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Antibody Purification Methods

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ABSTRACT

Antibodies (Abs) from the sera of patients with autoimmune diseases are reported to have different catalytic functions. Their recovery by efficient purification methods is, therefore, a crucial step. This article reviews different available methods for their recovery and emphasizes a new approach, namely adsorbents with immobilized histidine, which allows a good purification both in yield and purity of Abs, with the additional advantage of using gentle elution conditions. This, in turn, will ensure the recovery of intact (nondenatured) catalytically functional Abs, directly from the sera.

Index Entries: Autoimmune antibodies; catalytic antibodies; human serum; pseudobioaffinity chromatography; histidine ligand affinity; gentle elution conditions.

INTRODUCTION

Antibodies (Abs) are designed to vary their structure to fulfill their immunoprotective and immunoregulatory roles. Until recently, the biological function of the variable (V) domains of Abs was thought to be mediated exclusively by their ability to bind various antigens (Ags) through contacts at the complementarity determining regions (CDRs). Over the past few years, evidence has accumulated that Abs express catalytic functions under certain circumstances *(1,2)*. Thus, the V domains of certain Abs

can contain multiple ligand-binding sites with specific structural features. Different structural features may be responsible for different functions, such as catalytic activity and Ag binding.

The catalytic function of Abs is of particular relevance to autoimmune Abs. The first indication that catalytic Abs could be a factor in immunological dysfunction came from data that vasoactive intestinal peptide (VIP) is cleaved by Abs from the sera of patients with respiratory and gastrointestinal disorders *(1)*. Also, DNA-degrading (DNase) catalytic activity of Abs from sera of lupus, HIV, and rheumatoid arthritis patients was reported by Shuster et al. *(2)*. In addition to these catalytic functions, the Abs from sera of autoimmune disease patients have also shown specific binding to Vβ8 peptide fragments of the T-cell receptors. Such autoimmune binding phenomena may or may not result in catalytic cleavage of the autoantigen.

A prerequisite for in-depth study of the structural basis of the catalytic functions of autoimmune Abs is the availability of efficient and specific purification methods. The desired methods should not only ensure a good recovery, but also the conformational integrity of the purified Abs.

CONVENTIONAL METHODS

Until now, the studies elucidating the catalytic and receptor-binding functions of the autoimmune Abs all used well-established methods for recovering and purifying the Abs. The known methods for Ab purification employ protein A and certain other recently described adsorption methods, and are summarized in Table 1 *(4–14)*.

As seen in Table 1, methods that permit good IgG recovery from human sera all suffer from the drawback of being eluted at rather acidic pHs, which may result in some denaturation of the IgG and interfere in the quantitative/qualitative evaluation of their catalytic function. Buchner et al. *(15)* clearly showed that acidic pH can alter the conformation of the IgG. The acid-induced conformation was quite stable and irreversible. Even if the altered conformation does not influence the Ag-binding properties, it is possible that the catalytic function may be altered qualitatively or quantitatively.

Furthermore, when purifying catalytic Abs, one should ensure the absence of contaminating enzymes as well as contamination with other Abs whether the source of catalytic Ab be polyclonal human/animal sera or monoclonal culture supernatents or ascite fluid. In this context, Schultz recommends an additional ion exchange step after protein A purification of the catalytic Abs in order to eliminate any contaminating glycosidases and peptidases *(16)*.

