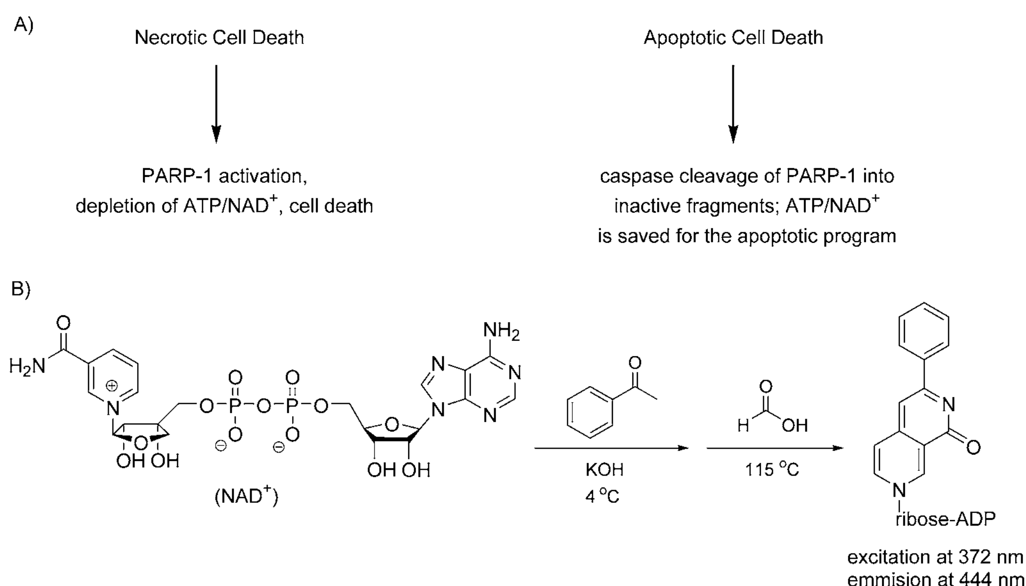
Direct Quantitation of Poly(ADP-Ribose) Polymerase (PARP) Activity as a Means to Distinguish Necrotic and Apoptotic Death in Cell and Tissue Samples

Karson S. Putt,[a] Gregory J. Beilman,[b] and Paul J. Hergenrother*[a, c]

The ability to regulate cell death is essential to the life of a multicellular organism, and two extremes on the cell-death spectrum are apoptosis and necrosis. Apoptosis is an energy-dependent cell-death program that enables the elimination of unwanted cells at distinct times and through defined mechanisms.[1–3] Because apoptosis occurs without spillage of cellular contents into the extracellular environment, this death does not lead to inflammation. In contrast, necrosis arises from blunt traumatic injury, oxygen depravation, or other gross cellular insult, and the rupture of the cellular membrane during necrotic death provokes an inflammatory response.[4] Both forms of cell death are observed in diseased tissue. For instance, much of the neurodegeneration observed in Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS) probably arises from premature apoptosis,[5–7] while damage seen in hemorrhagic shock and stroke patients can be due to massive necrotic death.[8] Although apoptosis and necrosis represent two fundamentally different forms of death, they can be quite difficult to distinguish experimentally. Typically, several hallmarks must be observed before it can be conclusively stated that apoptotic death has occurred.[9] These involve time-, labor-, and cost-intensive procedures such as immunoblotting, flow cytometry, and microscopy.[9]

The enzyme poly(ADP-ribose) polymerase-1 (PARP-1)[10–12] is differentially processed in apoptosis and necrosis, and therefore its activity can potentially be used as a means of distinguishing these two forms of cell death (Scheme 1 A). In response to mild DNA damage, PARP-1 catalyzes the formation of ADP-ribose polymers (from NAD^+) onto protein acceptors; this response is part of the machinery that allows the DNA to be repaired and the cell to be saved. In contrast, during more severe DNA damage, the cell activates the apoptotic cascade, and PARP-1 is cleaved by caspases into 89 and 24 kDa subunits; this separates the DNA-binding domain from the automodification and catalytic domains, thereby inactivating the enzyme.[13, 14] This PARP-1 inactivation most likely serves to prevent futile cycles of DNA damage and repair, preserving cellular energy for apoptosis. Finally, extreme DNA damage leads to massive PARP-1 activation that in turn depletes the cell of NAD^+/ATP and leads to necrotic cell death.[15] Thus, in cells that die by apoptosis, PARP-1 is cleaved and inactivated, while during necrotic cell death PARP-1 is highly activated (Scheme 1 A). While the Western blot for cleaved PARP-1 and the immunoblot for the presence of poly(ADP-ribose) are used as biochemical hallmarks for apoptotic and necrotic death,[16]



Scheme 1. A) Opposing roles of PARP-1 in necrotic and apoptotic death. B) Chemical quantitation of NAD^+.

