

Purification and Characterization of Bovine Serum Albumin Using Chromatographic Method

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Abstract

Purpose: Albumin is an abundant protein of blood and has many biopharmaceutical applications. The aim of this study was to purify bovine serum albumin (BSA) using produced rabbit anti-BSA antibody.

Methods: The polyclonal antibody was produced against the BSA in rabbits. Then, the pure BSA was injected to three white New Zealand rabbits. ELISA test was done to evaluate antibody production. After antibody purification, the purified antibody was attached to CNBr-activated sepharose and finally it was used for purification of albumin from bovine serum. Western blotting analysis was used for functional assessment of immunoaffinity purified BSA.

Results: The titer of anti-bovine albumin determined by ELISA was obtained 1: 256000. The SDS-PAGE showed up to 98% purity of isolated BSA and western blotting confirmed the BSA functionality. Purified bovine serum albumin by affinity chromatography showed a single band with molecular weight of 66 KDa.

Conclusion: Affinity chromatography using produced rabbit anti-BSA antibody would be an economical and safe method for purification of BSA.

Introduction

Separation is a keystone phase of downstream process that affects the final cost of chemical product. So this critical point needs more considerations because current bioseparation methods are not fully cost effective and operative in large scale production.¹ One of the proteins with worldwide consumption due to its structural stability and high level of abundance in plasma, is albumin. Many efforts have been accomplished to achieve high pure bovine serum albumin (BSA) during long period of time. The first effort for large scale purification of albumin and other plasma proteins developed about 60 years ago by Cohn and co-workers.² Plasma fractionation using ethyl alcohol is a dominant industrial method in the global albumin manufacture.³ In this process protein denaturation may occur, so other approaches were developed.

Among broad range of separation techniques, affinity approaches are the most selective methods for purification. So to achieve high quality of albumin product, novel ligands are required to design effective affinity approaches. Immunoaffinity via polyclonal antibody is an innovative idea that may increase the efficiency and yield of purification in industrial scale. The aim of this study was immunoaffinity purification of BSA using produced polyclonal antibody.

Materials and Methods

Immunization of rabbits with BSA

An amount of 300 microliter of BSA was mixed with an equal volume of complete Freund's adjuvant (CFA) and injected into three female New Zealand white rabbits (3-month-old, about 1.3 kg weight). The rabbits were fed regular diets. The research was confirmed by the Regional Medical Sciences Research Ethics committee of Tabriz University of Medical Sciences.

ELISA test was designed to determine the optimum titer of rabbit anti-BSA antibody.

Purification of rabbit polyclonal antibody

For purification of rabbit immunoglobulin, ion exchange chromatography (IEC) and protein G affinity chromatography were done. After column packing (hand-made with 12 mm diameter and 100 mm height), the sample was dialyzed and loaded onto columns. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (120 V, the concentration of stacking and resolving gels were 4 and 13 percent, respectively) was used for purity evaluation of the fractions.

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Immunoaffinity chromatography purification of the BSA using purified IgG

For preparation of immunoaffinity chromatography (IAC) column, purified antibody was attached to Cyanogen Bromide (CNBr) activated sepharose 4B beads.^{1,4} So, after the dialysis of the purified antibody against coupling buffer, the sepharose beads were washed several times by coupling buffer. After adding the antibody to the beads, the beads were blocked using glycine buffer. Then the column was washed with 1 mM HCl and acetate buffers, pH: 4.5 separately. The sample was loaded and related fractions were collected. Then the column was washed with 0.1 M glycine buffer, pH: 2.7 as elution buffer. Absorption of the fractions was read by spectrometry at 280 nm. SDS-PAGE analysis was used to evaluate purity of fractions.

Western blotting analysis

IAC purified BSA was mixed with sample buffer and separated by SDS-PAGE on reduced condition onto 12% gels. After blotting process, the PVDF membrane was blocked with the blocking solution and incubated with anti-BSA and HRP-conjugated anti-mouse IgG antibodies. The protein bands were visualized by ECL substrate.

Results

Evaluation of immunization

We used ELISA test for assessment of antibody production. The titer of anti-BSA was 1: 256000.

Albumin purification using immunoaffinity chromatography

Purified rabbit anti-BSA IgG was coupled to CNBr-activated sepharose 4B beads and used to purify albumin protein from bovine serum. The amount of 0.7 mg of bovine serum was loaded on the column. We got about 0.36 mg purified albumin. SDS-PAGE analysis showed the purity of protein was up to 98%. Also the single band with a molecular weight of approximately 66 KDa is related to BSA (Figure 1).

