

Poly(ADP-ribosyl)ation of Histone H1 Correlates with Internucleosomal DNA Fragmentation during Apoptosis*

(Received for publication, November 21, 1995, and in revised form January 22, 1996)

Yoo Sik Yoon‡, Jin Woo Kim‡, Ke Won Kang‡, Young Sang Kim§, Kyung Hee Choi¶, and Cheol O Joe‡¶

From the ‡Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejeon, 305-701, South Korea, the §Department of Biochemistry, Chungnam National University, Taejeon, 305-764, South Korea, and the ¶Department of Biology, Chungang University, Seoul, 156-756, South Korea

The biochemical role of poly(ADP-ribosyl)ation on internucleosomal DNA fragmentation associated with apoptosis was investigated in HL 60 human premyelocytic leukemia cells. It was found that UV light and chemotherapeutic drugs including adriamycin, mitomycin C, and cisplatin increased poly(ADP-ribosyl)ation of nuclear proteins, particularly histone H1. A poly(ADP-ribose) polymerase inhibitor, 3-aminobenzamide, prevented both internucleosomal DNA fragmentation and histone H1 poly(ADP-ribosyl)ation in cells treated with the apoptosis inducers. When nuclear chromatin was made accessible to the exogenous nuclease in a permeabilized cell system, chromatin of UV-treated cells was more susceptible to micrococcal nuclease than the chromatin of control cells. Suppression of histone H1 poly(ADP-ribosyl)ation by 3-aminobenzamide reduced the micrococcal nuclease digestibility of internucleosomal chromatin in UV-treated cells. These results suggest that the poly(ADP-ribosyl)ation of histone H1 correlates with the internucleosomal DNA fragmentation during apoptosis mediated by DNA damaging agents. This suggestion is supported by the finding that xeroderma pigmentosum cells which are defective in introducing incision at the site of DNA damage, failed to induce DNA fragmentation as well as histone H1 poly(ADP-ribosyl)ation after UV irradiation. We propose that poly(ADP-ribosyl)ation of histone H1 protein in the early stage of apoptosis facilitates internucleosomal DNA fragmentation by increasing the susceptibility of chromatin to cellular endonuclease.

Apoptosis is an active form of cellular suicide, a process that typically involves morphological changes such as the condensation of chromatin into clumps, nuclear fragmentation, and packaging of nuclear fragments into membrane-enclosed apoptotic bodies. These morphological changes are usually accompanied by biochemical changes including elevation of cytoplasmic Ca^{2+} and internucleosomal DNA fragmentation (1). Many of the chemotherapeutic drugs and radiation which cause DNA damage are known to induce apoptosis (2–5). It is well established that a variety of DNA damaging agents lower the cellu-

lar NAD^+ content by raising the specific activity of poly(ADP-ribose) polymerase (PARP)¹ (EC 2.4.2.30) that results from the conformational changes which occur when the zinc finger domains of the enzyme bind to the DNA strand breaks (6, 7). PARP is a nuclear enzyme which transfers the ADP-ribose moiety of NAD^+ to nuclear proteins including PARP itself and histones in cells (8). *In vitro* studies have also revealed that topoisomerase I (9), RNA polymerase II (10), DNA polymerase α (11), DNA polymerase β (12), and terminal deoxynucleotidyl transferase (13) are the substrates of PARP. Thus, poly(ADP-ribosyl)ation has been implicated in many biological responses including DNA replication (14), DNA excision repair (15), cell differentiation (16), and tumor promotion (17).

In recent years, the role of poly(ADP-ribosyl)ation in the cell death process has been discussed. The decrease in cellular PARP activity during the course of radiation-induced apoptosis in rat thymocytes was reported by Nelipovich *et al.* (18). Down-regulation of PARP activity in cells undergoing apoptosis was supported by the finding that C-nitroso compounds that inhibit PARP function provoke apoptosis in cultured mammalian cells (19). On the contrary, many studies proposed that the activation of PARP contributes to the induction of apoptosis. PARP activation was recognized during the cell death processes induced by DNA damaging agents such as alkylating agents (3), H_2O_2 (20), topoisomerase II inhibitors (21), adriamycin (22), and x-ray (23). Kaufmann *et al.* (24) attempted to explain these controversial results by suggesting that early during the course of apoptosis the activity of intact PARP is stimulated by DNA strand breaks, but proteolytic cleavage decreases the activity of PARP in the late course of apoptosis.

Poly(ADP-ribosyl)ation of nuclear proteins is one of the most dramatic post-translational modifications that occur during apoptosis. The potential correlation between poly(ADP-ribosyl)ation of nuclear proteins and apoptotic internucleosomal DNA fragmentation has been suggested (25, 26). These studies have described the suppressive effect of PARP inhibitors on the generation of DNA fragmentation associated with apoptosis. However, the target protein of PARP during apoptosis or the biochemical mechanism by which poly(ADP-ribosyl)ation affects the internucleosomal DNA fragmentation has not been addressed. In the present study, a correlation was found between internucleosomal DNA fragmentation and poly(ADP-ribosyl)ation of histone H1 nuclear protein during apoptosis mediated by DNA damaging agents. We propose that the poly(ADP-ribosyl)ation of histone H1 that occurs early during the course of apoptosis facilitates oligonucleosomal DNA fragmen-

* This work was supported in part by a grant from the Korea Science and Engineering Foundation and by a grant from Science Research Center for Cell Differentiation, Seoul National University, Seoul, Korea. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: Dept. of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejeon, 305-701, South Korea. Tel.: 82-42-869-4017; Fax: 82-42-869-2610.

