Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2012; 37 (2) ; 188–195.
doi: 10.5505/tjb.2012.69775

Research Article [Araştırma Makalesi]

Yayın tarihi 15 Nisan, 2012 © TurkJBiochem.com

[Published online 15 April, 2012]

A novel method for purifying a DNase from lysosomal fraction from human small intestine

[Özgün metodla insan ince bağırsağı lizozomal fraksiyonundan DNaz izolasyonu*]

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Registered: 9 September 2011; 23 January 2012

[Kayıt Tarihi: 9 Eylül 2011; Kabul Tarihi: 23 Ocak 2012]

ABSTRACT

Aims: The purification of an acid DNase related DNase II family from lysosomes of human small intestine (jejunum).

Materials and Methods: Two different methods: a column chromatography series, including phosphocellulose, CM-cellulose and Sephadex G-200 and a novel procedure that use immunoabsorbent technique were used in the purification.

Results: The purified DNase is an endonuclease that consisted of one polypeptide chain (monomer) with a molecular mass of 28-32kDa with an optimal pH and temperature of 6.0 and 30°C, respectively. The enzyme prefers native DNA on denatured DNA

Discussion: The catalytic properties of the purified enzyme are essentially the same as those of DNase II family such as independency of divalent ions which inhibited its activity at 10mM. The enzyme acts on dsDNA and ssDNA and generates 3'-phosphate and 5'-OH termini which was a characteristic of DNase II. The functions of the intestinal purified DNase from lysosomes were unclear, but lysosomes contained a set of enzymes required for degradation of food, and the enzyme may be necessary for degradation of nucleic acids within food, but small amounts of the enzyme were found in the nuclei and cytoplasm were detected. Several researchers suggested that DNase II family may be active in apoptosis and played a role as a barrier to transfection. The purified was designated SIDNase.

Key words: DNase II, small intestine, immunoabsorption, lysosomes, DLAD

Conflict of interests: There is no conflict in interest.

ÖZET

Amaç: İnsan ince bağırsağından (jejunum) asit DNaz II ailesi ile ilintili DNaz izolasyonu.

Materyal ve Metod: İzolasyonlar için iki farklı metod uygulanmıştır: Fosfoselüloz CM-selüloz ve Sephadex G-200 den oluşan bir kolon serisi ve immünabsorbsiyon tekniği kullanılarak geliştirilen yeni bir prosedür.

Sonuçlar: İzole edilen DNaz tek bir polipeptit zincirinden (monomer) oluşan bir endonükleaz olup, moleküler ağırlığı 28-32 kDa, fonksiyonu için optimal ısısı 30°C, pH ise 6.0 dır. Bu enzim natif DNA'yı denatüre DNA'ya tercih etmektedir.

Tartışma: İzole edilen enzimin katalitik özellikleri DNaz II ailesine ait enzimler ile temelde aynıdır. Mesela iki değerlikli iyonlardan bağımsız aktivite göstermesi, aktivitesinin 10mM da inhibe olması gibi. Bu enzim dsDNA ve ssDNA üzerinde DNaz II gibi 3'-fosfat ve 5'-OH terminali oluşturur. Bağırsaklardan izole edilen lizozomal DNaz'ın fonksiyonları tam olarak nitelenememiştir ancak lizozomler gıda parçalanması için bir set enzim içermektedir ve gıdada bulunan nükleik asitlerin parçalanması için gerekli olabilirler. Az miktarda enzim çekirdek ve sitoplazmada bulunmuştur. Bazı araştırmacılar DNAz II ailesinin apoptozda aktif olabileceğini ve görevinin transfeksiyonda bariyer oluşturmak olabileceğini öne sürmüşlerdir. İzolat SIDNaz olarak adlandırılmsiyonıştır.

Anahtar Kelimeler: DNaz II, ince bağırsak, immünabsorbsiyon, lizozom, DLAD

Çıkar çatışması: Çıkar çatışması yoktur.

Introduction

DNases have been classified, classically, into DNase I and DNase II families. DNase I enzymes (EC 3.1.21.1), have a pH optimum at approximately pH 7.0 and requires magnesium ion and many DNaes are purified that are related to DNase 1 family such as DNase1L1, DNase1L2 and DNase1L3 [1, 2].

