Structural Insights into Programmed Cell Death

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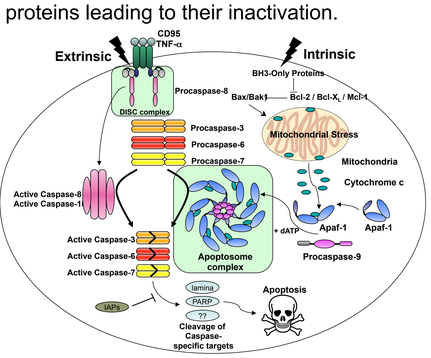
Abstract

Caspases are the protein hitmen of the cell, cutting key protein substrates and causing a controlled and coordinated cell death. Their regulation is critical for proper development, inflammation responses, and differentiation. Malfunctions in caspase activity and the apoptotic pathway play a role in cancer and neurodegenerative diseases. This study dives into the regulation of a particular executioner caspase, caspase-7, and how phosphorylation of this protein regulates its function. By taking an interdisciplinary approach utilizing molecular biology, protein biochemistry, enzymology, and crystallography we have shown that an engineered caspase-7 phosphorylation mimic inactivates the protein by sterically preventing substrate binding.

Background

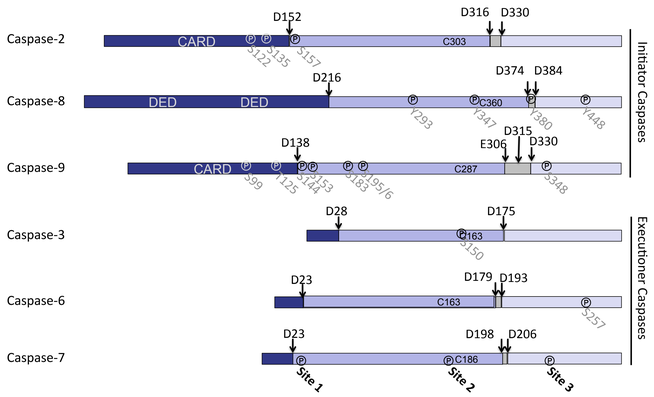
Apoptosis, or programmed cell death, is a critical cellular function for all multicellular organisms from the early stages of development until death. A wide variety of diseases have been linked to this apoptotic pathway including cancer, Alzheimer's, Huntington's, and autoimmune disease. The key players in this cascade are caspases, a class of cysteine proteases, proteins that cut other proteins leading to their inactivation.



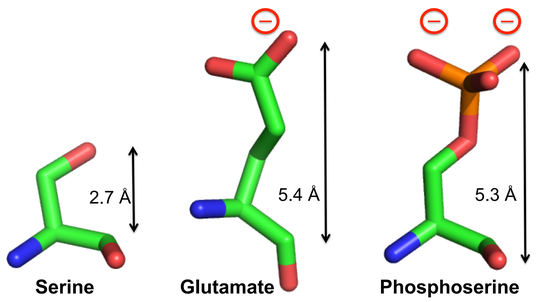
Regulation of the apoptotic caspases is pivotal in order to ensure that this cell death pathway is activated only when essential. Caspases are also important drug targets for several diseases, so there is additional need to probe caspase regulation.

Caspase Regulation by Phosphorylation

Phosphorylation, the enzymatic addition of phosphate to proteins, is extensively used throughout biology to regulate protein function. All apoptotic caspases are extensively phosphorylated. The map below shows the known sites of phosphorylation. We aim to understand how phosphorylation affects function of caspases at a molecular level.

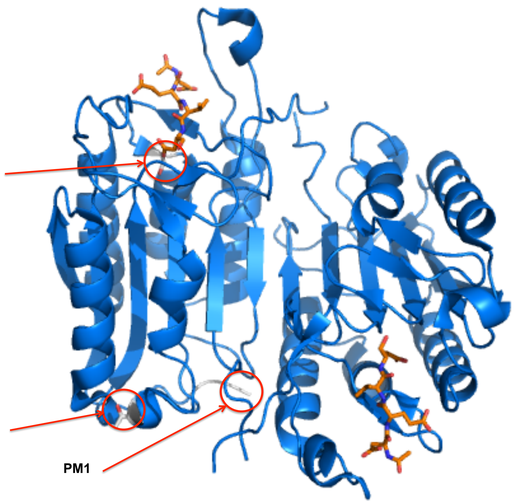


Glutamate as a Phosphomimic



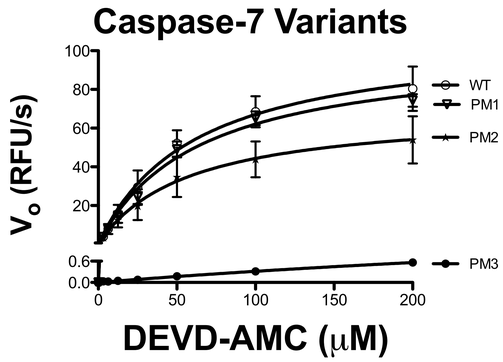
Adding an extra phosphate to a protein residue either blocks certain protein conformations through a steric clash or alters charged interactions. Both can affect protein function. To simulate these effects we can substitute the amino acid on the protein to mimic the addition of a phosphate. For example, serine amino acids are often converted by kinases to a phosphoserine. We can use molecular biology and protein biochemistry to insert a glutamate residue. This glutamate acts as an excellent phosphomimic by imitating the size of a phosphoserine as well as introducing a negative charge to the modified site.

