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SHORT COMMUNICATION

Separation of Whey Proteins by Anion-Exchange Membranes

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Abstract−Three strong anion-exchange membranes (CIM QA, Q100 and HiTrap Q) were investigated for the separation of the major proteins, which were contained in whey, such as α-Lactalbumin, BSA and β-Lactoglobulin. Experiments were performed to determine the optimum mobile phase composition for separating the whey proteins using the standard chemicals of the proteins. The mobile phase was buffer A (20 mM piperazine-HCl pH 6.4) and buffer B (buffer A+1 M NaCl) and the linear gradient elution changes of salt concentration were applied. The standard chemicals of the proteins were used to investigate the optimal mobile phase compositions with the three anion-exchange membranes. From the experimental results, it was found that HiTrap Q was the most effective in separating whey proteins.

Key words: Whey Proteins, Anion-exchange Membrane, Buffer, Optimal Mobile Phase Composition

INTRODUCTION

Milk proteins are the most important source of bioactive peptides. The composition of bovine milk includes water, fat, lactose and minerals, and up to 6% of the mass is made up by proteins and peptides, among them a number of high value substances [Lourdes et al., 2000]. In particular, milk contains two major protein groups, caseins and whey proteins, which differ greatly with regard to their physicochemical and biological properties. Normal milk contains 30-35 g/L proteins, approximately 80% of which are caseins with the remainder being the whey proteins [Korhonen et al., 1998]. Whey proteins can be acquired as a by-product in cheese manufacturing process. The required long-term stability in functional performance of these proteins is often lacking. Though the two major proteins are scientific magnitude material, the study of functional properties of whey proteins has attracted scientific interests for more than 20 years [Lieske et al., 1996]. In general, whey is dilute liquid composed of lactose, a variety of proteins, minerals, vitamins and fat. Whey contains about 6% solids of which 70% or more is lactose and about 0.7% is proteins [Gerberding et al., 1998]. Whey protein components are α-Lactalbumin, β-Lactoglobulin, immunoglobulins A, M and G, bovine serum albumin (BSA), lactoferrin and lactoperoxiedase. β-Lactoglobulin is the major whey protein in bovine milk. β-La has a molecular weight of 18.4 kDa, possesses 162 amino acid residues and its concentration is 2-4 g/L. α-Lactalbumin is an albumin which has 123 amino acid residues. It possesses a molecular weight of 14.2 kDa and its concentration in milk is 0.6-1.7 g/L [Ye et al., 2000]. Bovine whey proteins have potential applications in veterinary medicine, food industry and as supplements for cell culture media. Immunoglobulin G(IgG), Immunoglobulin A(IgA), lactoferrin and lactoperoxidase, present in bovine whey, have high pharmaceutical value [Hahn et al., 1998]. α-Lactalbumin can be used in infant formula and as a nutraceutical because of its high tryptophan content. β-Lactoglobulin is used in the production of confections [Andrew et al., 1998]. Oral administration of bovine IgG is known to be an effective treatment of various infections of newborn infants [Hutchens et al., 1990]. Lactoferrin and lactoperoxidase are known to act as antimicrobial factors [Strange et al., 1992].

Research on the separation and purification of whey proteins has been performed for the dairy industry for a long time. There were the researches of partitioning of whey proteins by aqueous two-phase systems [Jose et al., 2000; Marco et al., 1998; Luiza et al., 2000]. Also, some attempts have been made to isolate whey proteins by using membrane filtration [Gerd et al., 2000; David et al., 1998]. In this work, strong anion exchange membranes were used to separate the major whey proteins, such as α-Lactalbumin, β-Lactoglobulin and bovine serum albumin (BSA). The separation of whey proteins by using ion exchange membranes has been investigated by many researchers and several methods have been reported [Gerberding et al., 1998; Ye et al., 2000; Hahn et al., 1998; Kyoung et al., 1989; Seung et al., 2002]. Ion exchange separations take advantage of electrostatic interaction between surface charges on biomolecules, such as amino acids or proteins, and clusters of charged groups on membrane. An adsorbing biomolecule displaces counterions associated with the surface, discharging a complementary buffer salt in the process. Adequate buffering is required to shield native protein structures from changes in pH adjacent to exchange surfaces (Donnan effect) and pH effects were induced by sorption. Selection of an appropriate buffer is critical to the success of ion exchange membrane [Keith et al., 1995]. Large molecules (>1,000,000) such as plasmid DNA, are able to access charged groups which envelope large pores of membrane adsorbers, though they would commonly be excluded from cellulose-based ion exchangers. Mobile phases and buffers employed in ion exchange bioseparations are non-denaturing to hydrophilic proteins. Elution and recovery of biologicals using ion exchange are considered, as well as effects of additives and flow rate on performance.

