Purification of a Human Monoclonal IgM Antibody from Bioreactor Supernatant Using a Combination of Cation and Anion Exchange Chromatography

Mark I. Fitchmun, Alex Ermakov, and Michael McKnight, Hygeia Pharmaceuticals Inc., 6555 Nancy Ridge Dr., San Diego, California 92121,* MF Consulting, 3935 Southview, San Diego, CA 92117 (for correspondance).*

Introduction

For more than 15 years, hybridoma technology has provided access to unlimited quantities of well defined, homogeneous antibody reagents.

Monoclonal antibodies have become indispensable as biochemical reagents and for many processes in the biotechnology industry. They also have potential as therapeutics with diverse applications such as infectious diseases (AIDS, septic shock), transplantation, autoimmune diseases, and cancer. Availability of pure, reactive antibodies is a prerequisite for all these applications.

Today, purification of antibodies of IgG isotype is usually straightforward, resulting in reasonably pure and immunoreactive reagents. Purification of IgM antibodies has proven to be more difficult. Several methods have been proposed (see Knutson et al., J. Immunol. Methods, **136**, 151–157, 1991 for review), most of which result in poor yields and poor immunoreactivity of the recovered antibodies. Many available methods are also incompatible with GMP requirements and cost restraints associated with the production of pharmaceuticals and diagnostics.

Earlier, we described a human hybridoma cell line that secretes a human IgM monoclonal antibody, SK-1.45, with specificity for an adenocarcinoma associated antigen (Koda et al., Arch. Surg., **125**, 1591–1597, 1990). To provide the SK-1.45 antibody for clinical phase 1 trials, we developed a novel chromatographic procedure to purify human monoclonal IgM from bioreactor supernatants.

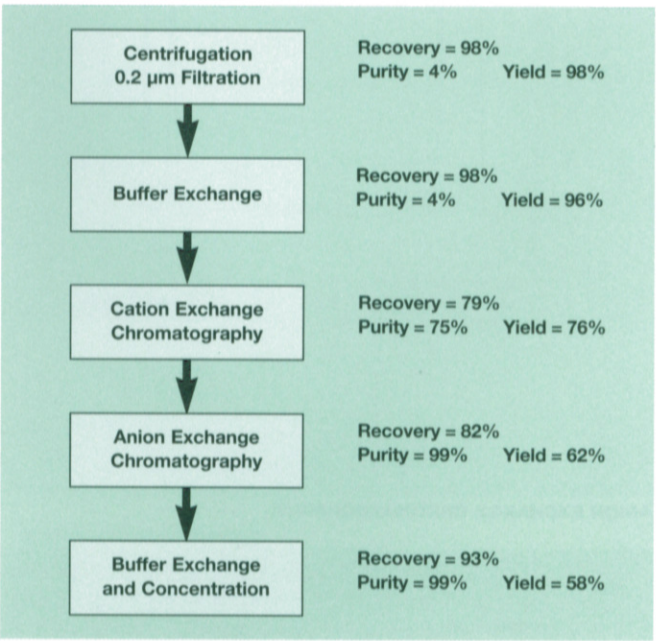
Discussion

SK-1.45 binds to both anion and cation exchange supports when applied at a neutral pH. It is therefore possible to attain a high degree of purity through a two step procedure involving cation and anion exchange chromatography. Initially, bioreactor supernatant is buffer exchanged into 50 mM Na℗, pH 7.2, via gel permeation chromatography on Sephadex® G-25 (Pharmacia) and fractionated using cation exchange chromatography, on the Macro-Prep® high S support (Bio-Rad Laboratories). Partially purified IgM is eluted with 50 mM Na℗, 100 mM NaCl, pH 7.2, diluted 1:1 with water, and loaded onto an anion exchange column packed with Macro-Prep high Q support (Bio-Rad Laboratories). Purified IgM is eluted with 30 mM Na℗, 250 mM NaCl, pH 7.0.

For SK-1.45, this procedure resulted in a final product which was > 98% pure as determined by SDS-PAGE and a yield of 60% as determined by ELISA. DNA levels were reduced by a factor of > 5,000 from the levels found in the bioreactor supernatant, and biological activity, binding to pure recombinant antigen, was fully retained.

This protocol was used to purify gram quantities of human IgM under GMP for use in clinical trials.