DNase II family (Acid DNase family) (EC 3.1.22.1) are one of the best characterized endonucleases that catalyze DNA hydrolysis in the absence of divalent cations at acidic pH and are found in a wide variety of animal tissues [1, 3, 4]. Three acid DNases have been identified in mammals so far: DNase 2a, usually referred to as DNase 2, [5], DNase 2b, also known as DLAD [6] and L-DNase II [7]. They are active and present in lysosome (but found in other organelles) and believed to act as a barrier to transfection for DNA or vectors entering the cell by endocytosis [8] and may degrade foreign DNAs and play a role in the replication of DNA [9].

Recent studies have identified many DNases that are active at neutral pH such as caspase-activated DNase (CAD) [10], and endonuclease G [11] that are found in mammals, though a few researchers have concentrated on insect acid [12].

The function of DNases within the small intestine is debated and very few researchers purified DNases from small intestine. DNase I was purified and studied from human small intestine in 1998 [13], while the present work purified a DNase II from the lysosomes of jejunum of the small intestine in human. The authors used two methods for the purification of the enzyme: a classical method that used affinity chromatography and the immunoabsorption technique [14]. The immunoabsorption technique was ignored by many researchers as impractical way that wastes a lot of the purified enzyme, or lead to the formation of denatured enzyme even when it offered a rapid way of purification, but the authors found it a good method with reasonable cost. The authors studied the characterization of the purified acid DNase and designated SIDNase.

Materials and Methods

Materials

500g of human (male, 50 years old) small intestine had been obtained from the operating theatre, Al-Essra hospital, Amman, Jordan. The authors were obtained a legally signed consent from the deceased family that permitted them to use pieces of his jejunum for research. Phosphocellulose, CM-cellulose and Sephadex G-200 were obtained from Merick. DNase I from bovine pancreas, DNase II from bovine spleen, snake (*Crotalus adamanteu)* venom Phosophodiesterase I (I.U.B. 3.1.4.1) and bovine spleen phosphodiesterase II (I.U.B. 3.1.16.1) were from Worthington Biochemical Co. Collodion bags were from Sartorius membrane-filter, Germany. Molecular weight markers are obtained from Boehringer, Mannheim, Germany. Motor driven tightly fitting glass/Teflon Potter Elvehjem homogenizer (30 ml) was used. All glassware were washed three times with distilled water to avoid contamination. All buffers are prepared in the same day of the experiment to avoid bacterial/yeast growth in stored buffers, and since pH depended on temperature, the pH must be measured in all solutions at 25°C.

Purification of lysosomes

Lysosomes were purified according to the method Kawashima et al [15] from human small intestine. 500g of human small intestine were minced with scissors, and suspended in 6 volumes of cold 0.3M sucrose bubbled with nitrogen gas and containing 50 ■g/ml leupeptin (a protease inhibitor). The suspension was transferred into a Teflon pestle and homogenized at 1,600 g at 4°C. The homogenate was centrifuged twice at 3,000g for 10 min and the supernatant was incubated for 10 minutes at 37°C in the presence of 5 mM $CaCl_2$ to induce mitochondrial swelling. The incubated sample was layered on an isoosmotic Percoll at a density of 1.23 g/ml in a Hitachi RP 30 rotor tube. The pH of the Percoll suspension was adjusted to 6.5 with hydrochloric acid. Centrifugation was performed for 15 minutes at 60,000g in a SW60Ti rotor (Beckman Ultra centrifuge tube) at 2°C. After centrifugation, the pellet was re-suspended in 30 ml of buffer A (0.05 M potassium phosphate buffer and 10%glycerol adjusted to pH 6.0). This suspension is the lysosomal fraction. The fraction was centrifuged at 15,000 g for 10 minutes and the pellets were washed by the above buffer to remove the contaminated Percoll. The washed pellets were used as the purified lysosomal fraction.

