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Rapid and Simple Isolation of Multigram Goat IgG from Serum Using Avid AL™ and Radial Flow Column

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ABSTRACT

Multigram quantities (2.5–10 g) of highly purified IgG were obtained within 4 h from serum by using Avid AL™ packed in a radial-flow column. Avid AL™ is an affinity gel containing a synthetic, low-mol-wt ligand capable of selectively binding IgG from serum of all animal species tested. By packing the gel in a radial-flow column up to 500 mL, a high flow rate of 50 mL/min can be achieved without adversely affecting the performance of the gel and the purity of the isolated antibody.

Index Entries: Immunoglobulins; affinity gels; radial flow columns; synthetic ligands; goat serum.

INTRODUCTION

Affinity chromatographic isolation of IgG from serum using immobilized bacterial immunoglobulin binding proteins, such as Protein A, has recently received popular acceptance (*1–5*). This popularity is mainly because of the generality of the procedure, which requires minimal sample preparation and relatively mild elution conditions (pH 3–5), and has the capability of isolating IgG from different species. Another immunoglobulin-binding protein is from group G streptococci (Protein G). This

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protein was recently introduced as a superior IgG-binding protein because, unlike Protein A, it does bind goat IgG. Furthermore, Protein G is more selective in IgG binding. It binds only IgG; Protein A binds IgM as well as IgA.

The disadvantages of using protein affinity ligands, such as Protein A or G, in preparing affinity gels are

1. The instability of these ligands on exposure to acid, bases, or water-miscible organic solvents (conditions commonly used for depyrogenation);
2. Susceptibility to proteolytic degradation;
3. Susceptibility to thermal denaturation.

In attempts to overcome these disadvantages associated with protein-based affinity ligands, Porath et al. (*7–11*) developed thiophillic gels that, under high-salt conditions, were able to adsorb most of the serum proteins, and the bound immunoglobulins were selectively eluted in low-salt buffer. The affinity ligands were low-mol-wt thioether sulfone containing compounds of the following general structure:

$$-CH_2-CH_2-SO_2-CH_2-CH_2-S-CH_2-CH_2OH$$

Nopper et al. (*12*) developed a thiophilic gel, using slightly different affinity ligands, that contained three sulfur atoms and had a structural formula of

$$-CH_2-S-CH_2-CH_2-SO_2-CH_2-CH_2-S-CH_2-CH_2OH$$

This gel is called 3 S gel (*12*). Ngo and Khatter, on the other hand, developed a series of affinity gels selective for binding immunoglobulins, using synthetic, nonpeptide, low-mol-wt, nitrogenous heterocyclic aromatic halide affinity ligands (*13*). The tentative structure for the affinity ligand is shown in Fig. 1. By judicious selection of the halogen and nucleophilic substituents on the pyridine rings, it was possible to fine-tune the adsorption and desorption characteristics and conditions for immunoglobulin purification. As an example, immunoglobulins can be adsorbed under high-salt conditions (1.5*M* NaCl) to the affinity gel containing the halogen fluorine and —OH as the nucleophilic substituent. On the other hand, if chlorine and ethylene glycol are substituted for the halogen and nucleophilic substituents, the immunoglobulins and albumin are both adsorbed under low-salt conditions (20 m*M*). The immunoglobulin can be selectively desorbed after the albumin is washed out by using buffer containing 0.5*M* K_2SO_4.

This article describes the application of a derivative of this new affinity gel known as Avid AL™ for the purification of immunoglobulin G from human and goat sera and tissue-culture medium of mouse hybridoma cells in conventional and radial flow columns under physiological phosphate-buffered saline conditions.

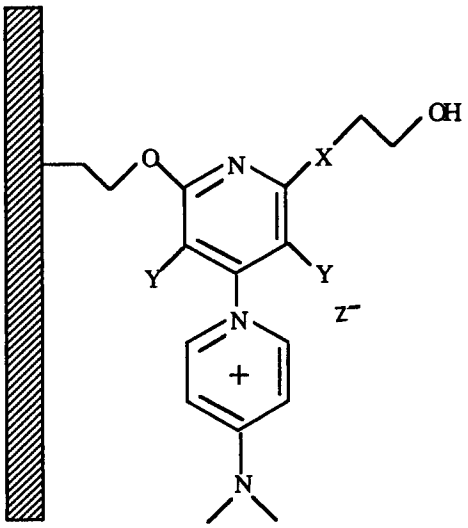

Fig. 1. Postulated structure of affinity ligand, where X = O, N, or S; Y = F or Cl; and Z^- is a counter anion.