¹ The abbreviations used are: PARP, poly(ADP-ribose) polymerase; 3-AB, 3-aminobenzamide; XP, xeroderma pigmentosum; PAGE, polyacrylamide gel electrophoresis.

tation by increasing the susceptibility of nuclear chromatin to cellular endonuclease.

EXPERIMENTAL PROCEDURES

Reagents—Nicotinamide, 3-aminobenzamide (3-AB), thymidine, cisplatin (cis-diamminedichloroplatinum(III)), adriamycin, histone H1, and micrococcal nuclease were purchased from Sigma. Mitomycin C was obtained from Boehringer Mannheim. [Adenylate-³²P]NAD⁺ was from Amersham. All other chemicals were of analytical grade.

Cell Lines and Culture Conditions—The HL 60 cell line was from American Type Culture Collection and was grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin G, and 100 µg/ml streptomycin. The xeroderma pigmentosum (XP) cell line (complementation group A, GM04312B) was obtained from Nigms Human Genetic Mutant Cell Repository (Camden, NJ) and was cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 100 units/ml penicillin G, and 100 µg/ml streptomycin.

Measurement of PARP Activity—PARP activity was measured by the method of Grube *et al.* (27). After induction of apoptosis, cells were permeabilized (2×10^6 cells/ml) in ice-cold permeabilizing buffer containing 10 mM Tris, pH 7.8, 1 mM EDTA, 30 mM 2-mercaptoethanol, and 4 mM MgCl₂. Cell suspension was incubated for 15 min at 4 °C. Aliquots of 10^7 cells were then collected by centrifuging at $200 \times g$ for 10 min and resuspended in 0.2 ml of permeabilizing buffer. To this permeable cell suspension, 0.1 ml of poly(ADP-ribose) polymerase reaction buffer containing 100 mM Tris, pH 7.8, 120 mM MgCl₂, 1 mM NAD⁺, and 10 µCi/ml [³²P]NAD⁺ was added. After incubation at 30 °C for 30 min, the reaction was stopped by the addition of ice-cold 10% trichloroacetic acid and 2% sodium pyrophosphate, and acid insoluble radioactivity was measured in a liquid scintillation counter (Beckman, LS 3801).

Examination of DNA Fragmentation—The apoptotic nature of cells was examined by agarose gel electrophoresis of their nuclear DNA, using the method of Warning (28). The quantification of DNA fragmentation was carried as described by McConkey *et al.* (29) with slight modifications. Cells treated with apoptosis inducer in a 100-mm culture dish were lysed in 0.33 ml of a buffer containing 5 mM Tris, pH 8.0, 20 mM EDTA, and 0.5% Triton X-100. After incubation for 15 min on ice, samples were centrifuged for 20 min at $27,000 \times g$ to separate the intact chromatin (pellet) from the fragmented DNA (supernatant). Pellets were resuspended in 0.33 ml of a buffer containing 10 mM Tris, pH 8.0, and 1 mM EDTA. Pellet and supernatant fractions were separately assayed for DNA content using the diphenylamine reagent containing 1.5% diphenylamine (w/v), 1.5% sulfuric acid (v/v), and 0.008% acetaldehyde (v/v) in glacial acetic acid (30). DNA fragmentation was quantified by measuring the ratio of the DNA content in supernatant fraction to the total (supernatant plus pellet) DNA content. For visualization of fragmented DNA, the supernatant fraction containing fragmented DNA was extracted once with an equal volume of phenol and once with chloroform, precipitated in 67% ethanol, 0.3 M sodium acetate at -20 °C for 18 h, and then resuspended in a buffer containing 10 mM Tris, pH 8.0, 1 mM EDTA, and 30 µg/ml RNase A, prior to electrophoresis in 1.8% agarose gel as described by Jones *et al.* (25).

Analysis of the Poly(ADP-ribosylation) of Histone H1—Cells were permeabilized for the poly(ADP-ribosylation) reaction as described earlier, and histone H1 was extracted by a modified version of the method of Cole (31). Briefly, perchloric acid was added to each cell sample in a 100-mm culture dish (5% final concentration). After vortexing for 3 min and centrifugation at $7,000 \times g$ for 5 min, the supernatant was saved and the pellet was re-extracted with 0.15 ml of 5% perchloric acid. Supernatant fractions were pooled and adjusted to 0.1 N HCl. The supernatant fractions were mixed with 4 volumes of acetone and stirred for 16 h at 4 °C. The pellet of histone H1 was collected by centrifugation at $12,000 \times g$ for 30 min and washed with acetone containing 0.1% HCl. Poly(ADP-ribosylation) of histone H1 was analyzed by 15% SDS-PAGE and subsequent autoradiography.

Micrococcal Nuclease Digestion of Nuclear DNA—Cells were permeabilized to exogenous nuclease by the modified method of Smets *et al.* (32). Cell preparations (10^7 cells/sample) either from control or UV-treated cells were permeabilized in 0.7 ml of a hypotonic buffer containing 9 mM HEPES, pH 7.8, 5 mM dithiothreitol, 4.5% (w/v) dextran (average M_w , 110,000), 1 mM EGTA, 4.5 mM MgCl₂, and 15 µg/ml digitonin for 20 min on ice. Ca²⁺ concentration was adjusted to 2 mM and specified concentrations of micrococcal nuclease were added. The reaction mixture was then incubated for 10 min at 37 °C. The DNA fragmentation was quantified by the method of McConkey *et al.* (29). The fragmented DNA by micrococcal nuclease digestion was collected for electrophoresis in 1.8% agarose gel (25).