Western blotting analysis

Western blotting analysis was done for functional assessment of IAC purified BSA. Figure 2 represents the Western blot analysis of the IAC purified albumin from bovine serum, showing the presence of a BSA protein band with a molecular weight of 66 KDa.

Discussion

BSA has many applications in diagnostic and immunological assays.⁵⁻⁷ For the first time Cohn et.al purified albumin based on pH and ethanol precipitations from serum. Many researchers have tried to improve this method by several modifications.⁸⁻¹³

In this research, the rabbits were immunized with BSA. Then the IEC and protein G affinity chromatography

were used to isolate and purify the rabbit anti-BSA antibody.

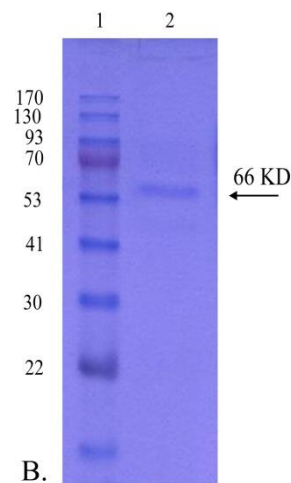


Figure 1. SDS-PAGE pattern of BSA purification by IAC using purified rabbit IgG.

SDS-PAGE was done under reduced conditions, the concentration of polyacrylamide gel was 13%. Lane 1: low molecular weight marker, Lane 2: fractions of elution process.

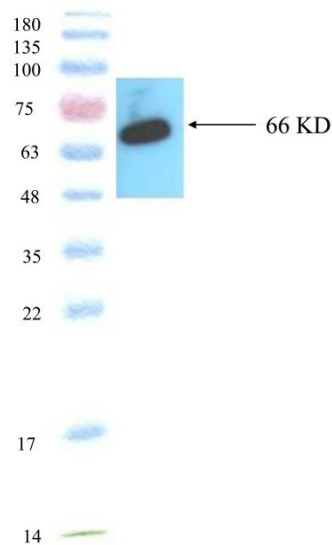


Figure 2. Western blot analysis of IAC purified BSA.

BSA protein was purified using produced antibody through IAC. SDS-PAGE was subjected onto BSA and electeroblotted onto PVDF membrane. Lane 1: prestained low molecular weight marker. Lane 2: the detected band showing purified BSA by IAC.

Chromatographic separations are bottleneck step in biopharmaceutical downstream protein processing.⁴ The affinity chromatography is a single step chromatography for purification of samples.¹⁴ The yield in this technique is high but, harsh elution conditions may affect the product function.¹⁵⁻¹⁷ The yield of purified IgG was about one-fourth of crude protein.

On the other hand the IEC as an alternative technique vastly used in antibody purification. IEC is considered as economical, cost effective and appropriate method which

is affected by physical and chemical factors including the protein properties, buffer type, pH, the flow rate, ionic strength and essence of counter ion.^{1,18} For achieving perfect condition of purification, these parameters must be optimized.¹⁹

In this study IEC and protein G affinity chromatography were applied to purify rabbit anti-BSA IgG. Then the purified antibody was attached to CNBr activated sepharose and used for albumin purification from bovine serum.

Immunoaffinity chromatography can be considered as an alternative method for albumin purification. Efficient affinity chromatography requires appropriate matrix. One of the suitable matrices which is widely studied and extensively used as activation reagent is CNBr and many researchers have used it for protein purification. Although CNBr has some drawbacks such as high toxicity that needs safety hazards consideration, but yet CNBr-activated sepharose immunoaffinity chromatography is an appropriate choice for protein purification.^{4,20,21} Since multi step chemical activation of the matrix must be taken part before usage, to eliminate these problems commercial pre-activated matrices are available. Finally, purified albumin as a single 66 kDa band with a purity of 98 % was achieved. The western blotting analysis confirmed the function of produced BSA.

Conclusion

In this study, protein G affinity and IEC chromatography were applied for polyclonal antibody purification against BSA. Then the produced antibody was coupled to the CNBr-activated sepharose 4B and used for the purification of albumin from bovine serum. The purity of prepared BSA was up to 98%. So immunoaffinity chromatography using purified anti-BSA antibody would be an economical and safe method for purify BSA.

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Ethical Issues

Not applicable.

Conflict of Interest

The authors have not declared any conflict of interest in this work.

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