Irreversible Binding of Poly(ADP)Ribose Polymerase Cleavage Product to DNA Ends Revealed by Atomic Force Microscopy: Possible Role in Apoptosis

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Abstract

During apoptosis, DNA undergoes fragmentation and caspase-3 cleaves poly(ADP-ribose) polymerase (PARP) into both a 24-kDa fragment containing the DNA binding domain and an 89-kDa fragment containing the catalytic and automodification domains. Atomic force microscopy revealed that recombinant full-length PARP bound to plasmid DNA fragments and linked them into chain-like structures. Autocatalysis of PARP in the presence of NAD⁺ resulted in its dissociation from the DNA fragments, which, nevertheless, remained physically aligned. A recombinant 28-kDa fragment of PARP containing the DNA binding domain but lacking the automodification domain irreversibly bound to and linked DNA fragments in the absence or presence of NAD⁺. Identical results were obtained on incubation of internucleosomal DNA fragments from apoptotic cells with the products of cleavage of recombinant PARP by purified caspase-3. The 24-kDa product of PARP cleavage by caspase-3 may contribute to the irreversibility of apoptosis by blocking the access of DNA repair enzymes to DNA strand breaks.

Introduction

PARP is a nuclear protein that is thought to function in the repair of DNA damage (1-10), DNA replication (11, 12), regulation of the cell cycle (13-16), and apoptosis (17-24). The zinc finger domain of the enzyme binds to DNA at sites of single- or double-strand breaks, resulting in catalytic activation (2, 25). With the use of NAD⁺ as substrate, PARP catalyzes the covalent attachment of chains of poly(ADP-ribose) to specific proteins in the vicinity of DNA strand breaks. The poly(ADP-ribose) chains are subsequently degraded with a half-life of 1 to 2 min, resulting in the release of the modified proteins from functional inhibition or activation (7-9, 26).

Mice lacking PARP as a result of gene disruption exhibit diverse phenotypes. Whereas animals of one strain show epidermal hyperplasia and obesity (27), those of another strain exhibit growth retardation, aberrant apoptosis, and increased sensitivity to DNA-damaging agents (28). HeLa cells expressing PARP antisense cDNA are defective in an early event of DNA repair (2), which results in reduced survival after exposure to DNA-damaging agents, altered chromatin structure, and an increase in gene amplification (29, 30).

PARP undergoes proteolytic cleavage early in apoptosis, before the appearance of internucleosomal DNA strand breaks (17-24). This cleavage is catalyzed by caspase-3 and generates an 89-kDa fragment containing the catalytic and automodification domains and a 24-kDa fragment containing the DBD of the protein (17, 20, 22). These fragments persist in apoptotic cells for up to several days after initiation of the death pathway (31), which suggests that they are not substrates for further proteolytic processing and that they may play a role in the death program.

We have now investigated the interaction of PARP and its proteolytic products with DNA by AFM, which reveals the topography of DNA and proteins at nanometer resolution. We have previously detected interactions between the DNA-Ku protein and DNA with the use of this technique (32).
Fig. 1. AFM images of the interaction of recombinant PARP with plasmid DNA. A, DNA fragments (~500 bp) prepared by EcoRI digestion of plasmid PUC18. One microliter (5 ng) of DNA was deposited on a freshly cleaved, atomically flat mica surface, which was then rinsed to remove the unbound DNA molecules and dried under nitrogen gas. Imaging was performed with a NanoScope IIIa instrument (Digital, Santa Barbara, CA) in tapping mode under ambient conditions. B, PARP-DNA complexes. Recombinant PARP was stored in a solution containing 50 mM Tris-HCl (pH 8.0) and 25 mM MgCl₂. The DNA fragments and PARP were incubated at a molecular ratio of 1:4 for 10 min at room temperature before imaging. Arrows indicate PARP molecules bound to DNA fragments. C, the effect of NAD⁺ on the interaction of PARP with DNA. PARP was incubated with DNA in the presence of 0.1 mM NAD⁺. D, the effects of 3-aminobenzamide and NAD⁺ on the interaction of PARP with DNA. PARP was incubated with DNA in the presence of 0.1 mM NAD⁺ and 1 mM 3-aminobenzamide.

AFM. Imaging of the PARP-DNA samples was performed on a NanoScope-IIIa AFM in tapping mode in air (Digital Instruments, Santa Barbara, CA). The cantilever used was the commercially available 125-μm Silicon cantilever from Digital Instruments. The resonant frequency of the cantilever was 300–400 MHz. The scanning frequency was set at 4 Hz.