Biochemical analysis

Cathepsin D [16] was used as lysosomal markers. Succinic-INT reductase [17], glucose-6-phosphatase [18] and catalase [19] were used as mitochondrial, microsomal, and peroxisomal markers, respectively.

Protein contents were determined by the modified method [20] of Lowry et al. [21] using Bovine serum albumin as a standard. Carbohydrates contents were determined by the method of Dubois et al. [22] using glucose as a standard.

The yield and the relative specific activity of marker enzymes for various cell organelles in the purified lysosomal fraction are shown in Table 1. The purified lysosomal fraction was very rich in cathepsin D, more than 30-fold over the homogenate. Succinic-INT reductase and glucose-6-phosphatase were below the level of detection.

Isolation of DNase by a classical method

The purified lysosomal fraction was loaded into phosphocellulose column (40 x 1.5 cm) that was previously washed equilibrated with buffer A till the A260 reached

Table 1. Purification of lysosomes from human small intestine by Percoll density gradient centrifugation

Purification (fold)	Yield (%)	Specific activity (units. mg^{-1})	Total protein (mg)	Total enzyme (units)	Enzyme
25.8	15	72.2	2.8	202.2	Cathepsin D
0.01	-	696.7	3.62	2522	Catalase
-	-	1075.2	3.1	3333.2	Succinic INT-reductase
-	-	30180	3.3	99594	Glucose 6-phosphate

zero. Enzyme fractions (30ml) were eluted with a NaCl gradient (0-2.0M in buffer A). The active fractions were pooled, desalted by dialysis and loaded into CM-cellulose column (20 x 1.5 cm) washed and equilibrated as described before with buffer A. The DNase activity was eluted with a linear NaCl gradient (0-1.0 M NaCl in buffer A). The active fractions were pooled, desalted by dialysis and concentrated by filtration through a collodion bag and loaded onto a Sephadex G-200 column (2.5 x 35cm) that was equilibrated as above. DNase activity was eluted and the peaked fractions were collected and stored at -20°C.

The homogeneity of the enzyme was determined by 10%SDS–PAGE. The total yield of protein was 2.5 $mg.L^{-1}$ of induced culture. All steps were carried out at 4°C. DNase activity, protein and carbohydrate concentrations were determined for all fractions.

Immunochemical procedures

Preliminary studies with the pooled peaked fractions from phosphocellulose column (considered as the crude extract) showed antigenic similarities between the semi-purified DNase with commercial DNase II from bovine spleen, so antibodies were raised against DNase II where produced in rabbits and used as immunoabsorbent for the purification of the DNase from human small intestine.

The antibody produced by the other commercial enzyme (DNase I from bovine pancreas) could not react with the crude extract and this procedure was as effective in purifying the DNase as the classical method that used affinity chromatography and not so expensive. The newly purified enzyme showed similarities to DNase II and probably can considered being a homologous to it.

Immunization was initiated by injecting 18 white male rabbits (albino from local markets) which were divided into two group with 100U/ul DNase I and DNase II, respectively (suspended in equal volume of complete Freund's adjuvant). A second injection of 50U/ul suspended in Freund's adjuvant was given a week later followed by two more injections (50U/ul each time) at weekly intervals. Antisera were collected from the marginal ear vein after 40 day. Additional antisera could be obtained by challenging the animal with similar dosage of the enzyme-Freund adjuvant suspension and bleeding it 1 to 2 weeks later.

The serum was diluted with 0.1%saline solution and a 3-ml aliquot was added to 0.5ml of one of the commercial DNases which was then stirred mechanically for 10 min before ammonium sulphate was added to it and stirred or 10 min.

The enzyme-antibody complex was precipitated after centrifugation at low speed and DNases activity was measured in the supernatant.The antisera was dialyzed (separately) for 2h against 4l of 0.1 $NaHCO_3$ containing 1M NaCl, and then coupled with CNBr-activated Sephadex G-200 at 25°C according to the method of Khon and Wilchek [23].

The Ouchterlony plates were prepared with 1·5%agar in 0·01 M buffer A containing 0·14 M sodium chloride and 0·02%sodium azide. After placing the antigen and antibody in the wells cut into agar plates, the plates were developed in a humid chamber at 0-5°C for 24 h. Immunoreactive bands were visualized according to the method of Canton *et al* [24] with some modifications.

