One-step purification of monoclonal IgM from cell culture supernatants

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CY13098-01May20-AN

One-step purification of monoclonal IgM from cell culture supernatants

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Introduction

The development of a general purification protocol for immunoglobulin M (IgM) has met with problems; the unique variation in the amino acid sequence of the variable regions gives different monoclonal antibodies and different chromatographic characteristics. This problem can be circumvented by using thiophilic interaction chromatography for the purification of IgM. The exact mechanism of thiophilic interaction between protein and ligand is not fully understood, but adsorption is promoted by water-structuring salts (1).

HiTrap™ IgM Purification HP columns are packed with a thiophilic adsorption medium consisting of the ligand 2-mercaptopyridine coupled to Sepharose™ High Performance. The column is well suited for purification of IgM, and the main application area for HiTrap IgM Purification HP is purification of monoclonal IgM from hybridoma cell culture supernatants.

Summary

- Monoclonal immunoglobulin M (IgM) from hybridoma cell culture supernatants was purified in a single chromatography step by using the HiTrap IgM Purification HP column.
- The binding of IgM to the HiTrap column was accomplished by adding ammonium or potassium sulphate (0.8 to 1.0 M or 0.5 M final concentration, respectively) to the IgM-containing cell culture supernatant.
- Bound IgM was eluted with 20 mM sodium phosphate, pH 7.5.
- The eluted material was estimated to contain more than 80% IgM, as judged by gel filtration.

Acknowledgments

We gratefully acknowledge Dr. Nils Linde, Medicago AB, Sweden, Imma Brogren, Dept. of Genetics and Pathology, Uppsala University, Sweden, and Anette Holtz, Phadia, Uppsala, Sweden, for their contributions to the work.

Methods and results

IgM purification

All chromatographic separations were performed on ÄKTAexplorer™ and ÄKTApurifier™ chromatography systems. Binding of IgM to the HiTrap IgM Purification HP column was obtained in all samples tested (purified human polyclonal IgM, purified murine monoclonal IgM, and cell culture supernatants from hybridoma cell lines expressing monoclonal murine IgM). Samples up to 100 ml of IgM-containing (10 µg/ml) cell culture supernatant were applied to the column, without detecting any IgM in the flow-through (Table 1). This gives a dynamic capacity of at least 1 mg IgM/ml medium. The IgM elutes when applying a salt-free buffer. Any remaining material on the column can be eluted with 30% isopropanol. See Figure 1 for a typical chromatogram.

Up to 100% of the applied IgM was recovered in the eluate (Table 1) from samples containing 0.5 M potassium sulphate or 0.8 M ammonium sulphate. The purity of the eluted IgM was 80% or higher, as judged by analytical gel filtration (Figures 3 and 4b).

Column:	HiTrap IgM Purification HP, 1 ml (0.7 × 2.5 cm)
Sample:	75 ml of cell culture supernatant containing α-S IgM, 0.5 M potassium sulphate
Binding buffer:	20 mM sodium phosphate, 0.5 M potassium sulphate, pH 7.5
Elution buffer:	20 mM sodium phosphate, pH 7.5
Cleaning buffer:	20 mM sodium phosphate, 30% isopropanol, pH 7.5
Flow rate:	1 ml/min

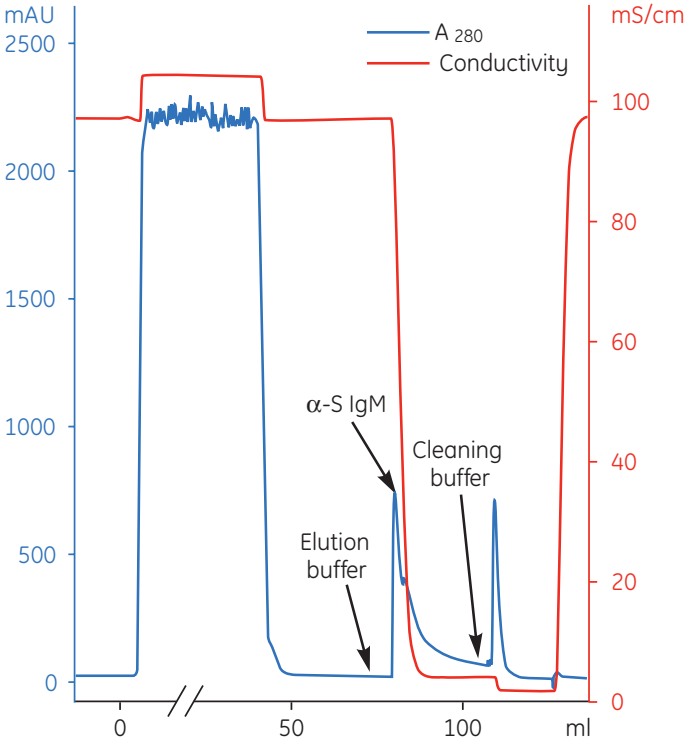
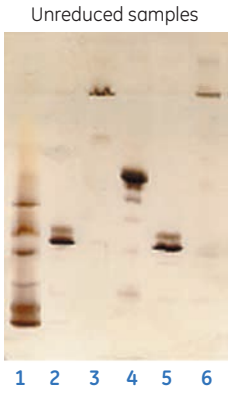


Fig 1. Purification of murine monoclonal α-Shigella (α-S) IgM on HiTrap IgM Purification HP.



Lane 1: Low Molecular Markers
Lane 2: Cell culture supernatant, starting material, diluted 20×
Lane 3: Human IgM
Lane 4: Human IgG
Lane 5: Flowthrough pool, diluted 20×
Lane 6: Eluted α-S IgM, diluted 8×

Fig 2. Non-reducing SDS-PAGE analysis on PhastGel™ Gradient 4–15, silver stained.

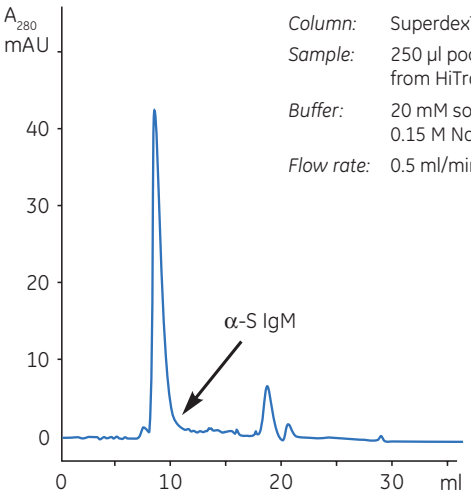


Fig 3. Gel filtration analysis of eluted α-S IgM.

Table 1. Recovery of anti-Shigella (α-S) IgM measured by ELISA

Sample	α-S IgM conc. (mg/ml)	Volume (ml)	Recovery (%)
Cell culture supernatant	0.01	100	100
Flowthrough pool, 0 to 90 ml	not detectable	90	0
Flowthrough pool, 90 to 100 ml	not detectable	10	0
Eluate	0.1 to 0.3	9	> 100
Cleaning eluate	approx. 0.1	1	approx. 10

Effects of increasing salt concentrations

If the recovery of IgM is not satisfactory, the ammonium sulphate concentration can be raised to 1.0 M in the sample. This improves the recovery of IgM at the expense of the purity, as more contaminants in the sample bind and co-elute. At ammonium sulphate concentrations of 1.2 M or higher, there is a strong increase in binding of contaminants from the cell culture medium (Dulbecco's Modified Eagle Medium with 5% Fetal Calf Serum) to the column (Fig 4).

References

1. Porath, J. and Belew, M. *Trends in Biotechnology*, **5**, 225–229 (1987).

Column: Superdex 200, 10 × 300 mm

Samples: **A.** 200 µl cell culture supernatant containing a murine monoclonal α-sheep red blood cell IgM was applied to the HiTrap IgM Purification HP column at different ammonium sulphate concentrations (0.8 to 1.2 M)