[a] K. S. Putt, Prof. P. J. Hergenrother
Department of Biochemistry, University of Illinois
Urbana, IL 61801 (USA)
Fax: (+1) 217-333-0363
E-mail: hergenro@uiuc.edu

[b] Dr. G. J. Beilman
Department of Surgery, University of Minnesota
Minneapolis, MN 55455 (USA)

[c] Prof. P. J. Hergenrother
Department of Chemistry, University of Illinois
Urbana, IL 61801 (USA)

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author: full experimental protocols.

 © 2005 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

respectively, such methods of analyses are neither convenient nor allow for precise kinetic measurements. Furthermore, it is impractical to use these blot techniques in a rapid diagnostic mode for assessment of cell death in tissue samples. Described herein is the application of a PARP-1 enzymatic assay to the direct quantitation of PARP activity from cellular lysates allowing for rapid and convenient detection and differentiation of apoptotic and necrotic cell death.

As a prelude to the apoptotic/necrotic evaluations, experiments were performed to test if PARP activity could be directly detected from whole cells. Thus U-937 cells were treated with either MNNG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) or H_2O_2, two DNA-damaging agents known to induce PARP activity,[17,18] for various times, and PARP activity was monitored. PARP activity was detected through a chemical quantitation of NAD^+ that has recently been utilized to assess the potency of PARP-1 inhibitors in vitro (Scheme 1 B).[19] As depicted in Figure 1, a large increase in PARP activity is readily apparent after addition of either MNNG or H_2O_2; hydrogen peroxide is known to be a stronger necrotic inducer than MNNG.[20,21] To determine if the observed NAD^+ processing could be ascribed directly to PARP activity, trials were also conducted in the presence of a known PARP-1 inhibitor. Addition of the inhibitor 4-amino-1,8-napthalimidine[22,23] completely abolished the PARP activity (Figure 1); this clearly implicates PARP in the NAD^+ hydrolysis. Although there are several PARP isozymes, PARP-1 accounts for the vast majority of cellular PARP activity.[10]

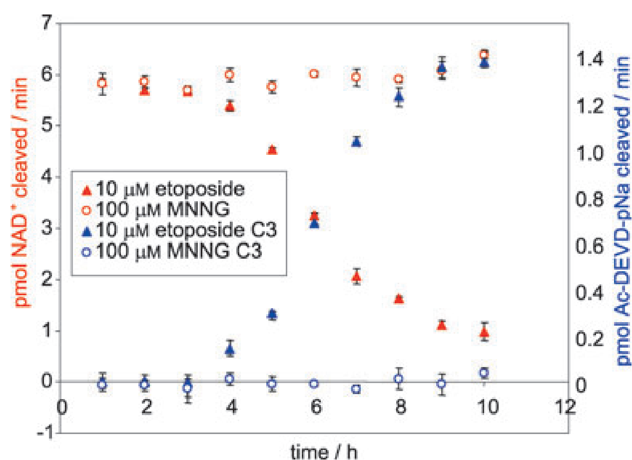


Figure 2. The PARP activity assay readily distinguishes apoptotic and necrotic cell death. Apoptotic death was induced with etoposide, and both PARP and caspase-3-like activity were monitored. As expected, a large increase in caspase-3-like activity was observed (closed blue triangles); the PARP activity assay showed a corresponding decrease in PARP activity (closed red triangles) consistent with the known cleavage and inactivation of PARP-1 in apoptosis. Induction of necrotic death with MNNG also gave high amounts of PARP activity (open red circles) and no caspase-3 activity (open blue circles). Error bars represent standard deviations from the mean.

