Published in final edited form as:

Nat Chem Biol. ; 8(7): 606–607. doi:10.1038/nchembio.1000.

Peptide Inhibitors:

Four of a kind beats a pair

Shawn B Bratton

the Department of Molecular Carcinogenesis at the University of Texas–M.D. Anderson Cancer Center, Science Park, Smithville, Texas, USA

Shawn B Bratton: sbbratton@mdanderson.org

Abstract

The proapoptotic cysteine protease caspase-6 participates in the neuropathology of several diseases. Unlike the active dimeric form, the caspase-6 zymogen forms a unique tetramer that can be stabilized by allosteric inhibitors, which prevents caspase-6 activation.

Apoptosis is a programmed form of cell death that is typically executed by caspases or cysteine-dependent aspartate-specific proteases¹. Caspases are activated in a hierarchical manner, wherein the upstream initiator caspases 2, 8, 9 and 10 process the downstream effector caspases 3, 6 and 7, which in turn proteolytically dismantle the cell. Initiator caspases are single-chain monomers that are activated through dimerization following association with their specific adaptor proteins. Effector caspases, in contrast, are obligate dimers that are activated through cleavage of their intersubunit linkers¹. Most efforts to design caspase inhibitors have, until now, targeted their active site cysteines with electrophiles, but achieving selectivity with this approach has been difficult. In this issue, Stanger *et al.*² demonstrate that procaspase-6 forms a unique tetramer that is resistant to activation. This complex can be stabilized for both the zymogen and active enzyme with peptides that bind an allosteric site²; these findings pave the way for the development of selective inhibitors of caspase-6.

Caspases contain either a long or short prodomain, followed by large and small subunits that are connected by an intersubunit linker (Fig. 1a)¹. Two major caspase cascades are initiated during apoptosis, depending on the type of cellular stress encountered. In the extrinsic pathway, ligation of death receptors such as Fas/CD95 results in the formation of a receptor–Fas-associated protein with death domain (FADD)–caspase-8 complex that promotes caspase-8 activation through dimerization. Similarly, in the intrinsic pathway, stressors such as DNA damage trigger mitochondrial release of cytochrome *c* and formation of an apoptotic protease-activating factor 1 ‘apoptosome’ complex that recruits and activates caspase-9. Active caspases 8 and 9 then process the downstream effector caspases 3 and 7 (ref. 1). Caspase-3 targets >800 protein substrates in the cell, including caspase-6, and is by far most responsible for dismantling the cell during apoptosis. Caspase-6 has comparatively few substrates, the most notable being lamin A, and is not directly activated by any established initiator caspase. Consequently, caspase-6 has been relatively undervalued and understudied.

Increasing evidence, however, suggests that caspase-6 may have a critical role in the pathogenesis of Alzheimer's disease³ and Huntington's disease^{4,5}. Indeed, caspase-6–

dependent cleavage of huntingtin protein is essential for the development of Huntington's disease in a mouse model, and transgenic mice expressing the cleaved N-terminal fragment of huntingtin protein likewise develop the disease^{4,5}. Moreover, whereas caspase-6-deficient mice were originally thought to develop normally, they now appear to have learning disabilities, and their neurons are resistant to excitotoxicity and growth factor deprivation⁶.

Despite the accumulating evidence in support of its role in neuropathology, one of the puzzling questions regarding caspase-6 is how it is activated in this context. As noted above, caspase-6 can be directly processed by caspase-3, downstream of either the extrinsic or intrinsic pathways⁷. However, if either of these pathways is activated sufficiently for caspase-3 to activate caspase-6, one would expect caspase-3 to cleave numerous substrates, resulting in immediate cell death. So how is caspase-6 activated in neurons during the much slower process of forming plaques, tangles and protein aggregates? One possible explanation is that, in addition to serving as a classical effector caspase, perhaps caspase-6 can also function as a type of hybrid or combined initiator-effector caspase. Indeed, a recent structure of the caspase-6 zymogen indicates that its intersubunit linker inserts into the active site of caspase-6. This orientation positions the ¹⁹⁰TEVD¹⁹³ cleavage site very near the active site cysteine, so that even a minor change in conformation should allow for catalytic attack of Asp193 (ref. 8). In other words, caspase-6 may self-activate through a highly unusual intramolecular cleavage event.