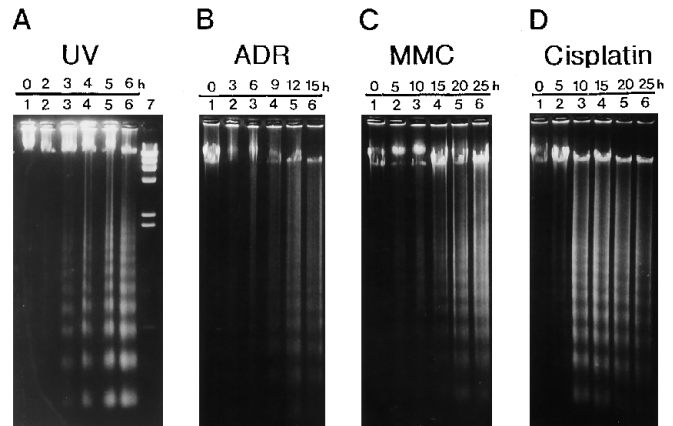


FIG. 1. Internucleosomal DNA fragmentation during apoptosis induced by DNA damaging agents. HL 60 cells were grown to log phase in 100-mm culture dishes and treated with 250 J/m² UV light (254 nm) (A), 10 µg/ml adriamycin (ADR) (B), 10 µg/ml mitomycin C (MMC) (C), or 10 µg/ml cisplatin (D). After incubation for the indicated time periods, DNA was extracted and analyzed by 1.8% agarose gel electrophoresis (lane 7 in A, λ DNA-HindIII digest).

RESULTS

Correlation between Internucleosomal DNA Fragmentation and Poly(ADP-ribosylation)—UV light, adriamycin, mitomycin C, and cisplatin are a well known group of agents that induce apoptosis in many different cell types (24, 33, 34). When HL 60 human premyelocytic leukemia cells were exposed to UV light, obvious internucleosomal DNA fragmentation was recognized by 3 h after exposure. Other chemotherapeutic drugs that induce DNA strand breaks such as adriamycin, mitomycin C, and cisplatin also induced evident internucleosomal DNA fragmentation by 9, 15, and 10 h following treatment, respectively (Fig. 1).

The change of PARP activity during the course of UV-mediated apoptosis was shown in Fig. 2A. The cellular PARP activity, as measured by the transfer of ³²P-labeled ADP-ribose moieties from NAD⁺ to cellular proteins, was elevated immediately after UV irradiation. The activity peaked at 2.5 h after UV irradiation and declined thereafter. However, the apparent increase in the amount of fragmented DNA was evident at 2.5 h after UV irradiation coinciding with the time point when the maximal PARP activity was observed. The observation of increased PARP activity before the commencement of DNA fragmentation has led us to investigate the possible involvement of PARP activation in the course of apoptotic DNA fragmentation. In Fig. 2B, we examined the effect of PARP inhibitors on DNA fragmentation induced by UV irradiation. Dose-dependent inhibition of UV-induced DNA fragmentation was observed with PARP inhibitors of 3-AB, nicotinamide, or thymidine. The internucleosomal DNA fragmentation induced by the chemotherapeutic drugs as well as UV light was completely inhibited by 3-AB, implicating a common requirement for PARP activation in the course of apoptosis mediated at least by the agents employed in this study (Fig. 3A). Interestingly, the morphological changes of apoptosis were also prevented by 3-AB treatment (Fig. 3B).

Poly(ADP-ribosylation) of Histone H1 in HL 60 Cells Undergoing Apoptosis—The activation of PARP elicits the modification of nuclear proteins by poly(ADP-ribosylation). When cells were irradiated with 250 J/m² of UV light and cellular proteins were analyzed for poly(ADP-ribosylation) by autoradiography following SDS-PAGE, two proteins of about 33 and 110 kDa were predominantly poly(ADP-ribosylated) (Fig. 4B, lane 5). The 33-kDa poly(ADP-ribose) acceptor protein had an electrophoretic mobility similar to that of histone H1 which migrates

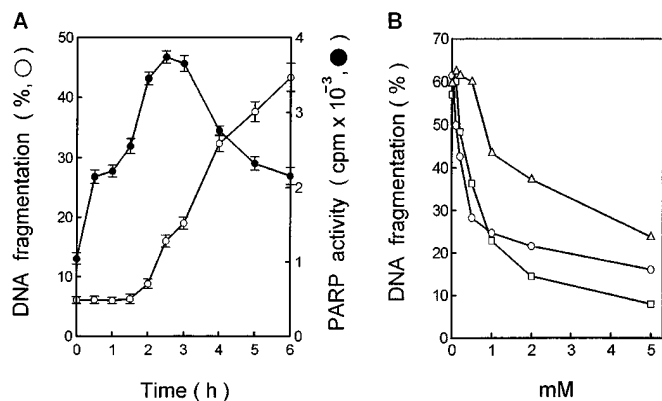


FIG. 2. *A*, time courses of PARP activity and DNA fragmentation during UV-induced apoptosis. HL 60 cells were irradiated with 250 J/m² of 254 nm UV light. After incubating for specified time periods, cells were harvested and divided into two aliquots. Each aliquot was, respectively, measured for PARP activity (●) and DNA fragmentation (○) as described under "Experimental Procedures." Each data point represents the mean value obtained from three separate experiments. *B*, effects of PARP inhibitors on DNA fragmentation induced by UV irradiation. HL 60 cells were treated with 250 J/m² of UV light and incubated for 3 h with various concentrations of 3-AB (□), nicotinamide (Δ), or thymidine (○).