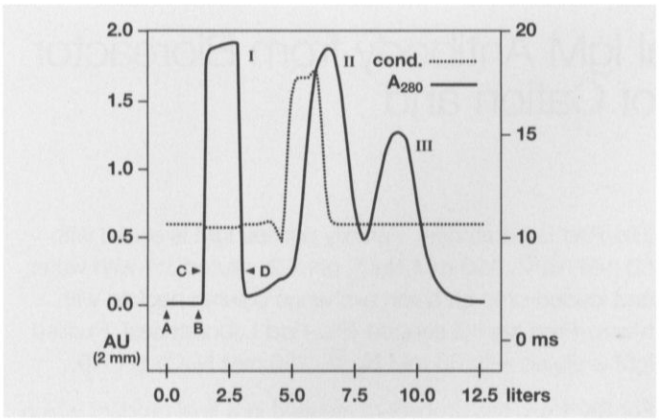
Procedure Overview



Recovery *= IgM recovered from preceding step.*
Purity *= Determined with SDS-PAGE.*
Yield *= IgM recovered from starting material.*

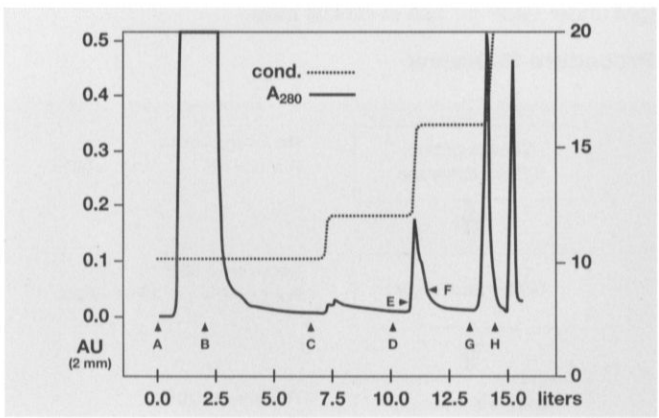
BIO-RAD

BUFFER EXCHANGE



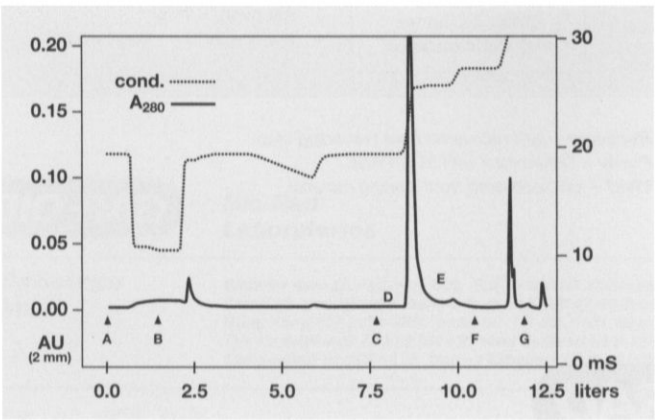
Resin: *6,000 ml Sephadex G-25 m*
Column: *Moduline, 2 x (9 cm x 50 cm) Amicon*
Flow rate: *100 ml/min*
Event table: *A–B Load filtered bioreactor sup*
B *50 mM Na Ⓟ, pH 7.2*
C–D *Collect protein fraction*
Note: *Peaks II and III are components of Hygeia's protein free tissue culture media. They contain no protein.*

CATION EXCHANGE CHROMATOGRAPHY



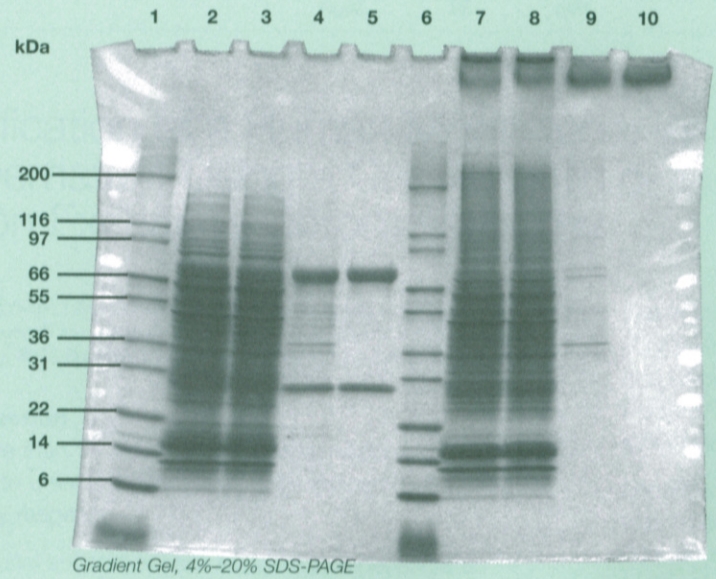
Resin: *2,000 ml Macro-Prep high S cation exchange support*
Column: *Vantage-S (9 cm x 50 cm), Amicon*
Flow rate: *75 ml/min*
Event table: *A–B Load protein fraction from previous run*
B 50 mM Na Ⓟ, pH 7.2
C 50 mM Na Ⓟ, 30 mM NaCl, pH 7.2
D 50 mM Na Ⓟ, 100 mM NaCl, pH 7.2
E–F Collect partially purified IgM
G 50 mM Na Ⓟ, 1 M NaCl, pH 7.2
H 1.0 M NaOH

