AN ALKALINE DEOXYRIBONUCLEASE FROM RAT LIVER NON-HISTONE CHROMATIN PROTEINS

P.J. O'Connor

Paterson Laboratories, Christie Hospital
and Holt Radium Institute, Manchester

Received April 30, 1969

SUMMARY

An alkaline deoxyribonuclease which preferentially degrades native DNA was shown to be associated with the chromatin acidic proteins. The non-histone chromatin proteins, which contain also an active DNA polymerase, therefore possess the enzymatic mechanisms for both synthesis and degradation of DNA.

INTRODUCTION

Recently the non-histone acidic proteins obtained from mammalian chromosomes have been shown to contain metabolically active components (Wang, 1967a). In particular, the DNA polymerising enzyme has been demonstrated as a component of these proteins from calf thymus (Wang, 1967b) and rat liver (Patel, Howk and Wang, 1967). The chromatin acidic proteins from calf thymus contain also the DNA terminal-addition enzyme (Wang, 1968).

Soluble extracts from homogenates of regenerating rat liver have been shown to contain an alkaline deoxyribonuclease (O'Connor, 1969) which is active with native DNA and distinct from the mitochondrial-associated alkaline nuclease active with RNA and denatured DNA (Curtis, Burdon and Smellie, 1966). During attempts to localise the deoxyribonuclease active with native DNA it was found that the enzyme active with denatured DNA was present in the cytoplasm and could be precipitated at pH 5, whereas, the substrate preference of the enzymes present in whole nuclei was

variable. In view of this, nuclei were separated into the nuclear sap proteins, DNA-histone and chromatin acidic proteins. This communication indicates that an alkaline deoxyribonuclease which preferentially hydrolyses native DNA is present in the chromatin acidic protein fraction obtained from the nuclei of normal and regenerating rat-livers.

METHODS

Normal and regenerating rat-liver nuclei were prepared by a slight modification of the procedure described by Widnell and Tata (1964). Male Wistar rats (220-240 g body weight) were used and partial hepatectomy was by the procedure of Higgins and Anderson (1931). The nuclei (yield, 80%) were extracted with 0.14M NaCl containing 0.02M Tris-HCl pH 7.5 and 5mM $MgCl_2$ to remove soluble nuclear sap proteins (Patel and Wang, 1964). The chromatin acidic proteins were obtained by the procedure of Patel, Howk and Wang (1967) and the nuclear sap proteins also were recovered by precipitation with ammonium sulphate. Both were resuspended in 0.1M potassium phosphate pH 8.0 containing 1mM EDTA and 2mM 2-mercaptoethanol and dialysed against the same buffer medium at 1mM potassium phosphate concentration. Insoluble material was removed by centrifugation at 10,000 x g for 20 minutes. Attempts to purify further the deoxyribonuclease from the chromatin acidic protein fraction, using Sephadex gel-filtration and hydroxylapatite chromatography, have resulted in large losses of activity.

Assays of deoxyribonuclease activity were carried out in a total volume of 0.5 ml containing: 30 μmoles glycine-NaOH pH 8.0, 3 μmoles $MgCl_2$, 0.5 μmoles 2-mercaptoethanol, 25 μg of either native or heat denatured calf thymus DNA (Sigma Type 1) and enzyme protein. The absorbance of the acid supernatant was determined at 260 mμ in microcuvettes and the amount of DNA rendered acid soluble was estimated using a molar absorbance coefficient of 10 200 (Burton, 1959). One unit of activity was defined as the amount of enzyme which released 0.1 μmoles acid-soluble nucleotide per hour.

Assays of DNA polymerase activity were carried out essentially as

described by Mantsavinos, (1964) using 20 mμmoles each of dGTP, dATP, dCTP and 20 mμmoles dTTP-2-^{14}C in the presence of 8 mM $MgCl_2$. After incubation for 1 hour the acid insoluble precipitate was collected on 3 cm Whatman GF/C discs and counted in a Nuclear Chicago gas flow counter (efficiency 35%) and corrected for the low level of non-specific absorption.

Proteins were determined by the method of Lowry et al. (1951).

RESULTS

The chromatin acidic proteins extracted from the nuclei of normal rats contained an active DNA polymerase and as previously described by Patel, Howk and Wang, (1967) the enzyme was more active with native DNA (Table I). The DNA polymerase of the nuclear sap proteins was equally active with native and denatured DNA. A similar difference in substrate preference between the bound and soluble polymerase has been reported also for exponentially growing mouse fibroblasts (Lindsay and Adams, 1968).