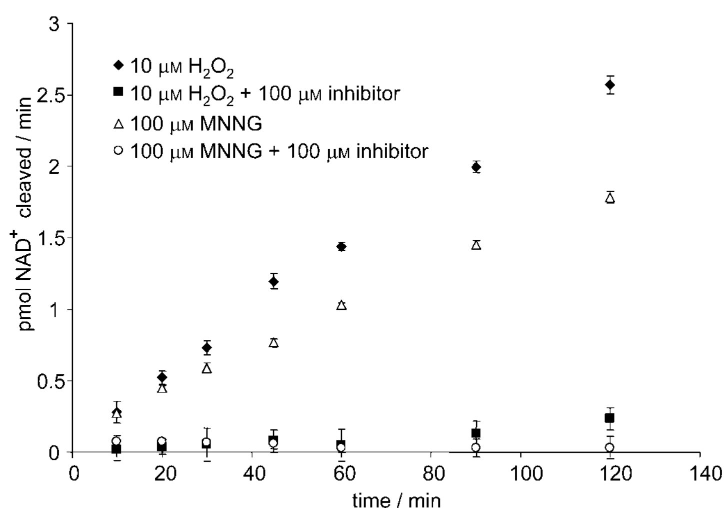


Figure 1. Direct quantitation of PARP activity. U-937 cells were treated with a DNA-damaging agent (H_2O_2 or MNNG), and PARP activity was quantitated in the presence or absence of the PARP-1 inhibitor 4-amino-1,8-napthalimidine. Error bars represent standard deviations from the mean.

To use the assay to quantitate apoptotic death, the U-937 cells were treated with etoposide (10 μM), a known apoptotic inducer. At various times cells were lysed, and any full length PARP-1 present was activated by the addition of 25 mM H_2O_2; this concentration of hydrogen peroxide does not interfere with fluorophore generation (see Supporting Information). As indicated by the data in Figure 2, upon treatment with etoposide, PARP activity drops off sharply over time; this is consistent with the known cleavage and inactivation of PARP-1 during apoptosis. This decrease is inversely correlated with the observed caspase-3 activity, which shows the expected increase in such apoptotic cells.

The assay was also useful for detecting necrotic cell death. Necrotic death was induced in U-937 cells by the addition of MNNG (100 μM), a known necrosis inducer.[16] After defined amounts of time, cells were lysed, and H_2O_2 was added to activate any full-length PARP that was present; PARP activity was then quantitated. As shown in Figure 2, upon treatment with MNNG, significant PARP activity is observed at all time points; this is indicative of full-length (and fully active) PARP-1. As expected, no caspase-3-like activity was detected in these necrotic samples. These activities correlate well with flow-cytometric analysis of cell death under the same conditions (see Supporting Information). Thus, this activity assay of PARP in whole-cell lysates is a simple, inexpensive, and convenient method for rapidly differentiating apoptotic and necrotic death. In addition to U-937 cells, the assay can also be successfully performed in HL-60 cells and SK-N-SH neuroblastoma cells, and probably many others.

As a final demonstration of this assay, PARP activity was directly detected from liver and muscle tissue of a pig that had hemorrhagic shock induced by a 35% bleed; necrotic cell death and PARP-1 activation are hallmarks of such trauma.[24,25] Baseline samples (taken before induction of shock) and samples after 90 minutes of hemorrhagic shock were analyzed. To assess PARP activity, tissue samples were homogenized, protein levels were normalized, and samples were added to the wells

 © 2005 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

of a 96-well plate. After addition of NAD^+, the plates were incubated at 37 °C for 30 minutes, at which point the PARP activity (based on the amount of NAD^+ remaining) was assessed by the fluorescence assay. As shown in Figure 3, post-shock samples from both tissue types displayed a marked increase in their PARP activity. Based on these results, it is quite likely that this direct PARP assay can be used in a diagnostic mode for the rapid and simple detection of necrotic death in animal tissue samples.

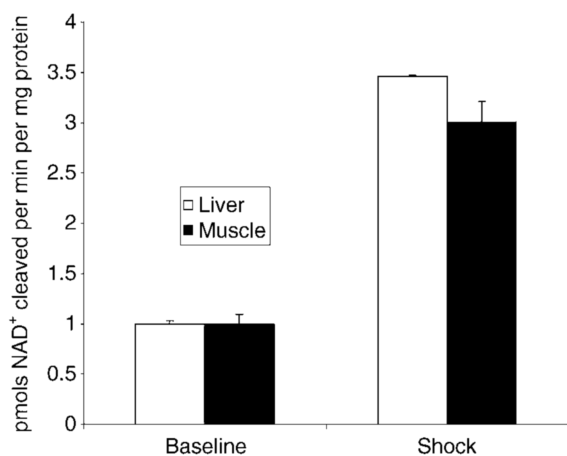


Figure 3. *Analysis of PARP activity from liver and muscle tissue of pigs before and after induction of hemorrhagic shock.*

Given the central role of apoptotic and necrotic cell death in a host of disease states, it is essential to have rapid and reliable protocols to assess cell-death pathways; current assays can be time-consuming, labor-intensive, and expensive. We have developed a rapid and simple method to distinguish apoptotic and necrotic cell death based on the direct assessment of PARP activity. This assay requires no specialized reagents and is also effective in whole animal tissue; this indicates that it might be useful as a research or clinical diagnostic tool. This direct PARP activity assay thus serves as a facile method to differentiate necrotic and apoptotic cell death.