ANION EXCHANGE CHROMATOGRAPHY



Resin: *2,000 ml Macro-Prep high Q strong anion exchange support*
Column: *Vantage-S (9 cm x 50 cm), Amicon*
Flow rate: *75 ml/min*
Event table: *A–B Load partially purified IgM (diluted 1:1 with water)*
B 30 mM Na Ⓟ, 160 mM NaCl, pH 7.0
C 30 mM Na Ⓟ, 250 mM NaCl, pH 7.0
D–E Collect purified IgM
F 300 mM Na Ⓟ, 1.6 M NaCl, pH 7.0
G 30% acetic acid, 20% isopropanol

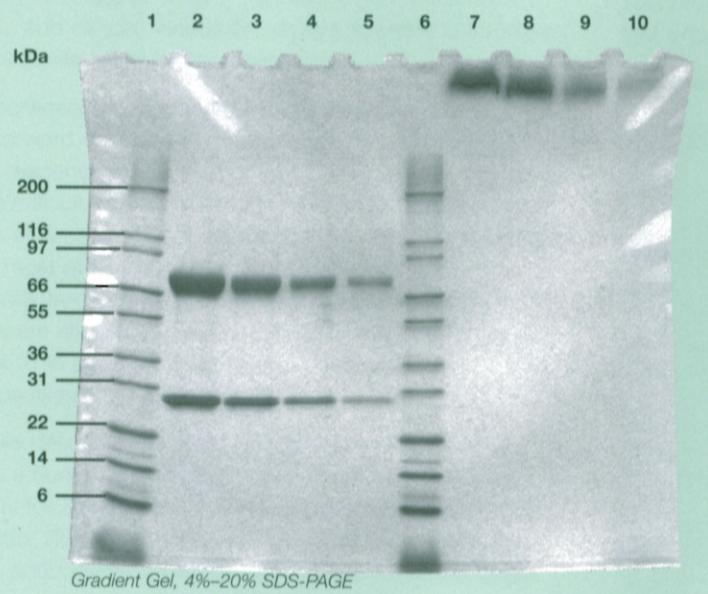
In Process Material



Gradient Gel, 4%–20% SDS-PAGE

Lane	Sample
1	Molecular weight standard
2	Bioreactor sup [reduced]
3	Buffer exchanged material [reduced]
4	Partially purified IgM from cation exchange [reduced]
5	Highly purified IgM from anion exchange [reduced]
6	Molecular weight standard
7	Bioreactor sup [non reduced]
8	Buffer exchanged material [non reduced]
9	Partially purified IgM from cation exchange [non reduced]
10	Highly purified IgM from anion exchange [non reduced]

Purified SK-1.45



Gradient Gel, 4%–20% SDS-PAGE

Lane	Sample
1	Molecular weight standard
2	Final product concentrated 2x [reduced]
3	Final product neat [reduced]
4	Final product diluted 1:1 [reduced]
5	Final product diluted 1:3 [reduced]
6	Molecular weight standard
7	Final product concentrated 2x [non reduced]
8	Final product neat [non reduced]
9	Final product diluted 1:1 [non reduced]
10	Final product diluted 1:3 [non reduced]

Results

Parameter	Starting Material	Final Product
Volume	7.5 liters	1.0 liter
Protein concentration	5 mg/ml	0.8 mg/ml
IgM concentration (ELISA)	0.3 mg/ml	0.8 mg/ml
Active SK-1.45 (Antigen ELISA)	0.19 mg/ml	0.81 mg/ml
Total SK-1.45	1.43 g	0.81 g
Purity	< 4%	99%
Endotoxin (LAL)	1.0 EU/ml	0.1 EU/ml
DNA	5 x 10-6 mg/ml	2 x 10-10 mg/ml
Activity (CDC for SK-1 positive cells)	100% kill	100% kill

Acknowledgements

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