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Purification and Characterization of Mongolian Mare Lactoferrin

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Abstract

The lactoferrin from mongolian mare colostrum has been purified by gel filtration (Sephadex G-100), affinity chromatography (Toyopearl-AF-Heparin-650M) in two steps. Mare lactoferrin-containing fractions were identified in the first peak among 3 peaks on Sephadex G-100 as first step, and purified lactoferrin was eluted with a step gradient of 0.5 M NaCl as a 3 step (gradient 0.1, 0.3, 0.5M). Eluted fractions were analyzed by 12% SDS-PAGE, and showed a single protein. Its molecular weight was estimated to be 82 kDa. N-terminal amino acid sequence was determined as APRKSVRWCTISPAEX-AKXA.

Key words : lactoferrin, mare milk, molecular weight, N-terminal sequence

Introduction

Mare milk has very unique composition rich in whey proteins and is very similar to human milk. Lactoferrin is an iron-binding glycoprotein and has multiple biological and chemical functions such as activation of the immune system, bacteriostatic, modulation of the inflammatory response in human and other animals (Nam and Yu 1998). Also, lactoferrin has attracted the attention of many researchers because of biological and biochemical functions related to the immune system of human and other animals (Jolles *et al.*, 1984; Nam *et al.*, 1998). Some of the bilogical functions of lactoferrin have been cosidered to be due to its iron-binding properties. The role of lactoferrin may be antimicrobial function, activation of the immune system, control of myelopoisis (Bollage and Rozycki, 1996) growth-stimulated enzyme of lymphocytes (Brock, 1995) and so on.

The lactoferrin concentration is higher than 2 mg/mL in human and is in the range of 0.02-0.2 mg/mL in bovine milk and in goat and pig milk and is lactoferrin concentration of horse milk is in the range of 0.2-2 mg/mL. In addition, colostral milk contains more lactoferrin than mature milk (Nam and Yu 1993).

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The physical and biochemical functions of human and bovine lactoferrin have been relatively studied, whereas the lactoferrin of horse milk has not been studying adequately. The lactoferrin in bovine milk was isolated and determinated N-terminal sequence (Jin *et al.*, 1995). In this study, the lactoferrin in Mongolian mare colostrums was isolated by gel filtration and affinity chromatography. Eluted fractions were analyzed by 12% SDS-PAGE, and showed a single protein. Its molecular weight was estimated to be 82 kDa.

Materials and Methods

Mare colostrum was obtained from Mongolia. The milk was stored frozen. Sephadex G-100 was from Sigma and Toyopeal-AF-Heparin-650M was from Toyopeal. BCA kit for protein assay was from Pierce, bovine lactoferrin and human lactoferrin were from Sigma, protein molecular standard marker was from Bio-Rad and other reagents were purchased from Sigma.

Preparation of whey proteins

The milk was thawed at 4°C, centrifuged at 3,000 × g for 1 h for defat and then the skim milk was adjusted to pH 4.6 with 1 M HCl. The caseins were precipitated by centrifugation at 20,000 × g, 4°C for 30 min.

Isolation of mare lactoferrin

The whey protein was precipitated by 70% ammonium sulfate and dialyzed against 0.01 M sodium phosphate buffer (pH 6.8) for 24 h and lyophilized.

The whey proteins were applied to a column of Sephadex G-100 (2.5 × 65 cm) and eluted with the same buffer and the flow rate was 30 mL/hr and the lactoferrin-enriched fractions were applied to Toyopeal-AF-Heparin-650M affinity chromatography (0.5 × 1 cm) and eluted by step gradient with the same buffer containing 0.1 M NaCl, 0.3 M NaCl, and 0.5 M NaCl. The flow rate was 30 mL/h. The lactoferrin enriched fractions were pooled and dialyzed against the same buffer for 24 h and lyophilized. Protein concentration was monitored at 280 nm by a spectrophotometer.

Electrophoresis

Sodium dodecylsulfate-polyacrylamide (12%) gel electrophoresis (SDS-PAGE) was performed. To detect protein bands after electrophoresis, the gel was stained in Coomassie Brilliant Blue R-250 for 1 h and was destained in Destaining solution I (methanol, 500 mL : glacial acetic acid, 100 mL : H_2O, 400 mL) for 1 hr and in Destaining solution II (methanol, 50 mL : glacial acetic acid, 70 mL : H_2O, 880 mL) for 12 h.