The method for sample preparation for AFM imaging was as described previously (32). Briefly, PARP or its cleavage products were incubated with the DNA fragments at a relative molar ratio of 4:1 for binding reaction. Then 1 μl of solution was deposited on an atomically flat mica surface and rinsed gently with 0.2 ml of distilled water to remove excess molecules. The sample was then briefly dried in the gentle flow of N₂ gas, and mounted on the AFM for imaging.

Results and Discussion

Fragments (average size, ~500 bp; length, ~170 nm) of plasmid DNA that had been digested with EcoRI (Fig. 1A) were exposed to purified recombinant PARP protein. AFM images showed PARP binding to the ends of DNA and physically joining multiple fragments into chainlike structures (Fig. 1B). Branch-like structures were also

Fig. 2. AFM images of the interaction of the PARP DBD with DNA. In A, the recombinant DBD of PARP was incubated with plasmid DNA fragments as in Fig. 1A. The geometric volume of the DBD is approximately one-third that of PARP. Arrows indicate the PARP DBD. In B, the PARP DBD was incubated with DNA in the presence of 0.1 mM NAD⁺ as in Fig. 1C. Again, the arrows indicate the PARP DBD that remained on the DNA fragments.
Fig. 3. A, cleavage of purified PARP by purified caspase-3. Purified recombinant PARP (0.1 μg) that had been incubated or not (control) with purified recombinant caspase-3 was resolved by SDS-PAGE and visualized by silver staining. Arrows, positions of intact PARP and its 89- and 24-kDa cleavage products. B, agarose gel electrophoresis of internucleosomal DNA fragments purified from apoptotic R1.1 mouse lymphoma cells. DNA fragments of 180, 360, and 540 bp (Lanes 1–3, respectively) were purified as described in “Materials and Methods.” Lane M, DNA size markers.

observed, consistent with previous observations suggesting that PARP binding can induce changes in DNA structure (34). Automodification [poly(ADP-ribosyl)ation] of PARP results in its detachment from DNA (7–9, 26). We, therefore, investigated the effect of incubating PARP and the DNA fragments with NAD+ under conditions conducive to PARP automodification (7–9). AFM of this preparation revealed that, although PARP was no longer bound to the DNA fragments, the latter remained aligned in chains exceeding 170 nm in length (Fig. 1C). When automodification of PARP in the presence of NAD+ was prevented with the use of 3-aminobenzamide to inhibit catalytic activity, PARP remained associated with the aligned DNA ends (Fig. 1D).

We obtained similar results with DNA digested with HindIII, which generates blunt, rather than overlapping, ends (data not shown). How-
ever, analysis by SDS-PAGE of incubation mixtures containing DNA (digested with EcoRI or HindIII), PARP, and NAD+ revealed that ligation of the DNA fragments had not taken place (data not shown); therefore, hydrogen bonding interactions were likely responsible for holding fragments together after dissociation of the automodified PARP from the DNA.

We next investigated the interaction with DNA of a 28-kDa recombinant fragment of PARP that contained the DBD but lacked the automodification domain (33). AFM images showed that the PARP DBD also bound to the ends of DNA fragments and joined them into chains (Fig. 2A). However, the addition of NAD+ did not result in release of the DBD from DNA (Fig. 2B).

To substantiate the relevance of our model system to events occurring in apoptotic cells, we repeated the experiments with recombinant PARP that had been digested with recombinant caspase-3 and with DNA isolated from cells undergoing apoptosis. SDS-PAGE analysis confirmed that incubation of PARP with caspase-3 resulted in complete processing to its 89- and 24-kDa fragments (Fig. 3A). Internucleosomal DNA fragments of 180, 360, and 540 bp were purified from R1.1 mouse lymphoma cells undergoing apoptosis induced by etoposide (Figs. 3B and 4A). AFM images of the caspase-3-generated PARP fragments that were incubated with apoptotic DNA revealed the 24-kDa DBD fragment linking the DNA strands into chainlike structures (Fig. 4B). Addition of NAD+ to the incubation mixture had no effect on the interaction of the 24-kDa PARP cleavage product with the apoptotic DNA fragments (Fig. 4C).

Our data both demonstrate the usefulness of AFM for investigating the interactions of proteins with DNA and provide support for a role for PARP cleavage by caspase-3 in apoptosis. We speculate that the irreversible binding of the 24-kDa fragment of PARP to DNA may serve to inhibit DNA repair by physically limiting access of repair proteins to the ends of the DNA fragments generated during the death program.

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References


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