Vol. 108, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 30, 1982 Pages 576-584

NUCLEAR DEOXYRIBONUCLEASE ACTIVITIES IN NORMAL AND XERODERMA PIGMENTOSUM LYMPHOBLASTOID CELLS

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Received August 6, 1982

Deoxyribonuclease activities were examined in isoelectric focusing fractions of non-histone chromatin-associated and nucleoplasmic proteins of isolated nuclei of normal human and xeroderma pigmentosum, complementation group A, lymphoblastoid cells using parallel procedures. In the nucleoplasm of both cell lines, a very similar series of both DNA endo- and exo-nuclease activities were found; in chromatin a series of similar endonuclease but no exonuclease activites were present. Several differences were observed in the xeroderma pigmentosum cells, however, notably a striking increase in DNA endonuclease activity in a chromatin fraction at pI 4.6 against linear duplex DNA and a decrease in a chromatin endonuclease activity focusing at pI 7.8.

INTRODUCTION

The autosomal recessive disease, xeroderma pigmentosum (XP), is characterized by marked sensitivity to ultraviolet (UV) light and by the development of secondary skin changes and of large numbers of skin cancers early in life (1-4). Extensive studies have shown that most cases of XP are associated with a cellular defect in the initial, incision step in excision repair of thymine cyclobutane dimers, the chief photoproduct of UV light in DNA (1-6). In addition, cultured XP cells have been shown to be defective in excision repair of damage produced by certain drugs and chemical carcinogens (4,7-13). To date, no specifically defective excision repair enzyme has been isolated or purified in XP cells even though a defective apurinic endonuclease activity has been reported in extracts of XP cells (14,15). UV light specific DNA endonucleases have been partially purified in several types of mammalian cells (16-18); however no deficient activity has yet been identified in XP cells.

We have recently developed a system for simultaneously examining multiple DNA endonucleases in mammalian cell nuclei, and have found a series of such

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Abbreviations: XP, xeroderma pigmentosum; UV, ultraviolet; XPA, XP (complementation group A).

0006-291X/82/180576-09$01.00/0

nucleases in the chromatin proteins of mouse melanoma cells (19). We now report a similar analysis of DNA endonucleases as well as exonucleases in nuclei of normal human lymphoblastoid cells and lymphoblastoid cells from a patient with XP, complementation group A (XPA), in which several abnormalities appear to be present.

MATERIALS AND METHODS

Normal (GM 1989) and XPA (GM 2345) lymphoblastoid cell lines (transformed with Epstein-Barr virus) were obtained from the Institute for Medical Research, Camden, New Jersey. The cell lines were grown in suspension culture at 37°C in sealed flasks in RPMI 1640 medium buffered to physiological pH with Hepes' buffer (Grand Island Biological Co., GIBCO), supplemented with 15% fetal calf serum (GIBCO), and were harvested under conditions of maximal proliferation. Cultures were routinely tested for mycoplasma (20) and steps were taken to insure that the cells were not exposed to UVL and that other light exposure was minimal. Cell nuclei were isolated following the method of Berkowitz et al. (21). Chromatin-associated and nucleoplasmic proteins were extracted from the purified nuclei as previously described (19,22) and subjected to isoelectric focusing (19). Fractions collected from the isoelectric focusing columns were assayed for DNA endonuclease and exonuclease activity against calf thymus DNA (19,23) and ^{3}H-poly d(A-T) (24) respectively. Peaks of endonuclease activity were pooled, dialyzed into 50 mM potassium phosphate, pH 7.1, 1 mM dithiothreitol, 1 mM Na_2EDTA, 40% ethylene glycol, and stored unfrozen at -20°C. The protein content of each fraction was determined by the method of Lowry et al (25).

DNA endonuclease activity was measured using a DNA polymerase primer assay and linear calf thymus DNA (Worthington Biochemical Corp) as substrate (19,23). DNA endonuclease activity was also assayed using circular, duplex, supercoiled PM2 bacteriophage DNA as substrate. The enzymatically treated PM2 DNA was subjected to electrophoresis on 0.9% agarose gels which were subsequently stained with ethidium bromide, photographed under ultraviolet light (19) and the negatives scanned using a Zeineh Soft Lazer Scanning Densitometer (LKB). Endonuclease activity, as measured by the number of enzyme-induced breaks per DNA molecule, was detected by the conversion of superhelical DNA (Form I), to nicked circular DNA (Form II) and linear unit length DNA (Form III). Quantitation of the amount of cut versus superhelical DNA was made from integral analysis of densitometric tracings of negatives of photographs of the gels, and the number of breaks per DNA molecule was estimated by the Poisson formula, number of breaks = -ln x, where x = fraction of superhelical molecules (26,27).

