INHIBITION OF DEOXYRIBONUCLEASE IN AN EXTRACT OF RAT LIVER NUCLEI BY POLY(ADP-RIBOSE)[1]

Michiyuki Yamada, Minako Nagao, Masanao Miwa* and Takashi Sugimura

Biochemistry Division, National Cancer Center Research Institute, Tsukiji, Chuo-ku, Tokyo, and *Department of Molecular Oncology, Institute of Medical Science, University of Tokyo, P.O. Takanawa, Minato-ku, Tokyo, Japan.

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SUMMARY

A deoxyribonuclease inhibited by poly(ADP-Rib) was found in an extract of rat liver nuclei. Of the compounds tested, (i.e. poly(ADP-Rib), poly(A), NAD, ADP-Rib, rRNA, and tRNA) only poly(ADP-Rib) and poly(A) inhibited deoxyribonuclease activity. The deoxyribonuclease activity was separated by DEAE-cellulose column chromatography into two distinct fractions, only one of which was inhibited by poly(ADP-Rib) and poly(A). Both fractions degraded poly[d(A-T)] exonucleolitically.

INTRODUCTION

Burgoyne et al. (1) reported that the endonuclease activity of isolated rat liver nuclei causes fragmentation of nuclear DNA and is involved in DNA synthesis by isolated nuclei. Previously, we demonstrated that the fragmentation of the nuclear DNA in isolated rat liver nuclei was prevented by poly(ADP-Rib)[2] formation in the nuclei, but not by the addition of NAD or ADP-Rib(2). Burzio and Koide also presented evidence that the template activating system for DNA synthesis was inhibited by treating isolated nuclei with NAD (3).

We studied the mechanism by which poly(ADP-Rib) formation in isolated nuclei inhibits fragmentation of nuclear DNA. This paper reports that a DNase which was inhibited by poly(ADP-Rib) was found in an extract of rat liver nuclei.

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2. The abbreviations used are: ADP-Rib, adenosine diphosphate-ribose; poly(ADP-Rib), polymer of ADP-Rib.

MATERIALS AND METHODS

Preparation of extract of rat liver nuclei. Rat liver nuclei were isolated as described previously (2). They were stirred with 0.2M potassium phosphate buffer (pH 7.5) containing 2 mM 2-mercaptoethanol for 60 min at 4° and then centrifuged at 14,000 g for 10 min. This extraction process was repeated twice. The supernatant fluids were combined and centrifuged at 105,000 g for 60 min, and DNase activity was found in the supernatant fraction.

Separation of two nucleases. The nuclear extract was brought to 85 % saturation with ammonium sulfate. The resulting precipitate was dissolved in 0.01M potassium phosphate buffer (pH 7.5) containing 2 mM 2-mercaptoethanol and 10 % glycerol (w/v). The solution was dialyzed against the same buffer for 3 hr with one change of the outer medium, and then centrifuged at 105,000 g for 60 min to remove insoluble material. The solution (containing 4.8 mg of protein) was placed on a DEAE-cellulose column (1 x 2.2 cm) previously equilibrated with 0.01 M potassium phosphate buffer (pH 7.5) containing 2 mM 2-mercaptoethanol and 10 % glycerol. The column was washed with the same buffer, and then eluted with a linear gradient of phosphate concentration obtained by placing 20 ml of 0.01 M phosphate buffer (pH 7.5) containing 2 mM 2-mercaptoethanol and 10 % glycerol in the mixing chamber and 20 ml of 0.43 M phosphate buffer (pH 7.5) containing 2 mM 2-mercaptoethanol and 10 % glycerol in the reservoir. Fractions of 1.2 ml were collected at a flow rate of 8 ml per hr.

Assay of DNase. DNase was assayed by measuring the conversion of H^3-labeled poly[d(A-T)] to acid-soluble products. The reaction mixture (50 µl) contained 2.5 µmoles of Tris-HCl (pH 8.0), 0.4 µmole of $MgCl_2$, 0.05 µmole of dithiothreitol, 317 ng of H^3-labeled poly[d(A-T)] (radioactivity, 5,600 cpm), and an appropriate amount of the test fraction. The reaction mixture was incubated for 30 min at 37° and then 40 µl of a cold solution of DNA (1.2 mg per ml) and 10 µl of cold 50 % trichloroacetic acid were added. After keeping the mixture 10 min at 0°, the precipitate was removed by centrifugation, and 80 µl of the supernatant fluid were used to measure radioactivity. Radioactivity was determined with 10 ml of dioxane-naphthalene-PPO-POPOP scintillator solution in a Beckman liquid scientillation counter.

Gel filtration analysis of the reaction product. Gel filtration analysis of the product of DNase digestion was performed as described by Birnboim (4). After digestion, the mixture was placed on a Sephadex G-100 column (1.5 x 22 cm) equilibrated with 0.5 M NaCl-10 mM Tris-HCl (pH 8.0) and eluted with the same buffer at a flow rate of 30 ml per hr. Fractions of 1 ml were collected.

