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Electroseparation of bovine lactoferrin from model and whey solutions

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ABSTRACT

Bovine lactoferrin (LF), a 80 kDa iron-binding glycoprotein, has been reported to have important nutraceutical and biological properties such as anti-inflammatory, antimicrobial and immunostimulatory activities. However, the large scale utilisation of LF requires a cost-effective purification process. The aim of this study was to evaluate the feasibility of separating lactoferrin from whey using electrodialysis with an ultrafiltration membrane (EDUF) system and to study the effect of pH on this protein migration rate during EDUF treatments. Initially, to set the optimum conditions for electroseparation of this molecule, its electrophoretic mobility was measured according to the pH (pH 3–12). LF had an optimal electrophoretic mobility at pH 3.0 of 1.5×10^{-8} m^2 V^{-1} s^{-1} in a 2 g/L KCl solution and of 3.0×10^{-8} m^2 V^{-1} s^{-1} in distilled water. Thereafter, a 0.1% LF solution at pH 3.0 was treated by EDUF with an ultrafiltration membrane of 500 kDa molecular weight cut-off. A migration rate going up to 46% was obtained after 4 h of treatment. Finally, the EDUF process was applied on lactoferrin-enriched-whey solutions at pH values of 3.0, 4.0, and 5.0. The highest migration rate for lactoferrin was obtained at pH 3.0 with a migration yield of 15%. Thus, it appeared that the EDUF process could allow the separation of large proteins, such as LF, from a solution. Furthermore, the selectivity of EDUF was decreased in whey solution due to concomitant migration of β-lactoglobulin or other whey proteins. However, the fraction obtained at pH 3.0 presented β-lactoglobulin percentages close to the ones obtained for WPI by ion-exchange technology.

1. Introduction

Bovine lactoferrin (LF), a 80 kDa iron-binding glycoprotein, has been reported to have important nutraceutical and biological properties [1] such as anti-inflammatory, antimicrobial and immunostimulatory activities [2–4]. This protein plays a fundamental role in iron metabolism as an iron-transport molecule [2]. LF has also been reported to have other biological functions such as antitumoral and transcriptional regulation and proteolytic and enzymatic activities [5]. Moreover, LF can be used in infant formulas as well as in meat to improve preservation [6]. However, with an average concentration of 0.1% of LF in whey [7], the large scale use of LF requires a purification process combining good selectivity, high extraction yield and low cost of production.

Due to LF large potential applications, many processing technologies have been developed to isolate high purity fractions [8–12]. Cation-exchange chromatography is already used for the production of LF at industrial scale [13]. This technology has the advantage of producing LF with a high degree of purity (>90% dry basis). However, the limitation of this technology for large scale applications lies with its high cost and its relatively low yield [14,15]. Pressure-driven membrane processes have been developed but their use is limited by their low selectivity and membrane fouling that develops with time [6,16,17]. The separation of LF from a whey protein mixture was also carried-out by an electrically enhanced crossflow microfiltration process [17]. The application of an electric field improved the separation of LF in comparison with the conventional pressure driving filtration technique. However, the use of this process resulted in a decrease in the level of purity of LF obtained, due to the migration of other whey proteins and to the occurrence of protein–protein interactions in whey [14].

Electrodialysis with ultrafiltration membrane (EDUF), an electrically driven membrane separation technology, could overcome these disadvantages of low selectivity and solution contamination. Indeed, in this system, the ultrafiltration membrane is stacked as a molecular barrier in a conventional electrodialysis cell and the only driving force of the separation process is an external electric field. In consequence, the process allows the separation of molecules according to their molecular size and their electrical charge. This technology has been shown to be efficient for the separation and purification of charged molecules with low molec-

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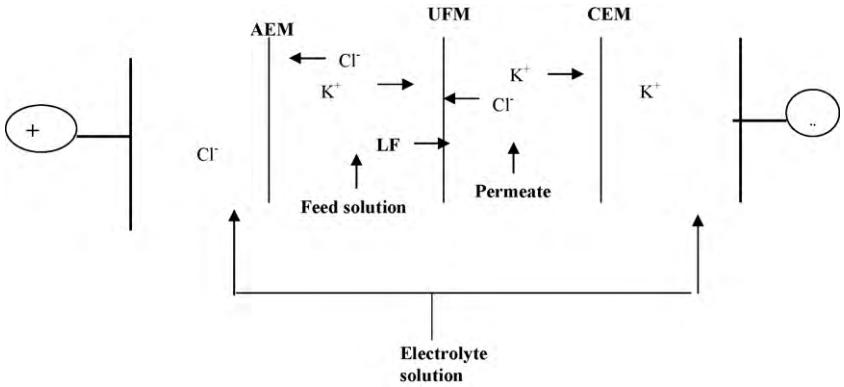

Fig. 1. Configuration of the electrodialysis cell with ultrafiltration membrane for the separation of lactoferrin. AEM: anion-exchange membrane; UFM: ultrafiltration membrane; CEM: cation-exchange membrane, LF = lactoferrin molecule.

ular weights (MW) such as tobacco polyphenols (MW ≤ 610 Da, [18]), green tea catechins (MW ≤ 458 Da, [19]), bioactive peptides (MW ≤ 3315 Da, [20]; MW ≤ 1200 Da, [21]) and chitosan oligomers (MW ≤ 800 Da, [22]). EDUF has never been used for the separation of whole proteins particularly for proteins having high molecular weights such as LF (80 000 Da). However, since LF is positively charged at whey pH 6.2–6.4 [23], the EDUF might be an effective means to separate this molecule from whey.

