CHROMATIN-ASSOCIATED DNA ENDONUCLEASE ACTIVITIES IN HeLa CELLS[1]

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Summary: Isolated HeLa nuclei were treated with buffer containing 0.14 M NaCl to remove nucleoplasmic DNase activities. A 1.0 M NaCl extract of the residue contained DNase activity, which was separated by column chromatography on DEAE-cellulose into four fractions, each with DNA endonuclease activity. The fractions differ in thermostability and in sensitivity to a variety of inhibitors.

Introduction

Initiation of DNA replication may be controlled by DNA endonucleases (1, 2), which by "nicking" the supercoiled double helix provide free 3'-OH end groups for DNA and RNA polymerase action, permit relaxation of the tertiary twists of DNA which hinder the access of enzymes during replication, and allow unwinding of the replicating portion of the genome. One approach towards identification of enzymes which have this function is to examine DNase activity associated with nuclei of eukaryotic cells, on the assumption that localization of these enzymes in proximity to intact DNA signifies a role in replication. A number of such DNases have been found, e.g. in the nuclei obtained from rat liver (3-7), calf thymus (8),

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mouse liver (9), the sea urchin embryo (10), and from cells cultured from mouse embryo (11), but reports from different laboratories show little consistency regarding the properties, nature of the nucleolytic activity and intranuclear localization of these enzymes. The evidence is strong that at least two DNases are present in the nucleoplasm of rabbit bone marrow (12, 13) and HeLa cells (14), but the question remains whether more than one DNase is present in the chromatin. This report presents evidence that four DNA endonucleases, all of which can increase the priming activity of DNA for DNA polymerase, can be demonstrated in HeLa chromatin.

Materials and Methods

HeLa S3 cells were cultivated in suspension under conditions standard in this laboratory (15). Mycoplasmal contamination was excluded by weekly tests (16).

Nuclei were isolated from 10^{10} exponentially growing cells and freed from cytoplasmic contamination by the detergent method of Berkowitz et al (17). The nuclear pellet was extracted three times with 0.14 M NaCl in TED (0.05 M Tris HCl, 1 mM Na_2EDTA, 1 mM DTT) at pH 7.5 and the extract retained for other studies. The residue was solubilized in 1.0 M NaCl in TED pH 7.5, which was then diluted to 0.14 M NaCl with TED to precipitate the nucleohistones. The dialyzed supernatant fluid was applied to a column of CM-Sephadex as described previously (14). The column wash fractions containing DNase activity were combined, dialyzed against TED pH 7.1 containing 40% ethylene glycol and applied to a DEAE-cellulose column (7 cm long, 0.5 cm diameter) equilibrated with the same buffer. Elution was accomplished with a linear gradient of 0-0.5 M NaCl in equilibration buffer (total volume 60 ml), and 1.5 ml fractions were collected.

DNase activity was assayed by the ability of the enzyme preparation to increase the priming efficiency of calf thymus DNA (Worthington) in a subsequent DNA polymerase (M. lysodeikticus, Sigma) reaction (18). One unit of DNase activity was defined as the amount of enzyme in the assay system (18) which increases priming activity of DNA by 100% in 3 hrs. RNase activity was measured by the extent of hydrolysis of [^{3}H]poly rC (Specific activity 15 μCi per μmole Pi) and [^{3}H]poly rA (Specific activity 85.6 μCi per μmole Pi) both obtained from Miles Labs, using 6 mM uranyl nitrate-0.4 M $HClO_4$ as precipitant. DNA polymerase activity was estimated by the method of Fansler and Loeb (19). Yeast transfer RNA and N^6, O^2-dibutyryladenosine-3'-5'-cyclic phosphate were obtained from Calbiochem.