as a group of bands of apparent molecular mass 30–35 kDa (35). The 33-kDa poly(ADP-ribose) acceptor protein showed the same electrophoretic mobility as that of commercially available histone H1. Histone H1 is a major target protein modified by PARP (36, 37). In an attempt to confirm histone H1 poly(ADP-ribosyl)ation, we extracted histone H1 from UV-treated cells (31), and analyzed its state of poly(ADP-ribosyl)ation. Histone H1 was markedly poly(ADP-ribosyl)ated by 2 h after UV irradiation (Fig. 4*B*). Fig. 4*A* shows that the amount of histone H1 protein in the cells was not changed by UV irradiation. The increase in the poly(ADP-ribosyl)ation of histone H1 was not a restricted phenomenon in cells undergoing apoptosis mediated by UV treatment. The treatment of cells with chemotherapeutic drugs such as adriamycin, mitomycin C, or cisplatin also resulted in an increase in the poly(ADP-ribosyl)ation of histone H1 protein (Fig. 5). Simultaneous treatment of 3-AB with these DNA damaging agents inhibited both internucleosomal DNA fragmentation and poly(ADP-ribosyl)ation of histone H1 (Figs. 3 and 5).

For further insight into the correlation between poly(ADP-ribosyl)ation of histone H1 and DNA fragmentation induced by DNA damaging agents, we examined whether the correlation is still established in XP cells (complementation group A, GM04312B), which are defective in introducing incision at the site of DNA damage (38). Complementation group A is one of the most severe forms of this genetic disease (39). There are reports that XP cells are defective in the synthesis of poly(ADP-ribose) in response to UV irradiation (40, 41). In contrast to HL 60 cells, the internucleosomal DNA fragmentation (Fig. 6*A*) and poly(ADP-ribosyl)ation of histone H1 (Fig. 6*C*) were both abolished in XP cells treated with UV light ranging from 100 to 300 J/m². None of the morphological characteristics of apoptotic cells were seen in XP cells after UV treatment (data not shown).

Effect of 3-AB on Micrococcal Nuclease Susceptibility of Chromatin in HL 60 Cells Treated with UV Light—Since histone H1 is a protein located within the internucleosomal space and modulates chromatin structure (42, 43), one might expect that the poly(ADP-ribosyl)ation of histone H1 influences native chromatin structure. This rationale was tested in Fig. 7 in which the chromatin DNA of intact or UV-treated HL 60 cells was examined for its susceptibility to micrococcal nuclease, an

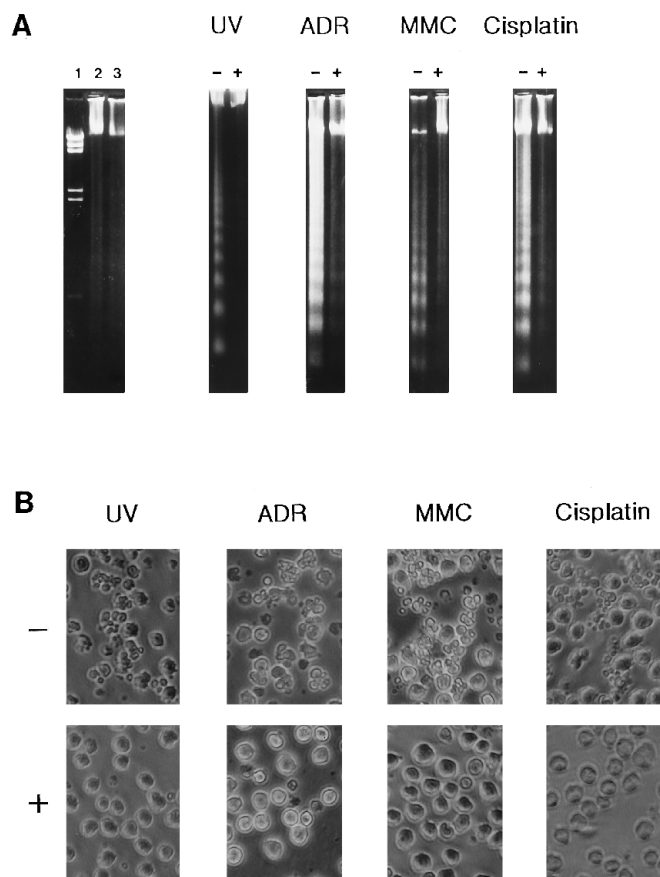


FIG. 3. **Effects of 3-AB on DNA fragmentation (A) and morphological characteristic of apoptosis (B) induced by DNA damaging agents.** Agarose gel electrophoresis of DNA extracted from control cells (lane 2) and from cells treated with 5 mM 3-AB for 15 h (lane 3). λ DNA-*Hind*III digest was used as molecular weight markers (lane 1). HL 60 cells were irradiated with 250 J/m² of UV light, and incubated for 3 h with (+) or without (–) 5 mM 3-AB. Cells were treated with 10 μ g/ml adriamycin (ADR) for 9 h with (+) or without (–) 5 mM 3-AB. Cells were treated with 10 μ g/ml mitomycin C (MMC) for 15 h with (+) or without (–) 5 mM 3-AB. Cells were treated with 10 μ g/ml cisplatin for 10 h with (+) or without (–) 5 mM 3-AB. Cells were incubated for the time periods necessary to initiate DNA fragmentation (see Fig. 1). Cell morphology was determined by phase contrast microscopy (\times 320).