Table 1
Different Chromatographic Methods Used for Antibody Purification

Ligand family	Ligand	Matrix	Antibody source	Price steps	Ends product	Remarks	Ref.
Microbial proteins	Protein A	Sepharose	Human serum		Purification of total IgG and subclass	IgG3 is not retained	4
	Protein G	Sepharose fast flow	Cell culture supernatent	Prepurification by ion exchange	Purification of MAbs	Desorption at pH 4.0 and 2.5; inactivation possible	5
Ion exchange	Aminoethyl	Zeta prep membrane	Cell culture supernatent	Microfiltration diafiltration	Purification of MAbs	Final product contamination with transferrine	6
	Carboxy methyl	Cellulose	Ascites fluid	Microfiltration	Purification of MAb IgG2b	Mild contamination	7
Thiophilic adsorption	β-mercapto-ethanol and other SH ligands	Agarose	Human serum or culture supernatent	–	Abs purification	Purified Abs were not of high purity	8
	β-mercapto-ethanol	Sepharose	$F(ab')_2$ fragments and Fc	–	Purification of Ab fragment	Recovery of about 90% of initial activity	9
Hydrophobic or aromatic adsorption	Phenyl	Bio-gel (Bio-rad)	Human serum	–	IgG purification	Adsorption at high salt concentration	10
	Aza-arenophilic	Sepharose	Human and other animal sera	–	IgG purification	Elution at pH 3.5, possibility of denaturation	11
	m-amino phenylboronic acid	Agarose	Human serum	–	IgG purification	C3 and C4 factors were found as contaminants	12
Metal chelates	IDA-NI(II)[a]	Sepharose	Culture supernatent and ascite fluid	Microfiltration	Purification of MAb	Binding site at Fc region of IgG1	13
	Tren-Cu(II)[a]	Novarose	Goat serum	–	IgG purification	IgG with ~95% purity scaled up operation	14

HISTIDINE LIGAND AFFINITY CHROMATOGRAPHY

Protein A and protein G seem to be the most prevalent ligands for Ab recovery, even for those with catalytic function *(1)*. Tables 2 and 3 compile the data for IgG/IgM recovery from human sera using either protein A or protein G coupled to Sepharose matrix *(4,5,17–22)*. It is to be noted that the desorption is invariably realized using acidic pH buffers, which may lead to certain protein structural modification *(15)*. It may be relevant, therefore, to compare the performance of these methods with a more recent approach proposed by Vijayalakshmi *(23)*, in which mild desorption conditions permit very efficient immunoglobulin G (IgG) recovery from pseudobioaffinity columns.

Figure 1 and Table 4 summarize the purification Abs (IgG/IgM) using histidine immobilized on Sepharose, flat nylon, nylon–polyvinyl alcohol (PVA) or silica membranes, and polyethylene vinyl alcohol (PEVA) hollow-fiber membranes *(24–28)*.

In all these cases, it was possible to recover high-purity IgG directly from the sera, without any prior fractionation. The Abs were eluted from the adsorbents under very mild conditions (addition of about 0.2 *M* NaCl to the starting buffer), which will ensure the structural integrity of the purified Abs. This is of relevance to the recovery of catalytically functional Abs.

Furthermore, during chromatography of human serum on affinity supports with immobilized histidine, IgG is not only separated from other human serum proteins, but it also fractionated into subsets. Thus, PEVA-based hollow-fiber membranes with immobilized histidine were able to adsorb all the IgG3 and part of IgG1 present in the sera, but IgG2 was not retained (Table 5). Therefore, a selective recovery of subclasses with specific functions can be envisaged *(25)*.

This feature was exploited for the recovery of Abs from patients with antiphospholipid syndrome, an autoimmune disease with thrombolytic complications. The antiphospholipid Abs involved in cardiovascular complications are known to show specific binding to a few protein cofactors such as β2 glycoprotein 1 and prothrombin, and these Abs are reported to belong to the IgG2 subclass *(27)*.

The sera from patients with clinical record of antiphospholipid syndrome were fractionated using a histidine-coupled PEVA hollow-fiber-membrane module. This resulted in a good recovery of antiphospholipid Abs with enriched specific binding to protein cofactors, β2 glycoprotein 1 and prothrombin (Fig. 2). Furthermore, the histidine-fractionated Abs (IgG) showed enhanced peptide-degrading and DNA-degrading catalytic activities (to be published).

