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Analysis of DNA fragmentation using a dynamic size-sieving polymer solution in capillary electrophoresis

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Abstract

Various natural and induced processes cause DNA fragmentation. Examples of these processes include apoptosis, enzymatic digestion, free radical production from ionizing radiation, photoscission by laser radiation and thermal degradation. Slab gel electrophoresis has been used most often to monitor such DNA damage. We have investigated with capillary electrophoresis the use of a new size-sieving polymer solution, TreviSol–CE (TS–CE), to monitor the DNA fragments produced from a variety of degradation processes. This polymer solution provides high run-to-run migration time and peak width reproducibilities and high separation efficiency of double-stranded DNA fragments in the 500 to 7000 base pair size range. Analysis of apoptotic DNA fragments suggested the presence of multiple nucleosomes within each cell type investigated. For irradiated DNA standards, peak-width-at-half-height and peak area were used to monitor the progress of DNA fragmentation. For both apoptotic DNA and irradiated DNA standards, fine structural features of fragmentation were revealed.

Keywords: DNA fragmentation; Buffer composition; DNA

1. Introduction

The natural process of apoptosis, a form of programmed cell death, is an important pathway in the development of numerous cell types of multicellular organisms. This process is regulated within the cell, where certain reactions are triggered to induce cell destruction. On a microscopic level, cell destruction begins with the collapse of the cell nucleus (condensation of the cytoplasm), followed by fragmentation of the cellular chromatin, and subsequent dispersion of the cell into discrete apoptotic bodies. Cellular chromatin is fragmented spatially, not sequentially, by one or more endogenous endonucleases, resulting in DNA fragments with lengths in multiples of approximately 200 base pairs (bp). This characteristic array of DNA fragments (the nucleosomal ladder) resulting from apoptosis has been visualized previously using slab gel electrophoresis.

In instances of non-programmed or externally-induced cell death, the lack of proper signal transduction in the pathway may not allow the specific apoptotic characteristics to be displayed [1]. Correspondingly, alternative DNA fragmentation relative

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to the nucleosomal apoptotic ladder has been reported recently [2]. One instance of externally stimulated cell death, ionizing radiation-induced DNA fragmentation, has been studied extensively [3]. Fragmentation can occur through direct ionization of the DNA molecule, or by indirect ionization processes involving water radiolysis species [4]. The ionization of an aqueous buffer medium produces hydrolysis products including hydrogen peroxide (H_2O_2), superoxide (O_2^-) and the hydrated electron. The metal-ion catalyzed conversion of O_2^- and H_2O_2 produces the highly reactive hydroxyl radical (·OH) which plays a key role in chemical reactions preceding the fragmentation of DNA in an aqueous buffer medium.

The study of cellular destruction and subsequent DNA fragmentation in vitro by natural processes, such as apoptosis or induced by external sources such as ionizing radiation, is extremely important in understanding cellular function and the lifetimes (survival rates) of various cell lines, as well as embryonic development and metamorphosis. Alternatively, the study of apoptosis may have therapeutic implications toward the control of degenerative diseases where decelerated cell death occurs, such as in cancer [5], or where accelerated cell death occurs, such as in Alzheimer's disease and human immunodeficiency virus (HIV) [6].

Traditionally, DNA fragmentation has been monitored using slab gel electrophoresis with rigid agarose or polyacrylamide anticonvective separation matrices. Separations performed in narrow-bore capillaries of 50–75 μm inner diameter (I.D.) with efficient Joule heat dissipation in capillary electrophoresis (CE) allow the use of higher applied field strengths for more rapid analyses. In addition, CE requires lower sample quantities (picograms or less) for analysis due to high mass sensitivity; is more easily amenable to on-line detection and automation of analysis; and demonstrates higher resolution of closely migrating fragments due to extremely high separation efficiencies. Therefore, CE would be an indispensable tool for the analysis and quantification of low levels of DNA fragmentation in general, and for the rapid assessment of potentially diseased states.

CE has been used previously to observe low levels of apoptotic DNA fragmentation in normal hybridoma cell cultures [7] and as a rapid means of analyzing whole digests of apoptotic immature rat thymocytes [8]. Nackerdien and Atha have shown that CE can be used to monitor ionizing radiation-induced physical changes in linear, double-stranded DNA fragments between 75 and 12 000 bp in length, in both the presence and absence of relatively high concentrations of ethidium bromide [9]. This damage was noted as peak broadening of DNA fragments larger than 1000 bp.