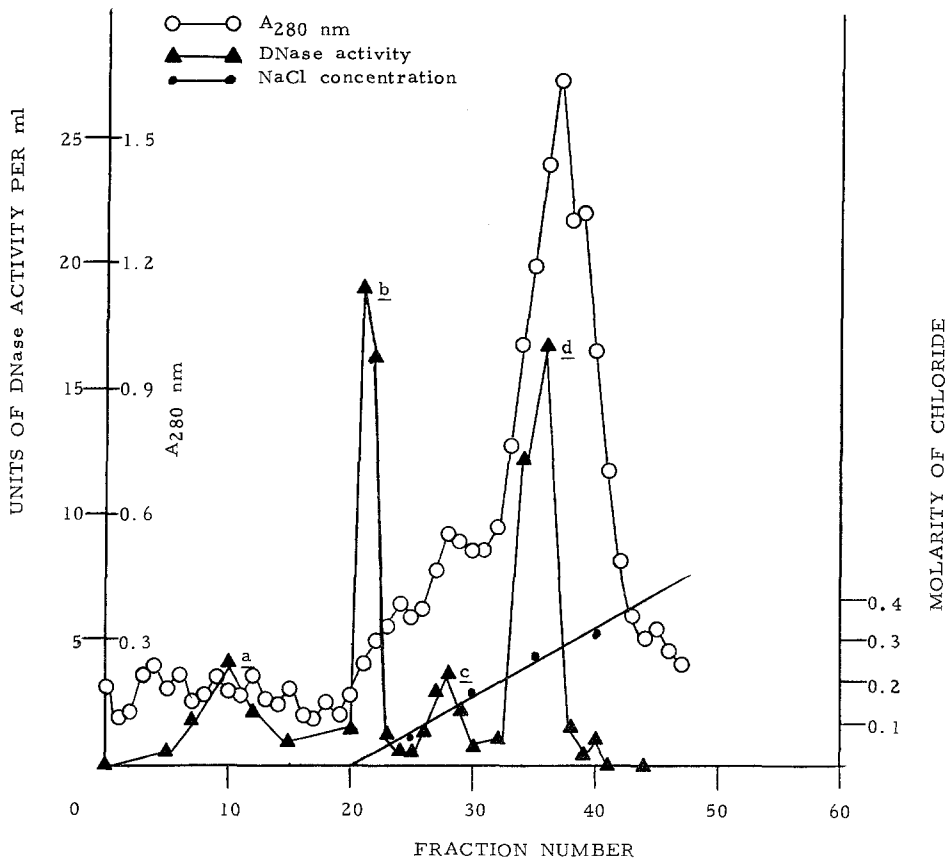

Figure 1: Elution from DEAE-cellulose of chromatin-associated DNase activity of HeLa cells. 2800 units (4.6 mg protein) of DNase activity A (ref. 14) were applied; enzyme recovery was 50.1%.

Results

DNase activity extracted from chromatin of exponentially growing HeLa cells was not retained by CM-Sephadex, and eluted as a single run-through peak, previously referred to as DNase A (14). When this material was applied to DEAE-cellulose, four peaks of DNase activity were obtained, two minor (a and c) and two major (b and d) (Fig. 1). Peak a was not the result of column overloading, since when reapplied to DEAE-cellulose it again eluted in the buffer wash. Chromatography of another portion of

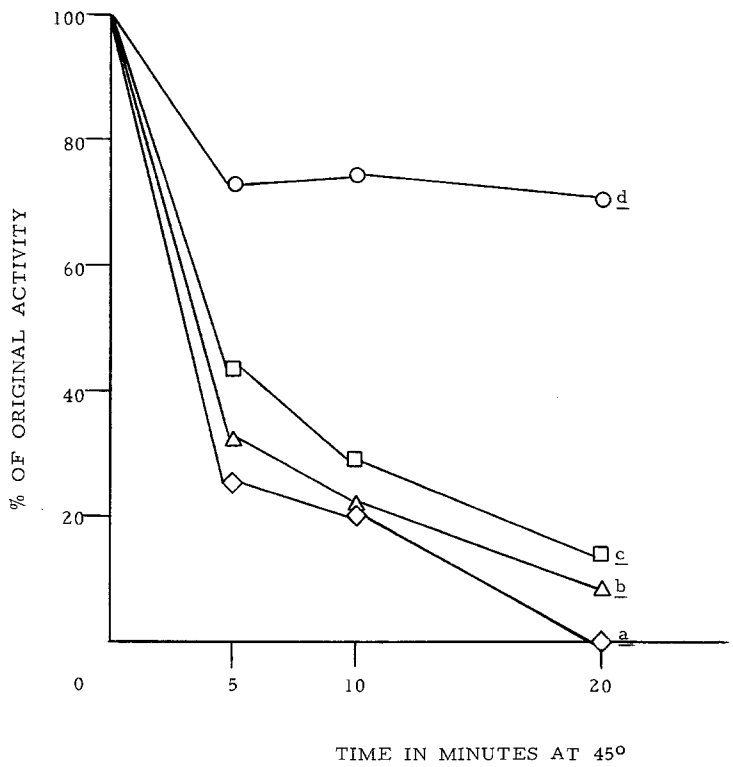

Figure 2: Stability at 45° of chromatin-associated DNases in presence of added protein (1 mg/ml of bovine serum albumin previously boiled for 15 min to inactivate contaminating DNase activity). Designations a-d refer to peaks of DNase activity shown in Fig. 1.

the enzyme preparation on DEAE-cellulose at pH 8.5 showed an identical profile of enzyme activity, but the recovery was lower.

DNA polymerase activity could not be detected in any of the fractions, and fractions a and d were free of RNase activity. At this stage of purification RNase activity was found in fractions b and c. Fractions a-d had low DNA exonuclease activity as measured by hydrolysis of labeled DNA or poly d(A-T) to acid-soluble products, and were most easily detected by the DNA-activation assay (18) which measures DNA endonuclease activity. Table 1 shows that the ratio of endo- to exonuclease activity varied from 4.1 to 21.2 for these chromatin-associated DNases, whereas nucleoplasmic DNases of HeLa cells have such ratios in the range 0.21-0.50 (14).