Phosphorylation Sites: PM1, PM2, and PM3



In cancer cells caspase-7 is phosphorylated by p21 activated kinase 2 (PAK2) at three sites. To examine the structural effects of this phosphorylation we constructed three phosphomimic (PM) versions of caspase-7.

Site 3 controls function

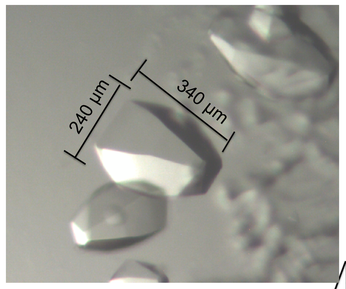


	Catalytic Efficiency (Normalized)
Wild Type	10
PM1	7.2
PM2	7.0
PM3	0.010

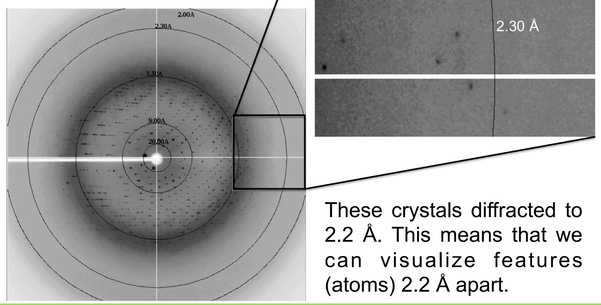
The phosphomimic at site 3 (PM3) decreases the catalytic efficiency by four orders of magnitude showing that is the critical site for function.

X-ray crystallography provides a molecular snapshot of PM3

To elucidate the mechanism of inhibition at site 3, we determined a three-dimensional x-ray crystal structure of PM3. We grew crystals of PM3 and performed an x-ray diffraction experiment at the NSLS synchrotron light source.

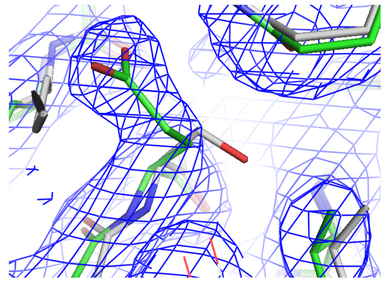


Although the crystals are micron sized, the repeating arrangement of PM3 molecules allows constructive diffraction of the x-rays. The diffracted x-rays (spots on the image below) provides detailed information about the atomic positions in PM3.

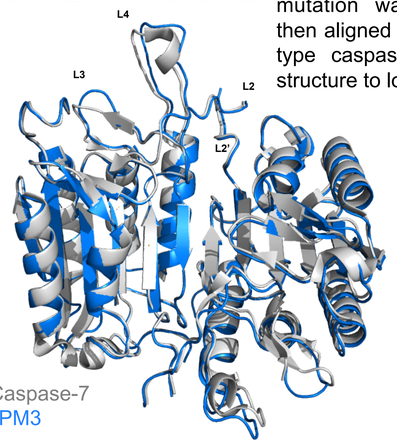


These crystals diffracted to 2.2 Å. This means that we can visualize features (atoms) 2.2 Å apart.

X-ray Crystallography Allows Visualization of Atomic Details

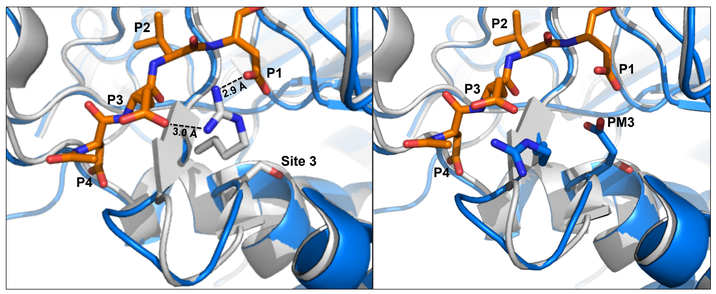


After obtaining the diffraction images, we were able to calculate maps of atomic density, shown here as a blue cage. This enabled us to build a structure of the protein. Examination of the phosphomimic at site 3 showed clear density for a glutamate residue (green/red sticks) and not a serine (white/red sticks), indicating the phosphomimetic mutation was successful. We then aligned the structure of wild type caspase-7 with our PM3 structure to look for differences.



Wild Type Caspase-7
Caspase-7 PM3

The wild type caspase-7 (gray) binding a peptide substrate (orange) was aligned with PM3 (blue). The left panel (below) indicates that an arginine makes several critical bonds with the substrate, anchoring it in the substrate-binding groove. The phosphomimic PM3 (blue) disrupts the necessary geometry to bind substrate. In addition, the glutamate introduces a negative charge into the P1 pocket that clashes with the aspartic acid.



Conclusions

Our interdisciplinary approach to this has allowed us to uncover the molecular mechanism by which nature turns off the function of caspase-7, preventing it from promoting cell death. By phosphorylating the PM3 site, a critical arginine residue is displaced. Thus, caspase-7 can not bind substrate and is not active. This understanding paves the way for new caspase-7-directed drugs that utilize the same mechanism.

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