In this paper, we demonstrate the use of TreviSol–CE[1] (TS–CE) polymeric size-selective solution (TS–CE, patent pending) in CE for the enhanced separation of a variety of DNA degradation products. Metabolic DNA fragmentation was measured by analyzing DNA extracted from ^{60}Co γ-irradiated ML-1 human leukemia cells or by analyzing DNA extracted from human lymphoblastoid nuclei that had been treated with the DNA cleavage enzyme, micrococcal nuclease. Radiation-induced physical changes to a linear array of DNA fragments were monitored by exposing two commercially-available DNA standards, the 1 kbp ladder and the *Hae III* digest of ΦX174, to 0 to 100 gray (Gy) of ^{60}Co γ-rays.

2. Experimental[1]

2.1. Cell cultures

Human myeloblastic leukemia cells (No. GM03798) from NIGMS Human Genetic Mutant Cell Repository (Camden, NJ, USA) were grown in suspension at 37°C and 5% CO_2 and harvested in log phase for the enzymatic digestions. Cells were grown in RPMI (Roswell Park Memorial Institute) medium supplemented with 15% fetal bovine serum (Sigma, St. Louis, MO, USA), penicillin (100 U/ml) and streptomycin (100 μg/ml). ML-1 human leukemia cells were cultured under the same conditions as

[1]Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment are the best available for the purpose.

above, except for the addition of 50 μg/ml gentamycin to the RPMI medium.

2.2. Production of apoptotic DNA

ML-1 cells (10 ml) were grown in suspension until they had a density of about 10^6 cells/ml. The cells were exposed to 20 Gy ionizing radiation and then incubated for 48 h at 37°C, 5% CO_2 prior to DNA isolation. The cell density was determined and cell viability was estimated by Trypan Blue staining. The cells were then centrifuged at 100 *g* for 5 min. The cells were fixed in 10 ml of 3.7% formaldehyde in 1×PBS (phosphate-buffered saline) for 1 h. The cells were centrifuged as above, then re-suspended in 10 ml of 80% ethanol and stored at 4°C.

The cells were tested for the presence of apoptotic cells using the TACS-2 TdT in situ apoptotic detection kit (Trevigen, Gaithersburg, MD, USA) according to the manufacturer's protocol [10]. DNA from the treated cells was isolated using the TACS Apoptotic DNA Laddering Kit (Trevigen) according to the manufacturer's protocol. The isolated DNA was redissolved in 10 mmol/l Tris–EDTA buffer. The DNA:protein ratio was 1.77:1 as monitored using UV absorbance spectroscopy.

2.3. Production of enzymatic DNA fragments

Human myeloblastic leukemia cells were harvested and incubated for 15 min on ice in the presence of 10 mmol/l Tris–HCl, 10 mmol/l NaCl, 5 mmol/l $MgCl_2$, pH 7.4. The swollen cells were lysed by dropwise addition of 10% Nonidet P-40 to a final concentration of 0.5% and the released nuclei were sedimented by centrifugation at 300 *g* for 5 min. Nuclei were re-suspended in 10 mmol/l Tris, 50 mmol/l NaCl, 24 mmol/l KCl, 0.75 mmol/l $CaCl_2$, pH 7.4. Micrococcal nuclease (Boehringer Mannheim: 15 000 U) was then added at 10 U/A_{260} and the preparation incubated at 37°C for 1, 2 and 5 min, respectively. The digestions were terminated by adding 0.1 ml volumes of 0.1 mol/l EDTA, pH 8. DNA from the treated cells was isolated using the TACS Apoptotic DNA Laddering Kit (Trevigen) according to the manufacturer's protocol. The isolated DNA was redissolved in 10 mmol/l Tris–EDTA buffer. The DNA:protein ratio was 1.80:1 as monitored using UV absorbance spectroscopy.

2.4. Radiation of DNA standards

The 1 kbp and ΦX174/*Hae III* DNA standards (Life Technologies, Gaithersburg, MD, USA) were diluted with HPLC-grade water to final concentrations of 0.09 μg/μl and 0.10 μg/μl, respectively. Polypropylene vials of DNA standards were irradiated with a ^{60}Co γ-source at a dose rate of 14 Gy/min over ice. For the 1 kbp DNA ladder, 60 Gy radiation was applied for 4.1 and 15 min. The ΦX174/*Hae III* DNA standard was irradiated at 20 or 40 Gy increments at a constant time period of 4.1 min.

