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Batch Adsorption of Whey Protein onto Anion Exchange Mixed Matrix Membrane Chromatography

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Abstract. Mixed matrix membrane (MMM) chromatography is an advanced method for whey protein fractionation. The adsorption capacity of MP500 anion exchange MMM chromatography was evaluated by batch experiments using simulated whey containing a single whey protein component and real whey at pH 6. The binding preference for whey proteins was demonstrated to follow the order: β-lactoglobulin > bovine serum albumin > α-lactalbumin. MP500 based MMM could be used to recover β-lactoglobulin very selectively from whey to produce a single protein component for food ingredient and related applications.

Keywords: membrane chromatography, whey protein, anion exchange membrane

1. Introduction

Whey is a water-like liquid or serum that separates from the curds during cheese or casein manufacturing. The major protein components in whey are β-lactoglobulin (β-Lac), α-lactalbumin (α-Lac) and bovine serum albumin (BSA). In cheese or casein production, only 10 to 20% of raw milk is utilized to obtain the final product, while 80 to 90% of the raw milk yields whey as a waste product [1]. Enormous amounts of whey are produced annually and it has now evolved from a waste product to a value-added ingredient for the food industry. Its potential as a nutritional protein source has been recognized and its functional properties have become more widely utilized. It has been proposed that the use of individual whey proteins could be more valuable than their use as a mixture [2].

Membrane based processes may represent an interesting alternative method for whey protein fractionation, as they have been well integrated within the dairy industry. However, conventional membrane filtration based on size difference is unable to effectively separate all whey protein components. Alternatively, membrane chromatography has been used, incorporating an adsorptive membrane having certain functionality that operates according to the principle of a chromatography process [3, 4].

The concept of mixed matrix membrane (MMM) chromatography is an alternative that provides a simple method for the preparation of adsorptive membranes yet results in high quality membrane chromatography performance. MMMs are prepared by incorporating an ion exchange resin (or any adsorptive resin) into a membrane polymer solution prior to membrane casting. The chemical modifications necessary for incorporating adsorptive ligands, as normally used in conventional preparation methods, are thus decoupled from the membrane preparation step. In our previous study [3], anion exchange MMM was developed using an ethylene vinyl alcohol (EVAL) base membrane with a Lewatit® MP500 anion resin as an adsorbent particle in the membrane. In the current study, the binding preference of major acidic whey proteins to MP500 based MMM was further investigated.

2. Methods

2.1. Membrane preparation

MMMs were prepared following procedures reported previously using Lewatit MP500 anion resin as the embedded adsorbent [3]. A homogenous ethylene vinyl alcohol (EVAL) polymer solution, consisting of 15 wt% EVAL polymer and 15 wt% 1-octanol in dimethylsulfoxide was used. Ground MP500 resin (43 μm) was added to the prepared polymer solution to make a 50% weight fraction (relative to the EVAL content in the polymer solution) and this mixture was stirred until a homogeneous casting slurry was obtained. A flat sheet MMM was cast on a glass plate from the slurry by a conventional casting process as previously described [3] and the wet MMM was then freeze-dried to remove water without affecting the structure of the membrane. The thickness of the membrane after the drying was around 200 μm.

2.2. Preparation of whey

Whey was prepared from skim (0.05% fat) milk by acid precipitation of casein at pH 4.8. Precipitated casein was discarded and the whey supernatant was centrifuged at 17 902 *g* at 4°C for 20 min. Final whey filtration was achieved with a 0.45 μm membrane filter. The whey was adjusted to pH 6 using 0.5 M sodium hydroxide.

In batch binding experiments, two sets of whey solution were used. The first set was the whey prepared as above, with serial dilution of concentration using binding buffer (20 mM sodium phosphate pH 6). The second set was a simulated whey solution consisting of the major whey acidic proteins β-Lac, BSA and α-Lac at a similar concentration ratio to whey but with the concentration of β-Lac undergoing a serial dilution while the concentrations of BSA and α-Lac were maintained at 0.15 mg mL^{-1} and 1.2 mg mL^{-1}, respectively.

2.3. Batch adsorption experiments and protein assay

A small rectangular piece of membrane with dimensions 12 mm × 12 mm (volume 2.9×10^{-2} mL) was cut from the membrane sheet. The membrane was first equilibrated for 6 h in binding buffer. After removing surface liquid by gentle contact with a tissue, the membrane was incubated with 1 mL of whey solution or simulated whey solution overnight at a room temperature (20°C).

The acidic protein concentrations in whey were assayed using a reversed phase chromatography (RPC) column following the method established by Palmano and Elgar [5], using a 1 mL Resource™ RPC column (GE Healthcare Technologies) at a flow rate of 2 mL min^{-1}. The assay was run on an AKTAexplorer™ 10 liquid chromatography system controlled by Unicorn™ 4.0 software (GE Healthcare Technologies) with 500 μL samples injected manually through a sample injection loop.

