



Comparison of the Affinity Chromatography and the Ion Exchange Chromatography in the Isolation of Bovine Immunoglobulin G

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Abstract

The purified Immunoglobulin G (IgG) is effectively used in passive immunization. There are various methods to isolate the IgG from serum, with its own advantages and disadvantages. In the current study, a comparative efficacy of the affinity chromatography using Protein A—Sephrose and ion exchange chromatography using DEAE Sephadex A50 was done with regard to yield and purity of IgG. Both methods were found equally effective in isolation of pure IgG with similar recovery, indicating that researcher can use either method to purify IgG depending on available resources.

Keywords

Affinity Chromatography, Ion Exchange Chromatography, Bovine Immunoglobulin G

Subject Areas: Agricultural Science, Microbiology, Veterinary Medicine

1. Introduction

Immunoglobulins are protein molecules that are produced by plasma cells in response to an immunogen. In 1890, von Behring and Kitasato reported the existence of antibody as an agent in the blood that could neutralize diphtheria toxin [1]. In the following year that agent was named as “Antikörper”, or antibodies. The immunoglobulin molecule consists of two heavy and two light chains. The light chain may have either a κ (kappa) or a λ (lambda) chain. There are five constant domain/chains. On the basis of constant domains, immunoglobulins can be di-

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vided into 5 isotypes, IgM, IgG, IgA, IgD, and IgE [2]. All isotopes are monomers except IgM and IgA. IgM is pentamer while IgA is dimer. IgG is the most versatile and major immunoglobulin in the blood, colostrum and extracellular fluid in human [3]. IgG plays an important role in controlling the disease. It controls invading pathogens via opsonization. The opsonization facilitates phagocytosis of invading organism by immune cells and helps in antibody dependent cell mediated cytotoxicity (ADCC). It activates the classical pathway of complement system and can neutralize the bacteria, virus or toxins. It also plays an important role in intracellular antibody mediated proteolysis [4]. The IgG is frequently used in passive immunization. The attainment of passive immunity in newborn calves occurs through the oral consumption and subsequent absorption of immunoglobulin soon after birth [5]. Low blood immunoglobulin concentrations are directly related to calf morbidity and mortality [5]. To increase the immunity of calves, purified IgG is given either in form of oral or parental administration. In the current study, we compare the two methods to isolate purified IgG from bovine serum that can be used in passive immunization.

2. Materials and Methods

2.1. Animals

Holstein Friesian cows housed at the Dairy Farm, South Dakota State University, Brookings, SD, U.S.A. were used in this study. All animals were healthy and vaccinated with “ViraShield” vaccine (Novartis Animal Health US, Inc), contained Bovine Rhinotracheitis-Virus Diarrhea-Parainfluenza 3-Respiratory Syncytial Virus Vaccine, Killed Virus-Campylobacter Fetus-Leptospira Canicola-Grippotyphosa-Hardjo-Icterohaemorrhagiae-Pomona Bacterin. Animal handling and blood collection methods were approved by the SDSU Institutional Animal Care and Use Committee.

2.2. Isolation of Immunoglobulin

Eighty (80) ml blood was collected from three cows in serum collecting vacuumed tubes. To separate serum, tubes were centrifuged at 2000 rpm for 30 minutes at 4°C. Following centrifugation, 8 ml serum was aspirated and sodium azide was added as a preservative to achieve final concentration as 0.1%. The 8 ml serum from each cow was divided in to two tubes with 4 ml each (one for affinity chromatography and other for ion-exchange chromatography). A 45% ammonium sulfate solution was added to each tube in 2:3 ration (v/v) to precipitate the immunoglobulin, followed by centrifugation at 10,000 rpm for 20 minutes at 4°C. The immunoglobulin pellet was dissolved in PBS (1/3 volume of original serum). This Ig solution was dialyzed using 75/100 Kd dialyzing tube (Sigma-Aldrich, St. Louis, MO, USA). The dialysis was done with PBS at 4°C for 24 hrs by replacing PBS every 6 hrs with continuous stirring.

2.3. Isolation of IgG Using Protein A—Sepharose

Total two grams of Protein A—Sepharose (Sigma-Aldrich, St. Louis, MO, USA) was allowed to come to equilibrium in 10 mM phosphate buffer saline (pH: 8) containing 0.1% sodium azide. The chromatography column (15 cm × 0.5 cm) was packed with equilibrated Protein A—Sepharose up to 2/3 of the length. The column was activated using 10 ml acid buffer (0.5 M glycine with HCL, pH: 2.8). Following activation, the column was neutralized using 200 ml 0.1 M phosphate buffer (pH: 8). The 1 ml dialyzed serum was applied over the column and elute was discarded. The column was washed 3 times with 0.1 M phosphate buffer (pH: 8). The IgG was eluted using 10 ml, 0.1 M sodium citrate (pH: 4) containing 0.1% sodium azide. The obtained elute was measured for protein concentration by Nanodrop, ND-1000 spectrophotometer (Thermo scientific, Wilmington, DE, USA) followed by confirmation of IgG using SDS PAGE.

