Global Mapping of the Topography and Magnitude of Proteolytic Events in Biological Systems

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Introduction
Proteases play central roles in numerous biological and pathological processes. Their importance is underscored by the fact that roughly 2% of the genes in the human genome encode proteases. Despite their essential functions, even the most well-studied proteinase pathways remain only partially understood, and a large portion of human proteases are uncharacterized with regard to endogenous substrates and biological function.

Identification of Known Markers of Apoptosis with PROTOMAP
Apoptosis was induced by incubation with the pan-kinase inhibitor staurosporine (STS) for 4 hours. These methods have all been applied, with varying degrees of success, to identify protease substrates in numerous biological contexts. While protease substrates can, indeed, be identified using these methods, they all suffer from serious drawbacks: iterative display techniques such as DNA- or RNA-display rely on overexpression of substrates at superphysiological concentrations. 2D-GE is conceptually straightforward, but is severely limited by sensitivity and reproducibility. Furthermore, substrates identified by iterative display or 2D-GE require laborious follow-up experiments involving multiplexing and immuno-detecting to identify the topography and sites of proteolytic cleavage. N-terminal labeling methods suffer from expression of substrates at subphysiological concentrations. 1D-GE is conceptually straightforward, but is severely limited by sensitivity and reproducibility. Furthermore, substrates identified by iterative display or 2D-GE require laborious follow-up experiments involving multiplexing and immuno-detecting to identify the topography and sites of proteolytic cleavage. N-terminal labeling methods suffer from expression of substrates at subphysiological concentrations.

Here, we present a novel proteomic method for the global identification and description of proteolytic events in native biological settings. Tabled PROTOMAP, for PROtein TOpography and Magnitude Analysis Platform, this system uses Competitive 1D SDS-PAGE fractionation followed by 1D LC-MS/MS to identify protease activities in total cell lysates. Peptides from each protein are assembled into "peptographs" which enable relative visualization of the relative abundance and magnitude of cleavage events.

Experimental Design
PROTOMAP identifies 170 proteins that were not previously reported to be cleaved. Many previously-reported proteases were highly abundant, suggesting the previous approaches were limited in sensitivity.

Identification of Erlative Sites of Caspase Cleavage
In this example, a peptide was detected that spans the procaspase-3 cleavage site (glycine 175). Furthermore, spectra matching the cleavage site were detected.

Many Novel Cleavage Events were Discovered using PROTOMAP
Two examples: JUULB and MAP2K2 were not previously reported to be cleaved during apoptosis.

PROTOMAP Enables Estimation of the Magnitude of Cleavage
Based on the quantity of parental species remaining at the 4 hr time-point, proteins were classified into general categories for their rates of degradation: rapid (>50% degraded) and slow (<50% degraded).

The complete apoptosis dataset, as well as software and documentation for running PROTOMAP are available at: http://www.scripps.edu/chemphys/cravatt/protomap.

Conclusions
PROTOMAP is a novel 1D-GE/1D-MS/MS-based method for the detection of proteolysis on a protein-wide scale. Proteins are cell-fractionated by 1D-GE and then analyzed by 1D-MS/MS using total cell lysates. Peptides from each protein are assembled into "peptographs" which enable relative visualization of the relative abundance and magnitude of cleavage events.

PROTOMAP uses a globally-analyzed cleavage pattern associated with the various apoptotic pathways in Jurkat T-cells. This approach led to identification of many proteins not previously reported as being cleaved. Furthermore, in nearly every case, detailed information about the topography of cleavage was generated, often yielding explicit or implicit sites-of-cleavage. Our data revealed that the vast majority of proteins cleaved during apoptosis generate persistent fragments that often correlate with functional domains. Furthermore, a time-course analysis demonstrated that the majority of cleavage events occur in the early stages of apoptosis, prior to degradation of the parent protein. Collectively, these data indicate that generation of persistent fragments, corresponding to disease-protein domains, may be much more common during apoptosis than originally appreciated, and that generation of active effectors may be a principal function of apoptotic cascades.

In summary, PROTOMAP has several advantages over other methods currently in use, namely:
- Exquisitely sensitive
- Magnitude of cleavage and abundance of parent protein can both be estimated
- Detailed topography of cleavage event is generated, often yielding explicit or implicit sites-of-cleavage

We believe that PROTOMAP will serve as a powerful platform which can be applied to map the substrate profiles of any protease directly within the confines of the environment in which the enzyme is naturally expressed.