Purification by Immunoabsorption

The purification procedure was according to Neuwelt et al [25] with some modifications, and was shown in Table II. The crude extract was added to centrifuge tubes containing Sephadex G-200 conjugated antiserum (see Methods). 10 ml of 0.80%NaCl was added to each tube. The mixture was stirred for 30min and the unbound enzyme was removed by suspending the immunoabsorbent in 50ml of buffer A and stirring for 5min, and then centrifuged and the supernatant was discarded.

The enzyme-Sephadex G-200 mixture re-suspended in buffer A and the procedure was repeated. The enzyme was eluted and separated from Sephadex G-200 by the addition of buffer A. The peaked fractions were stored at -20°C.

Enzyme assay

The standard assay system contained (1ml) containing 0.25ml 50mM buffer A, 0.25g of Salmon testes DNA (native or denatured) and an appropriate amount of enzyme. After incubation at 30°C for 30min, the reaction was stopped by the addition of 4ml of 10%perchloric acid in chilled vessel. The mixture was centrifuged after 10 min in ice at 3,000g for 10min and then the absorption read at 260nm.

One unit of the enzyme was defined as the amount of the enzyme which yields 10 absorbance units of soluble components per minute in 1 ml of the reaction mixture. This unit corresponds to solubilization of 1umole of nucleotide per minute, assuming that the average atomic extinction coefficient of nucleotide is 10.000.

Influence of pH and temperature

To assess the effect of pH on the enzyme activity, DNase activity was measured at various pH values ranging from 4.0 to 9.0. Buffer A was replaced with acetate buffer (pH 4.5-5.5), Tris-HCl (pH 7.0-8.5), Glycine buffer (pH 8.5-10.0). The optimum temperature was determined according to standard assay at different temperatures ranging from 20 to 60°C at intervals of 5°C.

Activity of purified DNase on RNA

The standard assay system contained (1ml) containing buffer A, 0.25g of yeast RNA, and an appropriate amount of enzyme. After incubation for 15min at 30°C, the reaction was stopped by the addition of 1 m of uranyl reagent (0.57 %uranyl acetate in 25%perchloric acid) in chilled vessel. The mixture was centrifuged after 10 min and then 0.3 ml of the supernatant was diluted with 3ml of water and the absorption at 260nm was measured. One unit of the enzyme was defined as the amount of the enzyme which yields 10 absorbance units of uranyl reagent soluble components per minute in 1 ml of the reaction mixture. This unit corresponds to solubilization of 1umole of nucleotide per minute, assuming that the average atomic extinction coefficient of nucleotide is 10.000.

Estimation of native molecular mass

The molecular mass of the purified DNase was estimated by gel filtration using Sephadex G-200 column (1.5 x 30cm) previously calibrated with protein markers of known size: Protein markers of known sizes: ferritin from equine spleen (364kDa), glutamate dehydrogenase (350kDa), catalase from *Aspergillus niger* (240 kDa), myosin from rabbit muscles (205 kDa), alcohol dehydrogenase (150kDa), B-galactosidase from *Escherichia coli* (116 kDa), bovine serum albumin (67 kDa), fumarase from porcine heart (48 kDa), ovalbumin (45 kDa) and cytochrome C (12.5 kDa) were used as standards.

The subunit of the purified DNase was estimated by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) used 12%polyacrylamide gel as described by Laemmli et al. [26] as modified by Maizel [27]. Proteins were visualized with Coomassie blue R-250. Very faint band was shown and the purified DNase activity was located by running a separate lane with pooled active fractions from Sephadex G-200 which was cut out from the gel before staining. The lane was cut horizontally into 0.25mm pieces, submerged in the buffer, and assayed for enzymatic activity as in Methods. Protein markers as above were used as standards.

Terminal products generated by the purified DNase

The method of Koh, Waddell, and Aposhian [28] as modified by Ikeda and Takata [29] was used for determination of terminal products of the purified DNase.