This work focused on the comparison of the separation characteristics of whey proteins using the three strong anion-exchange

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Table 1. Molecular weights and isoelectric points for whey proteins

Protein	Molecular weights	Isoelectric points (pI)
α-Lactalbumin	14,000	4.2-4.5
β-Lactoglobulin	18,300	5.35-5.49
BSA	69,000	5.13

membranes (CIM QA, Q100 and HiTrap Q). The mobile phase composition was adjusted in the linear gradient with NaCl concentration.

EXPERIMENTAL

1. Separation Unit and Chemicals

The whey powder (from Bovine Milk) used in this experiment was purchased from Sigma Co. The standard chemicals of α-Lactalbumin, β-Lactoglobulin and bovine serum albumin (BSA) were purchased from Sigma Co. Table 1 shows the molecular weights and isoelectric points for whey proteins. Standard protein and the whey powder solutions were freshly prepared in water. The standard solution was made of 10 mg/ml each protein, the 100 mg/ml whey power and water. The water was filtered with HA-0.5 μm (Division of Millipore, Waters Co.) and was deionized prior to use. The sample for injection was filtered with PVDF 0.45 μm (Waters Co.). Sodium chloride and hydrochloric acid were purchased from Duksan Pure Chemicals Co. (Incheon, Korea). Piperazine was purchased from Sigma Co. Buffer A was 20 mM piperazine-HCl pH 6.4 and buffer B was made by addition of 1 M NaCl into buffer A.

2. Equipment

The strong anion-exchange membranes used in this experiment were CIM QA (BIAseparation Co.), Q100 (Sartorius) and HiTrap Q (Pharmacia). Monolithic Convective Interaction Media (CIM) QA disk has a diameter of 16 mm and a thickness of 3 mm. The base material of CIM QA is a macroporous glycidyl methacrylate-co-ethylene dimethacrylate (GMA-EDMA) polymer matrix and CIM QA disk bears quaternary amine. The membrane material of Q100 is cellulose. The adsorption area of Q100 is 100 cm^2 and the binding capacity of protein is 100 mg/Q100 unit. The ion exchange media packed in HiTrap ion exchange columns are based on Sepharose high performance. The column volume of HiTrap Q is 1 ml.

The analytical HPLC system in this experiment was Waters Model 600S liquid chromatography (Waters Associates, Milford, MA, U.S.A.) equipped with the Waters 515 Multi-solvent Delivery System with 486 Tunable Absorbance Analytical Detector, and an injector (50 μl sample loop) of Rheodyne. The data acquisition system was Chromate (Ver. 3.0, Interface Eng., Korea) installed in a PC. The flow rate of mobile phase was fixed at 4, 2 and 1 ml/min with CIM QA, Q100 and HiTrap Q, respectively. The wavelength was fixed at 260 nm and the injection volume was fixed at 20 μl. The experiment was performed at room temperature.

