An organic solvent-based protocol for depletion of abundant proteins in plasma of Leprosy patients

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Method Article

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Abstract

In present study protein was purified from human plasma with the method involving organic solvents. Organic solvents like chloroform, methanol and isopropanol remove the lipoproteins and glycoproteins whereas TCA and Acetone are known to deplete the abundant proteins like albumins and globulins from the plasma of the blood. Purified protein was subjected to the estimation by Bradford Assay (96 well plate method). In this process a dye called Brilliant Blue forms complex with the purified protein of plasma because of which the maximum absorption of dye shifts to 595nm. So, the absorbance was observed at wavelength of 595nm. Purified protein along with unpurified plasma was imposed on SDS-PAGE. SDS (sodium dodecyl sulphate) is an anionic detergent which first denatures the protein and then provides all the protein fragments a negative charge. After which polyacrylamide gel separates the protein fragments on the basis of their molecular mass. Results of SDS-PAGE concluded that the protein was purified when its bands were compared to that of unpurified plasma, which means the treatment with organic solvents is useful in purifying protein from plasma of leprosy patient.

Introduction

Human plasma contains various high molecular and low molecular proteins like Albumin, Globulin, Lipoproteins, and Glycoproteins etc. To get a pure protein of interest, the organic solvents are very useful in the plasma protein purification steps (Barik et al, 2019). Human blood plasma contains a plethora of proteins, encompassing not only proteins that have plasma-based functionality, but possibly every other human protein in minute amounts as well (Schenk et al, 2008). The protein concentration in plasma/serum is approximately 60–80 mg/mL of which about 50–60% are albumins and 40% globulins (10–20% immunoglobulin G, IgG) (Leeman et al, 2018). Generally, the organic solvents like TCA and Acetone are used to remove albumin and globulin proteins in plasma. The inorganic solvent ammonium sulphate is used to remove the globulins and albumins in the plasma (Barik et al, 2020). The organic solvents protocol was used to deplete high abundant proteins in plasma of leprosy patients and compared with the Healthy individuals.

Reagents

S.NO.

REAGENTS / CHEMICALS

COMPANY

1.

Glycine

Merck, India

2.

Tris base

SRL, India

3.

Sodium Dodecyl Sulphate (SDS)

Himedia, India

4.

Ammonium Persulphate (APS)

Sigma-Aldrich, USA

5.

Acrylamide

Sigma-Aldrich, USA

6.

Bis-acrylamide

Sigma-Aldrich, USA

7.

Distilled water

Milli Q, Millipore, USA

8.

Tetramethylethylenediamine (TEMED)

Sigma-Aldrich, USA

9.

Glycerol

Merck, India

10.

Bromophenol blue

Merck, India

11.

β -mercaptoethanol

Sigma-Aldrich, USA

12.

Coomassie brilliant blue

Merck, India

13.

Ethanol

Merck, India

14.

Acetic acid

Merck, India

15.

Trichloroacetic acid (TCA)

Sigma-Aldrich, USA

16.

Acetone

Merck, India

17.

High Performance Liquid Chromatographic (HPLC) Water

Merck, India

18.

Urea

Sigma-Aldrich, USA

29.

Thiourea

Sigma-Aldrich, USA

20.

CHAPS

Sigma-Aldrich, USA

21.

DTT

Sigma-Aldrich, USA

22.

ASB-14

Merck, India

23.

Bovine Serum Albumin

Sigma, USA

24.

Chloroform

Merck, India

25.

Methanol

Merck, India

26.

Isopropanol

Merck, India

27.

Bradford Reagent

Sigma-Aldrich, USA

Equipment

S.NO.

EQUIPMENT

MANUFACTURER'S NAME

1.

Refrigerated Centrifuge

Sigma, Germany

2.

Micropipettes

Thermo Finepippet, Finland

3.

Refrigerator

LG, India

4.

Ultra deep freezer (-80°C)

New Brunswick Scientific, USA

5.

Deep freezer (-20°C)

Krispcold, India

6.

Electronic balance

Aczet, India

7.

pH Meter

Merck, Germany

8.

Spectrophotometer

Spectra max-M2, USA

9.

Gel Doc XR+

Bio-Rad, USA

10.

Rocknig platform shaker

Labnet International Inc.,USA

11.

Vertical PowerPac HC (power supply)

Labnet International Inc.,USA

12.

Vertical Electrophoresis cell

Procedure

TCA/Acetone and Chloroform : Methanol : Isopropanol (2:1:1) method for Plasma Protein Purification.

1. 200µl of plasma was taken in an Eppendorf tube and 1ml of Chloroform: Methanol: Isopropanol (500 µl: 250 µl: 250 µl) was added and kept it for 15 minutes. (Invert the tube 10 times to mix the solution).
2. The tube was centrifuged at 12000 rcf for 10 minutes at 4°C and the supernatant was discarded.
3. Then, 800 µl TCA/Acetone (50%) was added and incubated overnight at -20°C.
4. The tube was centrifuged at 12000 rcf for 10 minutes at 4°C and the supernatant was discarded.
5. Add 1ml of Isopropanol in each Eppendorf tube and centrifuged at 12000 rcf for 10 minutes at 4°C and the supernatant was discarded.
6. Let the Eppendorf tubes (protein) dry for 15-20 minutes.
7. Then add 200µl of sample buffer for protein (SBP) in each tube and let the protein dissolve in it completely.

8. Purified protein was estimated by Bradford assay.
9. Then the purified protein samples of leprosy and healthy individual were checked in 12% SDS-PAGE.
10. The purified protein bands were observed by staining and de-staining procedure.
11. Clear gel images were observed through gel documentation system.

Troubleshooting

The method of TCA/Acetone was slightly modified to enhance the quality of depletion of high abundant proteins. TCA/Acetone was used to remove high abundant protein accompanied by chloroform, methanol & isopropanol which helped in removal of glycoproteins & lipoproteins. Resolution of purified plasma proteins was clearer as compared to that of un-purified plasma which establishes the fact that the modified method is usable in purifying plasma protein.

Time Taken

20 hours

Anticipated Results

Wells 1,2,3 (Figure 1) show the depletion of highly abundant proteins in leprosy patients. Wells 4,5,6 (Figure 1) show depletion of highly abundant proteins from healthy individuals.

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Acknowledgements

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Figures

Fig. 1 Visualization of gel in Gel Doc

- In wells 1, 2 & 3, leprosy patient purified plasma was loaded.
- In wells 4, 5 & 6, healthy control purified plasma was loaded.
- In well 7, leprosy patient unpurified plasma was loaded.
- In well 8, healthy control unpurified plasma was loaded.
- In wells 9 & 10, BSA (ladder) was loaded.

Figure 1

Visualization of gel in Gel Doc.