MATERIALS AND METHODS

Materials

Avid AL™ gel and prepacked radial flow Avid AL™ (AvidPak Avid AL™), 100- and 500-mL columns, were obtained by BioProbe International (Tustin, CA), goat serum was obtained from Sigma Chemicals (Louis, MO), and human serum from Irvine Scientific (Santa Ana, CA). The cell-culture medium was obtained from Techniclone International Inc. (Tustin, CA). Gradient polyacrylamide gels (10–15%) for use with the Phast system were obtained from Pharmacia. All other chemicals were of analytical grade.

Methods

IgG Purification in a Conventional Column

For purification of IgG from 1 mL of human serum, a 3-mL vol of Avid AL™ was packed in a disposable column (9×0.8 cm). The column was first washed with about 15 mL of 20% methanol in water and 15 mL of phosphate-buffered saline (PBS). Filtered serum (using a 0.45-μm filter) was diluted fourfold with PBS, pH 7.4, and passed through the column at a flow rate of 0.5 mL/min. The column was washed with the same buffer at a flow rate of 1 mL/min. The bound proteins were eluted with 0.1*M* sodium acetate, pH 2.8–3.0 (adjusted with concentrated HCl), at a flow

rate of 1 mL/min. Glycerol or ethylene glycol (20%) can be added in the elution buffer to increase the yield of monoclonal antibody, if necessary. The increase in yield by inclusion of ethylene glycol or glycerol is probably attributable to a decrease in the polarity of the solution. Three-milliliter fractions were collected in an LKB 2070 Ultrorac II fraction collector. The absorbance at 280 nm was determined for each fraction.

IgG Purification in a Radial Flow Column

Both 100- and 500-mL Avid AL™ Avid Pak radial flow columns were washed with five column volumes of a solution consisting of 20% methanol (v/v) and 1% acetic acid (v/v) in water at flow rates of 10 mL/min for the 100-mL column and 50 mL/min for the 500-mL column. Then the column was further washed with PBS at the same flow rate. For the 100-mL radial-flow column, 50 mL of filtered goat serum (using a 0.45-μm filter) diluted with 200 mL of PBS was purified through the column at a flow rate of 5 mL/min. The column was washed with eight column volumes of PBS at a flow rate of 10 mL/min. Bound proteins were eluted with 300 mL of 0.1*M* sodium acetate, pH 3.0, at a flow rate of 5 mL/min. In the case of the 500-mL radial-flow column, 250 mL of filtered goat serum was diluted with 1 L of PBS and passed through the column at a flow rate of 25 mL/min. The column was then washed with 10 column volumes of PBS at a flow rate of 50 mL/min. Bound proteins were eluted with 1.5 L of 50 m*M* sodium acetate, pH 2.8, at a flow rate of 25 mL/min. Eluted IgG was neutralized immediately with 1*M* Tris buffer containing 1*M* NaCl, pH 8.5 to pH 7.0–7.5. The effluent from all the chromatographic columns was monitored continuously at 280 nm with an LKB2238 Uvicord SII. The pH of the effluent was continuously monitored with LKB2195 pH/ion monitor. Sodium dodecyl sulfate-polyacrylamide gradient gel electrophoresis (SDS-PAGE) on 10–15% gel was carried out using the Pharmacia Phast system.

RESULTS AND DISCUSSION

The reaction of crosslinked Sepharose with halogenated pyridine and 4-dimethylaminopyridine yielded affinity gels with tentative ligand structures as outlined in Fig. 1. The chemistry and detailed procedure for preparing the affinity gels have been described previously (*13,14*). It has been shown that these series of gels bind immunoglobulins selectively under various conditions of binding buffers (*13*). An affinity gel called Avid AL™, available from BioProbe International Inc., was designed to bind immunoglobulin under PBS conditions at pH 7.4 and eluted with 0.05 or 0.1*M* sodium acetate buffers (pH 2.8–4).

