Crystal structure of baculovirus P35 reveals a novel conformational change in the reactive site loop after caspase cleavage.

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Running Title: Structure of cleaved baculovirus P35
SUMMARY

Baculovirus P35 is a universal suppressor of apoptosis that stoichiometrically inhibits cellular caspases in a novel cleavage-dependant mechanism. Upon caspase cleavage at Asp87, the 10- and 25-kDa cleavage products of P35 remain tightly associated with the inhibited caspase. Mutations in the α-helix of the reactive-site loop preceding the cleavage site, abrogates caspase inhibition and anti-apoptotic activity. Substituting Pro for Val71 located in the middle of this α-helix produces a protein that is cleaved at the requisite Asp87, but does not remain bound to the caspase. This loss-of-function mutation provided the opportunity to structurally analyze the conformational changes of the P35 reactive-site loop after caspase cleavage. We report here the 2.7 Å resolution crystal structure of V71P-mutated P35 after cleavage by human caspase-3. The structure reveals a large movement in the carboxy terminal side of the reactive-site loop that swings down and forms a new β-strand that augments an existing β-sheet. Additionally, the hydrophobic amino-terminus releases and extends away from the protein core. Similar movements occur when P35 forms an inhibitory complex with human caspase-8. These findings suggest the α-helix mutation may alter the sequential steps or kinetics of the conformational changes required for inhibition, thereby causing P35 loss of function.

INTRODUCTION

Apoptosis or programmed cell death is an active process of cellular self-destruction essential in normal development, tissue homeostasis, and defense against foreign pathogens, including viruses (1,2). Disruptions in the apoptotic program are associated with diseases such as AIDS, Alzheimer’s, cancer, and those caused by viruses (3). There is a multitude of signals that can trigger apoptosis. However, all signals culminate in the activation of a well-conserved family of aspartyl-specific cysteine proteases called caspases (4,5). Caspases are expressed as inactive proenzymes that are proteolytically activated via autoactivation, transactivation, or cleavage by other caspases (6,7). The activation of caspases represents the decisive commitment to apoptotic death suggesting that these enzymes are important targets for anti-apoptotic drugs.
During viral infection, host cells often respond by undergoing apoptosis as an innate defense mechanism. Consequently, viruses have evolved diverse anti-apoptotic genes to prevent the premature cell death of host cells and thereby promote virus replication (1). Baculovirus P35 is particularly interesting because not only can P35 prevent virus-induced apoptosis in insect host cells but it can also prevent cell death in phylogenetically diverse organisms when produced ectopically (10-13). The ability of P35 to act as a general apoptotic suppressor is correlated with the stoichiometric inhibition of the cellular caspases through cleavage of P35 and formation of a stable P35-caspase complex, which precludes subsequent caspase protease activity (14-16).

Previously, we reported the crystal structure of the active uncleaved P35 at 2.2 Å resolution (17). The crystal structure provided insight into P35’s multi-step mechanism of caspase inhibition. The most remarkable feature of the P35 structure is a large loop domain (residues 60-98) that protrudes above the central β-sheet core. The loop contains the caspase recognition site, 84-DQMD-87 (P$_4$-P$_1$ residues). The apex of the reactive site loop (RSL) consists of the caspase cleavage site, Asp87-Gly88, which is solvent exposed and fully accessible to the caspase target. The loop is maintained and stabilized by the single amphipathic α-helix (α1) that traverses and interacts with the top of the central β-sheet. Distortion of the α-helix by substituting a proline residue for valine 71, or replacing hydrophobic residues of the α-helix with charged residues, caused loss of caspase inhibition (17,18). Thus a distinct conformation of the RSL as mediated through α1’s interaction with P35’s β-sheet core is necessary for the inhibition of caspases.

Caspases recognize and cleave V71P-mutated P35 at Asp87, indicating the uncleaved mutant structure is comparable to that of wild type P35. However the mutant P35 fails to form a stable caspase-P35 complex and therefore does not inhibit caspase activity or apoptosis (17). Thus, caspase inhibition by P35 involves post-cleavage events (17). To determine the molecular mechanism by which these subsequent steps inhibit caspase and to reveal insights in the post-cleavage conformational changes in the RSL, we have determined the three-dimensional crystal structure of the non-functional P35 V71P-mutant after cleavage by its cognate caspase.
Comparison of the cleaved mutant P35 with the uncleaved wild type model, revealed two large conformational changes that provide valuable clues in identifying functionally important P35 domains. These changes also revealed a novel mechanism for cleavage-dependent protease inhibition.

