Purification of lactoferrin from low-fat bovine milk by cation-exchange chromatography

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Introduction

Lactoferrin is a globular glycoprotein found in secretory fluids of mammals. It controls the concentration of free iron, and is part of the innate immune system. Several mechanisms have been found for antibacterial and antiviral actions of lactoferrin. The protein binds specifically to various components on the cell surface of microorganisms. When lactoferrin binds the free iron it depletes the microorganisms of essential substances. Lactoferrin also binds to lipoproteins of cell membranes to compete with the entrance of virus particles into the cell. There are studies showing promising anticarcinogenic properties of lactoferrin.

The bioactive properties have created a large growing demand for lactoferrin produced from bovine milk for use in infant formula, dairy products, dietary supplements, skin and oral care, and other products. The global market for lactoferrin is more than 200 tons per year.

The Bovine milk lactoferrin has a molecular weight of approximately 77 kDa and a pI of 7.9. Purification can be achieved by cation-exchange chromatography directly from milk depleted of fat (<0.1%).

We have investigated the capture of lactoferrin on WorkBeads™ 40 S chromatography medium from pasteurized standard bovine milk containing 0.1 % fat.

Results

Pure bovine milk lactoferrin was used to investigate elution conditions and dynamic binding capacity ($Q_{B,10\%}$) on WorkBeads 40 S. A concentration of approximately 1 M NaCl was required for elution (Fig. 1). The binding capacity was around 78 mg/ml at 3 minutes residence time (Fig. 2). Lactoferrin was purified from bovine milk (Fig. 3) and from whey (see SDS-PAGE Fig. 3B). The separation gave excellent purity in a single step, and show promises for scale-up.

Column: BabyBio™ S 1 ml(pre-packed column)
Medium: WorkBeads 40 S
Flow: 2.5 ml/min
Sample: 35 ml 1 mg/mL Lactoferrin in 50 mM Na-phosphate, pH 6.8
Binding buffer: 50 mM Na-phosphate, pH 6.8
Elution buffer: 50 mM Na-phosphate, 1.2 M NaCl, pH 6.8
Elution: Gradient 0-100% Elution buffer in 20 column volumes (CV)

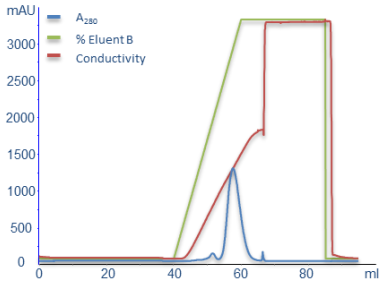

Figure 1 Determination of elution conditions on BabyBio™ S 1 ml column.

Column: 6.6 × 100 mm bed (Omnifit® glass column)
Medium: WorkBeads 40 S
Flow: 120-350 cm/h
Sample: 1 mg/mL Lactoferrin in 50 mM Na-phosphate, pH 6.8
Binding buffer: 50 mM Na-phosphate, pH 6.8
Elution buffer: 50 mM Na-phosphate, 1.2 M NaCl, pH 6.8
Elution: Step gradient 0-100% Elution buffer

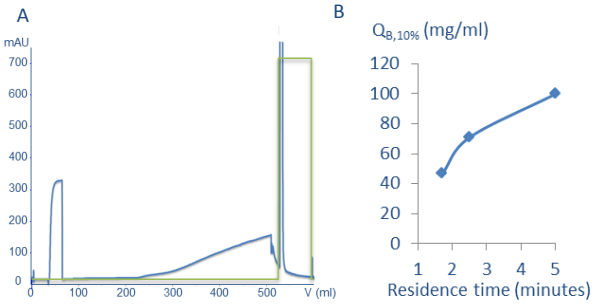

Figure 2 Dynamic binding capacity determination. (A) Example of frontal analysis of pure lactoferrin on WorkBeads 40 S. (B) Binding capacity *vs.* residence time.

Column: BabyBio S 5 ml(pre-packed column)
Medium: WorkBeads 40 S
Flow: 2.5 ml/min
Sample: 500 ml low fat milk (pH 6.6)
Binding buffer: 50 mM Na-phosphate, pH 6.8
Elution buffer: 50 mM Na-phosphate, 1.2 M NaCl, pH 6.8
Elution: Step gradient 50% and 100 % Elution buffer
Cleaning-in-place: 1 M NaOH

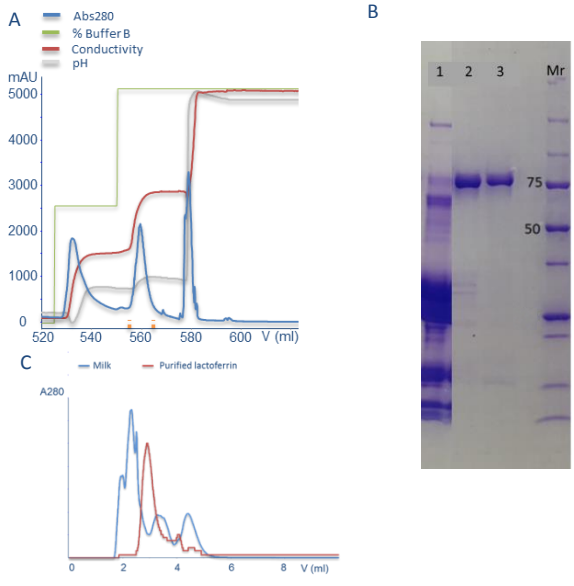

Figure 3 Purification of lactoferrin from bovine low-fat milk on BabyBio S 5 ml. (A) Elution by step gradient, second peak is lactoferrin (28 mg); (B) SDS-PAGE analysis 1. Fresh milk, 2. Lactoferrin purified from milk, 3. Lactoferrin purified from whey, M_r markers; 10-250 kD; (C) size-exclusion chromatographic analysis of the eluted lactoferrin.

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