2.5. Capillary electrophoresis

Separations were performed using a Hewlett–Packard CE system in the reversed polarity mode, the cathode (negatively charged electrode) at the capillary inlet and the anode (positively charged electrode) at the capillary outlet. Supelco CElect-H capillaries (Bellefonte, PA, USA) with a slightly hydrophobic internal covalent capillary coating were used for electrophoretic separations. The capillary dimensions were 35.5 cm×75 μm I.D. or 47 cm×50 μm I.D.. The effective capillary lengths from the inlet to the detector window were 27 cm and 38.5 cm, respectively. Detection was by UV absorbance at 260 nm with a bandwidth of 30 nm; the reference wavelength was set at 370 nm with a bandwidth of 100 nm. The response time of the detector was set at 1.0 s. The temperature of the capillary contained within a cartridge was maintained at 30°C with a Peltier heating/cooling device. Prior to each run, the capillary was flushed with TS–CE solution [a blend of polysaccharides in Tris–Phosphate–EDTA (TPE) buffer, pH 7] (Trevigen) for 4 min at the maximum pressure of the unit, 1 bar. The capillary was post-conditioned with Bio-Rad Capillary Wash Solution (aqueous, pH 2.5) for 1 min and with HPLC-grade water for 2 min. Samples were injected without pretreatment (at least in duplicate) electrokinetically at −2.5 kV for 5 s unless otherwise noted. Separations were performed at 5000 V (35.5 cm capillary length) for a resulting field strength of 141 V/cm or

at 3000 V (47 cm capillary length) for a resulting field strength of 64 V/cm.

3. Results and discussion

The polymeric size-selective solution TS–CE was employed in the present study for the enhanced separation of a variety of DNA degradation products using CE. A related matrix, TreviGel-500 (TG-500) comprised of a blend of polysaccharides has been adapted successfully for use in CE for the separation of DNA fragments in the 100 to 10 000 bp size range with high efficiency and unprecedented resolution, especially of intermediately-sized fragments [11]. TS–CE is comprised of a similar blend of polysaccharides as in TG-500 with inherently lower solution viscosity for enhanced reproducibility of electrophoretic migration.

The migration time precision of CE separations using the TS–CE size-selective polymer solution in a coated capillary was monitored by comparing non-irradiated ΦX174/*Hae III* DNA fragment migration times in fourteen consecutive same-day analyses (Table 1). The relative standard deviations (R.S.D.) of migration times over a DNA size range of 72 to 1353 bp were less than 0.4%. This precision is better than that previously reported using hydroxyethyl cellulose in CE for 100 and 400 bp DNA fragments, also without an internal standard [12]. A typical calibration curve for the ΦX174/*Hae III* standard for sizing DNA fragments is shown in Fig. 1. A second degree polynomial curve fit provided a coefficient of determination (r^2) of 0.9995. The average error of the bp assignments over the size of fragments for the ΦX174/*Hae III* DNA standard was calculated as 1.6%. A DNA standard, either ΦX174/*Hae III* or the 1 kbp ladder, was run at least in duplicate prior to each enzymatically digested or irradiated sample. A corresponding calibration curve was then constructed from the average migration times of the standard fragments versus fragment length in bp, to account for slight differences in day-to-day run migration times due to the condition of the internal surface of the capillary.

Table 1
Migration time precision of 14 consecutive runs of the ΦX174/*Hae III* DNA standard using 0.20% TreviSol–CE in TPE buffer, pH 7 at −140 V/cm and 30°C

Fragment size (base pairs)	Average migration time (min)	Standard deviation ($n=14$)	R.S.D. (%)
72	9.420	0.0288	0.306
118	9.570	0.0307	0.317
194	9.904	0.0324	0.327
234	10.08	0.0338	0.335
271	10.28	0.0357	0.348
281	10.31	0.0354	0.343
310	10.44	0.0357	0.342
603	10.68	0.0357	0.306
872	12.71	0.0339	0.267
1078	13.42	0.0357	0.266
1353	14.21	0.0485	0.341

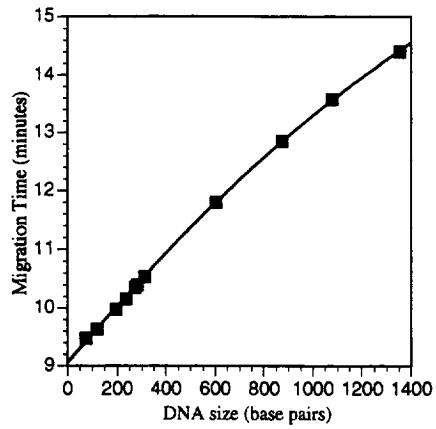

Fig. 1. Calibration curve of migration time in minutes versus DNA size in base pairs for the ΦX174/*Hae III* standard. The second degree polynomial curve fit provided a coefficient of determination (r^2) of 0.9995.