Native dsDNA was digested with the purified DNase in a standard assay system at 30°C until approximately 25%of the DNA became acid-soluble. The reaction was stopped by heating at 60°C for 20 min. As a control, DNA cut by DNase I was prepared in the same manner, except that the reaction proceeded at 30°C in buffer consisted of 20 mM Tris-HCl (pH 7.5) and 2mM $MgSO_4$. The limit digest products of the purified DNase could not be further degraded by phosphodiesterase II without prior bacterial alkaline phosphatase treatment, so the DNA was treated with bacterial alkaline phosphatase at 30°C for 10 min before being reacted with phosphodiesterase II. Phosophodiesterase I was a 3'-to-5' exonuclease, and hydrolyzed 5'mononucleotides from 3-hydroxyl terminated deoxyribo-oligonucleotides, while phosphodiesterase II was a 5'-to-3' exonuclease, hydrolyzed 3'phosphomononucleotides from oligonucleotides contained a 5'-hydroxyl terminus. It was suggested that the purified DNase limit digested products possess 5'-phosphorylated and 3' hydroxyl termini.

Results

Purification

The classical purification protocol rendered a homogenous preparation of DNase. The enzyme was purified to about 418-fold with a specific activity of 30180 units. mg^{-1} protein and a yield of 10.4%(Table 2), while the enzyme was purified 484-fold through immunoabsorbent procedure with a specific activity of 35000 units.mg^{-1} protein and a yield of 12.0%. (Table 3). The shape of the peak of Sephadex G-200 (in both cases) suggested homogeneity of the enzyme (Fig.1).

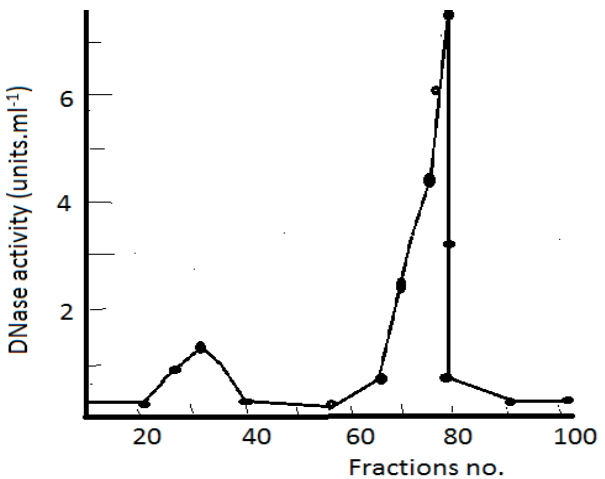

Figure 1. Elution profile of DNase II on Sephadex G-200 column ((2.5 × 35cm). Buffer: 0.05 M potassium phosphate buffer and 10%glycerol adjusted to pH 6.0. Flow rate, 1 ml/min; Column temperature, 4°C.

Optimal pH, temperature and stability

The pH optimum of the purified DNase is 6.0 and the enzyme was retained 85%of the activity at pH 7.0 and 5.0. The optimum temperature was at 30°C, but dropped quickly and disappeared completely at 45°C.

The enzyme in its purified form was stable up to 60 min at room temperature and for a week at 4°C. The addition of! 0%glycerol to the enzyme stabilized the enzyme substantially (up to 36 h at 4°C), and up to three weeks at -20°C, but any higher concentration will interfere with the chromatography steps. BSA (up to 10%) did not stabilized or activated the enzyme, and showed no effect at all.

Substrate specificity

The purified enzyme was at least 4 fold more active with native dsDNA than with heat denatured DNA. The enzyme has no activity against RNA. These results indicated that the purified enzyme is an endonuclease that cleaves DNA as a substrate

Divalent metals effects

The enzyme was independent of cation ions, and these ions would not affect its activity at low concentrations, but most divalent cations would inhibited the enzyme at various concentration (8mM or above), while EDTA had no effect on it (Table 4).

