Notes

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Isolation of a Deoxyribonuclease from Bovine Parotid Gland

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In the course of the study on the purification of a hypocalcemic substance from bovine parotid gland,[2] we found a fraction having a high activity of deoxyribonuclease (DNase). Ball reported that parotid gland of rat contained high levels of secretory DNase but the DNase was not purified.[3] Lundblad, *et al.* reported that partially purified bovine parotid DNase was immunologically different from pancreatic DNase [EC 3.1.4.5].[4,5] We undertook a study of the biochemical relationship of parotid and pancreatic DNase. The present paper describes the purification and some properties of DNase from bovine parotid gland.

DNase was routinely assayed in a reaction mixture (2.5 ml) containing 50 μmoles of Tris-HCl (pH 7.5), 20 μmoles of $MgCl_2$, and 0.5 mg of native brain DNA, which was prepared from bovine brain by the phenol–cresol method of Hastings and Kirby.[6] Incubation was performed at 37° for 10 min and stopped by addition of 1.0 ml of cold 12% perchloric acid. This mixture was allowed to stand in ice-water for 10 min and centrifuged at 3000 rpm for 10 min. The supernatant solution containing acid-soluble oligonucleotides was decanted, and its absorbance at 260 nm was read. An increase in absorption value of 0.1 is defined as 0.1 unit of enzyme activity. The method of estimating ribonuclease activity was essentially the same as that for the measurement of DNase activity except that commercial yeast ribonucleic acid (RNA) was used as substrate. Protein concentration was determined by measuring the absorbance of the enzyme solution at 280 nm or by the method of Lowry, *et al.*[7]

All the purification procedures were carried out in a cold room at about 5°. The saline extract of the acetone-dried powder, prepared from bovine parotid gland according to the procedure of Ito and Mizutani,[8] was brought to 0.5 saturation of ammonium sulfate. The resulting turbid fluid was centrifuged at 3000 × ***g*** for 10 min, and the clear supernatant solution was saturated with ammonium sulfate at pH 7. The precipitate was collected by centrifugation at 3000 × ***g*** for 10 min, dialyzed and lyophilized. This fraction (2 g) was chromatographed on diethylaminoethyl (DEAE)-cellulose as shown in Fig. 1. Fractions in tubes 80 to 110 in Fig. 1 contained DNase but did not contain RNase. Peak fractions (tubes 84 to 93 in Fig. 1) were pooled and further fractionated on Sephadex G-100 as shown in Fig. 2a. Pooled active fractions (tubes 57 to 62) appeared to be nearly homogeneous showing a single band by analytical disc gel electrophoresis[9] as shown in Fig. 3. In order to check the purity of fraction (tubes 57 to 62 in Fig. 2a), rechromatography on Sephadex G-100 was carried out and its

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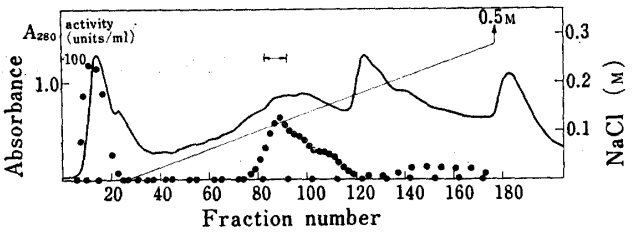

Fig. 1. Chromatography of Bovine Parotid DNase and RNase on DEAE-cellulose

The column (4.2×70 cm) was equilibrated with 0.05M Tris-HCl, pH 7.5. After the sample (2 g) was applied, the column was eluted with a linear gradient of 0 to 0.5M NaCl in the Tris-HCl buffer at 5°. Total elution volume was 6 liters. Flow rate was 20 ml per hr and fraction size was 20 ml. Aliquots (0.1 ml) of fractions were used to assay for DNase (⊙) and RNase (●). Protein was estimated by reading at 280 nm (—).

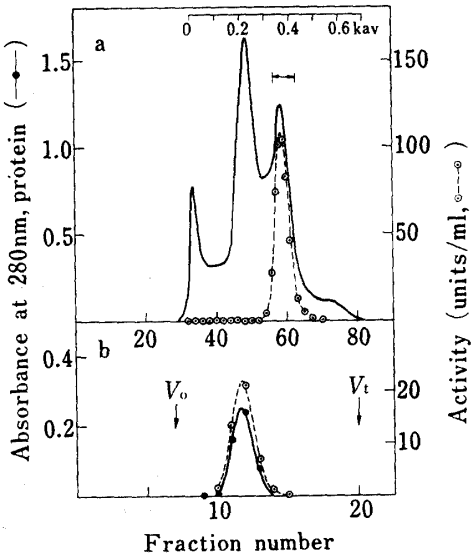

Fig. 2. Gel Filtration of Bovine Parotid DNase on Sephadex G-100

(a) DEAE-cellulose fraction (70 mg); column size, 1.7×120 cm); fraction size, 3.0 ml. (b) Sephadex G-100 fraction (2 mg) in (a); column size, 1.1×50 cm; fraction size, 2.5 ml. Protein was estimated by reading the absorbance at 280 nm (—). Ten microliters of each fraction was used in the assay for DNase (⊙).