The results of assays for deoxyribonuclease activity performed on the nuclear sap proteins and the chromatin acidic proteins are shown in Table I.

TABLE I. DEOXYRIBONUCLEASE AND DNA POLYMERASE ACTIVITY FROM NORMAL RAT LIVER NUCLEI

FRACTION	Specific Activity		Ratio of Activities
	Native DNA	Denatured DNA	Native DNA / Denatured DNA
Nuclear Sap Proteins			
Deoxyribonuclease	* 0.70	1.15	0.6
DNA polymerase	**0.94	0.79	1.2
Acidic Proteins			
Deoxyribonuclease	1.60	0.68	2.4
DNA polymerase	2.94	0.65	4.5

* units/mg Protein/hour

**mμmoles dTTP-2-^{14}C/mg Protein

It is evident that the deoxyribonuclease from the chromatin acidic protein fraction is 2-3 times more active with native DNA than with denatured DNA. The ratio of activities for this fraction has ranged in different preparations from 2.3 to 2.7 for normal rat livers. Similar activity ratios have been obtained for preparations of the chromatin acidic proteins extracted from regenerating rat-livers removed 38 hours after partial hepatectomy.

The deoxyribonuclease contained in the nuclear washings showed possibly a slight preference for denatured DNA (Table I). Since these proteins were were readily leached from the nuclei by low salt concentrations, they

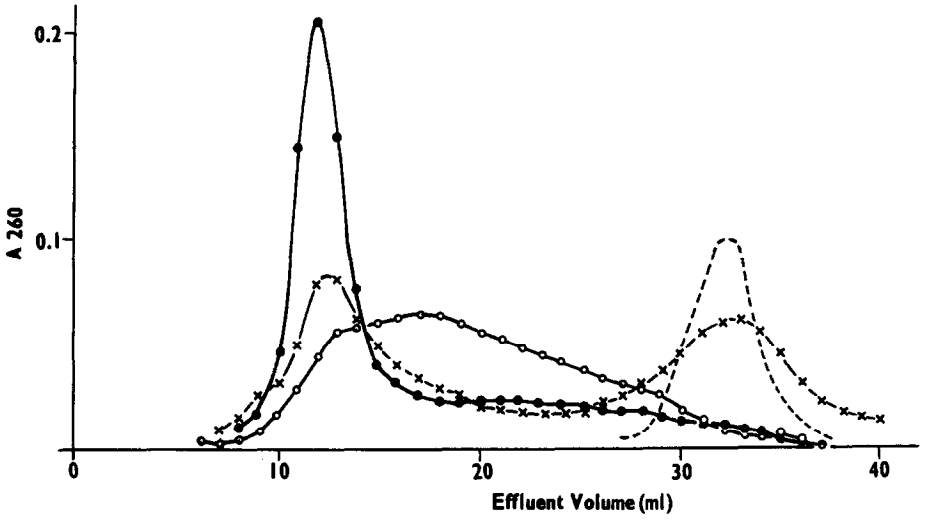

Fig. 1

Chromatography on Sephadex G-100 of DNA digested for 30 minutes by 50 μg snake venom exonuclease (x——x) and DNA digested for 30 minutes by 260 μg chromatin acidic protein fraction (o——o); the zerotime for the chromatin acidic protein digest is indicated by (•——•). The dotted line indicates the elution curve for ATP. Native DNA (50 μg) was digested by the chromatin acidic protein fraction for 30 minutes at 37°C in 1 ml of the medium used for the assay of deoxyribonuclease activity. The reaction was stopped by cooling in ice and the addition of 0.3 ml 4 M NaCl. The digest was thermally denatured and centrifuged to remove precipitated protein. A 1 ml portion of the supernatant was then applied to a 2 cm x 7.4 cm column of Sephadex G-100 which had been equilibrated with 0.015 M Sodium citrate (pH 7.1) containing 0.15 M NaCl. The effluent was collected in 0.5 ml fractions and the absorbance at 260 mμ was measured. These profiles were corrected for the absorbance due to 2-mercaptoethanol. Denatured DNA (50 μg) was digested by the snake venom exonuclease (Sigma Limited) for 30 minutes at 25°C in 1 ml of 60mM glycine-NaOH, pH 8.8 containing 6mM $MgCl_2$, treated and chromatographed as described above.

probably contained a mixture of diffused enzyme activities.