TABLE 1

Some Properties of Chromatin-Associated DNase Fractions

DEAE-cellulose fraction	Ratio of endo- to exonuclease activity*	Effect of tRNA#		Effect of 5mM dibutyryl 3'-5' cAMP#
		0.1mM	0.5mM	
a	8.5	63	49	24
b	4.9	47	14	49
c	4.1	56	26	64
d	21.2	72	47	84

*Obtained by dividing the activity in DNA-activation assay (endonuclease activity, ref. 18) by the hydrolytic activity on poly d(A-T) (exonuclease activity).

#Expressed as % of the value for untreated samples.

TABLE 2

Effect of Sulfhydryl Group Reactants on Chromatin-Associated DNase Fractions

Fraction	N-ethylmaleimide		p-chloromercuribenzoate	
	10 mM	20 mM	30 μM	100 μM
a	10	0	65	0
b	33	25	77	35
c	66	46	96	48
d	83	74	98	81

The results are expressed as percentage of the activity in the untreated samples.

The four fractions of DNase activity demonstrated here differed from one another with respect to a number of properties. They were inhibited to a different extent by yeast transfer RNA and by cyclic AMP (Table 1). Reagents which attack -SH groups also inhibited these DNases unequally (Table 2), and DNase d differed from the other enzymes as regards heat stability (Fig. 2).

Addition to the assay system of methylxanthines, such as theophylline and caffeine (2-25 mM), which inhibit phosphodiesterases (20) and the repair of UV damage to DNA (21), had no effect on any of these DNases. However, the significance of this in vitro observation is uncertain, since in vivo the xanthine derivatives may undergo metabolic conversions before they can inhibit DNA repair.

Discussion

The finding that DNase activity firmly bound to nuclear constituents can be fractionated into four components which show different properties but all have endonuclease activity suggests that the nucleus, at least in the HeLa cell, may contain a variety of enzymes which can break the phosphodiester bonds within the DNA double helix. This would indicate a complexity of reactions involving DNA greater than has been hitherto proposed. It is hoped that purification and study of these enzymes, at present in progress in this laboratory, will permit the construction of a detailed pathway for the initiation of DNA replication in mammalian cells.

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References

1. Becker, A. and Hurwitz, J., (1971) Prog. Nucleic Acid Res. Mol. Biol. (J.N. Davidson and W.E. Cohn, Eds.) 11, 423-459.
2. Ioannou, P., (1973) Nature New Biol. 244, 257-260.
3. O'Connor, P.J., (1969) Biochem. Biophys. Res. Comm. 35, 805-810.
4. Burgoyne, L.A., Wagar, M.A. and Atkinson, M.R., (1970) Biochem. Biophys. Res. Comm. 39, 254-259.
5. Barile, E., Brown, O. and Laszlo, J., (1971) Biochem. Biophys. Res. Comm. 43, 754-759.
6. Ishida, R., Akiyoishi, H. and Takahashi, T., (1974) Biochem. Biophys. Res. Comm. 56, 703-710.

7. Yamada, M., Nagao, M., Miwa, M. and Sugimura, T., (1974) Biochem. Biophys. Res. Comm. 56, 1093-1099.
8. Slor, H. and Lev, T., (1971) Biochem. J. 123, 993-995.
9. Hewish, D.R. and Burgoyne, L.A., (1973) Biochem. Biophys. Res. Comm. 52, 475-481.
10. Parisi, E. and De Petrocellis, B., (1972) Biochem. Biophys. Res. Comm. 49, 706-712.
11. Champoux, J.J. and Dulbecco, R., (1972) Proc. Nat. Acad. Sci. USA 69, 143-146.
12. Lindahl, T., Gally, J.A. and Edelman, G.M., (1969) Proc. Nat. Acad. Sci. USA 62, 597-603.
13. Lindahl, T., Gally, J.A. and Edelman, G.M. (1969) J. Biol. Chem. 244, 5014-5019.
14. Churchill, J.R., Urbanczyk, J. and Studzinski, G.P., (1973) Biochem. Biophys. Res. Comm. 53, 1009-1016.
15. Studzinski, G.P. and Gierthy, J.F., (1973) J. Cell. Physiol. 81, 71-84.
16. Studzinski, G.P., Gierthy, J.F. and Cholon, J.J., (1973) In Vitro 8, 466-472.
17. Berkowitz, D.M., Kakefuda, T. and Sporn, M.B., (1969) J. Cell Biol. 42, 851-855.
18. Studzinski, G.P. and Fischman, G.J., (1974) Anal. Biochem. 58, 449-458.
19. Fansler, B.S. and Loeb, L.A., (1973) in "Methods in Enzymology" (L. Grossman and K. Moldave, Eds.) 29E, 53-70, Academic Press, N.Y.
20. Robison, G.A., Butcher, R.W. and Sutherland, E.W., (1971) in "Cyclic AMP", 41-44, Academic Press, N.Y.
21. Rauth, A.M., (1967) Radiation Res. 31, 121-138.