The aim of this study was to evaluate the feasibility of separating LF in a model solution and in a lactoferrin-enriched-whey solution by electromigration in an EDUF process. The effect of pH on LF migration yields from lactoferrin-enriched-whey solutions was also studied. In order to determine optimal conditions for the electroseparation of LF, its electrophoretic mobility was measured as a function of pH (pH 3–12) prior to EDUF treatments.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

NaCl and KCl were obtained from Laboratories MAT (Quebec City, QC, Canada). HCl and NaOH (1.0 M) were obtained from Fisher Scientific (Montreal, QC, Canada).

2.1.2. Raw material

Commercial bovine lactoferrin (protein: 92.8%; moisture: 4.4%; minerals: 1.6%) used in this study (FD, lot number: 101 215 89) was graciously provided by DMV International (New York, USA). Reconstituted sweet whey (6% (w/v)) from whey powder (lot SAP 1708 127 00), with a protein concentration of 12.5%, was kindly provided by Agropur (Granby, QC, Canada). The whey solution was enriched with the commercial bovine lactoferrin powder at a concentration of 0.1% (w/v). The lactoferrin-enriched-whey solution was composed of the following proteins: β-lactoglobulin (3.9 ± 0.4 g/L), glycomacropeptide (GMP; 2.1 ± 0.8 g/L), α-lactalbumin (1.1 ± 0.2 g/L), lactoferrin (LF; 1.1 ± 0.2 g/L), bovine serum albumin (BSA; 0.1 ± 0.03 g/L) and IgG (0.1 ± 0.07 g/L).

2.1.3. Configuration of the electrodialysis with ultrafiltration membrane (EDUF) system

The electrodialysis cell was a MicroFlow type cell with an effective area of 10 cm^2 (Electrocell AB, Taby, Sweden). Membranes used were a CMX-SB cationic membrane (Tokuyama Soda Ltd., Tokyo, Japan), a Neosepta AMX-SB anionic membrane (Tokuyama Soda Ltd.) and a polyethersulfone ultrafiltration membrane (Millipore Worldwide Corporate Billerica, MA, USA) with a molecular weight cut-off (MWCO) of 500 kDa. The EDUF cell configuration defined three closed loops (Fig. 1). Each closed loop was connected to a separate external reservoir allowing continual recirculation of the solutions. The solutions were circulated using three centrifugal pumps (Iwaki Co. LTD, Tokyo, Japan) and flow rates were controlled using flowmeters (Gilmont Instrument Co, Barrington, IL, USA). The anode was a dimensionally stable electrode (DSA) and the cathode was a 316 stainless steel electrode. The anode/cathode voltage difference was supplied by a variable 0–30 V power source (model HPD 30-10SX, Xantrex, Burnaby, BC, Canada). The system was not equipped to maintain constant the temperature of the solutions.

2.1.4. Protocols

2.1.4.1. Electrophoretic mobility measurements. Solutions of 0.1% LF (w/v) in distilled water or in 2 g/L KCl solutions were prepared and adjusted at different pH (3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) with HCl or NaOH. A Zetasizer system (model 2000, Malvern Instruments Ltd., Worcs, United Kingdom) was used to measure the electrophoretic mobility of LF in solutions at different pH, according to the method of Brisson et al. [17]. All electrophoretic measurements were performed on triplicate independent samples.

2.1.4.2. EDUF treatments on a model solution. EDUF treatments were carried-out using 200 mL of LF solutions in a batch process using a constant voltage difference of 20 V. The duration of the treatments was 4 h. The electrode, permeate and feed compartments contained a 20 g/L NaCl aqueous solution (250 mL), a 2 g/L KCl aqueous solution (250 mL) and a LF aqueous solution (200 mL), respectively. LF solution was obtained by dissolving LF in a 2 g/L KCl solution to obtain a final concentration of 0.1% and to have a sufficient conductivity to allow current transfer. Permeate and feed solution flow rates were 200 mL/min while the flow rate of the electrode solution was 300 mL/min. The pH of LF solution and of the permeate were adjusted before each run at pH 3.0 with 1 M HCl or NaOH solutions and maintained constant, by addition of the same solutions, during treatments. Three replicates of each condition were performed using new ultrafiltration and ion-exchange membranes for each repetition. Samples of feed solutions and of permeates were drawn at the beginning of the process before applying external field and at 60 min intervals during the electroseparation process. The protein concentration was measured in the permeate samples.

2.1.4.3. EDUF treatments on a lactoferrin-enriched-whey solution. For these experiments, the electrode, permeate and feed compartments contained a 20 g/L NaCl aqueous solution (250 mL), a 6 g/L KCl aqueous solution (250 mL) and a lactoferrin-enriched-whey solution (200 mL), respectively. The same operating conditions as