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Immunoglobulin A Preparation from Human Pooled Plasma Using Plasma Fractionation and Ion Exchange Chromatography

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Abstract

Background: In this study we have prepared the IgA solution from normal human plasma using plasma fractionation and ion exchange chromatography.

Materials and Methods: Using fractionation of plasma with cold ethanol (starting with 8% ethanol), fraction III was prepared as a suitable source for IgA preparation. Then it was treated with caprylic acid for separation of impurities. For enrichment of IgA, ion exchange chromatography using Sephadex DEAE A-50 on a Pharmacia glass column was carried out.

Results: By fractionation of pooled human plasma and then treatment with caprylic acid, the amount of immunoglobulins showed an increase from 17% to 80% measured by immunoturbidimetric method. Then the ion exchange column chromatography by Sephadex DEAE A-50 was carried out. In washing solution 65 % of loaded IgG and only 17% of loaded IgA were found. In elution solution 32% of loaded IgG and 79% of loaded IgA were obtained, which showed the ion exchange chromatography is a promising method for preparation of enriched IgA solution.

Conclusion: Because of the similarity between IgA and IgG, techniques based on net charge and molecular differences would not give a high yield in IgA isolation. As IgA has a negative charge at the pH 8.1, Sephadex DEAE A-50 which is able to bind proteins at relatively high ionic strength was chosen for ion exchange chromatography. After column ion exchange chromatography, the concentration of obtained IgA was three times more enriched in comparison with the concentration of IgA in normal plasma.

Keywords: IgA, IgG, plasma, chromatography.

Introduction

A large variety of proteins exist in human blood plasma such as coagulation factors, immunoglobulins, albumin, α1-antitrypsin, antithrombin III, fibrinogen and some others [1-4]. Most of these proteins have important therapeutic uses. Clinical use of albumin the most abundant protein in the plasma for maintaining of the blood volume level is well known. Some clinical uses for other proteins are factor VIII for treatment of hemophilia A, factor IX for hemophilia B, immunoglobulin for passive prophylaxis and some types of immune thrombocytopenic purpura, antithrombin III for congenital deficiency, and α1-antitrypsin for treatment of hereditary deficiencies [5-8].

The most important method for blood plasma protein separation is Cohn process, which is based on fractionation of plasma using cold ethanol [9, 10]. In this method concentration of ethanol increases from zero percent to 40%. Another parameter for conditioning the plasma is the pH, which decreases from 7 to 4.8 (acidic pH) during fraction I to fraction V of separation at the constant temperature of about -5°C [11-14]. By plasma fractionation, fraction I, II, III, IV, and V can be obtained, which among these fraction III is a good intermediate source for isolation of immunoglobulin A.

Immunoglobulin A has an important role in mucosal immunity and can be used as a new immunotherapeutic choice. Isolation of immunoglobulin A is difficult due to similarity of IgA and IgG in terms of their net charge and molecular weight [15-17]. IgA has been isolated

by a method involving seven steps including ammonium sulfate precipitation of euglobulins, dialysis against veronal buffer, batch separation on DEAE-Sephadex A50 followed by cold ethanol and ammonium sulfate precipitation, and resolution on TEAE-cellulose and finally removal of residual IgG on a bromoacetyl cellulose absorbent.

Isolation methods for IgA may be designed in two main steps. The first step is the separation of IgA from non-immunoglobulin serum proteins, and the second step is the separation of IgA from other immunoglobulin classes by ion exchange chromatography. Another method for IgA isolating IgA is using Cohn fraction III residue after treating with zinc sulfate. The obtained IgA in the supernatant is further purified by DEAE-Sephadex, followed by desalination and lyophilization. In our study for the isolation of IgA by cold ethanol fractionation of human plasma, the obtained fraction III was used as intermediate source followed by treatment with caprylic acid and ion exchange chromatography.

Materials and Methods

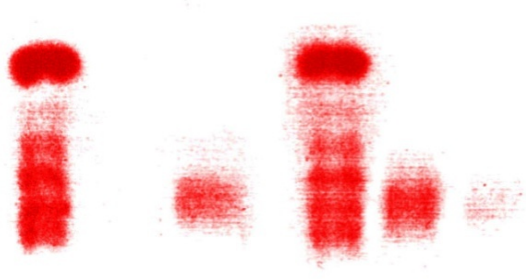
Materials

Tris(hydroxymethyl)aminomethane GR, fuming hydrochloric acid 37%, extra pure sodium chloride, tri-calcium phosphate dry extra pure, caprylic acid for biochemistry, sodium acetate tri-hydrate food grade, acetic acid glacial, and sodium chloride extra pure were prepared from Merck Company.

Methods

Fractionation of human plasma based on was performed using Kistler and Nitschmann method utilizing 96% (V/V) ethanol. First, fraction I which is a good source for fibrinogen and clotting factor VIII preparation was separated and then by increasing the concentration of ethanol to 19%, fraction II was precipitated and separated. The obtained fraction III was used for IgA isolation.

