The Role of Loop Bundle Hydrogen Bonds in the Maturation and Activity of (Pro)caspase-3[†]

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Supplemental Figure Legends

Supplemental Figure 1. V8 (endo-glu C) protease digests of (pro)caspase variants. (A) procaspase-3(D₃A), (B) procaspase-3(D₃A,E167A), (C) procaspase-3(D169A), (D) procaspase-3(D₃A,E173A), (E) procaspase-3(D₃A,Y203F), (F) wild-type caspase-3, (G) caspase-3(E167A), (H) caspase-3(E173A), and (I) caspase-3(Y203F). (J) Trypsin and V8 protease cleavage sites are mapped onto the caspase-3 structure, and the cleavages are summarized in Table 2. The structure was generated using the program *PyMOL* (Delano Scientific). The cleavages at K57, R64, and R207 (underlined) are by trypsin, and the cleavages at E98, E106, E173, D190, E248, and D253 (italicized) are by V8 protease. The prime (‘) indicates residues from the second heterodimer, “D₃A” refers to the procaspase, and “CP-3” refers to the mature caspase.

Supplemental Figure 2. Trypsin protease digests of (pro)caspase variants. (A) procaspase-3(D₃A), (B) procaspase-3(D₃A,E167A), (C) procaspase-3(D169A), (D) procaspase-3(D₃A,Y203F), (E) wild-type caspase-3, (F) caspase-3(E167A), (G) caspase-3(E173A), and (H) caspase-3(Y203F). Cleavage sites are summarized in Figure 3E and in Table 2.

Supplemental Figure 3. Quenching by iodide or acrylamide. Panels A-D and F-H: The Stern-Volmer quenching constants, K_{SV} , were determined for KI quenching over the pH range 3-9 as described in Materials and Methods for procaspase-3 (D₃A) (○), mature caspase-3 (○) and each (pro)caspase-3 mutant (●). The mutants are noted in each panel. The solid lines represent fits of the data as described in Materials and Methods. (E) K_{SV} from acrylamide quenching of procaspase-3(D₃A) (○), procaspase-3(D₃A,E167A) (▲), procaspase-3(D169A) (●), procaspase-3(D₃A,E173A)(◆), and procaspase-3(D₃A,Y203F) (■). (I) K_{SV} from acrylamide quenching of caspase-3(E167A) (▲), caspase-3(E173A) (◆), and caspase-3(Y203F) (■).

Supplemental Figure 4. Average emission wavelength, $\langle\lambda\rangle$, versus pH. For panels A-G, $\langle\lambda\rangle$ was determined over the pH range 2.4 to 9 for procaspase-3(D₃A) (○), mature caspase-3 (○) and each (pro)caspase-3 mutant (□). Mutants are noted on each panel. The solid lines represent fits of the data as described in Materials and Methods.

Supplemental Figure 1

Supplemental Figure 2

Supplemental Figure 3

A

B

C

D

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F

G

H

I

Supplemental Figure 4

A

E

B

C

F

D

G