3.1. Measurement of fragmentation of whole cell DNA generated by externally-induced apoptosis

ML-1 tissue cell culture was exposed to 20 Gy γ-radiation with a subsequent incubation period of 48 h and then the DNA was isolated from the cells. UV absorbance spectra of the extracted DNA revealed low protein contamination with a ratio of absorbances at 260 nm and 280 nm (A_{260}/A_{280}) equal to 1.77 (data not shown). Protein contamination would alter electrophoretic migration of the fragments, precluding the use of DNA standards to accurately size fragments. The electrophoretic sepa-

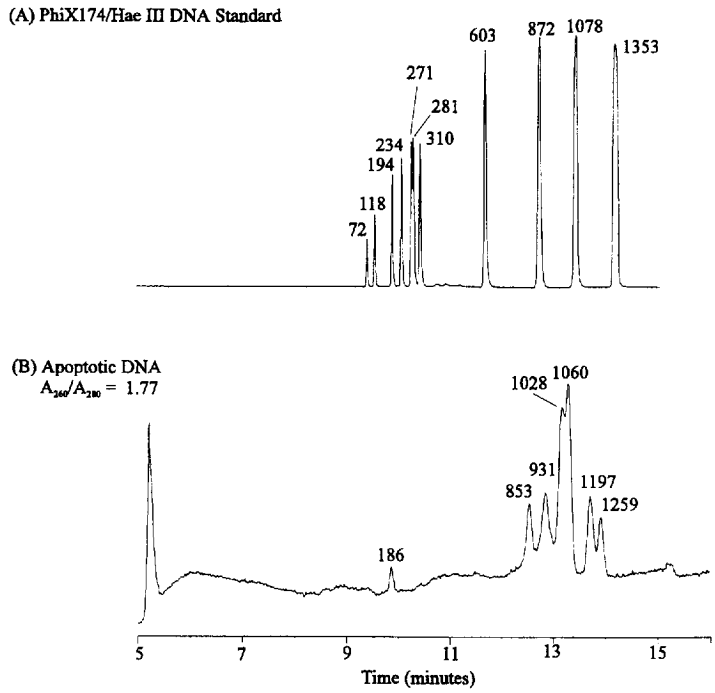

Fig. 2. Electrophoretic separation of (A) the ΦX174/*Hae III* DNA standard and (B) DNA fragments isolated from ML-1 tissue culture cells exposed to 20 Gy of γ-radiation from X-rays at 30°C, electrophoresed at −5 kV (−141 V/cm), in a 35.5 cm×75 μm Supelco CElect-H coated capillary using 0.20% TS–CE matrix solution, pH 7.0.

rations (run consecutively) of this sample and the ΦX174/*Hae III* DNA standard are shown in Fig. 2. Apoptotic DNA fragments were sized according to a calibration plot similar to Fig. 1, of average peak migration time versus fragment size of the ΦX174/*Hae III* DNA standard (run in duplicate). The calculated fragment sizes are indicated in Fig. 2. Several peaks representing distinct populations of DNA fragments in the 800 to 1300 bp size range were observed. In addition, an isolated peak corresponding to 186 bp was detected. The peak observed just after 5 min was outside of the calibration range and has not been identified definitively.

The peak broadness observed in Fig. 2B may be related to the nature of the nucleosomal cleavage process in general. Internucleosomal cleavage is spatially specific and not sequentially specific. Therefore, a narrow distribution of fragments closely related in size may correspond to a single nucleosome monomer or oligomer. Evans et al. observed comparable peak broadness of DNA digestion products of whole apoptotic rat thymocytes relative to co-injected ΦX174/*Hae III* DNA standard in CE using hydroxyethyl cellulose as the separation matrix [8]. These cells had been treated with either the synthetic glucocorticoid dexamethasone or topoisomerase II inhibitor etoposide (VP-16) in order to induce apoptosis.

In contrast to the results presented in Fig. 2B, Evans et al. observed the nucleosomal "ladder" for chemically treated immature rat thymocytes; these authors observed DNA fragments increasing in size, approximately 190, 380, 570 and 760 bp. Higher nucleosomal multiples were not resolved electrophoretically. The difference in results presented here may be related to the differences in the cell type investigated and/or the apoptotic induction method employed. Nucleosomal spacing is known to vary considerably between organisms, as well as within the chromatin of an individual cell type; nucleosomal repeat lengths may range from 140 to 240 bp [13]. The ML-1 cells used in this investigation were observed to be apoptotic after treatment by in situ analysis (data not shown). The regularity of DNA

fragment sizes depicted in Fig. 2B suggests that apoptosis occurred. The 186 bp fragment is believed to represent the average size in bp of differently sized mononucleosomes present; the peak broadness may indicate a distribution of fragment lengths. The base pair assignments in the 800 to 1300 bp size range may represent higher multiples of two different nucleosomes, indicating incomplete internucleosomal cleavage during irradiation. The peak assignments at 853, 1028 and 1197 bp, correspond to DNA fragments existing as 5-, 6- and 7-mers of a 170 bp nucleosome. The peak assignments at 931, 1060 and 1259 bp correspond to DNA fragments existing as 5-, 6- and 7-mers of a 180 or a 190 bp nucleosome. The peak that occurs just after 5 min may represent smaller internucleosomal fragments (10 to 50 bp in length) resulting from the action of the nucleases beginning to cut chromatin, as suggested previously by Solis-Recendez et al. [7].