Stanger *et al.*² have discovered yet another interesting aspect of caspase-6 biology; namely, that its zymogen is a tetramer at pH 8.0. Given the successful identification of allosteric inhibitors of caspase-7 (ref. 9), the authors used phage display in an effort to identify peptides capable of binding the caspase-6 zymogen outside of its active site. One peptide, pep419, bound the caspase-6 zymogen and stabilized the tetrameric complex, even under low-pH conditions that would otherwise favor dissociation of the tetramer into a dimer^{2,10}. Subsequent crystallographic studies revealed that pep419 formed a disulfide-linked antiparallel β -strand, sandwiched between each of the A-C and B-D chains of the zymogen, with Glu12 and His9 making key contacts with caspase-6 at His126 and Asp131, respectively (Fig. 1b)². Pep419 also shifted the equilibrium of active caspase-6 from its dimeric to its tetrameric form and correspondingly inhibited enzyme activity through a mixed noncompetitive mechanism, indicating that the peptide could stabilize the tetrameric complex, regardless of the processed state of the enzyme. Remarkably, compared to the zymogen, the peptide-bound complex showed no radical changes in active site conformation. Nevertheless, mutations of Glu12, His126 or both diminished the affinity of pep419 for caspase-6, prevented stabilization of the tetramer and failed to effectively inhibit active caspase-6 *in vitro* and in cultured cells².

Although the current study has tremendous implications for the treatment of Alzheimer's and Huntington's disease, a number of unanswered questions remain. For example, how is caspase-6 activated in neurons during the progression of Alzheimer's or Huntington's disease? The zymogen may be primed for 'self-activation' owing to the placement of its intersubunit linker, but something more is required to trigger cleavage. Also, does caspase-6 inhibition *in vivo* result primarily from stabilization of the zymogen or the active caspase-6 tetramer, and is the zymogen tetramer resistant to processing by active caspase-3 in the more classical caspase cascade? There are also practical hurdles to overcome in terms of drug design and delivery. Designing small molecules that can selectively stabilize the caspase-6 tetramer and cross the blood-brain barrier will be challenging. Nevertheless, Stanger *et al.*² provide the first proof of principle that caspase-6 (and perhaps other caspases) can be selectively inhibited through noncovalent binding of molecules to an allosteric site.

References

1. Fuentes-Prior P, Salvesen GS. *Biochem J.* 2004; 384:201–232. [PubMed: 15450003]
2. Stanger K, et al. *Nat Chem Biol.* 2012; 8:655–660. [PubMed: 22683611]
3. Guo H, et al. *Am J Pathol.* 2004; 165:523–531. [PubMed: 15277226]
4. Graham RK, et al. *Cell.* 2006; 125:1179–1191. [PubMed: 16777606]
5. Tebbenkamp AT, et al. *Hum Mol Genet.* 2011; 20:2770–2782. [PubMed: 21515588]
6. Uribe V, et al. *Hum Mol Genet.* 2012; 21:1954–1967. [PubMed: 22262731]
7. Slee EA, et al. *J Cell Biol.* 1999; 144:281–292. [PubMed: 9922454]
8. Wang XJ, et al. *EMBO Rep.* 2010; 11:841–847. [PubMed: 20890311]
9. Hardy JA, Lam J, Nguyen JT, O'Brien T, Wells JA. *Proc Natl Acad Sci USA.* 2004; 101:12461–12466. [PubMed: 15314233]
10. Baumgartner R, et al. *Biochem J.* 2009; 423:429–439. [PubMed: 19694615]

Figure 1.

The caspase-6 zymogen forms a tetramer that is stabilized by pep419. **(a)** In this structure of the caspase-6 dimer, the large and small subunits are shown in red and orange, respectively. The intersubunit linker is shown in magenta; the single β -sheet, containing the $^{190}\text{TEVD}^{193}$ cleavage site, is inserted into the active site of the enzyme. The region of the large subunit, containing the residues that interact with pep419, is shown in blue. **(b)** The A-C and B-D chains of two caspase-6 zymogen dimers face one another to form the tetramer, which is stabilized by pep419 (green). Renderings of the dimeric caspase-6 zymogen (Protein Data Bank code 3NR2)⁸ were performed using UCSF Chimera.