Table 2. Classical purification of the purified DNase from lysosomal fraction from human small intestin

Purification (fold)	Yield (%)	Specific Activity (Units.mg^{-1})	Total Protein (mg)	Total Activity (units)	Volume (ml)	Purification Step
1	100	72.2	40	2889	20	Lysosomal fraction
9.65	48.2	696.8	2.0	1393.6	16	Phosphocellulose
14.9	22.7	1075.2	0.61	655.9	10	CM-cellulose
418.0	10.4	30180	0.01	301.8	6	Sephadex G-200

Table 3. Immunoabsorbent purification of the purified DNase from lysosomal fraction from human small intestine

Purification (fold)	Yield (%)	Specific Activity (Units.mg^{-1})	Total Protein (mg)	Total Activity (units)	Volume (ml)	Purification Step
1	100	72.2	40	2889	20	Lysosomal fraction
9.65	48.2	696.8	2.0	1393.6	16	Phosphocellulose
36.95	21.1	1525.1	0.7	1067.6	16	Immunoabsorbent chromatography
484.7	12.1	35000	0.01	350.0	8	Sephadex G-200

Table 4. Effect of divalent metal ions on the purified DNase activity from the pooled fractions from Sephadex G-200

Relative activity (%)						
20.0 mM	10.0	8.0	4.0	2.0	1.0	Salt
					100	Control
0	0	25	80	100	100	$MgS0_4$
0	0	10	60	100	100	$MnSO_4$
70	85	90	100	100	100	$BaCl_2$
0	5	70	100	100	100	$CdSO_4$
78	90	95	100	100	100	$CaCl_2$
0	10	65	80	100	100	$CoCl_2$
0	0	0	55	100	100	$CuSO_4$
0	0	3	45	100	100	$Fe_2 (SO4)_3$
0	0	0	50	100	100	$ZnCl_2$
100	100	100	100	100	100	EDTA

Estimation of molecular mass

The enzyme was eluted as a single-chain polypeptide from Sephadex G-200 column with a molecular mass of about 28kDa. SDS-PAGE showed a very faint single band (that was identified by assaying the gel after cutting it as described in Methods), which gave the impression that DNase was a monomer with a molecular mass of 32kDa (Fig. 2). This deviation was expected since SDS-PAGE had its limitations and most proteins would give estimates within a few percentage of their actual weight which may be due to a non-globular native protein shape [30].

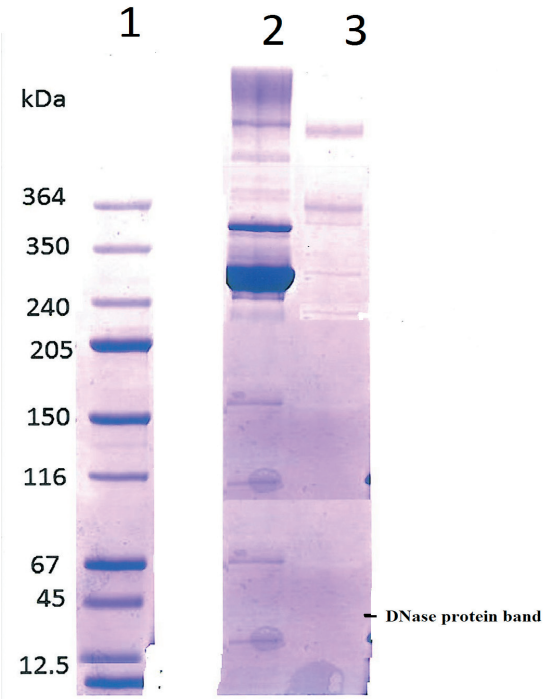

Figure 2. SDS-polyacrylamide gel with three lanes; Lane M: the standards, Lanes 2 and 3 the pooled peak of CM-cellulose and Sephadex G-200, respectively. The arrow indicates the band that showed DNase activity.

Terminal products produced by the purified DNase

Phosphodiesterase II was cleaved all the hydrolysis products formed by the purified DNase, but these products were cleaved with phosphodiesterase I after they were treated with alkaline phosphatase.

DNase I digested products are cleaved by phosphodiesterase I, but not by phosphodiesterase II. This was a clear indication that the purified DNase produced oligonucleotides with a phosphate group at the 3'-termini and a hydroxy group at the 5'-termini, which was a characteristic of DNase II [31, 32, 33].