These preliminary data, allow us to foresee a simple and efficient method for the recovery of Abs directly from sera, with their structural

Table 2
Isolation of Immunoglobulin Classes and Subclasses by Chromatography on Protein A-Sepharose

Species (class or subclass)	Application conditions pH	Application conditions Buffer	Elution conditions pH	Elution conditions Buffer	Comments	Ref.
Human whole IgG	7.0	0.1 *M* NaH_2PO_4	3.0	0.1 *M* glycine-HCl		*17*
IgG2	7.0	0.2 *M* NaH_2PO_4	4.7	0.2 *M* phosphate/	90–95% pure;	
IgG1		adjusted to pH 7 with 0.1 *M* citric acid	4.3	0.1 *M* citric acid	≤5% contamination with other IgG isotypes	*4*
IgG3	7.0	0.1 *M* phosphate	7.0	0.1 *M* phosphate	IgG3 in effluent, further purified on Sephadex G-150	*18*
IgM	7.5	Phosphate-buffered saline	2.5	0.1 *M* glycine-HCl	>80% of IgM in G-20 void volume peak was isolated	*19*

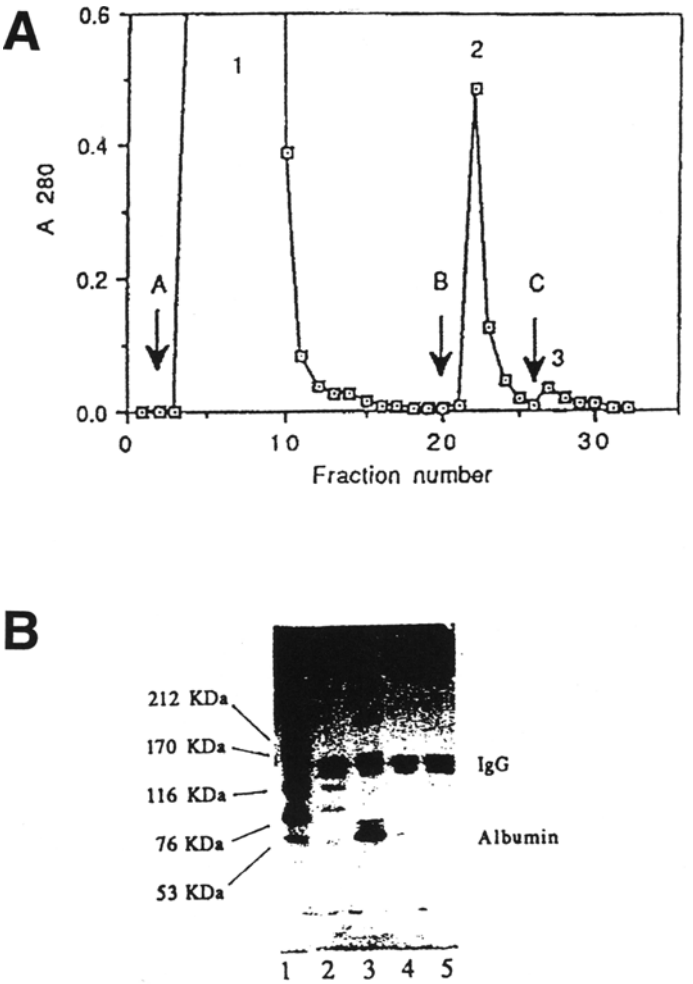

Fig. 1. **(A)** Typical chromatogram of untreated human serum on histidine-immobilized support (data from PEVA hollow-fiber membrane with immobilized histidine is shown as an example). Conditions: **(A)** 0.025 *M* Tris-HCl buffer pH 7.4; **(B)** A + 0.1 *M* NaCl; **(C)** A + 0.2 *M* NaCl; sample: 1.5 mL human serum; fraction vol: 6.0 mL. The numbers 1–3 indicate the protein peaks obtained. **(B)** SDS-PAGE analysis under nonreducing conditions of the peaks from the above chromatography. Lane 1: molecular mass markers; lane 2: reference IgG from human plasma obtained from Institut Merieux, France; lane 3: peak 1; lane 4: peak 2 and 5 = peak 3. (Reproduced from Bueno et al, 1995. J. Chromatog. B 667, 57–67.)