N-terminal sequence determination

The purified lactoferrin was transferred with PVDF membrane (Hoefer TE 22 Mighty Small, Amersham Pharmacia Biotech, USA) after SDS-PAGE and N-terminal sequence was determined by Procise 491 protein sequencer.

Results and Disscussion

Purification of lactoferrin from mare whey protein

The purification of mare lactoferrin was performed by 2 step chromatography. Fig. 1 shows the chromatography of mare lactoferrin on sephadex G-100 and this gel filtration resulted in 3 peaks. The lactoferrin-enriched fraction was identified in the peak I after SDS-PAGE anaysis. The lactoferrin containing fractions (15-28 fractions) were pooled, and applied to Toyopeal-AF-Heparin-650M column. Other proteins of whey were serum albumin, lactoglobulin and lactalbumin.

Affinity chromatography was carried out by step gradients of 0.1 M NaCl, 0.3 M NaCl, and 0.5 M NaCl with the same buffer. Fig. 2 shows 3 peaks in this experiment, pure lactoferrin was purificated in the third peak with the same buffer contained 0.5 M NaCl and subjected to analyze by SDS-PAGE (Fig. 3).

The molecular weight is expected 82 kDa on electrophoresis, Therefore, the molecular weight of lactoferrin from Mongolian horse was very similar to the mass 81 kDa of lactotransferrin which was obtained from E. Wettstein (Jin *et al.*, 1995), whereas this mass of lactoferrin has some similarities from KN goat milk lactoferrin (81 KDa), human lactoferrin (77 kDa), and bovine lactoferrin (85 kDa) (Hashizume *et al.*, 1983; Law and Reiter, 1977; Machaicki, 1991).

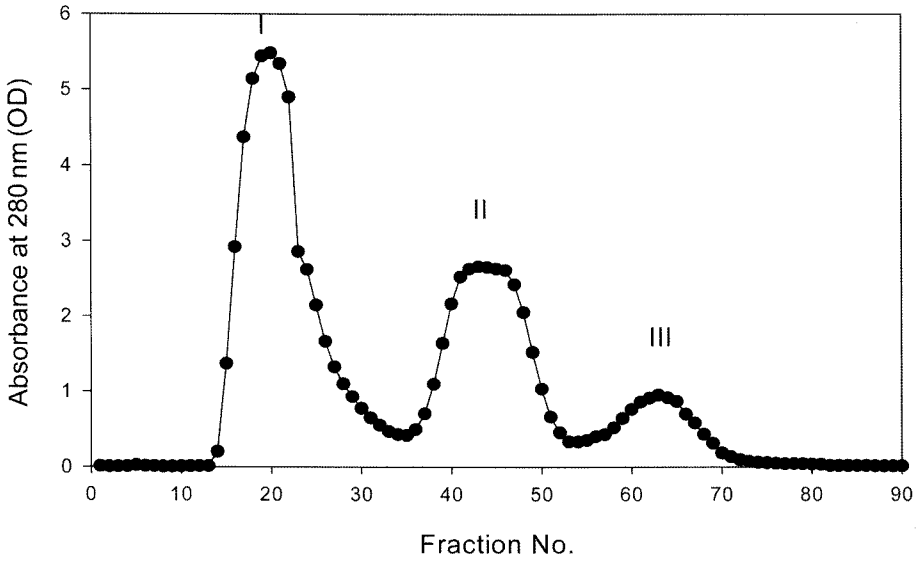

Fig. 1. Chromatography of lactoferrin from mare whey protein on Sephadex G-100. Whey protein dissolved in 0.01 M sodium phosphate buffer (pH 6.8) was applied to the column. The column was eluted with the same buffer and the flow rate was 30 mL/h. Detection was performed by UV absorbance measurement at 280 nm.