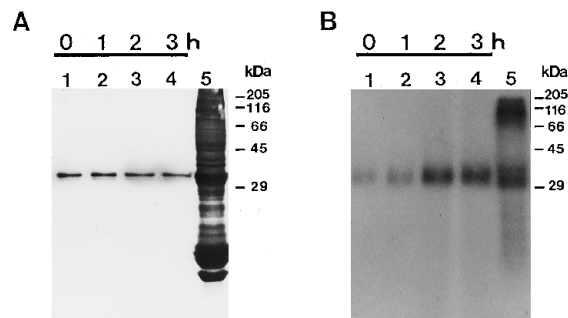


FIG. 4. **Poly(ADP-ribosyl)ation of histone H1 during UV-induced apoptosis.** HL 60 cells were irradiated with 250 J/m² of UV light and incubated for 0, 1, 2, or 3 h (lanes 1–4, respectively). The ³²P-labeled ADP-ribose moiety of NAD⁺ was attached to cellular proteins in a permeabilized cell system. Histone H1 was extracted (32), analyzed by 15% SDS-PAGE (A), and subjected to autoradiography (B). Total cellular proteins were also prepared at 3 h after UV irradiation by washing 2 times with phosphate-buffered saline and sonication for 3 min with W-385 sonicator (Heat systems-Ultrasonics), and were examined for poly(ADP-ribosyl)ation (lane 5).

endonuclease known to cleave internucleosomal DNA (44), after incubation with or without 3-AB. Chromatin DNA of HL 60 cells was examined at 2 h after 250 J/m² UV treatment when

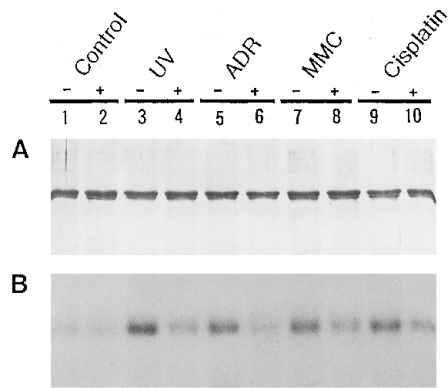


FIG. 5. Poly(ADP-ribosyl)ation of histone H1 during apoptosis mediated by UV and chemotherapeutic drugs. Cells were treated with 250 J/m^2 of UV light, $10 \mu\text{g/ml}$ adriamycin (ADR), $10 \mu\text{g/ml}$ mitomycin C (MMC), or $10 \mu\text{g/ml}$ cisplatin and incubated for 3, 9, 15, or 10 h, respectively, in the presence (+) or absence (-) of 5 mM 3-AB. Histone H1 was extracted from cell samples and analyzed on 15% SDS-PAGE (A). Poly(ADP-ribosyl)ation of histone H1 was visualized by autoradiography (B).

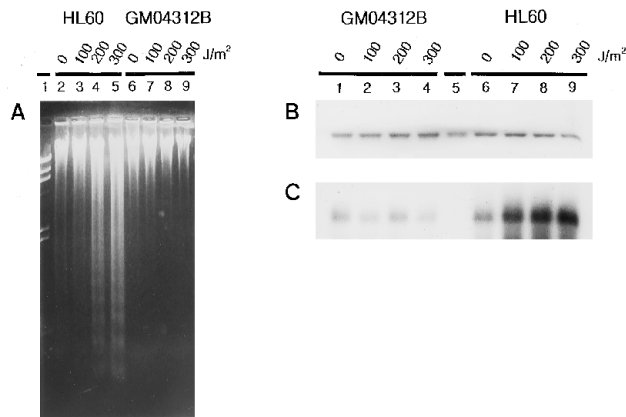


FIG. 6. DNA fragmentation and histone H1 poly(ADP-ribosyl)ation after UV irradiation in HL 60 and GM04312B cells. HL 60 and GM04312B cells were grown to log phase and irradiated with UV light. After incubation for 3 h, cell preparation was divided into two aliquots. One aliquot was prepared for agarose gel electrophoresis (lane 1, λ DNA-HindIII digest; lanes 2-5, HL 60; lanes 6-9, GM04312B) (A). The other aliquot was prepared for the examination of histone H1 poly(ADP-ribosyl)ation. Histone H1 was extracted and analyzed by 15% SDS-PAGE. The mobility of commercially obtained histone H1 was also analyzed (lane 5). After electrophoresis, the gel was stained with Coomassie Blue (B) and exposed to x-ray film for autoradiography (C).

the cellular proteins were fully poly(ADP-ribosyl)ated but DNA remained unfragmented (see Fig. 2A). Chromatin DNA of UV-treated HL 60 cells was susceptible to micrococcal nuclease digestion yielding a ratio of fragmented DNA content to total DNA content of up to 20% after 4 milliunits/ml treatment of micrococcal nuclease for 10 min (Fig. 7A). However, chromatin DNA of UV-treated cells became resistant to micrococcal nuclease digestion by 3-AB which inhibited the poly(ADP-ribosyl)ation of histone H1 (see Fig. 5). It seems highly unlikely that the DNA fragmentation was produced by the action of endogenous endonuclease, since Fig. 7B (lane 3) demonstrates that the chromatin DNA of UV-treated cells remains unfragmented during 2 h after UV irradiation. However, the chromatin DNA becomes susceptible to the exogenous micrococcal nuclease added early during the course of apoptosis (lane 8).