Table 1
A Comparison of the Binding Affinities
of Immunoglobulin G (IgG) Binding Gels[a]

	IgG Binding GELS		
Species	Avid AL™	Protein A	Protein G
Human	S	S	S
Rabbit	S	S	S
Mouse	S	S	S
Guinea pig	S	S	S
Bovine	S	S	S
Pig	S	S	S
Sheep	S	W	S
Goat	S	W	S
Horse	S	W	S
Rat	S	W	M
Chicken	S	W	W

[a]The immunoglobulin G (IgG) binding affinity: S, strong binding; M, medium binding; W, weak to no binding. Data adapted from ref. *14*.

Comparative studies (*14*) using serum from different animals have shown that Avid AL™ was able to purify IgG from all the species tested, including IgG from chicken serum (Table 1). In this regard Avid AL™ has broader species-specificity than either Protein A gel or Protein G gel. Since the Avid AL™ ligand is not a protein, it is not attacked by proteolytic enzymes. It is also stable in acid (0.1*M* HCl) or base (0.1*M* NaOH) for at least 12 h at room temperature (*14*). Furthermore, it was also shown that Avid AL™ can tolerate repeated autoclaving (at least up to three times) (*14*).

Goat IgG was highly purified using Avid AL™ in both conventional and radial-flow columns. The purity of the isolated IgG is shown in the SDS-PAGE (10–15% gradient gel) (Fig. 3). Immunoglobulin G from serum of other animals has been purified using the same experimental protocol. A typical chromatogram for purifying IgG from serum on a column of 3 mL is shown in Fig. 2.

A rapid, simple, and relatively inexpensive procedure for purifying IgG from goat serum is of great value for the manufacture of immunodiagnostic tests. Goat is still the animal of choice for producing a large volume of polyclonal antibodies. Current methods for purifying IgG from goat serum using protein G gel are very expensive. Moreover, protein G gel is rapidly destroyed by proteases, denaturants, or autoclaving.

We have developed a rapid, simple, and inexpensive procedure for purifying a multigram quantity of IgG from goat serum with 4 h by combined use of a radial-flow column and Avid AL™. Figure 4 shows the

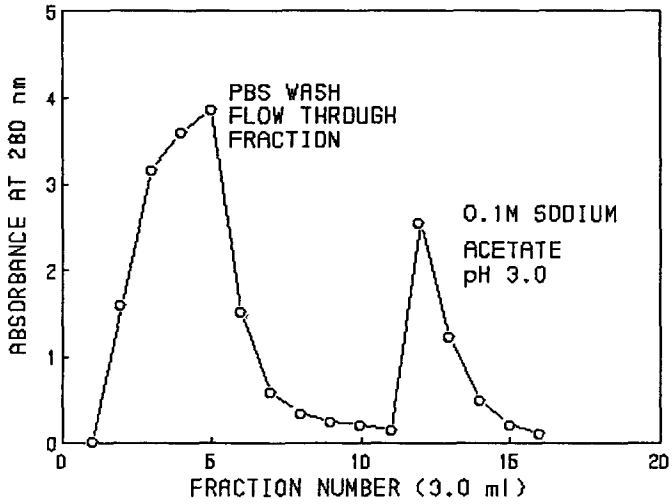

Fig. 2. Affinity chromatogram for purification of human serum IgG on Avid AL™ gel with PBS loading conditions. Gel volume was 3 mL. Serum was diluted fivefold with PBS, pH 7.4, and was applied at room temperature. Fractions of 3 mL were collected. Elution was carried out with 0.1*M* sodium acetate, pH adjusted to 3.0 with HCl.

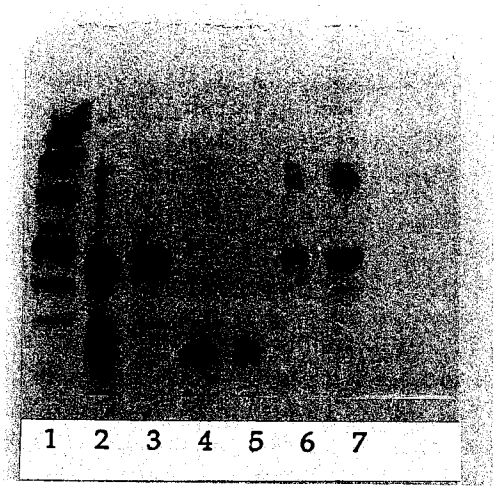

Fig. 3. SDS-PAGE (10–15% gradient gel) in reducing and nonreducing conditions of fractions obtained from 250 mL of goal serum fractionated by 500 mL Avid AL™ AvidPak radial flow column with PBS loading conditions.

