Circular Zymogens of Human Ribonuclease 1

Ian W. Windsor,[1,3] Crystal J. Graff,[1] and Ronald T. Raines[1–3]*

[1]Department of Biochemistry and [2]Department of Chemistry, University of Wisconsin−Madison, Madison, Wisconsin 53706, USA
[3]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

*Correspondence to: Ronald T. Raines, Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, United States. E-mail: rtraines@mit.edu

Content	Page

Table SI. *Catalytic Efficiency and Thermostability of RNase 1 Zymogens at pH 7.4*

Zymogen	k_{cat}/K_M ($M^{-1}s^{-1}$)	T_m (°C)
RNase 1[a]	$(2.1 \pm 0.2) \times 10^7$	57 ± 2
3G	$(2.8 \pm 0.6) \times 10^5$	50.4 ± 0.4
2G	$(4.6 \pm 0.2) \times 10^4$	48.9 ± 0.3
1G	$(3.2 \pm 0.2) \times 10^5$	48.1 ± 0.1
0G	$(2.1 \pm 0.2) \times 10^5$	46.9 ± 0.2
Str1	$(8.5 \pm 0.2) \times 10^4$	nd[b]
Str2	$(5.8 \pm 0.1) \times 10^3$	47.5 ± 0.1
Str3	$(1.1 \pm 0.1) \times 10^3$	42.6 ± 0.1
Str4	$(5.7 \pm 0.1) \times 10^2$	42.0 ± 0.1

[a]Values are from ref. 1.
[b]Not determined.

Table SII. *Catalytic Efficiency of Three RNase 1 Zymogens at pH 5.0*

Zymogen	k_{cat}/K_M ($M^{-1}s^{-1}$) (inactive)	k_{cat}/K_M ($M^{-1}s^{-1}$) (activated)	Relative Activity
Str2	$(2.9 \pm 0.1) \times 10^3$	$(5.2 \pm 0.2) \times 10^5$	180
Str3	$(7.3 \pm 0.1) \times 10^2$	$(7.0 \pm 0.3) \times 10^5$	960
Str4	$(9.2 \pm 0.3) \times 10^2$	$(2.4 \pm 0.4) \times 10^6$	2600

Table SIII. *Energy of Top-10 Scoring Models of the Str2 RNase 1 Zymogen and Its Complex with HIV-1 Protease as Calculated with Rosetta Software*

Model	Str2 (REU)	Complex (REU)
1	–197.697	–522.837
2	–196.236	–518.158
3	–193.333	–511.494
4	–192.159	–466.197
5	–191.876	–457.239
6	–191.655	–456.408
7	–191.556	–454.677
8	–191.252	–453.330
9	–191.203	–451.303
10	–190.923	–451.246

REU = Rosetta Energy Units

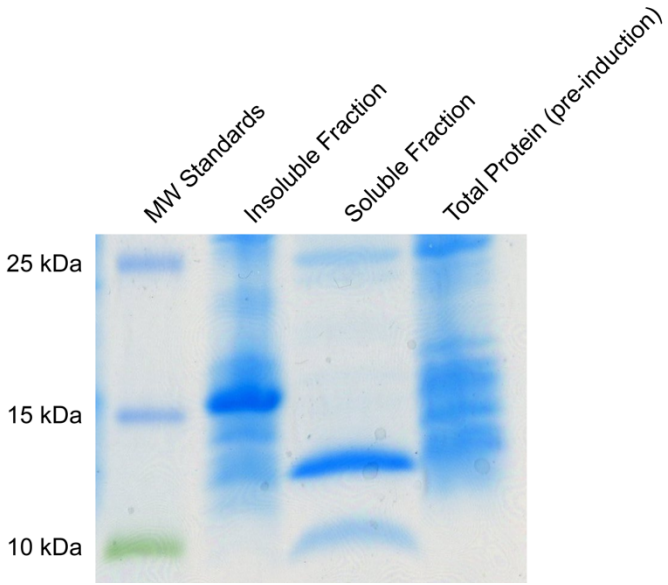

Figure S1. Image of the analysis by SDS–PAGE of the production of the 3G zymogen. MW standards were product 26623 from ThermoFisher Scientific (Waltham, MA). Induction of expression of the single open-reading-frame zymogen construct results in a single major band in the insoluble fraction (from the circular zymogen) and two major bands in the soluble fraction (from intein fragments), neither of which are observed pre-induction.

