, Taiwan

EXPERIMENTAL

Expression and purification of *Drosophila* cathepsin B

The *Drosophila* cathepsin B gene was amplified from cDNA libraries using the primers 5'-CGGATCCGACCCGA-TGAATCTATTGCTCCTG-3' (BamHI restriction site underlined) and 5'-GATCTCGAGTTACAGCTTGGGCAGACCCGCC-3' (XhoI restriction site underlined). The PCR amplification program was performed by 29 cycles of 30 s at 95°C, 30 s at 54°C and 1 min at 72°C. The PCR product was cloned into a pET-32a(+) vector (Novagen) to obtain a *Drosophila* cathepsin B construct containing a proregion and a mature protein. The sequence of the recombinant genes was verified by DNA sequencing (Mission Biotechnology).

E. coli BL21-Gold[®] (DE3) cells (Stratagene) harbouring a plasmid with the *Drosophila* cathepsin B gene were cultured in Luria–Bertani medium containing 50 mg/ml ampicillin at 37°C until the D_{600} value of the culture was 0.6. The protein was then expressed by adding IPTG (final concentration = 1 mM). Whole cells were then harvested by centrifugation at 4000 g for 20 min, and lysed by sonication. The lysates were centrifuged at 16000 g for 20 min at 4°C. The supernatant was removed and the precipitate was resuspended in 20 ml of 6 M guanidine buffer [50 mM Tris/HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA, 10 mM DTT and 6 M GdnHCl (guanidinium chloride)]. The solution was then diluted into 1 litre of 50 mM Tris/HCl (pH 8.5), 150 mM NaCl, 5 mM EDTA, 10 mM reduced glutathione, 1 mM oxidized glutathione and 0.5 M arginine overnight. After refolding, the protein solution was concentrated and dialysed against 25 mM NaH₂PO₄ (pH 7.0) and 0.5 M NaCl at 4°C. To autoproces cathepsin B, the protein solution was adjusted to pH 4.5 with acetic acid, and 5 mM EDTA and 5 mM DTT were added into a solution to incubate at 37°C for 1 h.

Proteins were purified by an AKTAprime system with a HiPrep[®] Sphacryl S-100 high-resolution gel-filtration column (Amersham Biosciences). The running buffer was 100 mM sodium acetate buffer (pH 5.0), containing 1 mM EDTA and 2 mM DTT. Each fraction was identified using Z-Phe-Arg-AMC substrate (Calbiochem) to confirm the cathepsin B activity.

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² Correspondence may be addressed to either of these authors (email lsipc@life.nthu.edu.tw or sthsu@gate.sinica.edu.tw). The structural co-ordinates reported will appear in the PDB under accession code 2L95.