Table 3
Use of Protein G for Affinity Chromatography

Species	Application buffer	Elution buffer	Comments	Ref.
Human IgG	0.05 *M* Sodium phosphate containing 0.25 *M* NaCl, pH 7.6	Glycine-HCl, pH 2.0–3.0	79% recovery	*20*
Human IgG	Optimized around pH 5.0	Variable	Detailed analysis of effect of pH on binding of human IgG to protein G-Sepharose	*21* *22*
Human (monoclonal)	0.1 *M* glycine-sodium hydroxide, pH 9.0	Gradient elution, pH 4.0–>2.5	~50% recovery	*5*
		Single-step elution at pH 2.5	~70% recovery	*5*

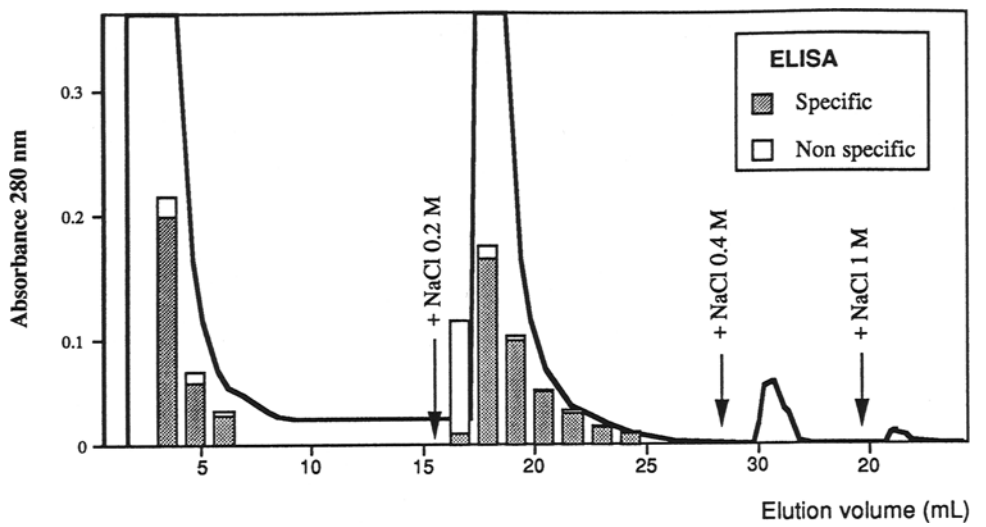

Fig. 2. Separation of serum from a patient with antiphospholipid syndrome on PEVA hollow-fiber membrane with immobilized histidine. The IgGs present both in the nonretained and in the eluted peaks were assayed for β2 glycoprotein 1 and prothrombin-specific antibodies by conventional ELISA methods. Data are shown for ELISA assay with prothrombin as an example. Specific, specific to prothrombin; nonspecific, no prothrombin binding.

Table 4
Purification of Abs on Different Supports with Immobilized Histidine

Ligand	Matrix	Antibody source	Prior steps	End product	Remarks	Ref.
Histidine	Sepharose	Human serum culture supernatent or ascite fluid	Precipitation	IgG subclass from human sera or MAb purification	Adsorption and desorption at physiological conditions; no risk of any denaturation	*24; 25*
	PEVA[a]–Hollow-fiber membrane	Human serum from healthy and autoimmune patients	None	IgG total or subclass selective recovery	do	*26* *27*
	Flat membranes silica, nylon, and so on	Human and other animals' sera	None	IgG purification	do	*28*

[a] PEVA, polyethylene vinyl alcohol.

Table 5
Subclass Composition in Fractions from Chromatography on H-B-PEVA[a] Using Salt Gradient Elution (IgG4 not Detected)

Peak	IgG_1	IgG_2	IgG_3
Passthrough	+	+	−
0.1 *M* NaCl	+	−	+
0.2 *M* NaCl	+	−	+

Adapted with permission from ref. *26*.
[a]B-PEVA stands for histidyl-bisoxirane poly (ethylene vinyl alcohol).

integrity intact, which, in turn, will be useful in studies for the understanding of their structure–function relations.

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