Sodium acetate buffer 0.05 M was used for dissolution of fraction III and it was stirred for 1 hour at room temperature. Then the solution was treated at room temperature by caprylic acid 1.5% (w/w) for 1 hour. After adding tri-



N (normal plasma), E_2 (second elution), E_1 (first elution), P (immunoglobulin precipitate), W (washing)

Figure 1: The electrophoretic analysis of immunoglobulins solution.

calcium phosphate 0.4% (w/w) the mixture was mixed for about 1 hour and it was kept at 4° C for 12 hours. Then the yellowish suspension was centrifuged at 16000 rpm by Hermle BHG model ZK401 with rotor A8.24 at 20° C for 30 minutes. First concentration of ethanol at 0° C was increased to 30% and then the solution was stirred for about half an hour at -10° C. The solution was centrifuged at 16000 rpm for half an hour at 0° C which resulted in obtaining precipitated immunoglobulins.

For ion exchange column chromatography the precipitated immunoglobulins were dissolved in 0.1M tris-0.05M NaCl buffer and by adjusting pH at 8.1 this solution was applied to the column for ion exchange chromatography. The column XK26 from Pharmacia was packed with the gel DEAE A-50 Sephadex, and the column was equilibrated with the buffer 0.1M tris-0.05 NaCl at pH 8.1. The immunoglobulins solution was applied to the column and it was eluted by 0.1M tris-0.2M NaCl at pH 8.1 at room temperature and then the ionic strength was increased using buffer 0.1M tris-0.35M NaCl for elution.

Using a fraction collector of 2211 Superrac, Pharmacia LKB, the 5ml fractions were collected and were monitored by UV absorption technique using Uvicord SII, Pharmacia LKB. All peaks were recorded using a 2210 recorder, Pharmacia LKB.

Determination of protein concentration was carried out using a Philips PU 8750 UV/Vis scanning spectrophotometer. Determination of IgA concentration was performed by immunoturbidimetric test using a Cobas Mira photometer. Identification of immunoglobulins was carried out by instrument of Vario 6, Elphor.

Results

In isolation of IgA from human plasma, the fraction I including fibrinogen and cryoprecipitate was removed by adding ethanol at concentration of 8% and the precipitate was removed. The supernatant which is a mixture of fraction II and III was obtained by increasing the concentration of ethanol to 19%, and after centrifugation, by addition of 10% ethanol the fraction III was obtained.

For separation of impurities from immunoglobulins, the solution was treated with caprylic acid. The technique which was used for the determination of the amount of immunoglobulins was immunoturbidimetric measurement, which showed the increased amount of immunoglobulins from 17% to 80%.

The solution which was loaded to the ion exchange column chromatography packed with the DEAE A-50 Sephadex consisted of 41% IgG, 26% IgA, and 33% other immunoglobulins.

Using ion exchange column chromatography three peaks were obtained. The first peak was related to washing and the second and third peaks were the elution peaks. Washing was done using buffer 0.1M tris-0.05M NaCl at the pH 8.1, and 65% of loaded IgG and only 17% of loaded IgA were found in washed solution. The first elution by buffer 0.1M tris-0.2M NaCl at the same pH resulted in getting a second peak with 32% of loaded IgG and 79% of loaded IgA. In order to remove the rest of immunoglobulins from the column, the ionic strength of buffer was increased by increasing the molarity of sodium chloride in buffer 0.1M tris-0.35M NaCl; the third peak was obtained containing 3.1% of loaded IgG and 2.5% of loaded IgA. The electrophoretic analysis confirmed that the most part of immunoglobulins were eluted in the first elution. The electrophoresis diagram of immunoglobulins solution is illustrated in figure 1.

Discussion

One of the most common immunodeficiency diseases is selective IgA deficiency which is marked by the complete absence of the IgA immunoglobulin in the blood. Selective IgA deficiency may happen one in five hundred individuals, but this figure could vary depend on different ethnic and racial groups.

Considering the similarity between IgA and IgG, implementation techniques based on net charge and molecular weight differences would not give a high yield in IgA isolation[18-21]. In this study IgA immunoglobulin was isolated from human plasma by plasma fractionation with cold ethanol. The obtained fraction III was treated by caprylic acid for separation of impurities, and then ion exchange chromatography was carried out. As mentioned above IgA has a negative charge at the pH 8.1; therefore Sephadex DEAE A-50 which is able to bind proteins at relatively high ionic strength was chosen for ion

exchange chromatography. The obtained IgA immunoglobulin concentration was three times more in comparison with the concentration of IgA in normal human plasma.

Conclusion

Because of the similarity between IgA and IgG, techniques based on net charge and molecular differences would not give a high yield in IgA isolation. As IgA has a negative charge at the pH 8.1, Sephadex DEAE A-50 which is able to bind proteins at relatively high ionic strength was chosen for ion exchange chromatography. After column ion exchange chromatography, the concentration of obtained IgA was three times more enriched in comparison with the concentration of IgA in normal plasma.

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