3. Results and discussion

3.1. Binding preference of whey protein in batch adsorption

At pH 6, all negatively charged whey proteins, such as β-Lac, α-Lac and BSA, have the ability to interact with an anion exchange membrane. However, in previous studies [3, 4], β-Lac showed most selective binding toward a strong anion exchange membrane and could even displace other positively proteins bound onto the anionic membrane. The binding strength of three major proteins on the anion exchange membrane could be postulated according to this order: β-Lac > BSA > α-Lac [4, 6]. To verify this order, a series of batch adsorptions using whey and simulated whey was conducted. Figure 1 shows the amount of individual proteins bound to the anion exchange MMM from a series of initial whey solutions with different dilution factors. As illustrated in Figure 1, β-Lac is preferably bound to the anion exchange membrane and for the small area of membrane used in this experiment, it has a specific capacity of about 1 mg for β-Lac (or capacity of 21 mg β-Lac mL^{-1} MMM). It can be seen that when the whey was diluted 4 times (whey 4X), nearly 100% β-Lac was bound, 90% BSA bound and less than 10% α-Lac bound. In whey 8X, because β-Lac in solution was less than 1 mg, all the β-Lac was bound first and at this stage the remaining membrane capacity was available for another protein, as shown by an increase in binding percentage (almost 80% for α-Lac and 90% for BSA).

A further experiment using a simulated whey solution with serial dilutions of β-Lac concentration (i.e. α-Lac and BSA concentration kept constant) was also conducted for further verification. Figure 2 shows the binding capacity of the MMM for this simulated whey solution. The specific β-Lac capacity was above 1.5

mg, which was higher than for the real whey solution previously. This could be due to the absence of small ions, minor proteins or peptide components in this simulated whey, which minimized the competition for β-Lac binding to the membrane. The binding pattern was similar to that shown in Figure 1: after one stage, when there was insufficient β-Lac to take up the entire MMM capacity, the residual capacity was taken up preferentially by BSA. In simulated whey solution 3, almost 50% of BSA bound compared to α-Lac, of which only 20% bound. The rest of the solutions showed almost 90% of BSA bound and the percentage of α-Lac bound gradually increased. These results confirm that the anion exchange MMM followed a binding preference order of β-Lac > BSA > α-Lac, as reported in previous studies [4, 6].

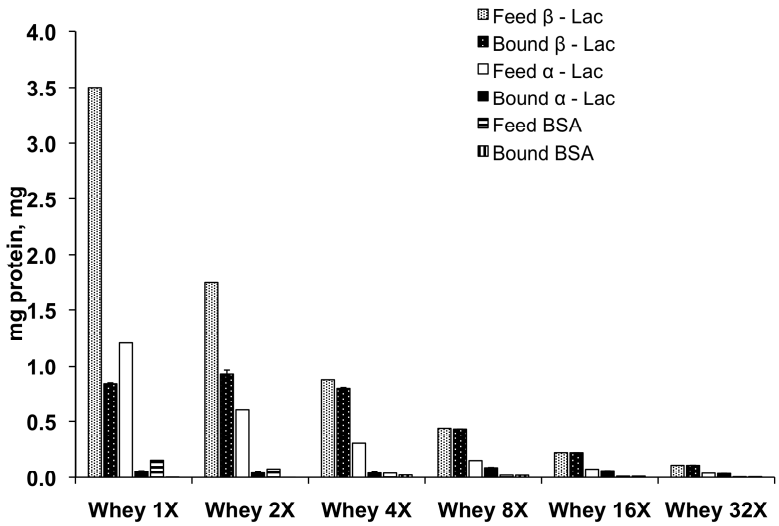

Figure 1: Individual proteins bound onto the anion exchange MMM using 1 mL of whey solution with different initial concentrations. Whey was diluted from full strength (1X) with binding buffer in a serial dilution, as indicated by 2X, 4X, etc. Error bars are ± one standard deviation (n=3).

Figure 2: Individual proteins bound onto the anion exchange MMM using 1 mL of simulated whey solution with different initial β-Lac concentrations. The concentrations of α-Lac and BSA were kept constant at 1.2 and 0.15 mg mL^{-1}, respectively. Error bars are ± one standard deviation (n=3).

4. Conclusion

β-Lac had the highest binding preference in MP500 based MMM chromatography compared with other acidic whey proteins. The binding preference for whey protein components onto this MMM was

demonstrated to follow the order: β-lactoglobulin > bovine serum albumin > α-lactalbumin. Selective binding by anion exchange MMM towards β-Lac will add another avenue for the purification of this protein to be used in food ingredient and other applications.

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