Chemicals. [Methyl-^{3}H thymidine] poly[d(A-T)] was purchased from Miles Laboratories, poly(A) from Cal Biochem, Inc., NAD and ADP-ribose from Sigma Chemical Co. rRNA of rat ascites hepatoma AH-130 cells and tRNA mixture of Escherichia coli were gifts from Dr. M. Izawa and Dr. S. Nishimura of this Institute, respectively.

Pure poly(ADP-Rib) was prepared by incubating NAD with calf thymus nuclei, as described previously (5).

RESULTS

Effect of poly(ADP-Rib) on DNase activity of the nuclear extract. As shown in Fig. 1, the degradation of poly[d(A-T)] in the first 30 min was proportional to the incu-

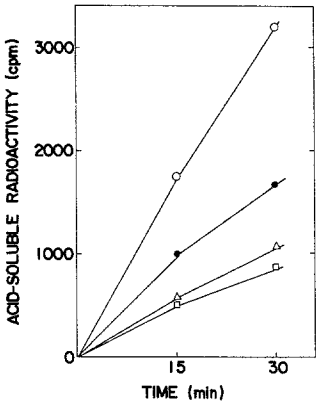

Fig. 1. Effect of poly(ADP-Rib) on DNase activity in an extract of rat liver nuclei. Assay conditions were as described under "Materials and Methods". Nuclear extract containing 21.5 μg of protein was used as enzyme. —○— , without poly(ADP-Rib); and with —●— , 0.025 A_{260} nm unit; —□— , 0.125 A_{260} nm unit; and —△— , 0.25 A_{260} nm unit of poly(ADP-Rib).

bation period, and attained more than 60 % degradation of the added poly[d(A-T)]. Additions of various amounts of poly(ADP-Rib) inhibited the DNase activity of the nuclear extract to up to 30 % of the control value (Fig. 1).

The effects of various nucleotide compounds on DNase activity were tested (Table I). Of the compound tested, poly(ADP-Rib) and poly(A) were inhibitory, poly(A) being the stronger inhibitor. However, ADP-ribose, NAD, rRNA and tRNA were not inhibitory.

Specificity of poly(ADP-Rib) for nuclear DNase. Fig. 2 shows that the DNase activity in the nuclear extracts was separated by DEAE-cellulose column chromatography into two distinct regions, Peak I and Peak II. One of the two DNases was not adsorbed on the DEAE-cellulose under the experimental conditions while the other was eluted with an increased concentration of phosphate. Poly(ADP-Rib) and poly(A) both inhibited the activity in Peak II but not Peak I (Table I). Thus poly(ADP-Rib) specifically inhibits Peak II enzyme. This suggests that the inhibition is not due to an interaction between poly[d(A-T)] and poly(ADP-Rib).

Exonucleolytic mode of degradation. The modes of action of the two DNases were

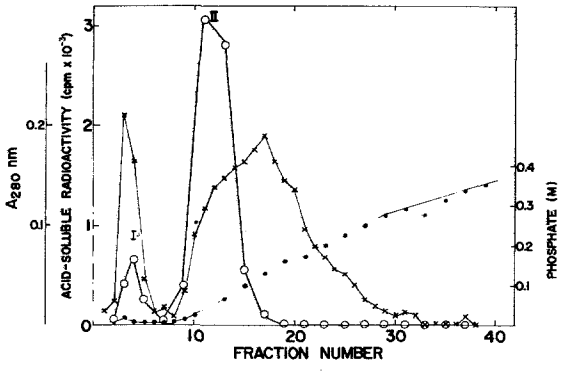

Fig. 2. Chromatography of the nuclear extract on a DEAE-cellulose column. —O—, DNase activity (a 25-µl aliquot of each fraction was used for the assay). —X—, absorbance at 280 nm; —●—, concentration of phosphate. See text for details.

studied. When the Peak I and Peak II enzymes had converted 10 % and 14 %, respectively, of the poly[d(A-T)] to acid-soluble materials, the reaction products was developed on a Sephadex G-100 column with TMP as a marker. Fig. 3A and B show the elution profiles of the reaction products of the Peak I and Peak II enzyme, respectively. In both cases, there were two discrete peaks of radioactivity. The position of the major peak after the void volume corresponded to that of remaining substrate, while that of the minor peak eluted with TMP added as marker corresponded to mononucleotide. The minor peaks produced by the Peak I and Peak II enzymes accounted for 76 % and 87 %, respectively, of the acid-soluble materials. The positions of the major peaks of the mixtures with Peak I and Peak II enzymes were the same as that of the control without enzyme. These results show that both the Peak I and II enzymes attack poly[d(A-T)] exonucleolytically.