Acknowledgements

This work was funded in part by the National Science Foundation (NSF-CAREER Award to P.J.H.) and the University of Illinois, in addition to the Office of Naval Research (Grant # N0001-14-02-1-0093 to G.J.B.).

Keywords: apoptosis · bioorganic chemistry · enzymes · necrosis · PARP-1

[1] N. B. Blatt, G. D. Glick, *Bioorg. Med. Chem.* **2001**, *9*, 1371 – 1384.
[2] M. O. Hengartner, *Nature* **2000**, *407*, 770 – 776.
[3] Z. Huang, *Chem. Biol.* **2002**, *9*, 1059 – 1072.
[4] G. Majno, I. Joris, *Am. J. Pathol.* **1995**, *146*, 3 – 15.
[5] R. M. Friedlander, *N. Engl. J. Med.* **2003**, *348*, 1365 – 1375.
[6] M. P. Mattson, *Nat. Rev. Mol. Cell Biol.* **2000**, *1*, 120 – 129.
[7] M. Vila, S. Przedborski, *Nat. Rev. Neurosci.* **2003**, *4*, 1 – 11.
[8] C. B. Cairna, *Curr. Opin. Crit. Care* **2001**, *7*, 437 – 443.
[9] A. Saraste, K. Pulkki, *Cardiovasc. Res.* **2000**, *45*, 528 – 537.
[10] S. Smith, *Trends Biochem. Sci.* **2001**, *26*, 174 – 179.
[11] A. Burkle, *ChemBioChem* **2001**, *2*, 725 – 728.
[12] W.-M. Tong, U. Cortes, Z.-Q. Wang, *Biochim. Biophys. Acta* **2001**, *1552*, 27 – 37.
[13] A. I. Scovassi, G. G. Poirier, *Mol. Cell. Biochem.* **1999**, *199*, 125 – 137.
[14] Y. A. Lazenik, S. H. Kaufmann, S. Desnoyers, G. G. Poirier, W. C. Earnshaw, *Nature* **1994**, *371*, 346 – 347.
[15] A. A. Pieper, A. Verma, J. Zhang, S. H. Snyder, *Trends Pharmacol. Sci.* **1999**, *20*, 171 – 181.
[16] E. B. Affar, M. Germain, E. Winstall, M. Vodenicharov, R. G. Shah, G. S. Salvesen, G. G. Poirier, *J. Biol. Chem.* **2001**, *276*, 2935 – 2942.
[17] K. K. Cole, J. R. Perez-Polo, *J. Neurochem.* **2002**, *82*, 19 – 29.
[18] A. Pogrebniak, I. Schemainda, P.-R. Fleischer, V. Nussler, M. Hasmann, *Eur. J. Med. Res.* **2003**, *8*, 438 – 450.
[19] K. S. Putt, P. J. Hergenrother, *Anal. Biochem.* **2004**, *326*, 78 – 86.
[20] e. B. Affar, R. G. Shar, A. K. Sallaire, V. Castonguay, G. M. Shah, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 245 – 250.
[21] T. C. Jagtap, A. Chandele, B. A. Chopde, P. Shastry, *J. Chem. Neuroanat.* **2003**, *26*, 109 – 118.
[22] G. J. Southan, C. Szabo, *Curr. Med. Chem.* **2003**, *10*, 321 – 340.
[23] G. Costantino, A. Macchiarulo, E. Camaioni, R. Pellicciari, *J. Med. Chem.* **2001**, *44*, 3786 – 3794.
[24] M. Paxian, I. Bauer, H. Rensing, H. Jaeschke, A. E. Mautes, S. A. Kolb, B. Wolf, A. Stockhausen, S. Jeblick, M. Bauer, *FASEB J.* **2003**, *17*, 993 – 1002.
[25] J. A. Watts, R. M. Grattan, B. S. Whitlow, J. A. Kline, *Am. J. Physiol. Gastrointest. Liver Physiol.* **2001**, *281*, G498 – 506.

Received: September 15, 2004
Early View Article
Published online on November 17, 2004

 © 2005 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim