Non–Affinity Based Purification Platform for Neutral/Basic IgMs

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Abstract

The biological properties of IgM antibodies make them very effective vehicles for in vitro diagnostics and therapeutics. However, purification of IgM antibodies is far more complex than that for IgG antibodies. Furthermore, affinity chromatography is not ideal for purifying IgMs due to the required elution conditions. Here we propose a non–affinity based platform for IgM purification of neutral and basic IgMs. This strategy utilizes a cation exchange (CEX) resin, Nuvia™ S, for capture and a mixed-mode media, CHT™ Ceramic Hydroxyapatite, for polish purification. We optimized the protocol with three different IgMs and present reducing and nonreducing PAGE images of the purified samples. This strategy is simple, scalable, and efficient and thus well suited for purification of diagnostic IgMs.

Introduction

Immunoglobulin M (IgM) is the first antibody to be produced in an immune response. The use of IgM antibodies for in vitro diagnostics and therapy is on the rise, especially for cancer, infectious diseases, and stem cell therapies. Although IgM is composed of pentameric complexes of Ig monomers, purification strategies used for IgG cannot directly be translated to IgM purification because of the multiple differences between IgG and IgM (Gagnon et al. 2008). IgMs are very sensitive to pH conditions, making them highly susceptible to degradation or precipitation, are soluble in a narrower range of conditions, and are more heavily glycosylated than IgGs. IgM purification is made even more complex by the antibodies' large size, labile nature, and complex physicochemical properties. In addition, the harsh elution conditions required by affinity chromatography are not compatible with process-scale IgM purification.

To overcome these challenges, we designed a scalable non–affinity based platform for manufacture of diagnostic IgMs by removing major process- and product-related impurities. This strategy exploits the binding of IgMs to ion exchange and hydroxyapatite-based media. We show that with optimization of the buffer pH and conductivity we can purify neutral and basic pentameric IgMs from expression-related impurities, such as transferrin (TF) and serum albumin (SA), and product-related impurities, such as free IgM light chain (LC) and monomeric IgMs, with Nuvia S Resin and CHT Media.

Purification of Neutral/Basic IgM Antibodies

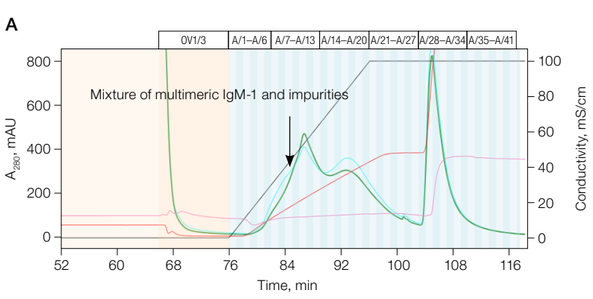
Three different IgMs were expressed in a HEK transient expression system resulting in culture supernatants with low titers. The calculated pIs for these IgMs were IgM-1: 7.5, IgM-2: 7.2, and IgM-3: 7.0. The IgMs were capture purified using the cation exchange resin Nuvia S and were further polish purified using the mixed-mode media CHT Ceramic Hydroxyapatite Type II, 40 µm (CHT-II-40), which incorporates affinity and cation exchange interactions.

Optimization of the Neutral/Basic IgM Capture with Nuvia S Resin

Optimization of the capture of three IgMs at pH 5.0 and a pH 1.5 lower than their respective pIs, with a constant linear gradient of 500 mM NaCl, was performed. The results shown reflect one representative, IgM-1, with a pI of 7.5. At pH 5.0, the multimeric IgMs and the impurities (TF, SA, LC, and monomeric IgMs) were seen in the same fractions (Figures 1A and 1B), making this pH nonideal for the capture purification. At pH 6.0, the contaminants were seen in the flowthrough while the multimeric IgMs were lower in the elution fractions (Figures 2A and 2B). Therefore, a pH of 1.5 lower than the three IgMs' respective pIs was selected for final purification.

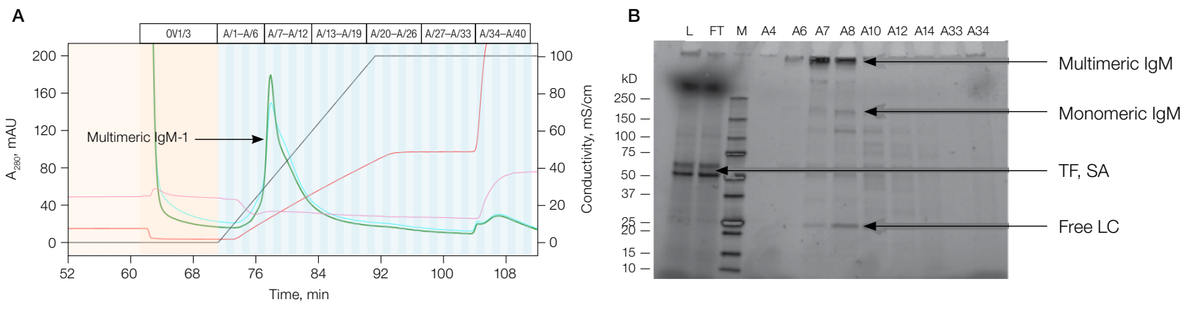
Optimization of the Neutral/Basic IgM Polish with CHT-II-40 Media

Biomolecules can bind to CHT by affinity and/or cation exchange interactions. A phosphate gradient is used to elute biomolecules that interact by affinity; a salt gradient can be used to break the cationic binding. The use of only a phosphate gradient of 20–500 mM $NaPO_4$ resulted in the monomeric and multimeric IgM fractions being eluted in the same sample (data not shown). A high salt gradient of 1.5 M NaCl was incorporated before the phosphate gradient elution. This resulted in the multimeric IgM being eluted in a separate fraction than all the impurities (data not shown).
To optimize the salt gradient further, similar elutions with 400 and 1,000 mM NaCl were performed before the 500 mM $NaPO_4$ gradient. The multimeric IgM eluted in a separate fraction with minimal contaminating proteins at 1 M NaCl (Figures 3A and 3B).



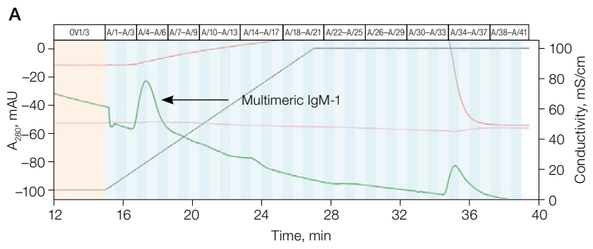
Column: Nuvia S, 1 ml Foresight™
Sample: IgM-1 cell culture supernatant, adjusted to pH 5.0 with 0.2 M acetic acid, 3 mg IgM-1 in 66 ml
Flow rate: 120 cm/hr
Equilibration: 50 mM sodium acetate, pH 5.0, 10 column volumes (CV)
Postloading wash: 50 mM sodium acetate, pH 5.0, 10 CV
Elution: Buffer A: 50 mM sodium acetate, pH 5.0
Buffer B: 50 mM sodium acetate, pH 5.0, 0.5 M NaCl
A to B linear gradient, 0–100%B, 20 CV

Fig. 1. Purification of IgM-1 on Nuvia S at pH 5.0. A, chromatography run of IgM-1 on Nuvia S at pH 5.0 with a constant linear gradient of 500 mM NaCl; **B**, fractions collected from the chromatography run in 1A were analyzed by nonreducing PAGE. Each of the fractions (22.5 µl) were loaded on the gel. A4–A18, elution fractions from 1A.



Column: Nuvia S, 1 ml Foresight
Sample: IgM-1 cell culture supernatant, adjusted to pH 6.0 with 0.2 M acetic acid, 3 mg IgM-1 in 62 ml
Flow rate: 120 cm/hr
Equilibration: 50 mM sodium acetate, pH 6.0, 10 CV
Postloading wash: 50 mM sodium acetate, pH 6.0, 10 CV
Elution: Buffer C: 50 mM sodium acetate, pH 6.0
Buffer D: 50 mM sodium acetate, pH 6.0, 0.5 M NaCl
C to D linear gradient, 0–100%D, 20 CV

Fig. 2. Purification of IgM-1 on Nuvia S at pH 6.0. A, chromatography run of IgM-1 on Nuvia S at pH 6.0 with a constant linear gradient of 500 mM NaCl; **B**, fractions collected from the chromatography run in 2A were analyzed by nonreducing PAGE. Each of the fractions (22.5 µl) were loaded on the gel. A4–A34, elution fractions from 2A.