result is shown in Fig. 2b. The peak of protein coincides with the peak of the activity of DNase. Table I summarizes the results of a typical purification of bovine parotid DNase from 10 kg of bovine parotid glands. The over-all purification was about 210-fold with an over-all yield of about 5%. This purified DNase was free of ribonuclease [EC 2.7.7.16], nonspecific phosphodiesterase [EC 3.1.4.1] (substrate sources, bis(*p*-nitrophenyl) phosphate and RNA) and phosphomonoesterase [EC 3.1.3.1] (substrate source, *p*-nitrophenyl phosphate) activities. Its pH optimum was about 6.6 using 0.05M phosphate buffer. The purified DNase was more active against native brain DNA than heat denatured brain DNA substrates

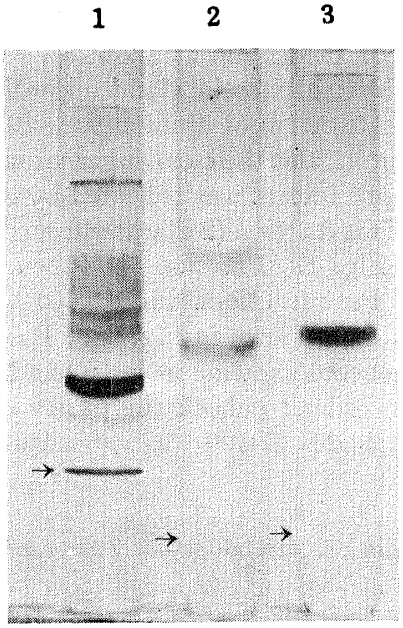

Fig. 3. Disc Gel Electrophoresis of Bovine Parotid DNase

(1) ammonium sulfate fraction,
(2) DEAE-cellulose fraction,
(3) Sephadex G-100 fraction,
Arrows indicate the BPB marker.

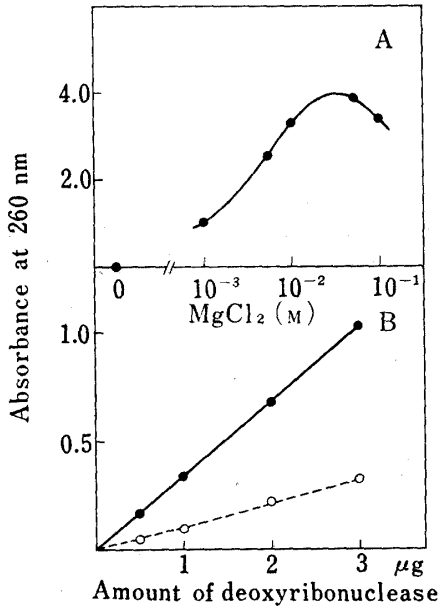

Fig. 4. Catalytic Properties of Bovine Parotid DNase

Fig. 4A shows magnesium-dependence of the DNase activity. Fig. 4B shows the substrate preference of the DNase. Denatured DNA was prepared by the heating at 98° for 10 min of native DNA and rapid cooling. native DNA (—●—); denatured DNA (---○---)

Table I. Purification of Bovine Parotid DNase

Fraction	Total protein (g)	Total activity (units)	Specific activity (units/mg)	Yield of DNase (%)
Crude extract from the gland	800	416×10^3	0.52	100
Extract of acetone-dried powder	53	106×10^3	2.0	25
Precipitate with ammonium sulfate (0.5—1.0 saturation)	8	92×10^3	11.5	20
DEAE-cellulose	0.62	36×10^3	58	9
Sephadex G-100	0.21	22×10^3	105	5

and had requirement for magnesium ion, as shown in Fig. 4. A result of analysis of a complete digest of the DNA with purified DNase according to the procedure described earlier[10] showed that the digest was composed of oligonucleotides from mononucleotide to pentanucleotide and the mononucleotide fraction contained 5′-dAMP, 5′-dCMP, 5′-dTMP and 5′-dGMP, which were assigned by their chromatographic behaviour on AG 1×2 columns. The approximate molecular weight of the parotid DNase was estimated to be 38000 by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis using the method of Dunker and Rueckert[11] and the molecular weight of pancreatic DNase (Miles Laboratories, Grade I) was also estimated to be 38000. The sugar in purified parotid DNase was determined by the phenol–H_2SO_4 method[12] and the content was 3.0% as calculated from a calibration curve based on glucose and this result is coincident with that of pancreatic DNase.[13] Rundblad, *et al.* reported that DNases from pancreas and parotid glands were immunologically different. However we could not obtain the biochemical results which showed differences between pancreatic DNase and parotid DNase. It was concluded that parotid DNase was very similar to pancreatic DNase.

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A Simple Synthesis of Amino-containing Bunte Salts by the Reaction of Aminothiols with Chlorosulfonic Acid

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Organic thiosulfates, so-called "Bunte salts," were first prepared by Bunte in 1874.[2] Bunte salts are remarked as surfactants,[3] intermediates in organic syntheses,[4] protectors

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