DISCUSSION

Our data suggest a correlation between internucleosomal DNA fragmentation associated with apoptosis and poly(ADP-ribosyl)ation of histone H1. It is well established that the free

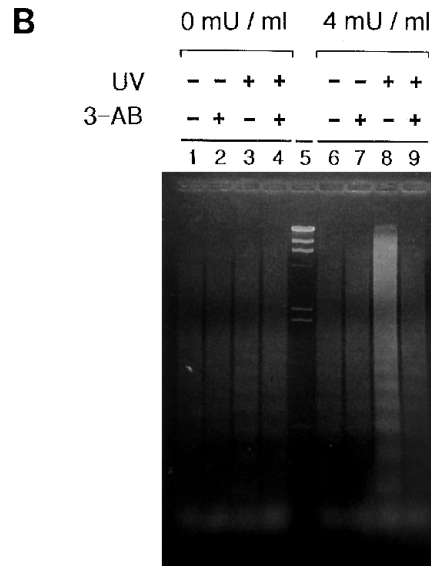
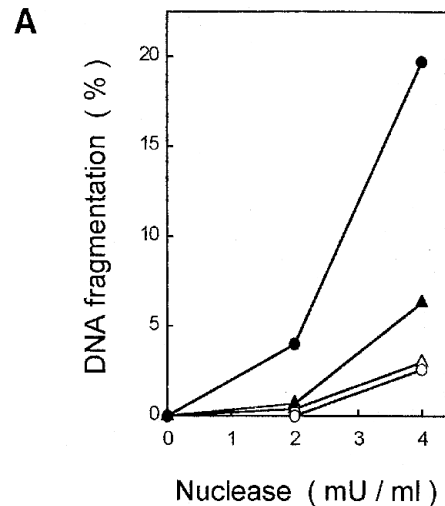


FIG. 7. Reduced susceptibility of chromatin DNA to micrococcal nuclease by 3-AB during apoptosis mediated by UV irradiation. Micrococcal nuclease digestibility of nuclear chromatin was examined in control HL 60 cells (▲), cells exposed to 5 mM 3-AB (△), cells treated with 250 J/m^2 of UV (●), or in cells exposed to 250 J/m^2 of UV and 5 mM 3-AB (○). Cells were incubated for 2 h and were permeabilized to allow the accessibility of their chromatin to micrococcal nuclease. The measurement of DNA fragmentation was done using diphenylamine reagent as described under "Experimental Procedures." The results of a typical experiment of three replicates is shown (A). DNA fragments were analyzed by 1.8% agarose gel electrophoresis (B). Lane 5 is λ DNA-HindIII digest, and the material shown at the bottom of each lane indicates undigested RNA.

ends of DNA are strong activators of PARP and that PARP activity is increased by DNA damaging agents which introduce DNA strand breaks (6, 7). Many studies have described the relationship between PARP activity and the cell death process. The consumption of NAD^+ by PARP activation was considered to be the main cause of cell death after DNA damage (45). Activated PARP cleaves NAD^+ into nicotinamide and ADP-ribose resulting in a depletion of NAD^+ . The reduction of the cellular NAD^+ level slows down glycolysis and other energy-generating reactions leading to the depletion of cellular ATP. Accordingly, the activation of PARP reduces the energy supply thereby slowing down cellular metabolism including macromolecular synthesis. This in turn disturbs cellular homeostasis resulting in an eventual cell death. However, the requirement

of energy-rich nucleotides differs between apoptosis and necrosis. Unlike necrosis, apoptosis is an energy-requiring process which needs macromolecular synthesis (46), and the depletion of intracellular ATP does not regularly precede the onset of irreversible morphological changes that occur during apoptosis. Despite an increased PARP activity during apoptosis, no massive depletion of NAD⁺ and ATP occurred. For example, apoptotic internucleosomal DNA fragmentation in L1210 cells occurred in 2 days after cisplatin treatment, but the levels of NAD⁺ and ATP were not significantly decreased until 3 days after the treatment, suggesting that the reduction of the cellular energy level was not the cause but the result of cell death (47).

While the negative (18, 19) and positive (3, 20–23) roles of PARP activity on apoptosis have been independently presented, the monitoring of cellular PARP activity during UV-induced apoptosis in HL 60 cells (Fig. 2A) suggests an explanation that integrates the two conflicting interpretations. We observed a rapid increase in PARP activity before the initiation of DNA fragmentation, but with the commencement of internucleosomal DNA fragmentation, the cellular level of PARP activity declined. This observation is supported by the study of Kaufmann *et al.* (24) that reported proteolytic cleavage of PARP in HL 60 cells undergoing apoptosis. During the course of chemotherapy-induced apoptosis in human leukemia cells, it was demonstrated that PARP was cleaved into a 25-kDa fragment containing the DNA-binding domain and a 85-kDa fragment containing the automodification and catalytic domains. Based on these observations, they suggested that the initial consumption of NAD⁺ that ordinarily occurs early during the course of apoptosis is attributed to the increase in the activity of intact PARP which is stimulated by DNA strand breaks. However, proteolytic cleavage decreases the activity of PARP and the 85-kDa fragment retains only basal PARP activity necessary for the late course of apoptosis. More recently, it was found that a protease of the CED-3/interleukin converting enzyme family is responsible for the specific cleavage of PARP during apoptosis (48, 49). The involvement of PARP activity in the induction of apoptosis mediated by DNA damaging agents is reinforced by the data showing that the inhibitor of PARP inhibits internucleosomal DNA fragmentation associated with apoptosis (Figs. 2B and 3A). Our data are consistent with the report showing that 3-AB inhibits DNA fragmentation associated with apoptosis in HL 60 cells at concentrations higher than 2 mM (26). Other inhibitors of PARP were also observed to inhibit DNA fragmentation in apoptotic cells induced by UV light (Fig. 2B), suggesting an obligate role of PARP activity in the apoptotic DNA fragmentation.

