Non-Isotopic Technique for the Identification of Endonucleases Involved in Apoptosis


A characteristic feature of apoptosis is the degradation of genomic DNA into discrete oligonucleosome fragments by the action of a Ca\(^{2+}\)/Mg\(^{2+}\)-dependent endonuclease. The endonuclease relevant to apoptosis has not been identified, but several possible enzymes have been described (2,4–6). One technique to identify potential nuclease activities is zymography, where protein extracts are resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in gels containing DNA (7). After electrophoresis, proteins are allowed to renature by washing the gel with buffer and then activated by addition of cations. Nuclease activity is then determined by staining the gel with ethidium bromide (EtBr) and visualizing the DNA with UV transillumination. Nuclease activity of a given molecular weight is identified as cleared areas of enzyme digestion in the gel not stained by EtBr. One potential problem with this approach is that histones bind to the DNA in the gel and produce false-positive nuclease activity by interfering with binding of EtBr to DNA (1,3). To circumvent this problem, the use of radiolabeled DNA in the gel and visualization by autoradiography has been reported (5).

Here we describe a simple non-isotopic technique where the gel is stained with silver to visualize DNA.

Protein extracts containing histones were prepared by extraction of rat liver nuclei with high salt TE buffer (1 M NaCl, 50 mM Tris-HCl, pH 7.5, 1 mM EDTA) and analyzed for nuclease activity by the zymographic method of Rosenthal and Lacks (7) using the Mini-PROTEAN\textsuperscript{®} Gel System (Bio-Rad, Hercules, CA, USA) with a 12% polyacrylamide gel containing 250 µg/mL calf thymus DNA. Bovine pancreatic DNase I was used as a standard. After separation by SDS-PAGE, gels were washed overnight in several changes of TE buffer. Gels were then soaked in TE buffer containing Ca\(^{2+}\) (2 mM) and Mg\(^{2+}\) (5 mM) for 24 h at 37°C, stained with EtBr (0.5 µg/mL TE) and photographed under UV transillumination (Figure 1A). Before silver staining, the gel was soaked in 5% SDS on a rotary shaker (for 2 h) to remove protein. The gel was then silver stained according to the Silver Stain Kit modified protocol (Bio-Rad), omitting the acetic acid in the fixation step, photographed on a light box (Figure 1B) and dried onto filter paper. The silver stains the DNA incorporated in the gel, and nuclease activities are identified as clear spots on a dark brown background.

The nuclease activity of DNase I (lane 2) and the false-positive histone staining of histone H1 (lane 1) appear as cleared areas in the gel when visualized by EtBr staining (Figure 1A). In the silver-stained gel, however (Figure 1B), DNase I nuclease activity (lane 2)
is clearly distinguished from false-positive histone activity (lane 1). A comparison of the sensitivity of EtdBr staining and silver staining is shown in Figure 2. Nuclease activity in as few as 50 pg of DNase I was visualized with either EtdBr or silver staining, but the silver-staining technique produced slightly more visible spots for 500, 100 and 50 pg of DNase I.

This silver-staining technique can be used to discriminate real nuclease activity from false-positive histones without requiring the use of isotopically labeled DNA and autoradiography. The silver-staining procedure requires only 3 h (2 h in SDS and 1 h of silver staining) and can be accelerated by performing the SDS wash at a higher temperature. Additionally, unlike isotopically labeled gels, the silver-stained gel can be dried and stored as a permanent record. This procedure should be useful for researchers attempting to study nucleases involved in apoptosis.

REFERENCES

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