Recently, the crystal structure of P35 complexed with human caspase-8 has been determined (19). This structure reveals the presence of a thioester covalent adduct between the requisite Asp87 of P35 and the caspase active site cysteine. Additionally, the amino-terminus of P35 swings out and interacts with the caspase active site. Surprisingly, the structure of V71P loss-of-function P35 mutant reported here is strikingly similar that seen in wild type protein when complexed with caspase. Our findings suggest that the V71P mutant may alter the kinetics or sequential order of movements in P35 needed for caspase inhibition.

**EXPERIMENTAL PROCEDURES**

*Protein expression and purification*—Baculovirus P35 V71P mutant was cloned into an *E. coli* strain BL21(DE3) using the pET 22(+)-b vector expression system (Novagen, Madison, WI) resulting in expression of protein with C-terminal His-tagged extensions as described previously (16). Proteins were purified by nickel (Ni\(^{2+}\)) affinity chromatography and further purified by ion-exchange chromatography. Cleaved V71P-mutated P35 was obtained by mixing the V71P P35-His\(_6\) (1 mg ml\(^{-1}\)) with purified caspase-3-His\(_6\) (0.1 mg ml\(^{-1}\)) in reaction buffer (100 mM HEPES pH 7.5, 0.1\% CHAPS, 10\% sucrose, 1 mM EDTA, 10 mM DTT) (20). The reaction mixture was incubated for 2 days at room temperature. After incubation, the reaction mixture was dialyzed in 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 20 mM HEPES (pH 7.0) and the caspase-cleaved V71P P35 mutant was isolated using ion-exchange chromatography, co-eluting as cleaved but still associated 25 kD and 10 kD subunits. The protein was dialyzed in 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM NaN\(_3\), 20 mM HEPES (pH 7.0) and then concentrated to 5-8 mg ml\(^{-1}\) prior to crystallization trials.

*Crystallization and data collection*—Caspase-3-cleaved V71P P35 mutant was crystallized using hanging-drop vapor diffusion at 25°C in 100-400 mM sodium chloride, 9-
13% polyethylene glycol (Mr 20,000), buffered at pH 6.0 with 100 mM MES. Crystals of the trigonal space group P3₂2₁ appeared in 2-3 days and grew to the size of 200 µm x 200 µm x 50 µm. The unit cell parameters are: \( a = b = 75.81 \, \text{Å}, c = 120.69 \, \text{Å} \). There is one molecule per asymmetric unit (ASU) giving rise to a solvent content of 62.1% (\( V_M \) is 3.27 Å³/Da) (21). These crystals diffract to 4.0 Å at home source and 3.1 Å at the synchrotron source.

Addition of 1-8% 2-methyl-2,4-pentanediol to the above conditions induced the protein to crystallize in the orthorhombic space group P₂₁₂₁₂₁ with unit cell parameters \( a = 77.6 \, \text{Å}, b = 89.3 \, \text{Å}, c = 135.1 \, \text{Å} \). These crystals appeared as thin needles in 3-5 days and diffracted to 2.7 Å resolution at SSRL. The \( V_M \) coefficient was calculated to be 2.25 Å³/Da (44.8% solvent) assuming three molecules per ASU (21).

Crystals used for data collection were cryo-protected using 30% ethylene glycol or 30% 2-methyl-2,4-pentanediol in 11% polyethylene glycol (Mr 20,000), 100 mM sodium chloride, 100 mM MES, pH 6.0. The crystal was immediately mounted in a loop and frozen in a nitrogen stream at -170ºC. Data were collected on the MAR345 image plate system at the Stanford Synchrotron Radiation Laboratory. The 3.1 Å resolution data set from the trigonal crystal was collected on Beamline 9-1 while the 2.7 Å resolution data set from the orthorhombic crystal was collected on Beamline 7-1 at SSRL. Both data sets were processed with DENZO and SCALEPACK (22). Table I lists data collection and processing statistics.

*Phase determination*—The structure of the caspase-3-cleaved V71P P35 mutant from the trigonal P3₂2₁ crystal was determined by molecular replacement method with the CCP4 program AMoRe (23). The wild type P35 structure, was used as a search model (17). Data between 10-4 Å resolution were used in the rotation search yielding two equivalent peaks that corresponded to the two possible choices of origin in the P3₂2₁ space group. The rotation solutions were then applied in a translation search, resulting in a distinct solution with an \( R \)-factor and correlation coefficient of 48.6% and 0.35, respectively. After least squares fast-rigid-body refinement (AMoRe) of the wild type P35 model using data between 20 and 4.0 Å resolution, the \( R \)-factor and correlation coefficient improved slightly to 47.81% and 0.441, respectively.
Model building and refinement—The initial electron density map obtained from molecular replacement solution of the trigonal \(P_321\) crystal data set was readily interpretable. However, residues corresponding to the reactive site loop (62-97) were disordered and removed prior to the first cycle of CNS refinement (24). Model building was carried out using the molecular graphics program O (25). After the first round of least squares refinement with CNS, the conventional \(R\)-factor dropped to 37.52% and the \(R_{\text{free}}\) was 41.91% (5% of data set) for the recorded data between 30 and 3.1 Å resolution (26). The new density map allowed tracing of additional residues in the reactive site loop. After iteratively subjecting the model to several rounds of simulated annealing and group temperature factor refinement with CNS and manual re-building of the electron density map, the final conventional \(R\)-factor is 24.0% and \(R_{\text{free}}\) value is 29.5% for 95 and 5% of all recorded data, respectively. The structure is characterized by a high overall B factor (\(B_{\text{ave}}=75.6\) Å\(^2\)), consistent with the Wilson plot statistics that predict an overall B factor of \(\sim 70\) Å\(^2\). The high temperature factor is attributed to the limited crystal contacts that the trigonal monomer makes with monomers in the adjacent asymmetric units. Despite the high B factor, none of the 274 residues modeled plot in the disallowed region of the Ramachandran plot, and 98% of the residues lie in the most favored or additionally allowed regions as defined by the program PROCHECK (27).

The 3.1 Å resolution structure from the trigonal \(P_321\) crystal was used as a search model for the molecular replacement solution of the 2.7 Å resolution data set from the orthorhombic \(P2_12_12_1\) crystal (see above). The rotation search using data between 20 and 4 Å resolution did not yield any one peak that was significantly larger than the rest of the solutions. The top 20 rotation solutions were applied in the translation search for the first molecule, resulting in a distinct solution with a marginal \(R\)-factor and correlation coefficient of 52.2% and 0.241, respectively. The translation search for the second molecule in the asymmetric unit, yielded a distinct but poor solution with an \(R\) factor and correlation coefficient of 53.6% and 0.309, respectively. The translation search for the third molecule was performed and yielded a solution with an \(R\)-factor and correlation coefficient of 38.4% and 0.626, respectively. After the first round of refinement with CNS of the three molecules, the \(R\)-factor and \(R_{\text{free}}\) were 26.64% and 31.79%, respectively.
The model was subjected in several iterative rounds of simulated annealing refinement with CNS (24), applying non-crystallographic (NCS) restraints between the three subunits in the asymmetric unit (which was relaxed in the later stages), and manual model building with O (25). The final $R$-factor is 20.1% and $R_{\text{free}}$ is 25.7% for 95 and 5% of all recorded data, respectively. The three monomers in the asymmetric unit have a much lower overall B factor ($B_{\text{ave}}=36 \text{ Å}^2$) compared to the trigonal model. This is attributed to the tighter crystal packing observed in the orthorhombic model. All of the 882 residues plot in the most favored or additionally allowed regions of the Ramachandran plot as defined by the program PROCHECK (27).

**RESULTS**

The caspase-cleaved P35 V71P mutant crystallized in two different space groups: P3221 and P212121, which diffract x-rays to 3.2Å and 2.7Å resolution respectively. The structure solutions were determined by molecular replacement methods using the uncleaved wild-type P35 as search model. The solution of the trigonal crystal form consists of one molecule in the crystallographic asymmetric unit while the orthorhombic solution consists of three subunits in the asymmetric unit. The main core of the wild type P35 structure: the eight-stranded β-sheet, the α-helix that traverses the β-sheet, the cellulose binding domain (CBD)-like region, and the large hairpin loop and the helix turn helix region are conserved in the cleaved V71P mutant (Figure 1). The main-chain rms deviation between these regions in the wild type P35, minus the RSL (residues 87 to 101), and the corresponding regions of the cleaved V71P is 2.226 Å.

The first striking feature of the caspase-cleaved V71P mutant structure is observed in the C-terminal half of the reactive site loop (residues 88-98). These residues fold back and rearrange to form a new β-strand, βE’ (Figures 1 and 2). The new strand, βE’, augments the outside of a β-sheet formed by strands βD, βE, and βF. This conformational change is observed in both trigonal and orthorhombic crystal forms. The main-chain of βE’ hydrogen-bonds with βE main-chain atoms, while its side-chains interact with side-chains of α2 and main-chain and side-chain atoms of βD’. Figure 2 shows a representative 2Fo-Fc electron density map revealing the new strand βE’. The first residue observed in the electron density map for the C-
terminal half of the reactive site loop in the cleaved structure is Ser92 whereas residues 88-91 are disordered.

The swinging down of the C-terminal half of the reactive site loop results in a major relocation of its residues (Figure 1). One interesting rearrangement involves the hydrophobic residues Ile93, Tyr95, and Phe96. In the wild type structure these residues, Ile93, Tyr95, and Phe96 are completely solvent exposed in the C-terminal part of the RSL (Figure 3A). After cleavage by caspase and the rearrangement of the C-terminal half of the RSL in the V71P mutant, these residues become partially buried in a hydrophobic patch (Figure 3B). Ile93 and Tyr95 reside on the same side of the new βE and pack against Ile118, while Phe96, on the opposite side of βE, packs in the hydrophobic patch outlined by Ile53, Val56, and V103.

In contrast to the large movements observed in the C-terminal portion of the RSL, the region immediately preceding the scissile bond, Lys80-Asp87, moves very little. The hydrophobic interactions of Tyr82 with side-chains Tyr260 and Trp262 (in βL) seen in the wild type structure are conserved in the cleaved model. Of the caspase recognition sequence, 84-DQMD↓G-88, electron density unambiguously defines main chain and side chain atoms up to Gln85, while only main chain atoms of Met86 and Asp87 are observed. Asp87 shifts toward the βL-βK hairpin loop region (residues252-257) which itself shifts away from the RSL (Figure 1).

The second remarkable difference observed between the wild type structure and the cleaved V71P mutant structure is located at the protein’s amino-terminus. The short N-terminal strand βA (residues 2 to 5), which is buried in the hydrophobic core of the wild type structure, releases and is fully solvent exposed in the cleaved structure (Figure 1). In the trigonal crystal form, residues 1 to 5 are disordered while the main-chain atoms of residues 6 to 10 are observed to extend out and interact with a symmetry-related molecule, while in the orthorhombic crystal form residues 1 to 11 are disordered. The swinging out of the amino-terminus creates a cavity in the cleaved model where βA was inserted. In the orthorhombic crystal form, water molecules and a dithiothreitol (DTT) molecule, which covalently modifies Cys137, occupy this cavity. DTT is used in the reaction buffer (10 mM) and in the storage buffer (1mM).

Most of the more conservative changes seen between the cleaved structure and the wild
type structure are localized in loops of the N-terminal CBD-like region. Residues 97 to 101 in the C-terminal portion of the reactive site loop hydrogen-bonds with residues 163 to 166 in the loop between strands βF and βG. These residues in turn interact with residues 35 to 44 in the βC-βD loop, which in turn interacts with residues 8 to 12 in the βA-βB loop. Thus, the C-terminal segment of the reactive site loop acts as a restraint on these loops keeping them in a relatively fixed orientation. The cleavage of the reactive loop and its subsequent rearrangement removes the restraint and network of interactions in the wild-type structure that holds these loops in place. As a result the loops become more flexible and large movements are observed (Figure 4).