In the present study, we attempted to propose an explanation for the role of poly(ADP-ribosyl)ation on the induction of apoptosis. Nuclear proteins are poly(ADP-ribosyl)ated in order to modify their structures and functions. We have shown an increase of histone H1 poly(ADP-ribosyl)ation in HL 60 cells by apoptosis inducers (Figs. 4 and 5). The poly(ADP-ribosyl)ation of histone H1 was measured in permeabilized cells which allow rapid access of ³²P-labeled NAD⁺ to nuclei. It is, however, unlikely that the permeabilization process affects any undue effects on the poly(ADP-ribosyl)ation reaction, since the process is generally used to measure the nucleotide polymerizing reactions under near physiological conditions (50, 51). Electron microscopic studies showed that the permeabilized cells retained intact morphology throughout the processing and incubation (52). The link between histone H1 poly(ADP-ribosyl)ation and induction of apoptosis is reinforced by data in Fig. 6 showing GM04312B cells, which are unable to poly(ADP-ribosyl)ate histone H1 and are also lacking the ability to generate

internucleosomal DNA fragmentation. Among the known acceptors of poly(ADP-ribose), histone H1, a very basic protein located within the space of internucleosomal DNA region, particularly plays an important role in the formation and stabilization of the highly ordered solenoid structure of native chromatin (reviewed in Ref. 53). Internucleosomal DNA and bound histone H1 are hidden in the interior of the solenoid structure (42, 43). It is thus conceivable that the change of polarity of the basic portions of histone H1 by the association of highly negative poly(ADP-ribose) may reduce their affinity to the associated DNA and consequently affect the stability of solenoid structure. Evidence has been accumulated showing the correlation between the change of chromatin structure and poly(ADP-ribosyl)ation of histone H1. Electron microscopic studies have revealed that the solenoid chromatin structure was relaxed by the poly(ADP-ribosyl)ation of histone H1 protein exposing internucleosomal DNA regions from the interior of the structure (54, 55). De Murcia *et al.* (56) further demonstrated that the degradation of ADP-ribose units on poly(ADP-ribosyl)ated histone H1 by poly(ADP-ribose) glycohydrolase restored the solenoid chromatin structure. The change of chromatin superstructure by poly(ADP-ribosyl)ation of histone H1 might explain the internucleosomal DNA fragmentation that occurs during apoptosis of which the mechanism requires the accessibility of endonuclease to internucleosomal DNA. The data in Fig. 7 indicate the elevated level of micrococcal nuclease susceptibility of chromatin DNA during apoptosis. It was also shown that the micrococcal nuclease susceptibility was inhibited by the treatment of PARP inhibitor.

REFERENCES

1. Wyllie, A. H., Kerr, J. F., and Currie, A. R. (1980) *Int. Rev. Cytol.* **68**, 251–306
2. Sellins, K. S., and Cohen, J. J. (1987) *J. Immunol.* **139**, 3199–3206
3. Marks, D. I., and Fox, R. M. (1991) *Biochem. Pharmacol.* **42**, 1859–1867
4. Sinha, B. K., Yamazaki, H., Eliot, H. M., Schneider, E., Borner, M. M., and O'Connor, P. M. (1995) *Biochim. Biophys. Acta* **1270**, 12–18
5. Kaufmann, S. H. (1989) *Cancer Res.* **49**, 5870–5878
6. Ikejima, M., Noguchi, S., Yamashita, R., Ogura, T., Sugimura, T., Gill, D. M., and Miwa, M. (1990) *J. Biol. Chem.* **265**, 21907–21913
7. Rankin, P. W., Jacobson, M. K., Mitchell, V. R., and Busbee, D. L. (1980) *Cancer Res.* **40**, 1803–1807
8. Kreimeyer, A., Wielckens, K., Adamietz, P., and Hilz, H. (1984) *J. Biol. Chem.* **259**, 890–896
9. Ferro, A. M., and Olivera, B. M. (1984) *J. Biol. Chem.* **259**, 547–554
10. Taniguchi, T., Suzuki, S., and Shizuta, Y. (1985) *Biochem. Biophys. Res. Commun.* **127**, 526–532
11. Yoshihara, K., Itaya, A., Tanaka, Y., Ohashi, Y., Ito, K., Teraoka, H., Tsukada, K., Matsukage, A., and Kamiya, T. (1985) *Biochem. Biophys. Res. Commun.* **128**, 61–67
12. Ohashi, Y., Itaya, A., Tanaka, Y., Yoshihara, K., Kamiya, T., and Matsukage, A. (1986) *Biochem. Biophys. Res. Commun.* **140**, 666–673
13. Tanaka, Y., Ito, K., Yoshihara, K., and Kamiya, T. (1986) *J. Biochem. (Tokyo)* **155**, 19–25
14. Shall, S. (1972) *FEBS Lett.* **24**, 1–6
15. Durkacz, B. W., Omidiji, O., Gray, D. A., and Shall, S. (1980) *Nature* **283**, 593–596
16. Farzaneh, F., Zalin, R., Brill, D., and Shall, S. (1982) *Nature* **300**, 362–366
17. Troll, W., Garte, S., and Frenkel, K. (1990) *Basic Life Sci.* **52**, 225–232
18. Nelipovich, P. A., Nikonova, L. V., and Umansky, S. R. (1988) *Int. J. Radiat. Biol.* **53**, 749–765
19. Rice, W. G., Hillyer, C. D., Harten, B., Schaeffer, C. A., Dorminy, M., Lackey, D. A., III, Kirsten, E., Mendeleyev, J., Buki, K. G., Hakam, A., and Kun, E. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 7703–7707
20. Schraufstatter, I. U., Hinshaw, D. B., Hyslop, P. A., Spragg, R. G., and Cochrane, C. C. (1986) *J. Clin. Invest.* **77**, 1312–1320
21. Tanizawa, A., Kubota, M., Hashimoto, H., Shimizu, T., Takimoto, T., Kitoh, T., Akiyama, Y., and Mikawa, H. (1989) *Exp. Cell Res.* **185**, 237–246
22. Tanizawa, A., Kubota, M., Takimoto, T., Akiyama, Y., Seto, S., Kiriya, Y., and Mikawa, H. (1987) *Biochem. Biophys. Res. Commun.* **144**, 1031–1036
23. Manome, Y., Datta, R., Taneja, N., Shafman, T., Bump, E., Hass, R., Weichselbaum, R., and Kufe, D. (1993) *Biochemistry* **32**, 10607–10613
24. Kaufmann, S. H., Desnoyers, S., Ottaviano, Y., Davidson, N. E., and Poirier, G. G. (1993) *Cancer Res.* **53**, 3976–3985
25. Jones, D. P., McConkey, D. J., Nicotera, P., and Orrenius, S. (1989) *J. Biol. Chem.* **264**, 6398–6403
26. Bertrand, R., Solary, E., Jenkins, J., and Pommier, Y. (1993) *Exp. Cell Res.* **207**, 388–397
27. Grube, K., Kupper, J. H., and Burkle, A. (1991) *Anal. Biochem.* **193**, 236–239
28. Waring, P. (1990) *J. Biol. Chem.* **265**, 14476–14480
29. McConkey, D. J., Hartzell, P., Jondal, M., and Orrenius, S. (1989) *J. Biol. Chem.* **264**, 13399–13402