DNA exonuclease activity was assayed according to the method of Lindahl et al. which measures the ability of the enzyme to convert radioactively labeled DNA to acid soluble products (24).

All experiments were repeated 6-8 times using enzymes obtained from 6-8 different cell extractions.

RESULTS

A series of eight clearly separable DNA endonuclease activities active against native calf thymus DNA, but no DNA exonuclease activity, was found upon isoelectric focusing of normal human and XPA lymphoblastoid cell chromatin (Fig. 1). The activities were very similar between the two cell lines except for a major increase in activity in XPA cells focusing at pI 4.6 and a lesser decrease in activity in XPA cells at pI 7.8. XPA activities at pI 4.1 and at pIs 6.3 and 6.8 appear to correspond to normal activities at pIs 3.8 and 4.1 and at pI 6.6, respectively.

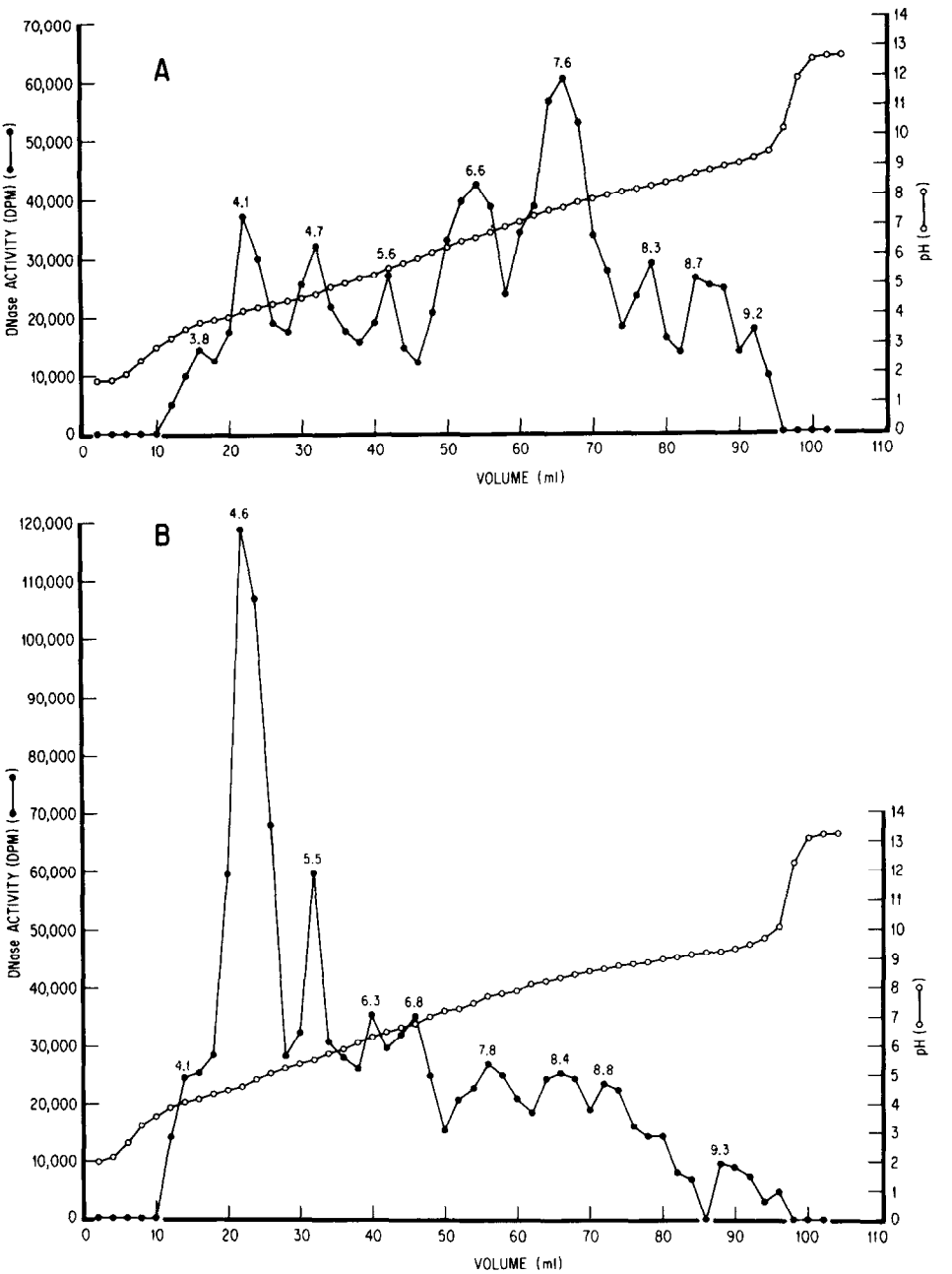

Figure 1: Isoelectric focusing patterns of chromatin-associated DNA endonucleases from : A, normal human and B, XP lymphoblastoid cells. ●—● , DNase activity on calf thymus DNA; ○—○ , pH gradient.

Fractions of chromatin-associated proteins were pooled according to their DNA endonuclease activities against calf thymus DNA, and each pooled fraction was tested for activity against PM2 DNA (Fig. 2). Fractions at lower pIs were much more active than those at higher pIs, but the XPA fractions at lower pIs were less active than the corresponding normal fractions. In particular, the

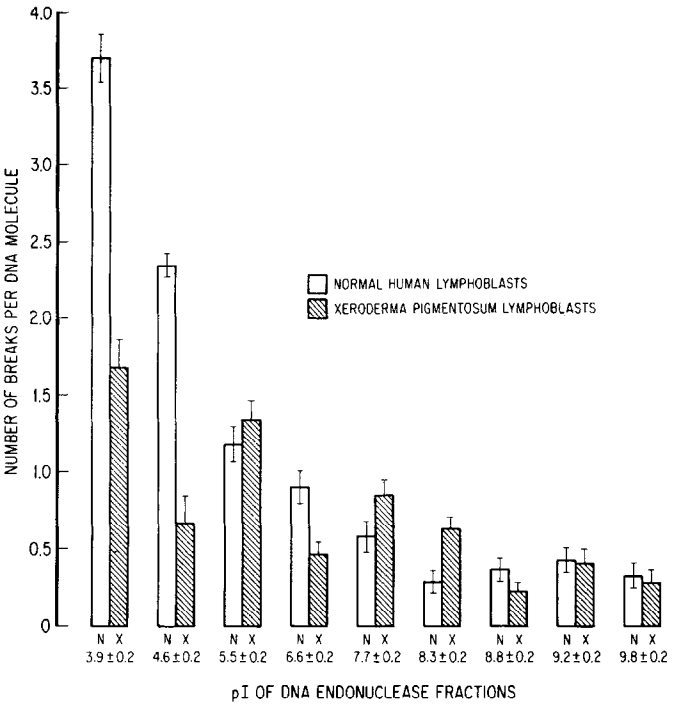