B. 200 µl HiTrap IgM Purification HP eluate, 0.8 M ammonium sulphate in sample and binding buffer

C. 200 µl HiTrap IgM Purification HP eluate, 1.0 M ammonium sulphate in sample and binding buffer

D. 200 µl HiTrap IgM Purification HP eluate, 1.2 M ammonium sulphate in sample and binding buffer

Buffer: 20 mM sodium phosphate, 0.15 M NaCl, pH 7.2

Flow rate: 0.4 ml/min

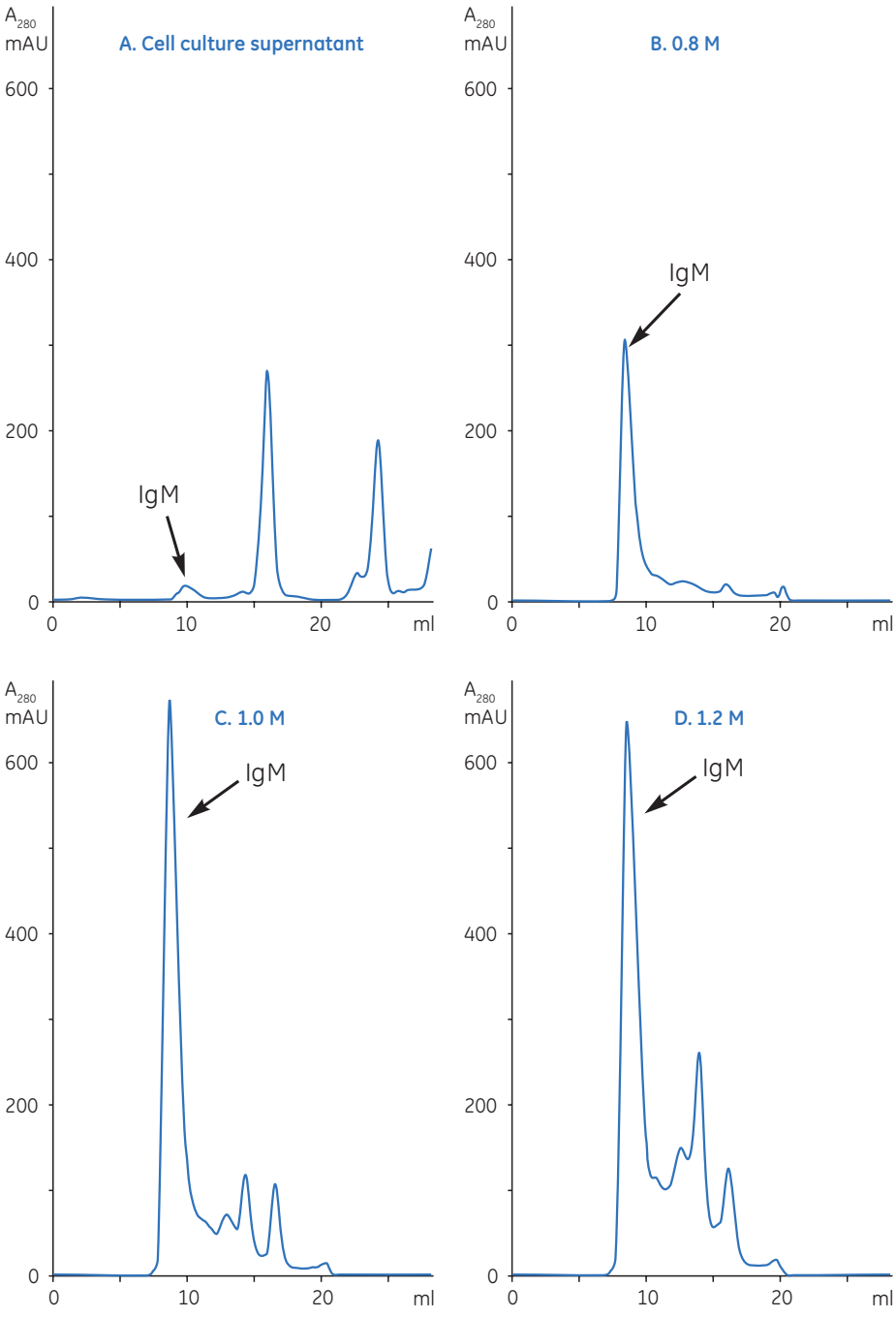


Fig 4. Gel filtration analysis of cell culture supernatant (A) and eluate (B-D) from HiTrap IgM Purification HP.

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18-1134-82 AB 12/2006