3.2. Measurement of fragmentation of whole cell DNA generated by micrococcal nuclease digestion

Intact lymphoblastoid nuclei were digested with micrococcal nuclease at 37°C for 1, 2 and 5 min time periods. The DNA that was isolated from the nuclei contained extremely low protein contamination with a DNA:protein ratio of 1.80:1. The electropherograms of the DNA digestion products and the ΦX174/*Hae III* standard are shown in Fig. 3. The peaks in Fig. 3B–D between 9 and 15 min, corresponding to the DNA digestion products, were not fully resolved but were highly reproducible. The 1 and 2 min digestion samples yielded similar electrophoretic

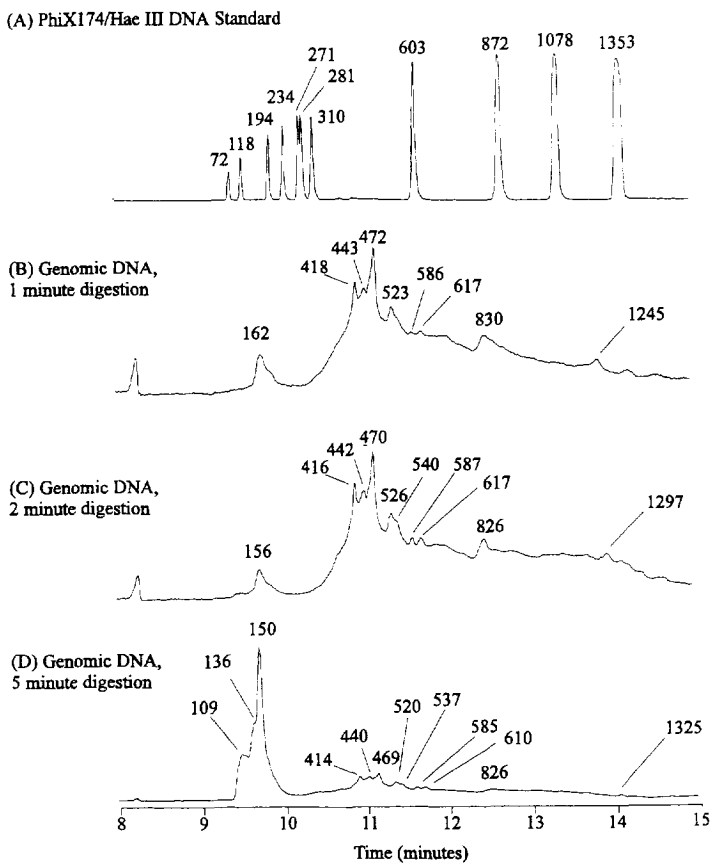

Fig. 3. Electrophoretic separation of (A) the ΦX174/*Hae III* DNA standard and cellular lymphoblastoid DNA digested with micrococcal nuclease enzyme for (B) 1 min, (C) 2 min and (D) 5 min. Separation conditions are the same as in Fig. 2.

separations with a broad distribution of fragments ranging from 400 to 1300 bp in size. After 5 min of enzymatic digestion, the larger fragments of 400 to 1300 bp were lower in proportion relative to the 160 bp fragment peak and two additional fragments of approximately 109 and 136 bp.

The complete nucleosomal ladder was not observed in Fig. 3B–D. The high reproducibility of base pair assignments between the 1, 2 and 5 min treated samples and the periodicity in fragment sizes, however, suggest that the fragmentation process was not random. Using two-dimensional slab gel electrophoresis, Todd and Garrard observed five mononuclosome classes for both bovine thymus cells and transformed mouse cells, upon treatment with micrococcal nuclease [13]. The mononucleosomes present in the investigations of these authors ranged in size from 140 to 205 bp, with approximately 10 bp increments. In Fig. 3B–C, the peaks corresponding to approximately 160 bp most likely represent the average size in bp of all nucleosomes present for this particular cell type. The 136 bp fragment in Fig. 3D may correspond to the further degradation of a single nucleosome to a core fragment of approximately 140 bp, where several bp fragments have been cleaved from each end [2]. A DNA fragment of approximately 100 bp also present in Fig. 3C has been cited previously during apoptosis in hybridoma cell cultures [7]. The peaks observed between 400 and 1300 bp would correspond to higher multiples of the various nucleosomes. As a result of multiple mononucleosomal sizes, a broad distribution of peaks is observed. It is anticipated that this distribution of partially resolved peaks observed using CE would have appeared as a "smear" using slab gel electrophoresis. Previously, a two dimensional slab gel electrophoresis method was necessary to reveal the various classes of mononucleosomes in mammalian nuclear chromatin [13]. The high resolving capabilities of CE in one-dimension of separation enable identification of specific fragment sizes, and hence further insight into the nature of such fragmentation processes.