Figure 2: Activities of chromatin-associated DNA endonuclease fractions (35 ug) incubated with PM2 DNA (0.15 ug) for 3 hours at 37°C. The number of breaks in non-enzymatically treated DNA has been subtracted from each value. Vertical lines represent ± standard error of the mean.

XPA activity at pI 4.6, which was markedly increased against calf thymus DNA, was markedly depressed against PM2 DNA compared with that of the corresponding normal fraction.

Isoelectric focusing of the normal and XPA nucleoplasmic fractions revealed a series of both DNA endo- and exo-nuclease activities (Fig. 3) which were quite similar between the two cell lines except for an increase in the XPA endonuclase activity at pI 4.6, a decrease at pIs 6.7, 7.7 and 8.3, and a decrease in the XPA exonuclease activity at pI 7.6. Each pooled nucleoplasmic fraction that did not have exonuclease activity (i.e., those with lower pIs) was assayed against PM2 DNA (Fig. 4). All three activities assayed were quite similar between the two cell lines, including a markedly elevated activity at pI 4.6.

The protein content of each pooled fraction was measured and compared with its DNA endonuclease activity against calf thymus DNA. The relative specific activities of the various pooled fractions do not markedly differ from the relative absolute activites, indicating that the differences observed between the two cell lines, particularly in the chromatin fraction at pI 4.6, are not due to major differences in bulk protein content of the respective fractions.