Discussion

Deoxyribonuclease II (DNase II) from lysosomes of human's small intestine (jejunum) was purified using two methods: a column chromatography series, including phosphocellulose, CM-cellulose and Sephadex G-200 and a novel procedure that used the immunoabsorbent technique with the advantage of utilized a commercial available enzyme (DNase II) which was injected in rabbits to produce antigens that cross-reacted with the enzyme of the interest. Although affinity chromatography is a powerful and widely used tool in enzyme purification, the use of antibody, or an immunoabsorbant, as the binding agent has been ignored by most researchers. This is due to the use of large amounts (milligrams) of pure enzyme to immunize a host animal; and because the antibodies, when used as an immunoabsorbant, bind the enzyme too strongly, often requiring 6 M urea for elution which lead to the formation of denatured enzyme even when it offered a rapid way of purification [3, 24, 25]..The authors were succeeded in obtaining a purified enzyme and the method seemed promising.

The specific activity obtained from the immunoabsorption technique was similar to the specific activity produced through classical method of preparation.

The purity of the final preparation assessed on the basis of SDS-PAGE and the production of a nonspecific antibody against the purified enzyme.

The enzyme was an endonuclease which had an optimal pH and temperature of 6.0 and 30°C, respectively and had one polypeptide chain (monomer) and a molecular mass of 28-32kDa. Most molecular masses of DNase II varied from 26 to 45 kDa (32, 34) and compared with other human purified DNases II: DNases from human urine [35], human gastric mucosa and cervix [36], human lymphoblasts [32], and from an enzyme with a DNase I-like structure exhibiting acidic DNase II-like activity from human [37] had 32 kDa, 38 kDa, 45 kDa and 35 kDa respectively.

The structures of DNase II family had not been elucidated but several researchers suggested that most of them consisted of a single polypeptide [35, 36] similar to the purified DNase from the lysosomes of small intestinal, but the DNase from the human liver consisted of three non-identical subunits [38].

The catalytic properties of the purified enzyme were essentially the same as those of all other well described as members of acid DNases family mentioned above. The enzyme was independent of divalent ions. Divalent ions at low concentrations had no effect on the enzyme, but the presence of Mg^{2+} and Mn^{2+} at 4mM would inhibit the activity by 20 and 40%, respectively and all activity would be disappeared at 10mM concentration.

Cadmium and Cobalt salts affected the enzyme at 8mM, and most the activity deteriorated rapidly at 10mM. Cupric, Ferric, and Zinc salts had strong inhibitory effect and would inhibit 40-55%of activity at 4mM and all the activity disappeared at 8mM.

Barium and Calcium seemed to had a very little effect on the activity of the enzyme.

The enzyme acts on both native (dsDNA) and denatured DNA (ssDNA), but acts preferentially on native dsDNA and generates 3'-phosphate and 5'-OH termini.

The purified DNase showed similar enzymatic properties to DLAD, a novel DNase II-like mammalian endonuclease [4, 6]. Both DLAD and the purified DNase II required no cofactors for their catalytic activities, exert their maximum activities under acidic conditions, divalent cations inhibited both enzymes and both produced DNA fragments with 3′-P/5′-OH termini. The main difference was that DLAD exerts its DNase activity under neutral to alkaline pH conditions while the purified DNase II was inactive at alkaline pH, and its optimal pH was 6.0.

The functions of the intestinal purified DNase from lysosomes were unclear, but lysosomes contained a set of enzymes required for degradation of food, and the enzyme may be necessary for degradation of nucleic acids within food, but small amounts of the enzyme were found in the nuclei and cytoplasm (unpublished data) were detected. Several researchers suggested that DNase II family may be active in apoptosis [39, 40] and played a role as a barrier to transfection. [10].

The purified DNase from lysosomes in human small intestine (Jejnum) was clearly a member of DNase II family and designated SIDNase.

Acknowledgement

The authors are grateful to the technical staff in the operating theatre, Al-Essra hospital, Amman, Jordan for their assistance and to Ms. Hebah M. Deebajah, Princess Haya Biotechnology Center, Jordan University Of Science And Technology.

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