2.4. Isolation of IgG Using DEAE Sephadex A50

The DEAE Sephadex A50 (GE Healthcare Bio-Sciences, Uppsala, Sweden) was swollen in 0.1 M tris buffer (pH: 8.3). The chromatography column (15 cm × 0.5 cm) was filled by DEAE Sephadex A50 up to 2/3 of the length. The 1 ml dialyzed serum was applied over the column and elute was discarded. The column was washed with 0.1 M tris buffer (pH: 8.3). The IgG was eluted using 10 ml, 0.1 M tris buffer containing 0.22 M sodium chloride (pH: 8.3). The obtained elute was measured for protein concentration by Nanodrop, ND-1000 spectrophotometer (Thermo scientific, Wilmington, DE, USA) followed by identification of IgG using SDS PAGE.

2.5. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with and without protein denaturation. For protein denaturation, samples were mixed with loading dye containing SDS and beta mercaptoethanol and samples were heated at 100°C for 10 minutes. For non-denaturation, samples were mixed in loading dye lacking beta mercaptoethanol and SDS and samples were not heated at 100°C. To separate protein, samples were run with 10% resolving gel. The protein bands were visualized using the coomassie blue staining.

3. Results

The IgG was isolated in pure form by the both affinity chromatography using Protein A—Sepharose as well as anion exchange chromatography using DEAE Sephadex A50. In both methods, the non-denatured IgG was observed at 150 kD (**Figure 1(a)** and **Figure 1(b)**), while the denatured gel revealed two bands with molecular size of 50 kD (heavy chain) and 25 kD (light chain) (**Figure 1(c)** and **Figure 1(d)**).

The yield of IgG by affinity chromatography and anion exchange chromatography revealed as 17.13 ± 2.08 mg/ml and 17.00 ± 1.60 mg/ml, respectively, which were significantly similar to each other ($p < 0.05$) (**Table 1**).

These results indicated that the both affinity chromatography and ion exchange chromatography are equally efficient in isolating the IgG from serum samples.

4. Discussion

The current study revealed that ion exchange chromatography using DEAE Sephadex A50 and affinity chromatography using Protein A—Sepharose are equally effective in isolating the IgG. The both technique isolated the

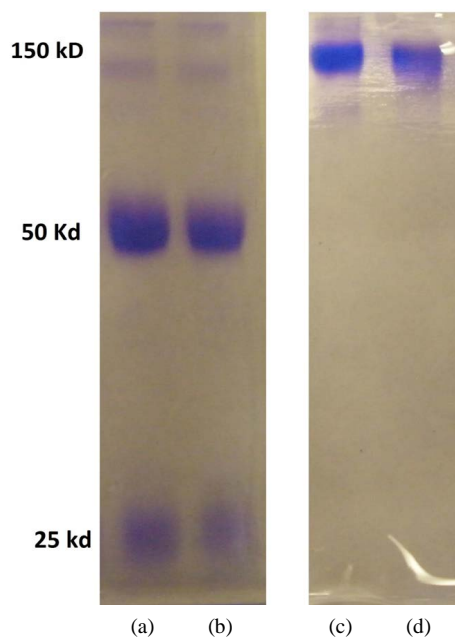


Figure 1. Characterization of Immunoglobulin G using SDS-PAGE gel. The immunoglobulin G (IgG) was isolated from bovine serum through affinity chromatography or ion exchange chromatography. The isolated IgG was non-denatured or denatured by heating the sample at 100°C for 10 minutes and examined through the SDS-PAGE. The denatured SDS-PAGE gel showing heavy chain of IgG with molecular size 50 kDa and light chain with 25 kDa isolated by affinity chromatography (a) or ion exchange chromatography (b), respectively. While non-denatured IgG showed the molecular size as 150 kDa isolated by affinity chromatography (c) or ion exchange chromatography (d), respectively.

Table 1. Concentration of IgG using affinity chromatography and exchange chromatography. The immunoglobulin G (IgG) was isolated from bovine serum through affinity chromatography or ion exchange chromatography. The concentration of isolated IgG was measured through Nanodrop, ND-1000 spectrophotometer.

Sample	The concentration of the IgG using Protein A—Sepharose/ affinity chromatography (mg/ml)	The concentration of IgG using DEAE Sephadex A50/ion exchange chromatography (mg/ml)
Sample 1	17.8	17.5
Sample 2	14.8	15.2
Sample 3	18.8	18.3
Average	17.13±2.08	17.00 ± 1.60

IgG in pure form with similar concentration. The recovery of IgG from 1 ml dialyzed serum protein using affinity chromatography and anion exchange chromatography was 17.13 ± 2.08 mg/ml and 17.00 ± 1.60 mg/ml, respectively. The actual concentration of serum IgG may be different, as some of IgG may be lost during isolation process.

The IgG is widely utilized in the immunological and dietary supplementation [6]. The study showed that oral consumption of bovine IgG reduced the viral gastroenteritis [7], along with dietary supplements, purified IgG also used in passive immunization [8] and various diagnostic assays [9]. There are various methods have been standardized to isolate the IgG, the choice of procedure generally depends upon intended used of IgG and resources available. The current study was conducted to compare the two methods that are widely used in the laboratories to isolate the substances. It will be worth interesting to compare other methods including HPLC (High-performance liquid chromatography) to isolate the IgG in different species. The comparative studies will be useful for future reference through which researcher can choose the best method for his/her work.

5. Conclusion

The current study indicated that affinity chromatography and ion-exchange chromatography using Protein A—Sepharose and the DEAE Sephadex A50 respectively have same efficiency to isolate the immunoglobulin G, in terms of purity and yield.

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