The cα of Gly162 in the βF-βG loop shifts 10Å past the vacated RSL restraint (Figure 4). The displacement in the βF-βG loop pulls the βC-βD loop which in turn pulls the βA-βB loop. The conformational change in the βA-βB loop destabilizes the amino-terminus (strand βA) causing it to disengage and swing up and away and become solvent-exposed in the cleaved structure.

The amphipathic helix α1, which initiates the reactive site loop (residues 60-98) in the wild type structure, is slightly kinked in the cleaved structure as a direct result of the Pro mutation at Val71. The bend in the helix shifts the residues before Pro71 (54 to 70) with only minimal movement in the main-chain of residues after Pro71 (71 to 84) (Figures 1 and 5). This distortion in helix α1 is stabilized by a new salt-bridge between Asp69 and Lys73 within the helix. Despite the distortion in helix α1, the interactions between the hydrophobic residues of helix α1 and the complementary hydrophobic residues of the β-sheet main core are still intact. In addition to the bending of helix α1, an additional turn is observed at the N-terminal portion of helix α1. This could account for the loop between strand βD and helix α1 in the wild type structure to pack closer to strand βG and form a new β-strand, βD’ (residues 55-58) (Figure 3). Thus, distortion of α1 may have caused a destabilization in the conformation of the reactive site loop, which could explain why the Val71 to Pro substitution fails to stably interact with caspase after cleavage. In fact, disruption in the conformation of helix α1 by Ala-Ser insertional mutagenesis at residue 74 or alteration of the size of the RSL by Ala-Ser insertion at residue 83 also results in a loss of anti-apoptotic activity (16).

Lastly, another disparity of note between the wild type and mutant structures is observed
in the side-chain of Tyr101. In the wild type structure, Tyr101 points toward the core of the protein and is buried in the hydrophobic pocket defined by Leu155, Leu167, Cys153, and Met45, and its phenolic oxygen hydrogen-bonds with the side-chain of Asn32. In the cleaved mutant structure, Tyr101 swings out 180° and points towards the surface of the protein with its phenolic oxygen exposed to the solvent (Figure 3). This results in a movement of 12.6 Å in the phenolic oxygen. This large movement is directly due to the movement on the C-terminal portion of the RSL.

Recently the crystal structure was reported of wild type P35 complexed with human caspase-8 (19). The structure reveals that P35 is trapped as a thioester covalent adduct between Asp87 (P1) of P35 and the nucleophile cysteine residue (Cys360) from caspase. During normal substrate cleavage, a water molecule would complete the hydrolysis of the thioester intermediate. However, the amino-terminus of wild type P35 is also released after cleavage and interacts with the active site histidine residue preventing access or activation of a water molecule (19). Interestingly, all conformational changes seen in P35 after cleavage by caspase-8 and subsequent formation of a covalent inhibitory complex are also observed in the caspase-3-cleaved V71P P35 mutant. The rms deviation between the 284 equivalent α-carbons of the cleaved P35 in the complex structure and the caspase-cleaved mutant is only 1.58 Å (Figure 5). The largest differences are observed in the disposition of the amino-terminus (residues 6 to 12) and the caspase-recognition sequence (residues 84 to 87). These differences are due to crystal contacts in the uncomplexed structure, yet the gross overall movements are similar. Otherwise, the two structures are virtually identical. One intriguing similarity observed in both structures is the kink in helix α1. The distortion in helix α1 in the cleaved mutant P35 is directly attributable to the valine to proline substitution at its central residue 71, yet a similar distortion is seen in the wild-type helix after cleavage with caspase (Figure 5). Similar dispositions are seen in the βC-βD and βF-βG loops in both P35 crystal structures which is likely the result of the movement of the reactive-site loop restraint described above. Additionally, the large movement seen in the Tyr101 side chain is also observed in both wild type and mutant structures.
DISCUSSION