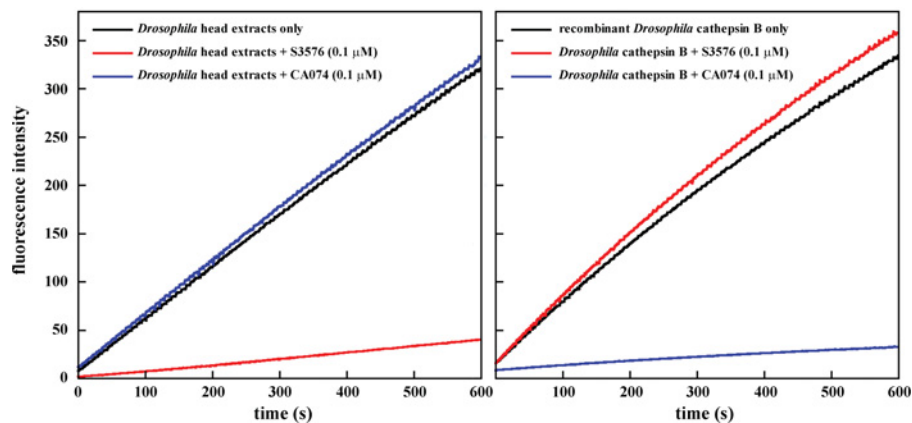


Figure S1 Progress curves for the inhibition of cathepsins

To identify the active substance in our *Drosophila* head extract, we used cathepsin B/L-specific inhibitors. A cathepsin L-specific inhibitor (S3576, {tert-butyl-[(2S)-1-{2-[2-(2-ethylamino)-2-oxoethyl]sulfanylcarbonylhydrazinyl}-3-(1H-indol-3-yl)-1-oxopropan-2-yl]carbamate}) shows strong inhibition against the *Drosophila* head extract (left-hand panel). The same use of S3576 does not inhibit recombinant *Drosophila* cathepsin B (right-hand panel). In contrast, the cathepsin B-specific inhibitor (CA074, [L-3-*trans*-(propylcarbonyl)oxirane-2-carbonyl]-L-isoleucyl-L-proline) exhibits opposite results in the same assay. This indicates that the enzymatically active substance in the *Drosophila* head extract is mainly cathepsin L.

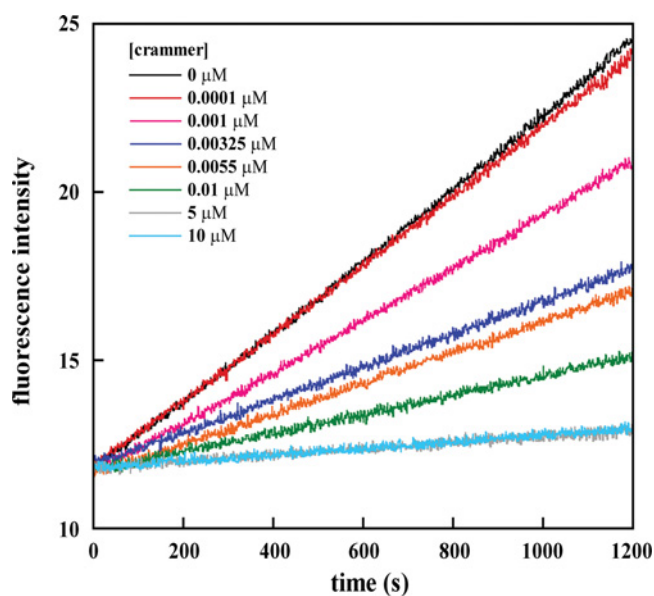


Figure S2 Progress curves for the inhibition of cathepsin L by crammer

Progress curves for the inhibition of *Drosophila* cathepsin L in the presence of various concentrations of crammer gave linear plots, suggesting a concentration-dependent inhibition of initial velocity of product formation. The crammer concentrations are labelled in the Figure.

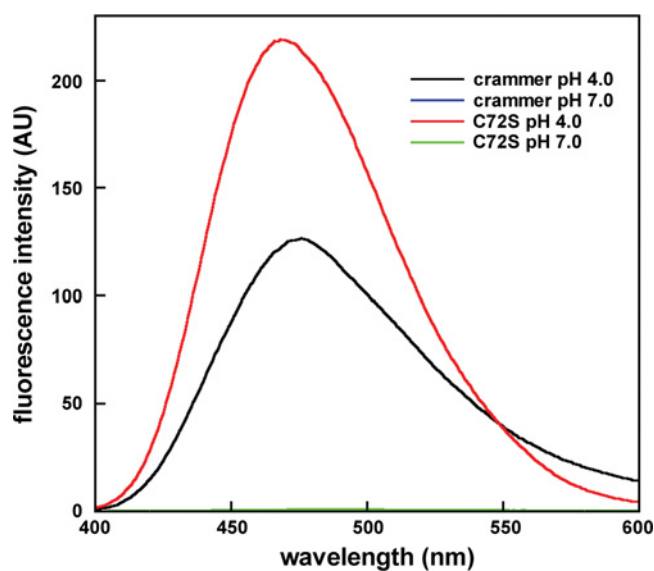


Figure S3 ANS binding

The exposure of hydrophobic core was investigated by ANS fluorescence spectra. The ANS intensity was recorded from 400 to 600 nm ($\lambda_{ex} = 365$ nm).

