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# Affinity chromatography: A versatile technique for antibody purification

Sushrut Arora [a], Vikas Saxena [b], B. Vijayalakshmi Ayyar [c,*]

[a] Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030, USA
[b] Center for Vascular and Inflammatory Diseases, School of Medicine, University of Maryland, Baltimore, MD 21201, USA
[c] Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030, USA

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ABSTRACT

Antibodies continue to be extremely utilized entities in myriad applications including basic research, imaging, targeted delivery, chromatography, diagnostics, and therapeutics. At production stage, antibodies are generally present in complex matrices and most of their intended applications necessitate purification. Antibody purification has always been a major bottleneck in downstream processing of antibodies, due to the need of high quality products and associated high costs. Over the years, extensive research has focused on finding better purification methodologies to overcome this holdup. Among a plethora of different techniques, affinity chromatography is one of the most selective, rapid and easy method for antibody purification. This review aims to provide a detailed overview on affinity chromatography and the components involved in purification. An array of support matrices along with various classes of affinity ligands detailing their underlying working principles, together with the advantages and limitations of each system in purifying different types of antibodies, accompanying recent developments and important practical methodological considerations to optimize purification procedure are discussed.



**Contents**



* Corresponding author.
*E-mail address:* vijiayyar@gmail.com (B.V. Ayyar).

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## 1. Introduction

Antibodies are inducible defensive components of host immune system programmed to identify and neutralize non-self-entities such as pathogens. Antibodies are specific components of adaptive immune system which not only directly engage in the elimination of foreign particles but also trigger other non-specific effector functions such as antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent cell-mediated phagocytosis to eradicate the threat to host's homeostasis.

An antibody is typified by the immunoglobulin G (IgG) molecule, the most abundant subclass found in the serum of mammals [1,2]. IgG finds extensive application in assay development and antibody-based therapeutics [3,4] owing to their relatively small size and stability during the isolation and purification processes. An IgG is represented as a Y-shaped structure consisting of four polypeptide chains, two heavy chains and two light chains, connected by disulfide bonds (Fig. 1). These heavy and light chains are named based on the difference in their molecular weights. A light chain has a molecular weight of ~25 kDa whereas a heavy chain has a molecular weight of ~50 kDa. Based on variability in their amino acids, both heavy and light chains are further divided into constant (C) and variable (V) regions. The constant region determines mechanisms used to destroy antigen and segregates antibodies into five major classes (IgM, IgG, IgA, IgD and IgE) depending on their type and immune function. Variable region is further subdivided into hypervariable (HV) and framework (FR) regions. The relatively conserved amino acid sequences make-up the FR region. Conversely, there is great amount of variability in the sequence of amino acids in HV regions (also known as complementarity determining regions (CDRs)). Combination of different amino acids in the CDRs confers to antibody its ability to recognize specific antigenic determinants (epitopes). In the heavy and light chains, there are three HVs/CDRs which are connected to each other by four FR regions. Functionally, antibodies are composed of a fragment antigen binding (Fab), responsible for specific binding to the antigen; and a fragment crystallizable (Fc), important for effector mechanisms, entwining specific recognition of antigen with non-specific effector mechanisms of the host immune system (Fig. 1).

Antibodies are categorized into polyclonal, monoclonal and recombinant forms. A polyclonal antibody is a mixture of antibodies to a given antigen produced by different B cells. Such a mixture contains antibodies with different affinities and specificities to different epitopes on the antigen. Monoclonal antibodies are produced from a single clone of a B cell, having singular epitope specificity. The antibodies or antibody fragments generated *in vitro* using molecular techniques are classified as recombinant antibodies.

Antibodies are being exploited more and more for the obvious benefits they offer. They are increasingly being utilized in various scientific endeavors including but not limited to basic research, chromatography, targeted delivery, imaging, diagnostics and therapeutics [4–14]. Most of these applications require homogeneous antibody preparations with high purity. Consequently, there is an ever-increasing demand for robust, simple, efficient and cost-effective purification methodologies for antibodies from complex mixtures such as plasma, serum, ascites fluid, cell culture medium, egg yolk, plant extracts, and bacterial and yeast cultures [15,16]. To this end, a variety of chromatographic methodologies based on different biochemical properties (e.g. size, solubility, charge, hydrophobicity and binding affinity) have been tested [15,16]. However, affinity chromatography based processes have been reported to be the most efficient and widely employed techniques owing to their exceptional specificity, ease of operation, yield and relatively high throughput [16,17].

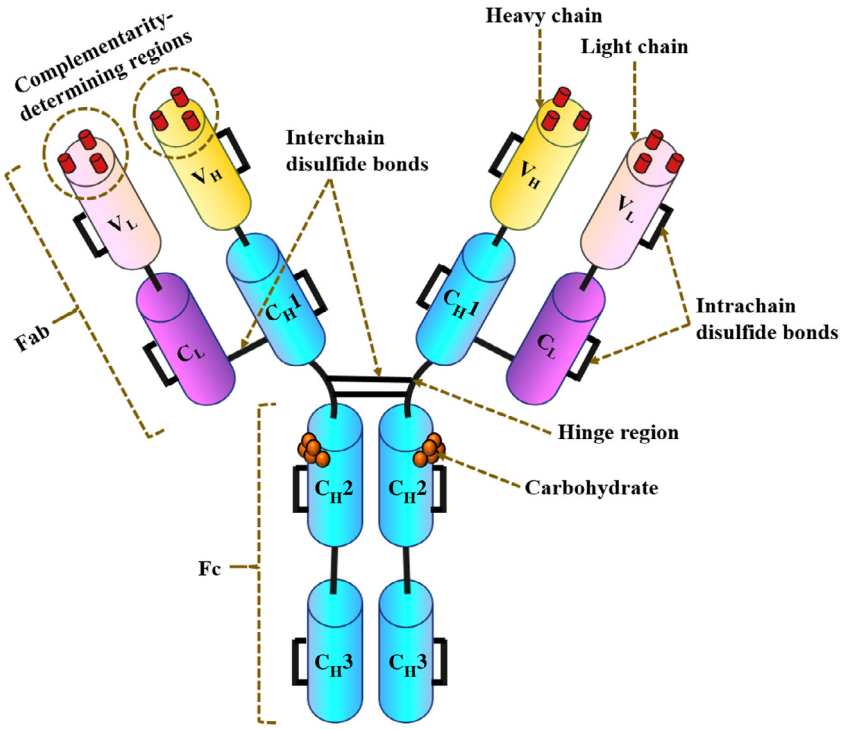


**Fig. 1.** Structure of a typical immunoglobulin, IgG. IgG are large molecules of approximately 150–155 kDa containing 2 pairs of heavy and light chains composed of different domains. The heavy chain consists of a variable domain ($V_H$) and three constant domains ($C_H1$, $C_H2$, and $C_H3$). The two heavy chains are connected by disulfide bonds (SS) in the hinge region. The light chain has one variable domain ($V_L$) and only one constant domain ($C_L$).