3.3. Measurement of fragmentation of small-fragment DNA standards generated by irradiation

The *Hinf I* fragments of the 1 kbp DNA ladder were exposed to 60 Gy γ-radiation for 4.1 and 15 min time periods. The electropherograms for these two exposed standards and the corresponding unexposed standard are shown in Fig. 4. The application of 60 Gy γ-radiation for 4.1 min increased corresponding peak-width-at-half-height ($w_{1/2}$) values by a factor of two or three for fragments less than 400 bp, relative to the unexposed standard (Fig. 4B). Partial resolution of the 506 and 517 bp fragments was not observed for the 4.1 min irradiated standard. The application of 60 Gy γ-radiation for 15 min caused increased degradation of the fragments, as evidenced by enhanced peak broadening and lower resolution of fragments relative to the unexposed standard (Fig. 4C).

We attribute this band broadening phenomenon to the presence of a distribution of DNA fragments, closely related in size. These fragments were presumably created by a variety of radiation-induced physical and chemical processes that could alter the size, shape and charge of the fragments. These radiation-induced processes may include cyclization, derivatization and untwisting at ends of DNA fragments. Longer exposure to radiation caused significant degradation of the smaller fragments, 75 to 220 bp in size; an extremely broad distribution of peaks was observed in this corresponding size range. The $w_{1/2}$ values were not proportional to fragment length in bp (data not shown). This suggests that the ionizing radiation induced fragmentation in a non-random, possibly sequence-dependent fashion. For both irradiated standards, the general electrophoretic migration pattern of the original *Hinf I* fragments was discernible.

Next, the ΦX174/*Hae III* standard was exposed to various dosages (0–100 Gy) of ^{60}Co-γ ionizing radiation for a constant time period of 4.1 min (Fig. 5). In monitoring degradation of DNA fragments less than 600 bp in length at 20, 40 and 60 Gy radiation, a general increase in $w_{1/2}$ was observed. In addition, two new peaks corresponding to two new subpopulations of DNA fragments, approximately 200 and 240 bp in length, were observed and are indicated by arrows in Fig. 5. The bp assignments of these newly observed peaks, were calculated based on a calibration curve similar to that shown in Fig. 1. The exposure of the smaller fragments to 100 Gy radiation resulted in an extremely broad, unresolved

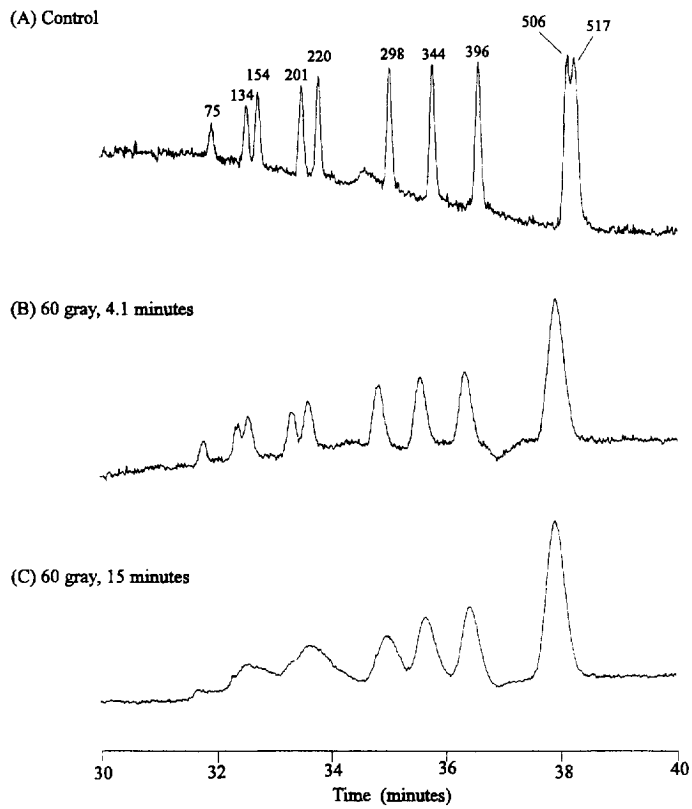

Fig. 4. Effect of γ-radiation on the *Hinf I* fragments of the 1 kbp DNA standard, (A) unexposed standard, (B) 60 Gy exposure for 4.1 min and (C) 60 Gy exposure for 15 min. The samples were analyzed at 30°C and electrophoresed at −3 kV (−64 V/cm), in a 47 cm×75 μm Supelco CElect-H75 coated capillary using 0.20% TS–CE matrix solution, pH 7.0.

distribution of fragments (Fig. 5E). The peaks corresponding to the four largest fragments are in significantly lower proportion relative to the smaller fragments.