Lane 1: Molecular weight marker reference proteins: myosin, phosphorylase, bovine serum albumin, ovalbumin, α-chymotrypsin, lactoglobulin, and lysozyme.
Lane 2: Unfractionated goat serum.
Lane 3: Unbound, flow-through fraction.
Lane 4,5. IgG eluted with 0.05*M* sodium acetate, pH 2.8; treated with nonreducing conditions.
Lane 6,7. IgG eluted with 0.05*M* sodium acetate, pH 2.8; treated with reducing conditions.

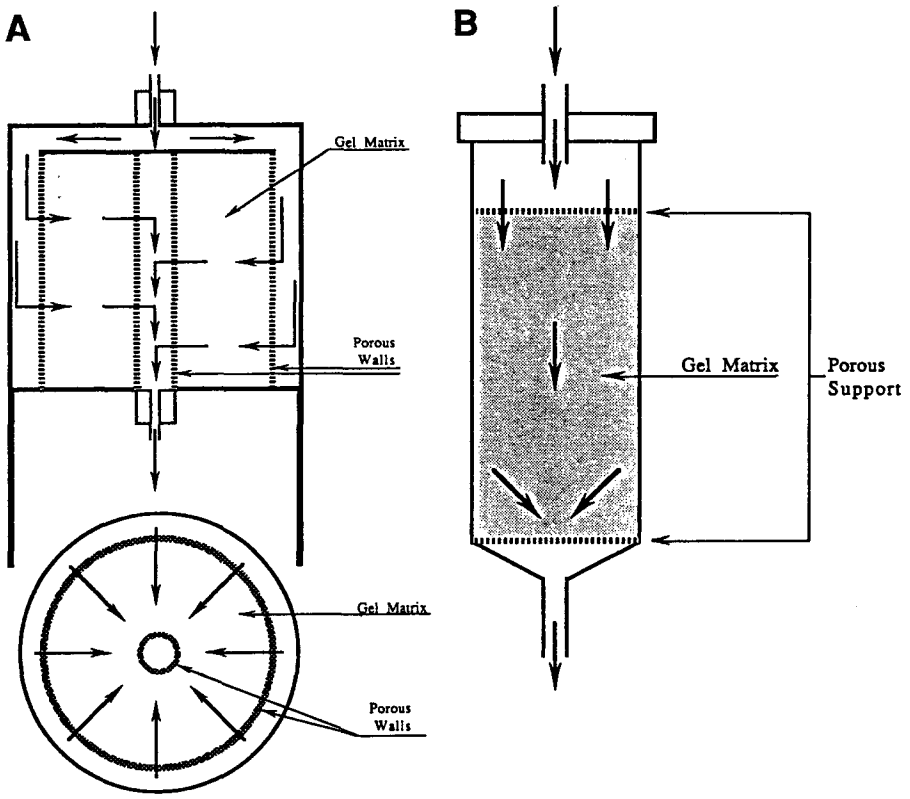

Fig. 4. Graphic presentation: **A:** Avid AL™ AvidPak radial flow columns and **B:** conventional axial flow column, indicating the direction of fluid flow.

direction of fluid flow in a radial-flow column and in a conventional column. In the conventional column the fluid flows axially; in the radial-flow column, the fluid is distributed evenly to the side of the gel column and flows laterally through the gel. In the radial-flow column the fluid is therefore applied over a larger surface area, and the effective column height is relatively small. This permits the sample separation to be carried out at high flow rates without generating significant back-pressure. The pressure drop is small. Scaling up the purification batch size can be achieved by simply increasing linearly the size of the column. A linear relationship between sample size and column length makes scaling up simple. Figures 5 A and B show chromatograms for the purification of goat IgG directly from diluted serum using 100- and 500-mL radial-flow columns containing Avid AL™. Similar results were obtained with either the 100- or the 500-mL Avid AL™ (Figs. 5 A and B). These results illustrate the linearity in scaling up the column.

