DNASES OF CELL NUCLEI: Mn^{2+}-DEPENDENT ENDONUCLEASE

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The presence of an Mn^{2+}-dependent endonuclease was demonstrated previously in rat liver chromatin, not connected with the Ca^{2+}, Mg^{2+}-dependent endonuclease [1, 4]. An enzyme with similar properties also is contained in rat brain cell nuclei [7]. The Mn-dependent endonuclease of rat liver cell nuclei has a molecular mass of 30 ± 5 kD and its isoelectric point is 10.4. It acts on native and denatured DNA and is activated more strongly by Mn^{2+} than by Mg^{2+} and Ca^{2+}, added either together or separately [1, 4]. During initial endonucleolysis of chromatin in isolated cell nuclei it cuts repeating sequences of DNA more rapidly than the Ca^{2+}, Mg^{2+}-dependent endonuclease [5]. Its activity is enhanced in hepatomas induced by diethylnitrosamine [2]. The aim of the present investigation was to characterize the Mn^{2+}-dependent endonuclease of rat liver chromatin and to compare it with Ca^{2+}, Mg^{2+}-dependent endonuclease.

EXPERIMENTAL METHOD

Preparations of Mn^{2+}-dependent endonuclease, purified 150 times, and of Ca^{2+}, Mg^{2+}-dependent endonuclease, purified 740 times, as described previously [1], were used. The substrates were rat liver DNA, isolated as described previously [4], single-stranded (SS) circular DNA of phage M13, its replicative form, and plasmid pBR322, isolated as described in [6]. Endonuclease activity was determined by electrophoresis of the DNA in agarose gel [1]. The unit of activity was taken to be the amount of enzyme degrading 1 μg DNA in 1 h at 37°C. The rate of formation of single-stranded breaks (SS-breaks) on supercoiled DNA of the replicative form of phage M13 was measured as the initial rate of accumulation of open circular DNA, and the rate of formation of double-stranded breaks (DS-breaks) as the initial rate of growth of linear DNA. PAG electrophoresis of the proteins with SDS was carried out as in [10]. The protein concentration was measured as in [11].

EXPERIMENTAL RESULTS

An essential condition for the study of physicochemical and catalytic properties of the enzyme is its functional homogeneity, and this was proved by electrophoresis. Preliminary experiments showed that neither Mn- nor Ca,Mg-dependent endonuclease is inhibited in the presence of 0.1% SDS. To determine the nuclease activity, the track in the gel at the end of electrophoresis was cut into pieces 2 mm long and placed in buffer containing 50 mM Tris-HCl, pH 7.7, 0.5 mM dithiothreitol, 25 mg/ml of bovine serum albumin fraction B, 5 mM $MnCl_2$, and 1 μg DNA of plasmid pBR322. The samples were incubated with constant shaking for 10 h at 37°C, after which the degree of polymerization of the DAN was determined by electrophoresis in 0.8% PAG. Hydrolysis of DNA was carried out only in the fraction corresponding to mol. mass of 31 kD (Fig. 1). Thus the results of determination of the mol. mass of Mn^{2+}-dependent endonuclease by methods of gel-filtration [1] and electrophoresis coincided. The preparation was functionally homogeneous: it contained no other endonucleases. Preparations of Mn^{2+}- and Ca^{2+},Mg^{2+}-dependent endonuclease were analyzed for the presence of concomitant activity. Neither RNase activity, as shown by inability to degrade yeast RNA, determined by the method in [3], ATPase activity, as shown by inability to remove the terminal phosphate from [γ-^{32}P]-ATP, determined by the method in [9], DNA-binding activity, as shown by inability to catalyze protease-sensitive binding of DNA, hindering its migration in agarose gel on electrophoresis [2], nor DNA-topoisomerase activities of types I and II, as shown by inability to relax circular supercoiled DNA [2], was found.

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TABLE 1. Activity of Mn^{2+}- and Ca^{2+},Mg^{2+}-Dependent Endonucleases Depending on Type of Substrate and Conditions of Reaction

Enzyme	Substrate	Breaks	Specific activity, $\times 10^3$ units/mg protein			
			A	B	C	D
Mn^{2+}-dependent endonuclease	Liver DS-DNA	DS	1,8	0,9	15,7	2,2
	RF M13 DS-DNA	SS + DS	28,8	12,6	34,9	15,8
	The same	SS	24,3	10,3	26,0	8,0
	" "	DS	4,5	2,3	8,9	7,8
	SC-DNA of phage M13	SS	58,4	<10	<10	<10
Ca^{2+}, Mg^{2+}-dependent endonuclease	Liver DS-DNA	DS	150	3,5	36,0	12,5
	RF M13 DS-DNA	SS + DS	828	54,6	36,8	25,0
	The same	SS	744	4,3	10,0	0
	" "	SS	83,5	50,3	26,8	25,0
	SC-DNA of phage M13	SS	1042	1028	<100	242

Legend. A) 2 mM $CaCl_2$ + 5 mM $MgCl_2$, pH 7.4; B) 5 mM $MgCl_2$ + 2 mM EGTA, pH 7.4; C) 5 mM $MnCl_2$, pH 7.4; D) 1 mM EDTA, pH 5.0.