The stoichiometric inhibition of caspases by P35 is a multi-step process that involves cleavage of the reactive-site loop and a subsequent formation of a very stable complex (15-19). The structure of wild type P35 shows a solvent-exposed reactive-site loop that projects from the main core of the protein. At the apex of the loop is the caspase recognition site 84-DQMD-87 and thus is easily accessible to the target caspase (17). While cleavage at Asp87 (P1) is required for anti-apoptotic activity, it is not sufficient for stable association and inhibition of caspases. Thus, a post-cleavage conformational change occurs to stabilize the interaction of P35 with caspases and accounts for its high affinity binding. The three-dimensional structure of a caspase-cleaved P35 V71P mutant reveals two significant conformational changes. The C-terminal segment of the reactive-site loop folds back down to the side of the protein and forms a new β-strand (Figure 1). This conformational change is characterized by the stabilization of hydrophobic residues (Ile93, Tyr95, and Phe96) that are solvent-exposed in the wild type pre-cleaved structure (Figure 3). The post-cleavage environment for these hydrophobic residues is provided by the regions that already exist in the pre-cleaved form (i.e. hydrophobic pocket for Phe96) or by conformational changes involving other regions in the protein (i.e. shifts α1-loop-α2 domain). This RSL movement indirectly causes the second large conformational change; P35’s amino-terminus releases from the protein core.

Similar conformational changes are also observed in wild-type P35 after cleavage and subsequent formation of the inhibitory complex with caspase-8 (19). The kink in helix α1 as well as the considerable movements seen in the carboxy terminal portion of the reactive-site loop and the release of the amino-terminus are observed in both cleaved wild type P35 (complexed with caspase-8) and V71P mutated P35 crystal structures. Additionally, the rearrangements in the βC-βD and βF-βG loops are also observed in both P35 crystal structures. Yet, given the similar post-cleavage conformational changes seen in wild-type and loss-of-function mutant, the P35 V71P mutant does not form an inhibitory complex with caspase (17). This suggests the possibility that the premature distortion of helix α1 prior to cleavage, by exchanging Val71 with Pro, may alter the sequential steps or kinetics of protein movements after
caspase-cleavage that are required for caspase inhibition. In the P35-caspase-8 crystal structure, the amino terminus releases and interacts with the active site, possibly blocking the thioester hydrolysis through solvent exclusion, or reorientation of active-site residues. The kinetics of the amino-terminal release may be detained in the V71P mutant thus allowing a water molecule to finish hydrolysis of the thioester between Asp87 and the active-site cysteine thereby allowing the V71P mutant to disengage from the enzyme. This would allow for caspase turnover and explain the loss of caspase inhibition by V71P mutated P35. Therefore, our findings propose that the kinetics or order of P35’s multi-step mechanism is essential for caspase inhibition, which needs further examination.

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FIGURE CAPTIONS

**Figure 1.** Structural comparison of V71P caspase-cleaved structure (right) to P35 wild-type pre-cleaved structure (left). The position of the mutated residue, 71, is labeled in the middle of helix α1. The regions that exhibit large conformational changes between the two structures are highlighted in yellow. The long arrow represents the conformational change that occurs after cleavage of the V71P P35 mutant by caspase. The amino terminus, which is disordered in the orthorhombic crystal form, is extended and interacts with a symmetry-related molecule in the trigonal crystal form. Very little deviations are seen in the P35 β-sheet core. All protein structure figures were generated using the program Bobscript (28) or Molscript (29) and rendered with Raster3D (30).

**Figure 2.** Stereo representative section of the final electron density map (contoured at 1σ) calculated to 2.7 Å resolution with coefficients of 2|Fo| - |Fc| and phases computed from the final refined model. The protein region corresponds the strands βE and the newly formed βE after the caspase-cleaved induced conformational change. The density is clearly defined for both main-chain and side-chain residues of βE.

**Figure 3.** Stereo view centered on the C-terminal region of the reactive site loop (red) of both the (A) wild-type P35 structure and (B) the V71P caspase cleaved structure. The hydrophobic residues Ile93, Tyr95, and Phe96, which are solvent-exposed in the wild-type structure (A), become partially buried in the cleaved mutant structure (B). Additionally, Tyr101, which is completely buried in the wild-type pre-cleaved structure, flips out 180° in the cleaved V71P mutant structure. Also, shown are the new interactions that are formed between residues Arg58, Lys94, Asp98, and His100 between the two structures.