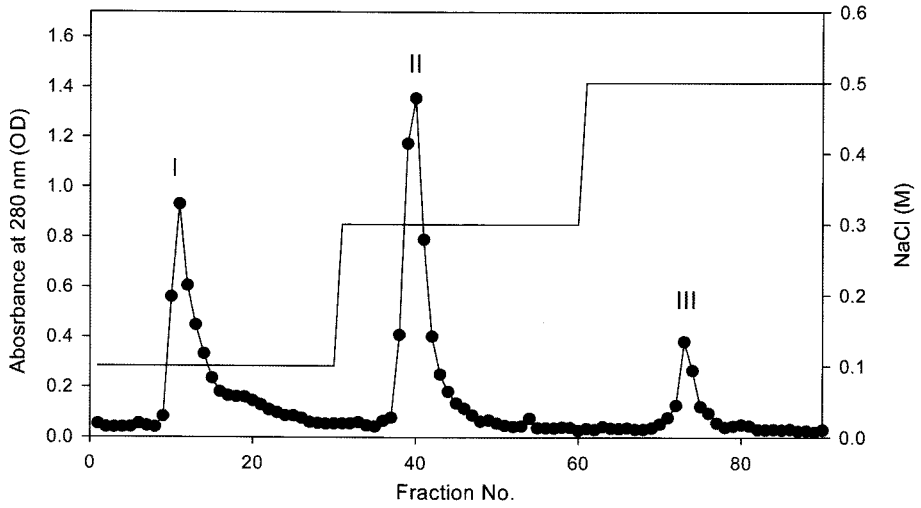

Fig. 2. Chromatography of lactoferrin on Toyopeal-AF-Heparin-650M column from Sephadex G-100 gel filtration. Lactoferrin-enriched fractions dissolved against 0.01 M sodium phosphate buffer (pH 6.8) was applied to the column. The column was washed with the same buffer and then eluted with a step gradient. The flow rate was 20 mL/h and detection was performed by UV absorbance measurement at 280 nm.

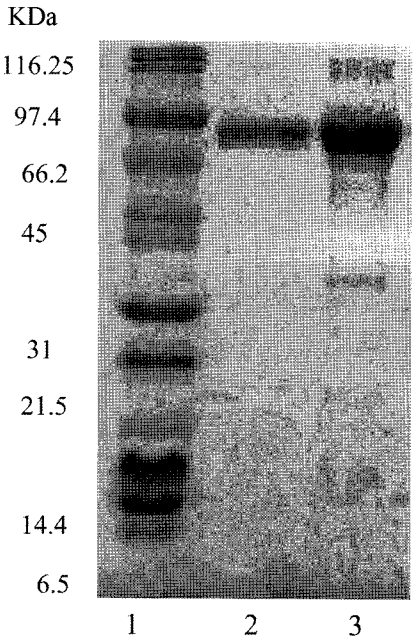

Fig. 3. Molecular weight determination of lactoferrin purified from mare whey on SDS-PAGE. lane 1. SDS molecular weight markers; 2. purified lactoferrin; 3. Bovine lactoferrin. *SDS molecular weight markers; 1. β-galactosidase (116,250); 2, phosphorylase b (97,400); 3. serum albumin (66,200); 4. ovalbumin (45,000); 5. carbonic anhydrase (31,000): 6.lysozyme (14,400); 7. aprotinin (6,500).

Identification of lactoferrin from mare milk and N-terminal sequence determination

To identify the lactoferrin, A 20 amino acid N-terminal sequence of mare lactoferrin was determined (Table 1) and it was APRKSVRWCTISPAEXAKXA. the identical homology of mare lactoferrin was 90% and 70% each compared with mare diferric lactoferrin and bovine lactoferrin. Therefore, purified sample from monglian mare milk was identified as lactoferrin. Also, mare lactoferrin has such a high identical homology as mare lactotransferrin (Jolles *et al.*, 1984) and KN goat lactoferrin (Law and Reiter, 1977).

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Table 1. N-terminal sequence of mare lactoferrin

	1									10										20
Mare lactoferrin	A	P	R	K	S	V	R	W	C	T	I	S	P	A	E	X	A	K	X	A
Mare diferric lactoferrin	A	P	R	K	S	V	R	W	C	T	I	S	P	A	E	A	A	K	C	A
Bovine lactoferrin*	A	S	K	K	S	V	R	W	C	T	T	S	P	A	E	S	K	K	C	A

*: Shimazaki *et al.*, (1991)

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