Previews

Chemical Ligation—An Unusual Paradigm in Protease Inhibition

Some protease inhibitors use uncommon mechanisms to restrain the activity of their target enzymes. A recent paper in *Chemistry and Biology* (Lu et al., 2006) demonstrates a curious mechanism of inhibition of a caspase, relying on principles of native peptide ligation.

Chemical ligation using activated ester intermediates has long been used as a tool in synthetic chemistry. More recently, native chemical ligation has emerged as a mechanism used by self splicing proteins, termed inteins, in which an N-terminal polypeptide covalently couples to a C-terminal polypeptide during autocatalytic excision of the central intein element (Paulus, 2000). A similar mechanism generates the essential C-terminal cholesterol modification required for the ability of *Hedgehog* to direct patterning of vertebrate and invertebrate embryos (Lum and Beachy, 2004). The essential feature of native chemical ligation is the presence of a thiol moiety on a Cys side chain that attacks the carbonyl of a suitably placed peptide bond, forming a labile thioester intermediate. This intermediate is then attacked by a nucleophile, and, if the nucleophile is an N-terminal amino acid, it may undergo an S-N acyl shift to generate the stable amide bond (Figure 1).

Intein structures have similarities to protease active sites, containing features capable of stabilizing the tetrahedral intermediate in peptide bond hydrolysis via a critical "oxyanion pocket." Of course, in the case of inteins, the attacking nucleophile is a protein side chain, not water, but the fundamental mechanisms of inteins and proteases are similar. Indeed, the cysteine protease bleomycin hydrolase also conducts native chemical ligation (Zheng et al., 1998), although this is rather inefficient and the enzyme acts in a catalytic mode, rather than the single efficient turnover observed in inteins. Consequently, it may not come as a surprise that caspases, another branch of cysteine proteases, participate in peptide ligation. The surprise comes when one considers that caspase-mediated chemical ligation may be an unusual strategy adopted by the baculovirus protein p35 to secure caspase inhibition, as discussed by Lu et al. (2006).

Caspases are conserved in multicellular animals, where they orchestrate the complex biochemistry known as apoptosis: the ordered dismantling of unwanted cells (Green and Evan, 2002). Their stringent regulation by endogenous inhibitors is considered pivotal in controlling cell fate decisions, and caspase inhibition is a strategy used by some viruses to defeat host cell attempts to suicide following infection (Stennicke et al., 2002). To date, caspase inhibitors belonging to three different classes have been extensively examined: (1) members of the inhibitor of apoptosis protein (IAP) family that utilize an unusual allosteric binding mechanism, (2) members of the classic serpin family, and (3) p35, which has its entirely own inhibitory mechanism and thus far is the only known inhibitor of its class. Superficially, serpins and p35 have a similar mechanism. Both are "suicide inhibitors" that offer themselves up as bait to an attacking protease but then trap the protease catalytic machinery, and both are cleaved as a result of the sacrifice. Although there are no high-resolution caspase:serpin structures, examination of the trypsin or elastase:serpin complex reveals a mechanism in which the catalytic machinery is radically distorted in a trapped
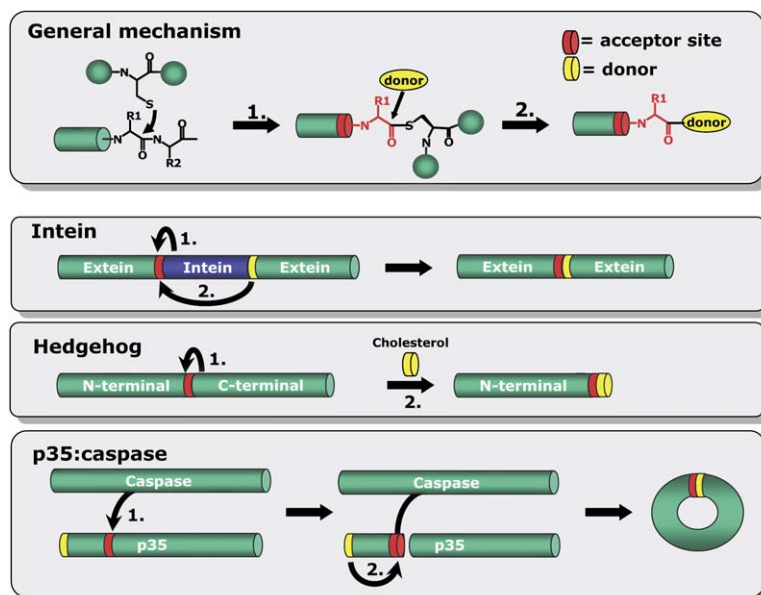

Figure 1. Cartoon Depicting the General Mechanism of Native Peptide Ligation and Showing Three Examples of Its In Vivo Use