This observation of new discrete subpopulations of DNA fragments is unprecedented, since previous studies in CE using hydroxyethyl cellulose as the separation matrix were only able to discern damage in DNA fragments larger than 1000 bp in length [9]; no visible damage of the smaller fragments was observed. The reproducibility and distinctness of the new subpopulations upon exposure to 20–60 Gy radiation is most likely related to energetically favorable cleavage as opposed to sequentially specific radiation-induced DNA fragmentation.

At higher radiation exposure, fragments larger than 500 bp may be more prone to radiation-induced crosslinks and strand breaks. This is a reasonable presumption from a statistical standpoint with a greater probability of radiation damage sites in larger fragments. From the reproducibility study cited previously (Table 1), the $w_{1/2}$ values for the 14 consecutive runs were plotted as a function of DNA fragment size in bp for fragments larger than 500 bp (Fig. 6). The corresponding data for the irradiated DNA standards in Fig. 5 were averaged for multiple injections and plotted concomitantly in Fig. 6. The reproducibility study data were used to establish statistically significant peak broadening for this standard due to DNA fragmentation. The error bars in this figure represent standard deviations in minutes. In the majority of instances, the average $w_{1/2}$ values for the fragments larger than 500 bp are outside of the range of the unexposed standards, demonstrating statistically significant peak broadening due to exposure to γ-radiation.

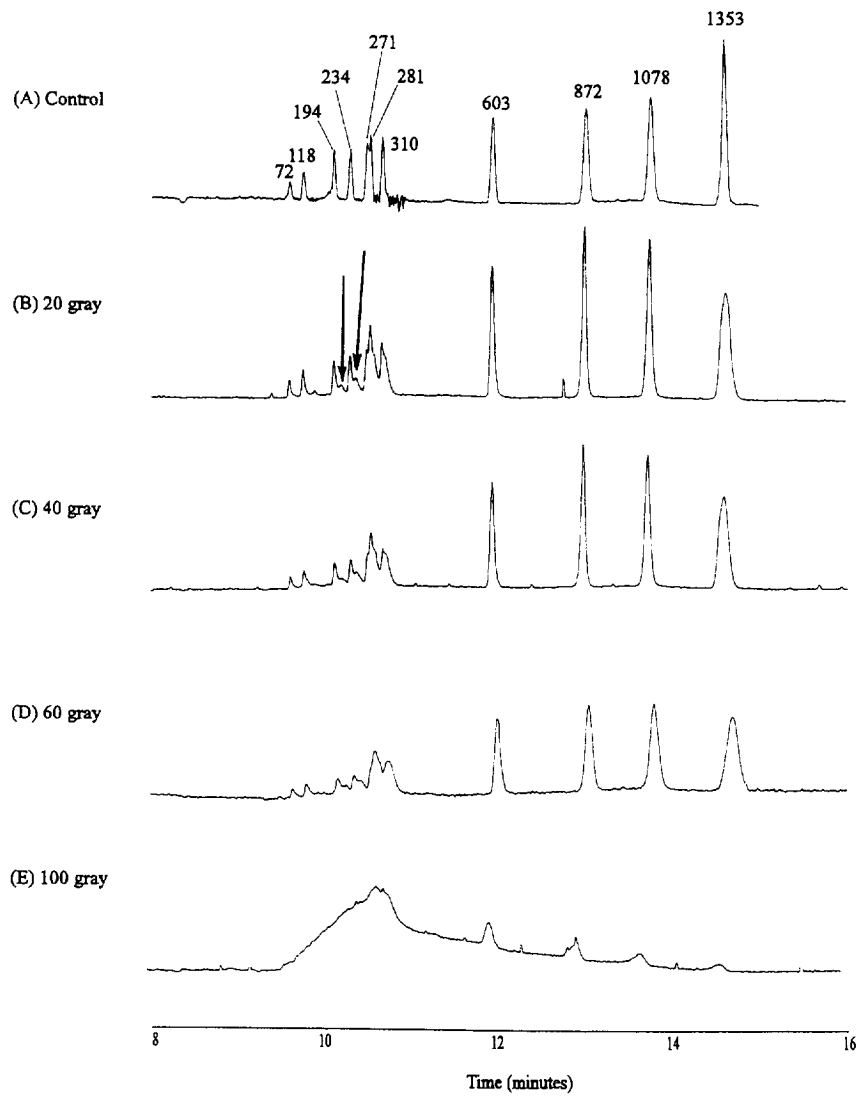

Fig. 5. Effect of γ-radiation on the ΦX174/*Hae III* DNA standard. (A) unexposed control, (B) 20 Gy exposure for 4.1 min, (C) 40 Gy exposure for 4.1 min, (D) 60 Gy exposure for 4.1 min, (E) 100 Gy exposure for 4.1 min. Separation conditions are the same as in Fig. 2.