**Figure 4.** Stereo superposition between the P35 wild-type (green) and V71P caspase-cleaved mutant (red) revealing the conformational changes observed in the βA-βB, βC-βD, and βF-βG
loops. View is looking from the top of P35, approximately rotated 90° about the horizontal toward the viewer from figure 2B. The disposition of the loops are highlighted in dark green (wild-type) and red (V71P) colors. The C-terminal portion of the reactive site loop interacts with, and fixes the βF-βG loop in the pre-cleaved wild-type structure. In the V71P mutant, after cleavage, the C-terminal portion of the reactive site loop relocates and no longer restrains the βF-βG loop, which pulls the βC-βD loop with it, which in turn pulls the βA-βB loop, causing the N-terminus to unpack from the proteins core. Part of the βC-βD loop undergoes dramatic rearrangement and forms one turn of a $3_{10}$ helix.

**Figure 5.** Stereo superposition between the wild type uncleaved P35 (black), V71P caspase-cleaved mutant (magenta) and cleaved wild type P35 (cyan) as complexed with caspase-8 (PDB coordinates 1I4E). (A) Front view and (B) Top view (90° rotation from (A)). The superposition reveals that the post-cleavage conformational changes observed in both cleaved forms of P35 are virtually identical (rms deviation is 1.58 Å). A bend in helix α1 (residues 60-79) is observed in both non-functional V71P mutant (magenta) and wild type (cyan) cleaved structures. Additionally, the dispositions of the βC-βD loop (34-42) and βF-βG (156-165) loop are very similar in both cleaved structures, but differ greatly to uncleaved wild type P35 (black).
### Table 1. Data Collection and Refinement Statistics

|                  | Space Group | X-ray source | Wavelength (Å) | Resolution (Å) | Measurements | Independent reflections | Completeness (%) | Rsym\(^*\) (%) | R-factor\(^\ddagger\) (%) | R-free\(^\ddagger\) (%) |
|------------------|-------------|--------------|----------------|----------------|--------------|-------------------------|-----------------|----------------|--------------------------|--------------------------|
|                  | P3\(_2\)21  | SSRL 9-1     | 0.98           | 3.1            | 80,534       | 7,577                   | 99.0            | 5.4            | 23.9 (31.4)              | 29.1 (38.4)              |
|                  | P2\(_1\)2\(_1\) | SSRL 7-1     | 1.08           | 2.7            | 183,500      | 25,271                  | 94.9            | 7.0            | 20.1 (23.8)              | 25.7 (30.1)              |

**Refinement Statistics**

|                  |                  |               |               |               |               |                       |                 |                |                          |                          |
|------------------|------------------|---------------|---------------|---------------|---------------|-----------------------|-----------------|----------------|-------------------------|-------------------------|
|                  | Resolution (Å)   | 15.0 - 3.1    | 30.0 - 2.7    |               |               |                       |                 |                |                          |                          |
|                  | Number of reflections \(|F| > 0\) | 7,530         | 25,271        |               |               |                       |                 |                |                          |                          |
|                  | R-factor\(^\ddagger\) (%) | 23.9 (31.4)   | 20.1 (23.8)   |               |               |                       |                 |                |                          |                          |
|                  | R-free\(^\ddagger\) (%)   | 29.1 (38.4)   | 25.7 (30.1)   |               |               |                       |                 |                |                          |                          |

**Root mean square deviation from ideal geometry**

|                  | Bond distances (Å) | Bond angles (°) | Average B factor of all atoms (Å\(^2\)) |
|------------------|--------------------|-----------------|------------------------------------------|
|                  | 0.019              | 2.1             | 75.4                                     |
|                  | 0.013              | 1.7             | 35.2                                     |

\(^*\)Rsym = \[
\frac{\sum_h \sum_i |I_h - \bar{I}_h|}{\sum_h \sum_i |I_{hi}|} \times 100 \]

where \(\bar{I}_h\) the mean of the \(I_{hi}\) observations of reflection h.

\(^\ddagger\)R-factor and R-free = \[
\frac{\sum |F_{obs}| - |F_{calc}|}{\sum |F_{obs}|} \times 100 \]

for 95% of recorded data (R-factor) or 5% data (R-free).
Crystal structure of baculovirus P35 reveals a novel conformational change in the reactive site loop after caspase cleavage
Wilfred P. dela Cruz, Paul D. Friesen and Andrew J. Fisher

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