Column: CHT Type II, 40 µm, 1 ml Foresight
Sample: Nuvia S elution pool, buffer exchanged to buffer E
Flow rate: 120 cm/hr
Equilibration: 20 mM sodium phosphate, pH 7.0, 1 M NaCl, 10 CV
Postloading wash: 20 mM sodium phosphate, pH 7.0, 1 M NaCl, 15 CV
Elution: Buffer E: 20 mM sodium phosphate, pH 7.0, 1 M NaCl
Buffer F: 500 mM sodium phosphate, pH 7.0, 1 M NaCl
E to F linear gradient, 0–100%F, 20 CV

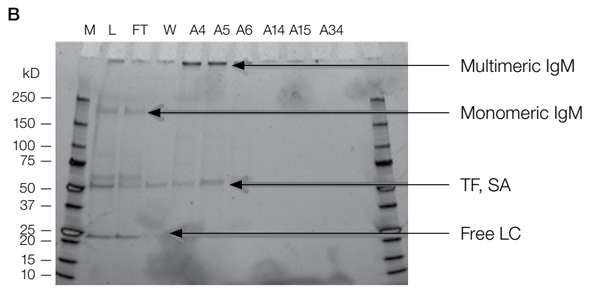


Fig. 3. Purification of IgM-1 on CHT-II-40. A, chromatography run of the IgM-1 on CHT-II-40 with a 20–500 mM phosphate and 1 M NaCl gradient; **B**, fractions collected from the chromatography run in 3A were analyzed by nonreducing PAGE. Each of the fractions (22.5 µl) were loaded on the gel. A4–A34, elution fractions from 3A.

Application of the Optimized Protocol for All Three Neutral/Basic IgM Purifications

The final optimized protocol for capture and polish purification yielded purified fractions of all three IgMs tested (Figure 4), making this an ideal platform for a non–affinity based purification of neutral/basic IgMs.

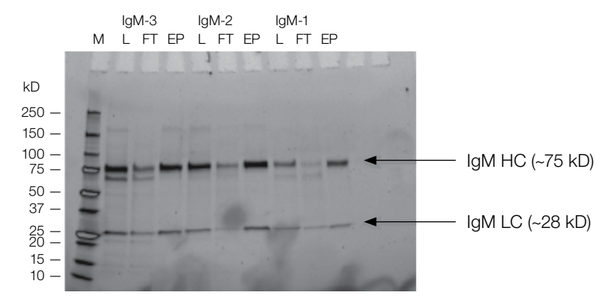


Fig. 4. Analysis of the neutral/basic IgMs purified on Nuvia S and CHT-II-40 Columns. Fractions collected from the chromatography runs of the optimized protocol as mentioned above were analyzed by SDS-PAGE. Each of the fractions (22.5 µl) were loaded on the gel.

For Figures 1–4: EP, eluted protein; FT, flowthrough; HC, heavy chain; L, load; LC, light chain; M, prestained protein standards; SA, serum albumin; TF, transferrin; W, wash; A_{260} (—); A_{280} (—); conductivity (—); %B, D, or F (—); pH (—).

Conclusions

The non–affinity based platform approach proposed here meets the purification requirements for manufacture of diagnostic IgMs. The process involves two steps and requires a narrow range of optimized conditions, making it suitable for scalable purification. Using these state-of-the-art media with high capacity and selectivity also helps overcome the affinity-based challenges of low binding and/or recovery during IgM purification. While the two-step purification is designed to meet purity requirements for manufacture of diagnostic IgMs, therapeutic targets generally require significantly lower levels of process- and product-related impurities. For such purposes, this platform could be extended with a suitable third purification step. Pending further testing, such types of purification platforms can also be used for purification of acidic IgMs, Fab fragments, diabodies, and bispecific antibodies.

Reference

Gagnon P et al. (2008). Purification of IgM Monoclonal Antibodies. biopharminternational.com/purification-igm-monoclonal-antibodies, accessed July 25, 2017.

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