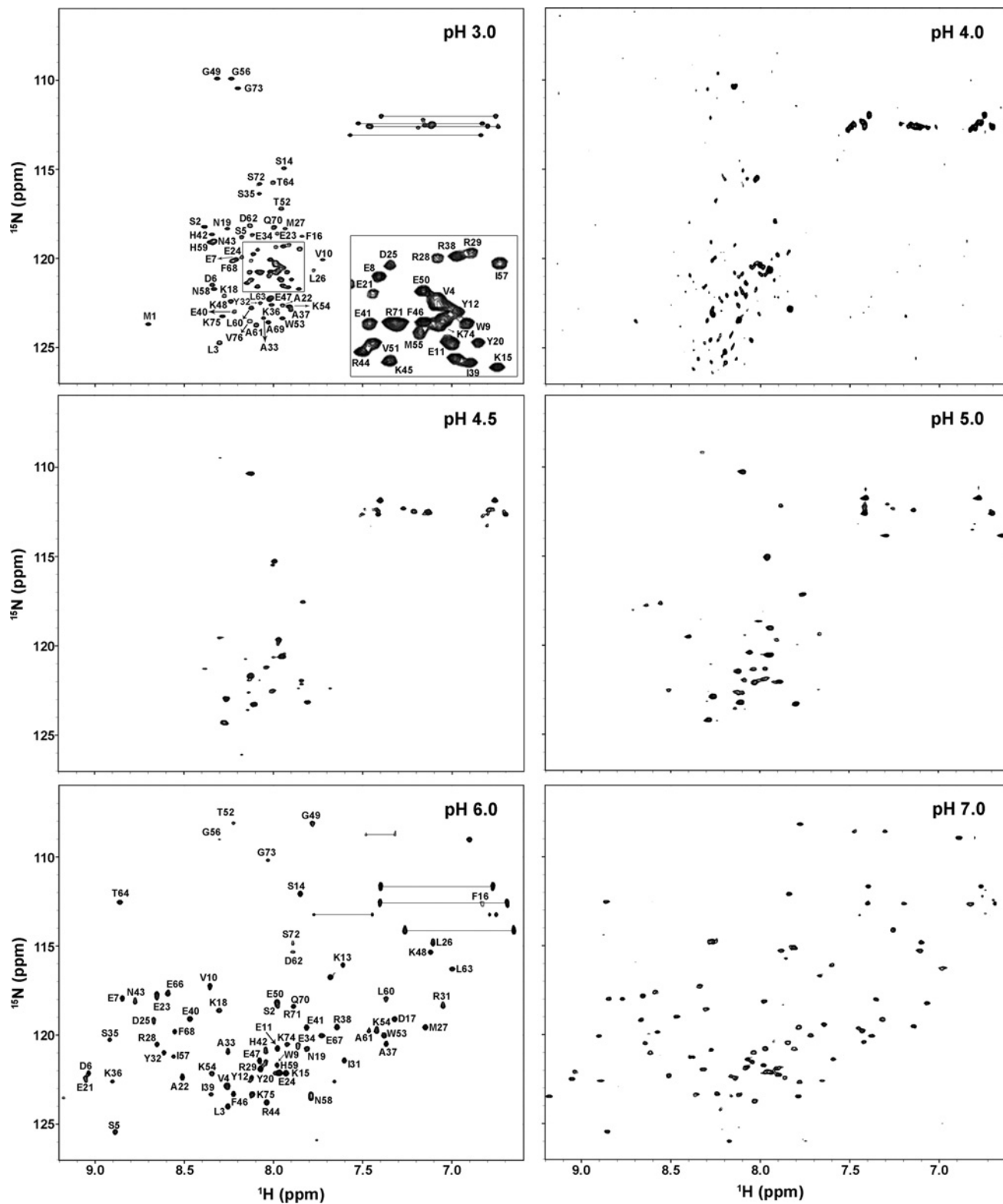


Figure S4 ^1H - ^{15}N -HSQC spectra of C72S as a function of pH

All spectra were recorded at 25 °C, and the assigned cross-peaks for crammer at pH 3.0 and pH 6.0 are labelled in the Figure. The insert in the upper-left-hand panel shows an expanded view of part of the spectrum.

Received 22 August 2011/29 November 2011; accepted 9 December 2011
 Published as BJ Immediate Publication 9 December 2011, doi:10.1042/BJ20111360

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