RESULTS AND DISCUSSION

Elution by linear or step-change in mobile phase composition may produce differential migration of concentrated solutes. The average velocity of each desorbed solute is proportional to its fractional equilibrium mobile phase concentration. Therefore, gradient elution has been normally used to remove adsorbed components from membranes. Gradient elution is convenient since it is difficult to determine a priori the modifier concentration required to selectively elute just the desired species. In this experiment, a gradient mode with change in NaCl concentration was used to separate whey proteins of α-Lactalbumin, β-Lactoglobulin and BSA [Du et al., 2002]. The standard chemicals of proteins were used and the strong anion-exchange membranes used in this experiment were CIM QA (BIAseparation Co.), Q100 (Sartorius) and HiTrap Q (Pharmacia). The mobile phase was buffer A (20 mM piperazine-HCl pH 6.4) and buffer B (buffer A+1 M NaCl). In this experiment, the sample of whey was injected only with filtered PVDF 0.45 μm without further pretreatment of ultrafiltration.

Generally, the retention times of proteins were shorter with increasing the amount of NaCl in buffer B. The elution order of the proteins was α-Lactalbumin, BSA, β-Lactoglobulin B and β-Lactoglobulin A. β-Lactoglobulin was composed of β-Lactoglobulin A and β-Lactoglobulin B. In Fig. 1, CIM QA was used to separate whey proteins. In this case, the ratio of the volume of buffer A to the volume of buffer B in the mobile phase (buffer A/buffer B) was changed as follows: 100/0-95/5 (0-10 min), 95/5-80/20 (10-30 min),

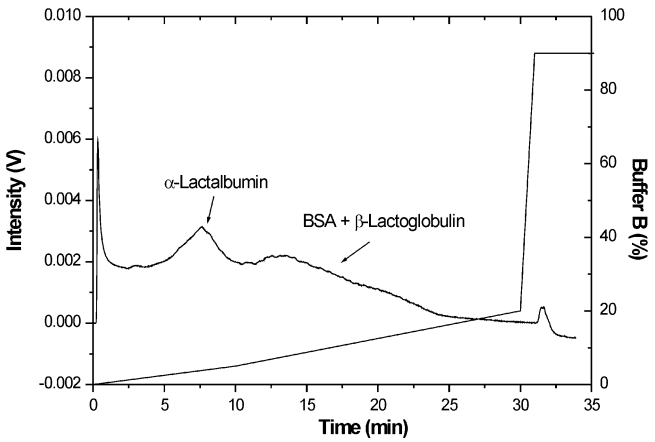

Fig. 1. Separation of standard chemicals of whey proteins using CIM QA [Column: CIM QA (Bioseparation), 4 ml/min, 20 μl, 260 nm].

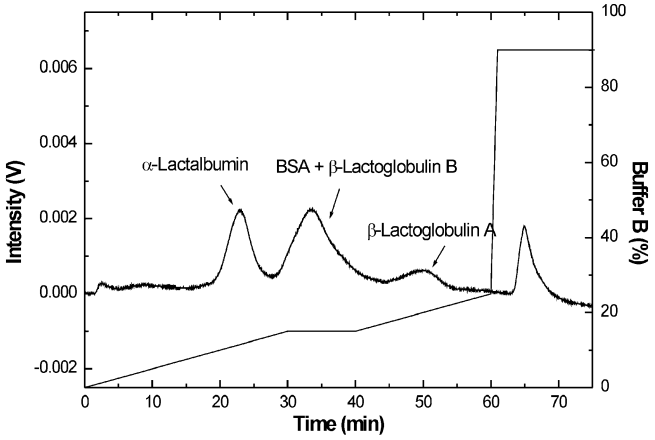

Fig. 2. Separation of standard chemicals of whey proteins using Q 100 [Column: Q100 (Sartorius), 2 ml/min].

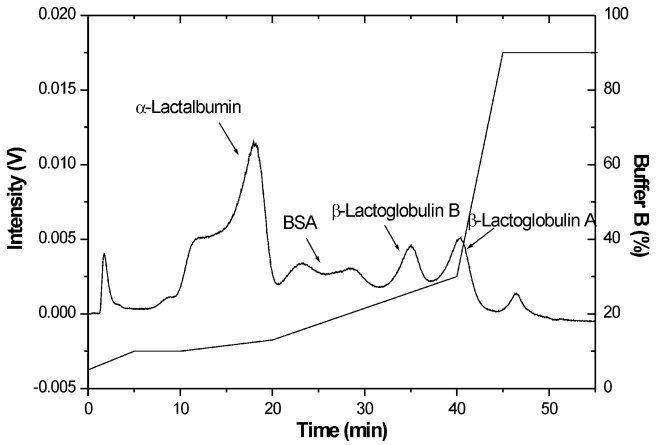

Fig. 3. Separation of standard chemicals of whey proteins using HiTrap Q [Column: HiTrap Q (Pharmacia), 1 ml/min].

