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ORIGINAL ARTICLE

Purification and characterization of deoxyribonuclease from small intestine of camel *Camelus dromedarius*

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Abstract The chromatography of deoxyribonuclease (DNase) from small intestine of camel *Camelus dromedarius* by DEAE-Sepharose separated three isoforms DNase 1, DNase 2 and DNase 3. The DNase 3 was purified to homogeneity by chromatography on Sephacryl S-200. The molecular weight of DNase 3 was 30 kDa using gel filtration and SDS-PAGE. The pH optimum of DNase 3 was reported at 7.0 using Tris-HCl buffer. The temperature optimum of DNase 3 was found to be 50 °C. The enzyme was stable up to 50 °C for one h incubation. The Km value was 28.5 µg DNA, where this low value indicated the high affinity of enzyme toward DNA as substrate. No activity of DNase 3 was determined in the absence of metal cations. Mg^{2+} and Ca^{2+} caused significant enhancement in the enzyme activity by 90 and 75%, respectively. The mixture of Mg^{2+} and Ca^{2+} caused 100% of enzyme activity. Ni^{2+}, Co^{2+}, Ba^{2+}, Zn^{2+} and Cd^{2+} showed very strong inhibitory effect on enzyme activity. In conclusion, the characterization of DNase 3 indicated that the enzyme is considered as a member of DNase I family. The low Km value of the DNA suggested that the high digestion of DNA of camel forage by small intestine DNase 3.

1. Introduction

Deoxyribonucleases (DNases) have been classified into DNase I and DNase II families. DNase I enzymes (EC 3.1.21.1) had neutral pH and required Mg^{2+} and Ca^{2+} cations. DNases I family included DNase1L1, DNase1L2 and DNase1L3 [5,25,28]. DNase II family (EC 3.1.22.1) hydrolyzed DNA at acidic pH and in the absence of Mg^{2+} and C^{2+} cations [5,30,32]. DNase II family included DNase 2a [17], DNase 2b [19] and L-DNase II [27].

DNase I hydrolyzed DNA to oligonucleotides with 5′-phospho and 3′-hydroxy termini [11,29]. DNase I is existed principally in tissues of the alimentary canal included intestine, pancreas and salivary glands, where it hydrolyzed DNA [9].

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DNase I was also detected in human small intestine [24]. Although the enzyme has been found in all other studied tissues its role in non-digestive tissues remains uncertain [26]. DNase I may be participated in apoptotic cell death [1,13,20]. DNase II is found in a wide variety of animal tissues. They are active and present in lysosome and believed to act as a barrier to transfection for DNA or vectors entering the cell by endocytosis [7,23] and may degrade foreign DNAs and play a role in the replication of DNA [2]. DNase II digested DNA of apoptotic cells after phagocytosis [12].

The feeding of camels depended on low quality natural forages, which existed in deserts and dry lands. Camels can survive in very dry environmental conditions with the minimal amount of water [3]. Most studies of digestion of camel focused on microbial digestion in the rumen [6,21,22]. We previously studied some of digestive enzymes in camel such as carbohydrases in pancreas and disaccharidase in small intestine [15,14]. In the present study, deoxyribonuclease, as digestive enzyme, from small intestine of camel *Camelus dromedarius* has been purified and characterized.

2. Materials and methods

2.1. Camel small intestine

Camel small intestine was obtained from Cairo slaughter house. The small intestine was saved directly into an ice box for transportation to the laboratory and transferred to frozen storage at −80 °C.

2.2. Deoxyribonuclease assay

Deoxyribonuclease (DNase) activity measurements were carried out according to Yasuda et al. [29]. The one ml reaction mixture consisted of 20 μg calf thymus DNA, 10 mM $MgCl_2$, 10 $CaCl_2$, 50 mM Tris-HCl buffer, pH 7.0 and 2–10 μg protein. The change in absorbance at 260 nm was followed at 30 s intervals. One unit of DNase activity was defined as the amount of enzyme which increases the O.D. 1.0 per min under standard assay conditions.

2.3. Purification of DNase from camel small intestine

2.3.1. Preparation of crude extract

The DNase crude extract was prepared by homogenization of 5 g camel small intestine in 15 ml of 20 mM Tris–HCl buffer, pH 7.0 using a homogenizer. The homogenate was centrifuged at 10,000 g and the supernatant was designated as crude extract. The crude extract was subjected to dialysis against the same buffer.

2.3.2. Column chromatography

The dialyzate was applied directly to a diethylaminoethanol (DEAE)-Sepharose column (10 × 1.6 cm i.d.) pre-equilibrated with the same buffer. The adsorbed material was eluted with a stepwise gradient of NaCl ranging from 0.0 to 0.2 M prepared in the same buffer at a flow rate of 30 ml/h and 3-ml fractions were collected. The pooled fractions (0.2 M NaCl) with the highest specific activity of DNase were concentrated through dialysis against solid sucrose and applied on a Sephacryl S-200 column (90 × 1.6 cm i.d.) previously equilibrated with the same buffer and developed at a flow rate of 20 ml/h, and 3.0 ml fractions were collected.

2.4. Protein determination

Protein was quantified by the method of Bradford [4]. Bovine serum albumin was used as the protein standard.

2.5. Molecular weight determination

Molecular weight was determined by gel filtration technique using Sephacryl S-200. The column (90 × 1.6 cm i.d.) was calibrated with cytochrome C (12,400), carbonic anhydrase (29,000), bovine serum albumin (67,000), alcohol dehydrogenase (150,000) and β-amylase (200,000). Dextran blue (2,000,000) was used to determine the void volume (Vo). Subunit molecular weight was estimated by SDS-polyacrylamide gel electrophoresis [10]. SDS-denatured phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,000) and α-lactalbumin (14,200) were used for the calibration curve.

2.6. Characterization of DNase 3

The effect of pH on DNase 3 activity was determined in the pH range from 4.5 to 9.0 using 50 mM sodium acetate buffer (pH 4.5–6.0), sodium phosphate buffer (pH 6.5–7.5) and Tris-HCl buffer (pH 7.0–9.0). The optimal temperature for DNase activity was determined by incubating the enzyme-substrate mixtures at various temperatures (10–80 °C) in 50 mM Tris-HCl buffer, pH 7.0. Thermal stability of DNase was measured in terms of residual activity after incubation of DNase at

Table 1 Purification scheme of DNase from small intestine of camel.

Purification step	Total protein (mg)	Total units[a]	Specific activity (unit/mg protein)	Fold purification	% Recovery
Crude extract	3.15	3375	1071	1.0	100
Chromatography on DEAE-Sepharose					
0.0 M NaCl (DNase 1)	0.78	1300	1666	1.55	38.5
0.1 M NaCl (DNase 2)	0.336	833	2479	2.31	24.6
0.2 M NaCl (DNase 3)	0.15	670	4466	4.16	19.8
Gel filtration on Sephacryl S-200					
(DNase 3)	0.06	525	8750	8.16	15.5

[a] One unit of DNase activity is defined as the amount of enzyme that increases the optical density 1.0 per min under standard assay conditions.