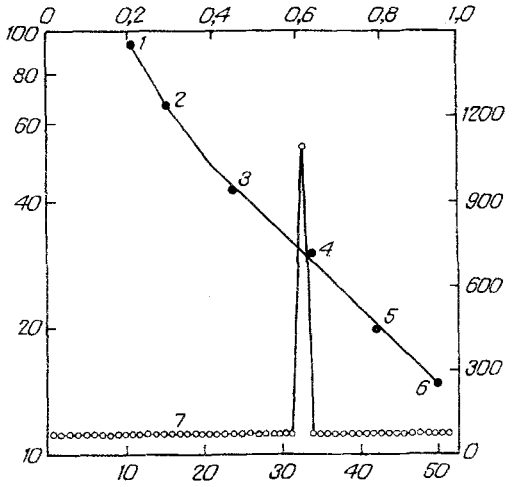

Fig. 1. Electrophoresis of preparation of Mn-dependent endonuclease in 15% PAG with 0.1% SDS. 1) Phosphorylase B (94 kD); 2) albumin, 67 kD; 3) ovalbumin (43 kD); 4) carbonic anhydrase (30 kD); 5) trypsin inhibitor (20.1 kD); 6) lactalbumin (14.4 kD); 7) endonuclease activity. Abscissa: below, nos. of fractions; above, relative mobility. Ordinate: on left, mol. mass (in kD); right, activity (in units/ml).

Figure 2 shows that the endonucleases differed in their pH-optimum. Mn^{2+}-dependent endonuclease was active over a wider pH range. These results agree with data on the wide pH-optimum of Mn^{2+}-dependent endonucleolysis in rat brain cell nuclei [7]. Activity of both endonucleases on DS-DNA depended on the type of substrate (Table 1). On the DS-DNA of phage M13 Mn^{2+}-dependent endonuclease in 5 mM $MnCl_2$ and Ca^{2+},Mg^{2+}-dependent endonuclease in 2 mM $CaCl_2$ + 5 mM $MgCl_2$ reduced the rate of formation of DS-breaks by about half compared with liver DS-DNA. The mechanism of action of the endonucleases depended strictly on the cations present. Whereas for Mn-dependent endonuclease the addition of $CaCl_2$ to $MgCl_2$ caused virtually no change in the percentage of DS-breaks compared with the total number (Table 2), for Ca^{2+}-, Mg^{2+}-dependent endonuclease it led to the transition from a 1-hit mechanism to a 2-hit mechanism. For Ca^{2+},Mg^{2+}-dependent endonuclease the same effect also is observed on replacement of Mn^{2+} ions by Ca^{2+} + Mg^{2+}, and of EDTA by Ca^{2+} + Mg^{2+}. The rate of formation of DS-breaks by the endonucleases in buffers with nonoptimal ionic composition was higher for linear DNA than for circular: by Mn^{2+}-dependent endonuclease – in Ca^{2+} + Mg^{2+}, Mg^{2+}, and EDTA, and by Ca^{2+},Mg^{2+}-dependent endonuclease – in Mg^{2+} and EDTA. On the whole, sensitivity of Mn^{2+}-dependent endonuclease to ions was lower than that of the Ca^{2+},Mg^{2+}-dependent type.

TABLE 2. Comparison of Properties of Mn^{2+}-Dependent and Ca^{2+}, Mg^{2+}-Dependent Endonuclease of Rat Liver Cell Nuclei

Parameter	Mn^{2+}-dependent endonuclease	Ca^{2+}, Mg^{2+}-dependent endonuclease
Mol. mass, kD:		
Gel-filtration	30±5 [1]	65±10 [1]
Electrophoresis	31	57*[8]
Active products of limited proteolysis	Not found [1]	45 and 35 [1]
Isoelectric point	10.4[1]	9.7[1]
pH-optimum	5.5	6.5
Percent of DS-breaks at beginning of hydrolysis in Ca^{2+}+Mg^{2+}; Mg^{2+}; Mn^{2+}; EDTA	16, 18, 26, 49	11, 92, 73, 100
Action on SS and DS-DNA	SS = DS	SS > DS
Content in nuclear extract, percent of total activity:		
Ca^{2+}+Mg^{2+}	4[1]	73[1]
Mn^{2+}	58[1]	28[1]
Activity in hepatomas and embryonic liver	Increased	Lowered

Legend. For Mg,Mg-dependent endonuclease of human lymphocytes [8].