and 80/20-10/90 (30-31 min), and the flow rate of mobile phase was 4 ml/min. Though only α-Lactalbumin was resolved from the other proteins, the resolution was not good. The coeluted peak of BSA and β-Lactoglobulin was followed.

Fig. 2 showed the chromatogram for the separation of whey proteins by Q100. The buffer A/buffer B was changed as follows: 100/0-85/15 (0-30 min), 85/15 (30-40 min), 85/15-75/25 (40-60 min), and 75/25-10/90 (60-61 min), and the flow rate was 2 ml/min. The resolution of α-Lactalbumin was better than that using CIM QA and β-Lactoglobulin A was also resolved. But BSA and β-Lactoglobulin B were coeluted.

The separation of whey proteins by HiTrap Q is shown in Fig. 3. The buffer A/buffer B was changed as follows: 95/5-90/10 (0-5 min), 90/10 (5-10 min), 90/10-87/13 (10-20 min), 87/13-70/30 (20-40 min), and 70/30-10/90 (40-45 min), and the flow rate was 1 ml/min. The resolutions for whey proteins were relatively excellent.

Real whey proteins were separated by the change of mobile phase composition obtained from Fig. 3. The injection volume was increased to 50 μl and the change of mobile phase composition was slightly modified to fit the larger injection volume and to improve the resolution of whey proteins. This result is shown Fig. 4. Though β-Lactoglobulin A and β-Lactoglobulin B were not satisfactorily separated, the other whey proteins were well resolved. The buffer A/buffer B was changed as follows: 95/5-90/10 (0-5 min), 90/10 (5-10 min), 90/10-70/30 (10-45 min), 70/30 (45-55 min), and 70/30-10/90 (55-60 min).

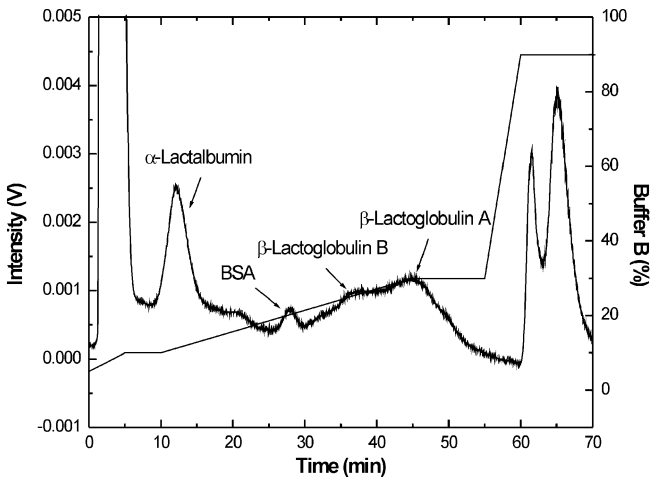

Fig. 4. Separation of the proteins contained in whey using HiTrap Q [Column: HiTrap Q (Pharmacia), 1 ml/min].

In this work, three strong anion-exchange membranes manufactured different from each other in the corporations were used to separate whey proteins. From the result, it was found that HiTrap Q (Pharmacia) was the most effective anion exchange membrane to separate whey proteins.

CONCLUSION

The separation characteristics of whey proteins of α-Lactalbumin, BSA and β-Lactoglobulin were investigated with the strong anion-exchange membranes on a gradient mode. The concentration of NaCl and the numbers of linear gradient steps were adjusted to find the optimum mobile phase with the commercially available anion-exchange membranes. It was experimentally confirmed that HiTrap Q was the most effective to resolve the whey proteins.

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