30. Burton, K. (1956) *Biochem. J.* **62**, 315–323
31. Cole, R. D. (1989) *Methods Enzymol.* **170**, 524–532
32. Smets, L. A., Loesberg, C., Janssen, M., and Van Rooij, H. (1990) *Biochim. Biophys. Acta* **1054**, 49–55
33. Cotter, T. G., Lennon, S. V., Glynn, J. M., and Green, D. R. (1992) *Cancer Res.* **52**, 997–1005
34. Ohmori, T., Podack, E. R., Nishio, K., Takahashi, M., Miyahara, Y., Takeda, Y., Kubota, N., Funayama, Y., Ogasawara, H., Ohira, T., Ohta, S., and Saijo, N. (1993) *Biochem. Biophys. Res. Commun.* **192**, 30–36
35. Lennox, R. W., and Cohen, L. H. (1989) *Methods Enzymol.* **170**, 532–549
36. Huletsky, A., Niedergang, C., Frechette, A., Aubin, R., Gaudreau, A., and Poirier, G. G. (1985) *Eur. J. Biochem.* **146**, 277–285
37. Quesada, P., Faraone-Mennella, M. R., Jones, R., Malanga, M., and Farina, B. (1990) *Biochem. Biophys. Res. Commun.* **170**, 900–907
38. Squires, S., and Johnson, R. T. (1988) *Mutat. Res.* **193**, 181–192
39. Robins, P., Jones, C. J., Biggerstaff, M., Lindahl, T., and Wood, R. D. (1991) *EMBO J.* **10**, 3913–3921
40. Berger, N. A., Sikorski, G. W., Petzold, S. J., and Kurohara, K. K. (1980) *Biochemistry* **19**, 289–293
41. McCurry, L. S., and Jacobson, M. K. (1981) *J. Biol. Chem.* **256**, 551–553
42. Allan, J., Hartman, P. G., Crane-Robinson, C., and Aviles, F. X. (1980) *Nature* **288**, 675–679
43. Clark, D. J., and Thomas, J. O. (1986) *J. Mol. Biol.* **187**, 569–580
44. Nelson, P. P., Albright, S. C., and Garrard, W. T. (1979) *J. Biol. Chem.* **254**, 9194–9199
45. Gaal, J. C., Smith, K. R., and Pearson, C. K. (1987) *Trends Biochem. Sci.* **12**, 129–130
46. D'Adamio, L., Clayton, L. K., Awad, K. M., and Reinherz, E. L. (1992) *J. Immunol.* **149**, 3550–3553
47. Sorenson, C. M., Barry, M. A., and Eastman, A. (1990) *J. Natl. Cancer Inst.* **82**, 749–755
48. Lazebnik, Y. A., Kaufmann, S. H., Desnoyers, S., Poirier, G. G., and Earnshaw, W. C. (1994) *Nature* **371**, 346–347
49. Tewari, M., Quan, L. T., O'Rourke, K., Desnoyers, S., Zeng, Z., Beidler, D. R., Poirier, G. G., Salvesen, G. S., and Dixit, V. M. (1995) *Cell* **81**, 801–809
50. Berger, N. A., Adams, J. W., Sikorski, G. W., Petzold, S. J., and Shearer, W. T. (1978) *J. Clin. Invest.* **62**, 111–118
51. Berger, N. A., Weber, G., and Kaichi, A. S. (1978) *Biochim. Biophys. Acta* **519**, 87–104
52. Berger, N. A. (1978) *Methods Cell Biol.* **20**, 325–340
53. Thomas, J. O. (1984) *J. Cell Sci. Suppl.* **1**, 1–20
54. Aubin, R. J., Frechette, A., de Murcia, G., Mandel, P., Lord, A., Grondin, G., and Poirier, G. G. (1983) *EMBO J.* **2**, 1685–1693
55. Niedergang, C. P., de Murcia, G., Ittel, M., Pouyet, J., and Mandel, P. (1985) *Eur. J. Biochem.* **146**, 185–191
56. de Murcia, G., Huletsky, A., Lamarre, D., Gaudreau, A., Pouyet, J., Daune, M., and Poirier, G. G. (1986) *J. Biol. Chem.* **261**, 7011–7017