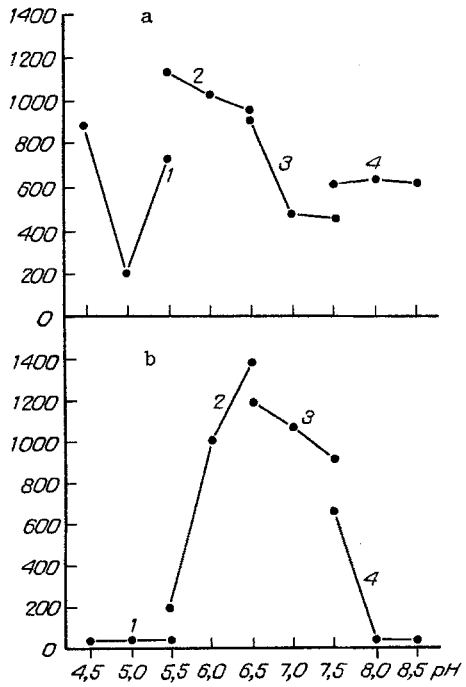

Fig. 2. Determination of pH-optimum of Mn-dependent endonuclease in presence of 5 mM $MnCl_2$ (a) and Ca,Mg-dependent endonuclease in the presence of 2 mM $CaCl_2$ + 5 mM $MgCl_2$ (b). Buffers: 1) Na acetate, 2) MES-NaOH, 3) PIPES-NaOH, 4) Tris-HCl. Ordinate, activity (in units/ml).

Activity of Mn^{2+}-dependent endonuclease on SS-DNA of phage M13 under conditions of 5 mM $MnCl_2$, pH 7.4, did not increase activity on DS-DNA of the replicative form of the phage and on DS-DNA of rat liver. These findings agree with the results of determination of activity of Mn-dependent endonuclease on native and denatured DNA, which we undertook previously [4]. Activity of Ca,Mg-dependent endonuclease under conditions optimal for it was greater on SS-DNA of phage M13 than on DS-DNA of the replicative form and on DS-DNA of rat liver. The more rapid cleavage of circular DS-DNA than linear can probably be explained by the action of the enzyme on regions of local denaturation of these molecules.

Data on the characteristics of Mn^{2+}- and Ca^{2+},Mg^{2+}-dependent endonucleases of rat liver cell nuclei are summarized in Table 2. Clearly, the enzymes differed in molecular mass, the presence of active limited proteolysis products, isoelectric point, pH-optimum, mechanism of action, activity on SS- and DS-DNA, and content in cell nuclei. On the whole Mn^{2+}-dependent endonuclease had weaker properties than Ca^{2+},Mg^{2+}-dependent endonuclease: cationic dependence, substrate specificity, and sensitivity to pH.

The writer showed previously that initial endonucleolysis of chromatin in isolated rat liver cell nuclei in the presence of 5 mM $MnCl_2$ was accompanied by more rapid degradation of DNA repeating sequences ($C_0t < 10^{-1}$) than endonucleolysis in 2 mM $CaCl_2$ + 5 mM $MgCl_2$ [5]. Since the method of isolation of the repeats included annealing and removal of nonreassociated ends of the duplex by nuclease S1, the length of the repeat which we determined was equal to the distance between the two breaks, regardless of whether they were SS or DS, and the average length of the repeat removed by 2-hit endonuclease is less than the average length of the repeat removed by 1-hit nuclease, if the number of breaks of phosphodiester bonds is the same. Table 2 shows that the 1-hit nature (percentage of DS-breaks) of both endonucleases of chromatin, accounting altogether for more than 80% of endonuclease activity of the cell nucleus, was higher in the presence of Mn^{2+} than in the presence of Ca^{2+} + Mg^{2+}. An increase in the degree of cleavage of repeats in the presence of Mn^{2+} cannot therefore be explained by a change in the mechanism of DNA hydrolysis by nuclear nucleases in the direction of an increase in their 2-hit character.

Knowing the ratio of activities of the principal nuclear endonucleases [1], and activity of Mn^{2+}- and Ca^{2+},Mg^{2+}-dependent endonuclease on circular DNA, determined in the present investigation, is can therefore be asserted that the repeat-specificity in the presence of Mn^{2+} is connected, not with a change in the overall mechanism of DNA hydrolysis by nuclear endonucleases, but can evidently be explained by the specificy of the nuclear nucleases toward the primary structure of DNA or the structure of chromatin in the region of the repeats.

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