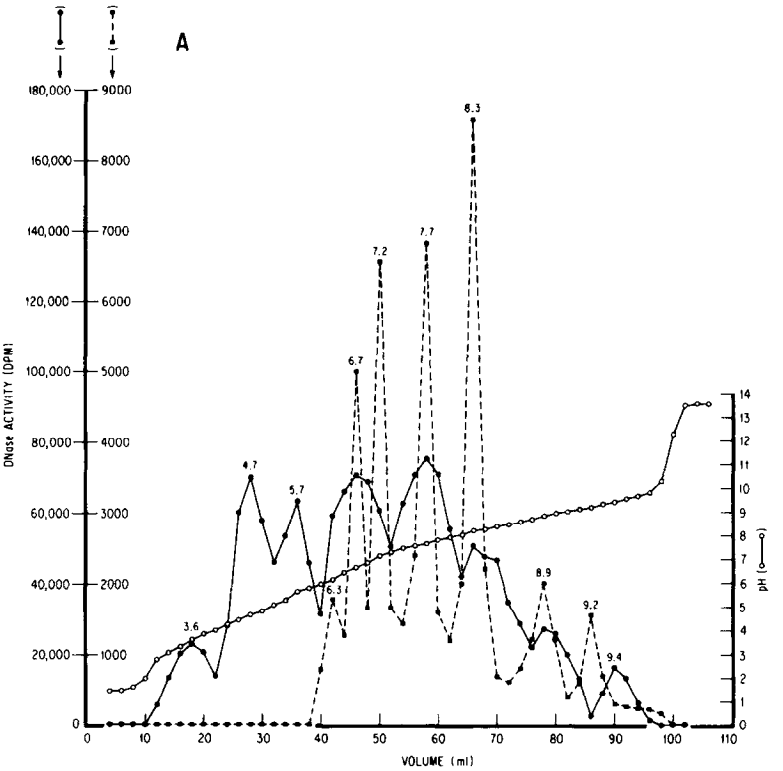

Figure 3: Isoelectric focusing patterns of nucleoplasmic DNases from: A, normal and B, XP lymphoblastoid cells. ●——● , DNA endonuclease activity on calf thymus DNA; ●- -● , DNA exonuclease activity on poly d(A-T); o——o , pH gradient.

DISCUSSION

The data presented here show several striking differences between normal and XPA nuclear DNases, particularly DNA endonucleases. The chromatin-associated DNA endonuclease activity at pI 4.6, which shows the most marked differences in XPA cells, is especially interesting because this fraction shows a marked increase in activity against calf thymus DNA and a decrease in activity against PM2 DNA. It is possible that the discrepancy in activity using the two different types of substrate may be due to differing requirements for optimal incubation conditions by the enzymes, to variable ability of the enzymes to act on supercoiled or otherwise distorted DNAs, or to a requirement of the enzymes for substrate sequences not present in PM2 DNA. It is of interest that we have recently found this fraction to have markedly increased activity against anthramycin treated DNA in normal cells, and that this increased activity is completely absent in XPA cells (28).

Although XP cell lines from the same complementation groups have been shown to be heterogeneous in their ability to repair certain types of alkylated DNA (11,29), and transformation with SV40 virus may induce a defect