A gel-filtration procedure (Birnboim, 1966) has been used to show that the deoxyribonuclease from the chromatin acidic protein fraction cleaves native DNA to produce polynucleotides of widely differing molecular weight (Fig.1). Exonuclease activity cannot be excluded, but it is evident from a comparison of the elution profiles obtained for the digestion products of the acidic protein deoxyribonuclease and the snake venom exonuclease, that the enzyme functions predominantly as an endonuclease.

DISCUSSION

The DNA polymerase activity associated with the chromatin acidic-protein fraction prepared here is similar to that previously described for rat liver (Patel, Howk and Wang, 1967). It is significant that the deoxyribonuclease activity which is associated with the chromatin acidic protein fraction is definitively more active with native DNA. If, during the process of isolating and extracting the nuclei, there had been a significant binding of the mitochondrial associated nuclease to the chromatin this would have lowered the activity ratio for the chromatin acidic-protein fraction. As this was not the case, the chromatin presumably contains a firmly bound deoxyribonuclease which preferentially hydrolyses native DNA and is obtained as a constituent of the chromatin acidic-protein fraction. The deoxyribonuclease activity which has been reported bound to the chromatin of mammalian cells (Cole, Swingle and Bailey, 1967) and the nuclei of sea urchin embryos (Solari, 1967) may be of a similar type.

The presence of a deoxyribonuclease in the chromosome raises many issues. In the normal cell the activity of this enzyme must be under rigorous control in order to preserve the integrity of the genetic message. Abnormal circumstances, causing release of activity, might provide an explanation for the loss of DNA such as that observed in the adrenal medulla of cold-shocked rats (Viola-Magni, 1966). The presence of a

chromosomal deoxyribonuclease may be also of considerable importance in studies which attempt to assess the template capacity of chromatin.

A recent report has shown that oligodeoxynucleotides increase the template activity of single stranded DNA for the E. coli DNA polymerase and that they are incorporated into newly synthesised DNA by covalent bonds (Goulian, 1968). The data are consistent with the incorporation of one oligomer per product chain and therefore suggest that the oligomer might be serving as a primer for the synthesis of a new chain. It is possible that a chromosomal deoxyribonuclease might provide a limited supply of oligodeoxynucleotides for the initiation of synthesis at multiple replication sites such as have been described for Chinese hamster chromosomes by Huberman and Riggs (1968). Alternatively, the enzyme might be used simply to degrade any DNA which becomes displaced from its proper position in the chromosomal structure and therefore prevent any unorganised replication of DNA.

REFERENCES

Birnboim, H., Biochim.Biophys.Acta 119, 198 (1966).
Burton, K., in Dawson R.M.C., Elliot, D.C., Elliot, W.H. and Jones, K.M., Data for Biochemical Research, Oxford University Press, 74 (1959).
Curtis, P.J., Burdon, M.G. and Smellie, R.M.S., Biochem.J. 98, 813 (1966).
Goulian, M., Proc.Natl.Acad.Sci. 61, 284 (1968).
Higgins, G.M. and Anderson, R.M., Arch.Path. 12, 186 (1931).
Huberman, J.A. and Riggs, A.D., J.Mol.Biol. 32, 327 (1968).
Lindsay, J.G. and Adams, R.L.P., Biochem.J. 108, 43 P (1968).
Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., J.Biol.Chem. 193, 265 (1951).
Mantsavinos, R., J.Biol.Chem., 239, 3431 (1964).
O'Connor, P.J., Biochem.J., 111, 32 P (1969).
Patel, G., Howk, R. and Wang, T.Y., Nature 215, 1488 (1967).
Patel, G. and Wang, T.Y., Exptl.Cell. Res. 34, 120 (1964).
Solari, A.J., J.Ultrastruc. Res., 17, 421 (1967).
Swingle, K.F., Cole, L.J. and Bailey, J.S., Biochim.Biophys.Acta 149, 467 (1967).
Viola-Magni, M.P., J.Cell Biol. 28, 9 (1966).
Wang, T.Y., J.Biol.Chem. 242, 1220 (1967a).
Wang, T.Y., Arch. Biochem.Biophys., 122, 629 (1967b).
Wang, T.Y., Arch. Biochem.Biophys., 127, 235 (1968).
Widnell, C.C. and Tata, J.R., Biochem.J. 92, 313 (1964).