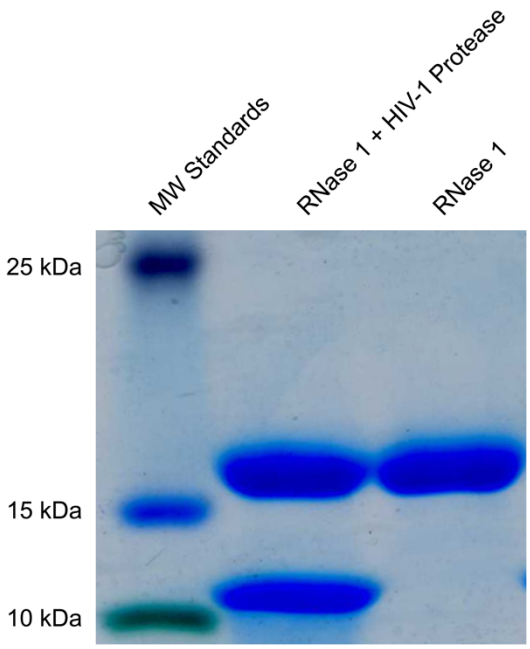

Figure S2. Image of the analysis by SDS–PAGE of the effect of HIV-1 protease on wild-type RNase 1. MW standards were product 26623 from ThermoFisher Scientific. After incubation for 3 h at 37 °C, no detectable cleavage of RNase 1 was observed.

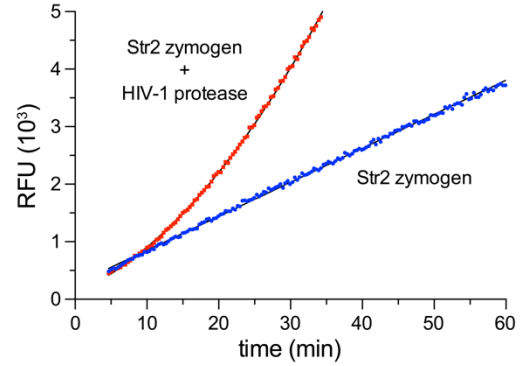

Figure S3. Graph showing activation kinetics of the Str2 zymogen. Hydrolysis of a fluorogenic substrate (20 nM) at pH 5.0 by Str2 zymogen (10 nM) led to a linear increase in product formation at <10% substrate turnover. The presence of HIV-1 protease (2.6 nM) led to a time-dependent increase in activity, which is fitted by a second-order polynomial.

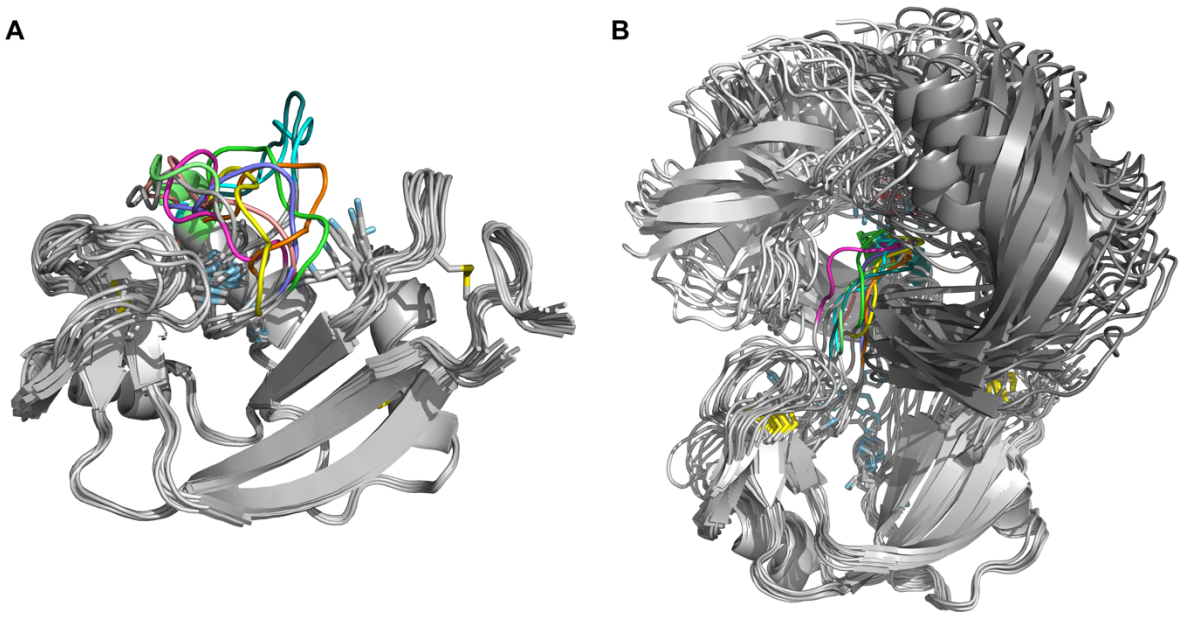

Figure S4. Images of the top-10 scoring molecular models of the Str2 zymogen and its complex with HIV-1 protease, as generated with Rosetta software. (A) Str2 zymogen. The linker (colored) is predicted to occupy multiple conformations, suggesting that this region is conformationally dynamic. (B) Protease·zymogen complex. The termini of the zymogen must unfold for HIV-1 protease to close its flaps on the substrate-containing linker (colored).

Reference

1. Johnson RJ, McCoy JG, Bingman CA, Phillips GN, Jr., Raines RT (2007) Inhibition of human pancreatic ribonuclease by the human ribonuclease inhibitor protein. J Mol Biol 368:434–449.