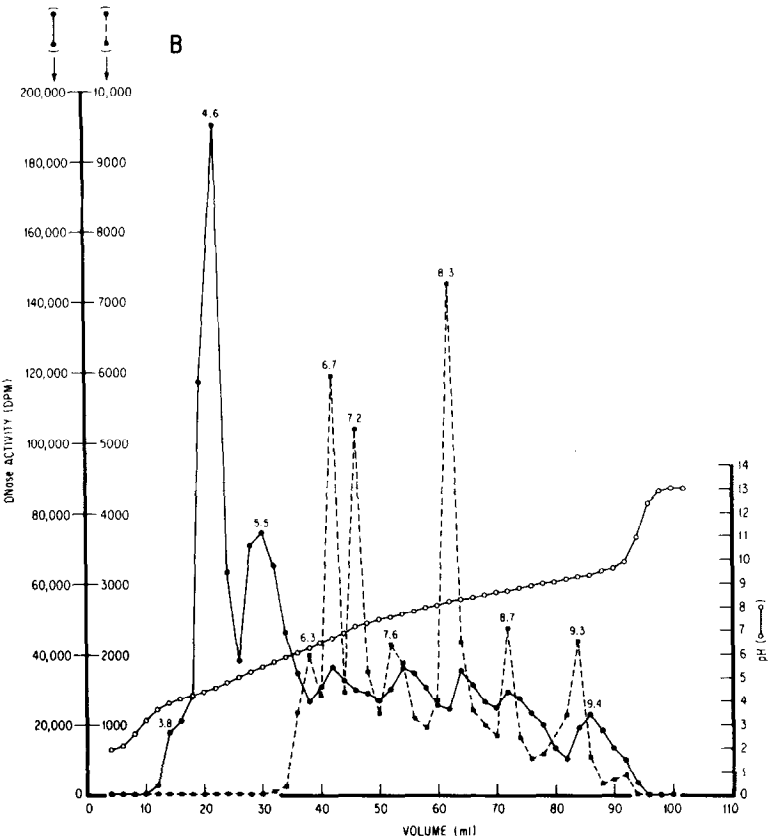

Figure 3--Continued

in repair of such DNA (12), these variations appear to be essentially restricted to O^6 alkylguanine residues (1). Moreover, both the increase in XPA DNA endonuclease activity of the chromatin fraction with pI 4.6 against calf thymus DNA and the decrease at pI 7.8 are at least as great as any quantitative difference we have previously observed even when comparing DNases from such disperate sources as human lymphoblastoid and mouse melanoma cells (Lambert et al, in preparation). We therefore believe that these differences are significant.

Whether, and in what ways, the chromatin and nucleoplasmic nuclease activities observed here may be related is not clear at present. It is possible that some of these may represent modified versions of the same proteins, which, if true, would itself be of interest, since such modifications are likely to affect both enzyme activities and their control mechanisms. Also, certain of these DNase activities may be similar to several previously reported mammalian DNases. For example, the chromatin-associated endonuclases at pI 4.7 in normal human and pI 5.5 in XP lymphoblastoid cells may be similar to chromatin-associated endonucleases from rat small intestinal

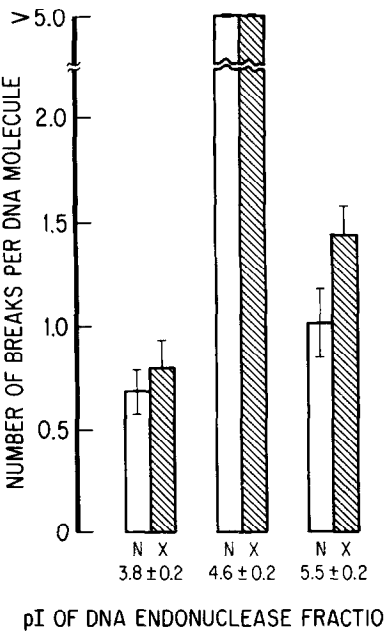

Figure 4: Activities of nucleoplasmic DNA endonuclease fractions (25 ug) incubated with PM2 DNA (0.15 ug) for 3 hours at 37°C. The number of breaks in non-enzymatically treated DNA has been subtracted from each value. Vertical lines represent ± standard error of the mean. □ normal human and ▨ xeroderma pigmentosum lymphoblastoid cells.

mucosa, pI 4.7, and from HeLa cells, pI 5.1 ± 0.2, which create single- and double-strand breaks in DNA (22,30). These fractions, along with the nucleoplasmic fractions at pI 4.6/4.7, may also be similar to DNA endonucleases which have been isolated from several mammalian cell types which show partially site-specific cleavage of duplex DNA and produce single and double strand cuts (31-34). The chromatin-associated endonuclease activities at pI 8.3/8.4 and 8.7/8.8 in the two lymphoblastoid cell lines may be similar to a chromatin-associated DNA endonuclease from bovine intestinal mucosa, pI 8.5 ± 0.1, which produces only single-strand scissions in duplex DNA (35). In addition, the activity at pI 6.6 shares some similarities with a single-strand specific endonuclease from KB cells, pI 6.4 ± 0.2 (36).

To date, no specific DNA endonuclease has been isolated and purified from XP cells which is different from that isolated from normal cells, although DNA repair abnormalities are known in XP cells (1-13). There is, moreover, some evidence that a defective nuclear protein other than a DNA endonuclease may be present in XP cells (37). The abnormalities in nuclear DNA endonuclease activities against undamaged DNA in XPA cells described here, particularly those at pIs 4.6 and 7.8 of chromatin, indicate that molecular changes in XP may be more extensive than has been previously suspected. These changes, whether primary or secondary, may account for these apparently conflicting results as well as for such changes in XPA as neurological defects and poor

growth of cells in culture under certain limiting conditions (Lambert et al, in preparation) that are not clearly related to defective DNA repair.

ACKNOWLEDGEMENTS

This work was supported in part by Basil O'Connor Grant #5-287 from the March of Dimes Birth Defects Foundation, the Dermatology Foundation and the Foundation of the UMDNJ.

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