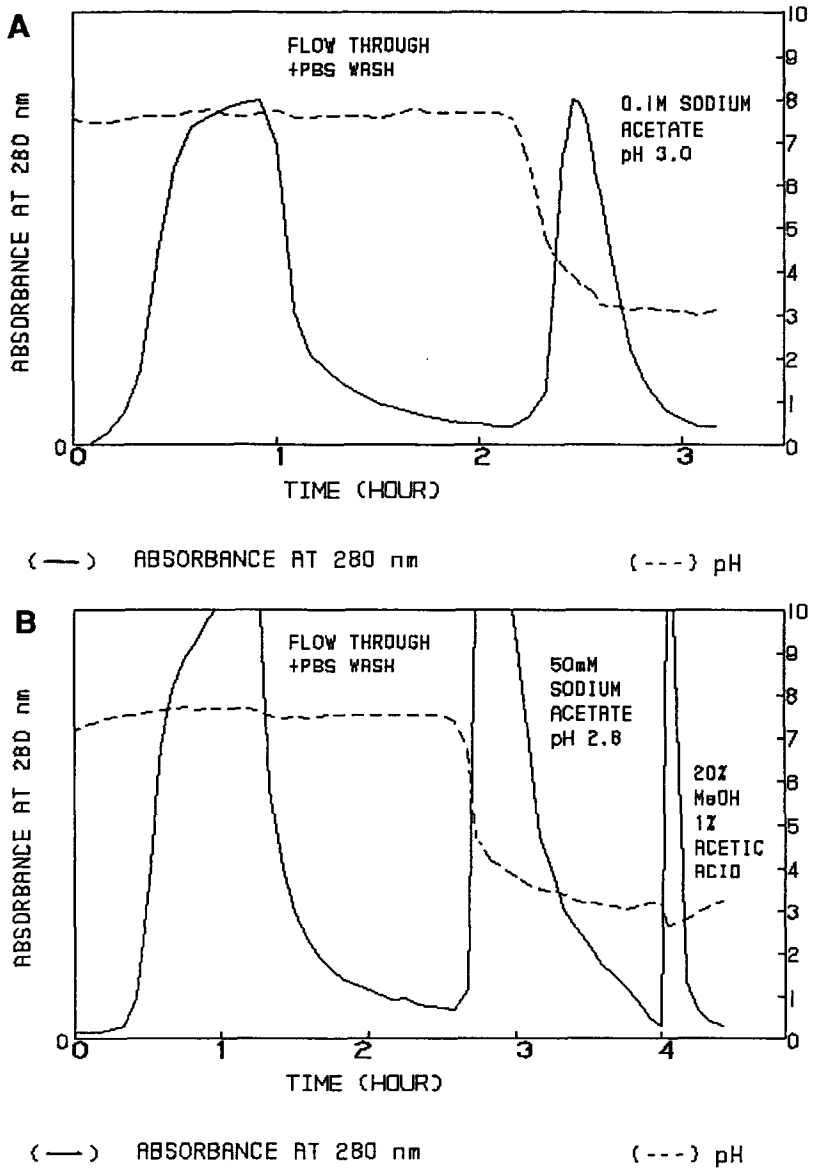

Fig. 5. Purification of IgG from goat serum by Avid AL™ AvidPak radial flow column with PBS loading conditions. **A:** Using a 100-mL Avid AL™ radial flow column, goat serum (50 mL) was fivefold diluted with PBS and passed through the column at 5 mL/min. The column was washed with PBS at a flow rate of 10 mL/min. Elution was carried out with 0.1*M* sodium acetate, pH adjusted to 3.0 with concentrated HCl. **B:** Using AvidPak Avid AL™ 500-mL radial flow column, goat serum (250 mL) was diluted fivefold with PBS and passed through the column at 25 mL/min. The column was washed with PBS at a flow rate of 50 mL/min. Elution was carried out with 50 m*M* sodium acetate, pH adjusted to 2.8 with concentrated HCl.

CONCLUSION

We have recently discovered that a number of affinity gels prepared with synthetic, low mol-wt ligands were able to mimic the binding properties of immobilized Protein A/G. Unlike Protein A/G, the synthetic affinity ligand can withstand acid, base, organic solvents, proteases, and autoclave treatments. When packed in a radial-flow column, 500 mL of this mimetic gel was able to isolate from goat serum approx 4 grams of goat IgG in a highly purified form in < 4 h.

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