The corresponding peak area data in Fig. 5 for the 20, 40 and 60 Gy irradiated samples as well as the peak area data from the reproducibility data in Table 1, were plotted as a function of DNA fragment size for fragments larger than 500 bp (Fig. 7). Peak area values were calculated using the Hewlett–Packard data analysis software and the values were averaged for multiple injections. The relatively large standard deviations for peak area values, represented by error bars in this figure, are related to the use of electrokinetic injection that generally provides higher peak efficiencies, but lower peak area reproducibilities versus hydrodynamic injection. These data quantify the DNA fragments remaining after irradiation, and complement the more qualitative data presented in Fig. 6.

As expected, peak area for each sample increased with DNA fragment size due to the presence of a greater number of DNA chromophores. However, peak area decreased for each fragment as the dosage

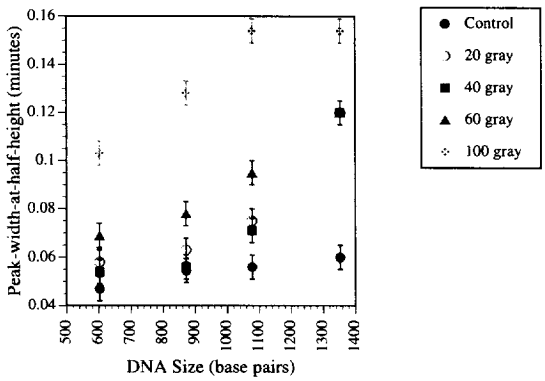

Fig. 6. Peak-width-at-half-height ($w_{1/2}$) as a function of DNA size in base pairs for unexposed ΦX174/*Hae III* DNA standard fragments from the reproducibility study in Table 1 and fragments of the standard exposed to 20, 40, 60 and 100 Gy radiation doses. Error bars indicate standard deviations.

of radiation was increased. The loss in peak area was calculated relative to the control and averaged for each fragment size; approximately 22, 40 and 74% loss in peak areas were observed due to 20, 40 and 60 Gy radiation exposure, respectively. The latter phenomenon is due presumably to the progressive loss of DNA chromophores in the form of significantly smaller DNA fragments at higher and higher radiation dosages.

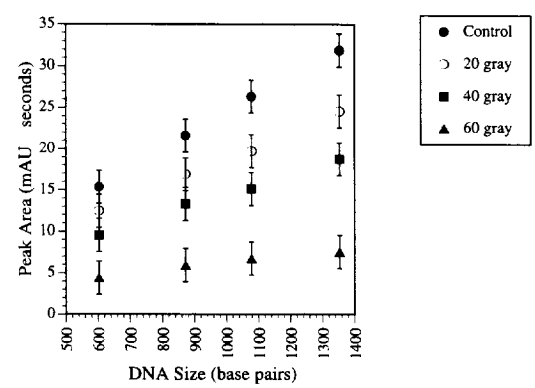

Fig. 7. Peak area (mAU s) as a function of DNA size in base pairs for unexposed ΦX174/*Hae III* DNA standard fragments from the reproducibility study in Table 1 and fragments of the standard exposed to 20, 40 and 60 Gy radiation doses. Error bars indicate standard deviations.

4. Conclusions

Ionizing radiation and enzymatic digestion were used independently to induce apoptotic fragmentation in human chromatin. Separation of DNA fragments using dynamic size-sieving CE generated nonrandom fragmentation patterns that were dependent on cell type and method of apoptotic induction. These data suggest the presence of multiple mononucleosomal fragment sizes in each instance. Future investigations will involve the analysis of apoptotic DNA fragmentation in a variety of cell types and use of a variety of external stimuli in order to further the understanding of the metabolic process of apoptosis.

For γ-irradiated commercial DNA standards, specific and random subpopulations were observed as a function of time and radiation dosage. Peak-width-at-half-height in dynamic size-sieving CE was used to assess the presence of various DNA fragment alterations, relative to an unexposed standard. Peak area values were used to quantify the significant (up to 74%) loss of DNA, supposedly due to irradiation, relative to unexposed standard.

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