DISCUSSION

This work provides the first evidence that DNase activity in an extract of rat liver nuclei is inhibited by addition of poly(ADP-Rib) or poly(A), but not by tRNA or rRNA. Two DNases activities were found in the nuclear extract, only one of which was inhibited by poly(ADP-Rib) or poly(A). Lindahl et al. found two exonucleases, DNase III and

TABLE I

Effects of Poly(ADP-Rib) and Various Nucleotide Compounds on DNases[a]

Addition		Nuclear Extract	Enzyme I	Enzyme II
	A_{260} nm unit	% of control		
None		100[b]	100	100
Poly(ADP-Rib)	0.025	63.4	100	65.4
	0.125	59.6	87.6	25.5
Poly(A)	0.030	34.4	104	12.5
	0.150	18.8		
NAD	0.027	105		
	0.135	110		
ADP-Rib	0.030	102		
	0.150	96.3		
rRNA	0.025	153		
	0.125	159		
tRNA	0.025	107		
	0.125	85.6		

a The reaction conditions were as described under "Materials and Methods", except that various amounts of nucleotide compounds were added as indicated. Nuclear extract (21.5 μg protein), enzyme I (7.1 μg protein), and enzyme II (4.1 μg protein) were used as enzyme preparations.

b The values for 100 per cent hydrolysis of poly[d(A-T)] to acid-soluble materials by the nuclear extract, and enzymes I and II were 1548 cpm, 422 cpm, and 1408 cpm, respectively, under the assay conditions.

DNase IV, in nuclei of rabbit bone marrow (6, 7), but it is still unknown whether polyribonucleotide inhibits their activities. Further investigations are required on the relationship between the DNases in rat liver nuclei and those of bone marrow nuclei.

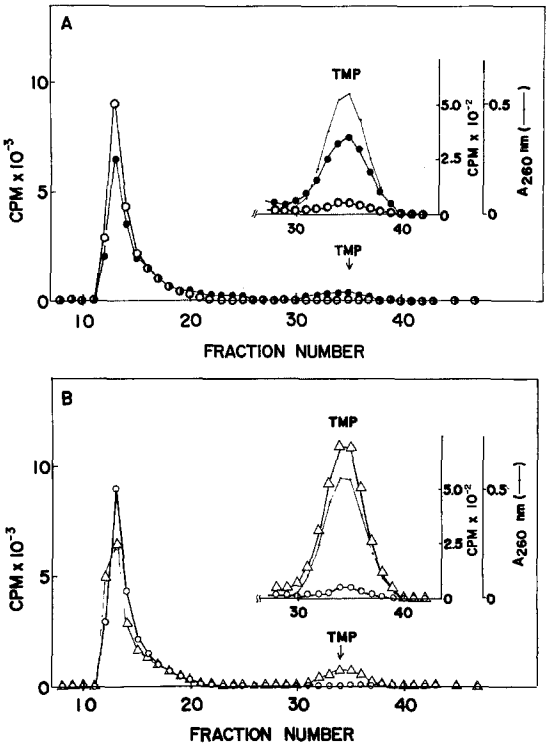

Fig. 3. Chromatography of the digestion products on Sephadex G-100. The reaction mixture used was 4 times that described under "Materials and Methods". The reaction was terminated by immersing the tubes in boiling water for 1.5 min, and then 20 µl of 5 M NaCl and 0.3 µmole of 5'-TMP were added. The mixture was applied to a Sephadex G-100 column and eluted as described in "Materials and Methods". —○—, profile after incubation for 46 min without enzyme; —●—, profile after incubation for 46 min with enzyme I (37 µg protein) rendering 10 % of the poly[d(A-T)] acid-soluble; —△—, profile after incubation for 12 min with enzyme II (16.5 µg protein) rendering 14.1 % of the poly[d(A-T)] acid-soluble.

The possibility that the phosphodiesterase of rat liver nuclei is concerned with the inhibition of DNase by poly(ADP-Rib) is ruled out, because the phosphodiesterase is inhibited by tRNA (8) while the DNase is not. Previously, we suggested that inhibition of the endonuclease, known to be present in rat liver nuclei (9), might decrease fragmentation of nuclear DNA by causing poly(ADP-Rib) formation in the nuclei (2). In the present work it was found that the two DNases in extracts of rat liver nuclei were exonucleases. However, it is well established that in bacteria a single homogeneous DNase may catalyze

either exo- or endonucleolytic cleavage of diester bonds depending upon the structure of its macromolecular substrate (10). Thus the possibility that the DNase inhibited by poly-(ADP-Rib) also has endonucleolytic activity or is identical with the endonuclease involved in fragmentation of DNA in rat liver nuclei remains to be elucidated.

The present findings also indicate that some physiological functions in the nucleus may be directly affected by poly(ADP-Rib) without modification of histone and other nuclear proteins by ADP-ribosylation (11, 12).

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