The general mechanism involves an activating domain that generates the essential thioester intermediate (1), which subsequently may be attacked by a donor nucleophile (2). In the general mechanism, the nucleophilic residue that mediates thioester formation is depicted as a remote residue; however, in both inteins and *Hedgehog*, the nucleophilic moiety mediating this reaction is situated at the R2 position. In the case of *Hedgehog*, this simply leads to generation of a C-terminal cholesterol ester; however, in the case of inteins, the intermediate generated upon nucleophilic attack by the donor may undergo N-S acyl shift, leading to the formation of a new peptide bond. The mechanism for p35 is slightly different, since the activating domain here is the protease caspase 8, and the circular product is the N-terminal region of p35 formed either during or after protease attack on the inhibitor. As for the inteins, a new peptide bond joining the N-terminal to the P1 Asp is formed by a subsequent N-S acyl shift.

complex (Dementiev et al., 2006; Huntington et al., 2000). In contrast to what is seen for the serpins, there is no distortion of the protease structure in the p35:caspase complex (Xu et al., 2001). Technically, the complex structure revealed an unusual inhibitory mechanism in which cleavage of p35 allows its N-terminal Cys to insert into the active site in such a manner that the thiol forms a hydrogen bond with the catalytic His of caspase 8, resulting in the imidazole ring rotating approximately 90° around the Cβ-Cγ bond. This conformation was considered to be the stable inhibitory form, since the N-terminal Cys of p35 apparently eliminated the ability of the catalytic His of the caspase to promote the formation of the nucleophilic water molecule required for hydrolysis of the acyl-enzyme intermediate.

In an elegant example of deductive logic, Lu et al. (2006) now demonstrate that the free N-terminal Cys of p35 not only facilitates anchoring of the inhibitor to the catalytic site but also serves as a nucleophile in the chemical ligation of the N-terminal Cys to the Asp of cleaved p35 (Figure 1). This means that what was assumed to be the end adduct containing the Cys2-His287 hydrogen bond was rather the polarization of Cys2 for a nucleophilic attack on the acyl-enzyme intermediate of caspase 8 with p35. The beauty of this mechanism is that, once the thioester in the acyl enzyme has been substituted for the thioester with Cys2, the system may then reach equilibrium between the two thioesters, chemically trapping the enzyme in a futile acylation/deacylation cycle. Eventually, when denatured, the system undergoes an acyl shift to form a chemically ligated circular product of p35, although this may not be productive under native conditions, since it would no longer deliver inhibitory capacity to the exhausted inhibitor.

The data presented now beckon the question of whether this particular mechanism only functions in the background of the p35:caspase 8 complex or whether the caspases have a general ability to catalyze this particular type of reaction in p35.

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Selected Reading

Dementiev, A., Dobo, J., and Gettins, P.G. (2006). J. Biol. Chem. *281*, 3452–3457.

Green, D.R., and Evan, G.I. (2002). Cancer Cell *1*, 19–30.

Huntington, J.A., Read, R.J., and Carrell, R.W. (2000). Nature *407*, 923–926.

Lu, M., Min, T., Eliezer, D., and Wu, H. (2006). Chem. Biol. *13*, 117–122.

Lum, L., and Beachy, P.A. (2004). Science *304*, 1755–1759.

Paulus, H. (2000). Annu. Rev. Biochem. *69*, 447–496.

Stennicke, H.R., Ryan, C.A., and Salvesen, G.S. (2002). Trends Biochem. Sci. *27*, 94–101.

Xu, G., Cirilli, M., Huang, Y., Rich, R.L., Myszka, D.G., and Wu, H. (2001). Nature *410*, 494–497.

Zheng, W., Johnston, S.A., and Joshua-Tor, L. (1998). Cell *93*, 103–109.

Growth Factor Withdrawal and Apoptosis: The Middle Game

In this issue of *Molecular Cell*, Maurer et al. (2006) detail molecular events that trigger apoptosis following growth factor withdrawal, linking irreversible mitochondrial permeabilization to activation of GSK-3 and subsequent phosphorylation, ubiquitinylation, and degradation of the antiapoptotic BCL-2 family member MCL-1.

The game of chess can be divided into three different segments: the opening, the middle game, and the end game. The combinations entailed in most openings and end games are finite enough to permit their memorization, and many are sufficiently established to have earned names. In between the opening and end game, however, the variety of combinations makes impossible

any human attempt to memorize or "solve" the game of chess, and the performance of supercomputers designed specifically for the task cannot yet regularly exceed the skill of the best human players. The variety of the middle game is arguably what keeps the game of chess exciting enough to have attracted so many generations of devoted students.

If chess may be used as a metaphor for the intrinsic (or mitochondrial) pathway of apoptosis, most of the attention has heretofore been focused on the opening and the end game. We know much about what initial stimuli result in apoptosis (e.g., DNA damage, oncogene activation, and growth factor withdrawal). We also have learned much about the steps that commit the cell to death (e.g., BAX or BAK oligomerization, mitochondrial outer membrane permeabilization, and caspase activation). However, in most cases, the connection between the opening and the end game has remained obscure.

Growth factor withdrawal has long been used as a standard opening in the study of apoptosis. It has been shown by many that withdrawal of a variety of growth