Use of Tri-Chloroacetic Acid /Acetone/polyethylene glycol for protein purification from HIV-1 infected human plasma

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Method Article

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Abstract

Human plasma contains high amount of abundant proteins like albumin and globulin. Normally, the proteins having potential for biomarkers are present in very low concentration in human plasma. To resolve the low concentration proteins in polyacrylamide gel, the removal of high abundant proteins from plasma are very essential. Polyethylene glycol is a nontoxic, water soluble synthetic polymer has several applications in chemical and biomedical industries. Various molecular variants of poly ethylene glycol is available and used in protein purification. The mechanism behind the use of high concentration of polyethylene glycol is it binds the molecule in more compact or interpenetrates forming a gel like network surrounding the molecule. Polyethylene glycol -6000 removes the high abundant proteins like Albumin and Globulin in the HIV -1 infected plasma samples and concentrates the low molecular weight proteins as the low molecular weight proteins are essential in biomarker study.

Introduction

Human blood plasma contains the several numbers of proteins which could be useful in biomarker discovery [1]. Several techniques for separation of human plasma proteins such as salting out, ion exchange chromatography, ethanol fractionation , TCA/ Acetone precipitation are being carried in different laboratories. Human plasma derived drugs separation was also achieved by polyethylene glycol [2].Polyethylene glycol has the significant role in refolding of active proteins[3]. Polyethylene glycol and ion exchange chromatography method was used for purification of haptoglobin from human plasma fraction that scaled up the proteins in a simple, low cost and fast manner[4].Polyethylene glycol was already used in biopharmaceutical industry as it enhance the pharmacologic and pharmaceutical properties of proteins[5].Low abundance plasma proteins was obtained by use of polyethylene glycol and immunoaffinity depletion[6]. After 50% TCA/Acetone precipitation with the HIV-1 infected human plasma proteins , several concentration of polyethylene glycol was used . The 16% polyethylene glycol was found to precipitate and purify the low abundance proteins as which can be resolved in 10% SDSPAGE.

Reagents

1. Tri-chloroacetic acid(Sigma Aldrich, USA)
2. Acetone(Merck,India)
3. Polyethylene Glycol-6000(Merck, India)
4. Urea (Sigma Aldrich, USA)

5. Thio Urea (Sigma –Aldrich, USA)
6. CHAPS(Sigma-Aldrich, USA)
7. ASB-14(Merck, India)
8. Dithithretol(Sigma –Aldrich,USA)
9. Bromophenol blue(Merck, India)
10. H2O (HPLC purified ,Merck, India)
11. Acrylamide(Sigma –Aldrich, USA)
12. Bis-acrylamide(Sigma-Aldrich, USA)
13. Tris base(SRL,India)
14. Hydrochloric acid(Merck, India, Cat. No:1.09063.1000)
15. Sodium dodecyl sulfate(Himedia, India)
16. Ammonium per sulfate(Sigma-Aldrich, USA)
17. TEMED(Sigma-Aldrich, USA)
18. Glycerol(Merck,India, Cat No.56-81-5)
19. β-Mercaptoethanol(Sigma-Aldrich, USA)
20. Coomassiae brilliant blue-R250(Merck, India)
21. Acetic acid(Merck, India, Cat No. 64-19-7)
22. Methanol(Merck,India, Cat No.67-56-1)

Equipment

1. -20 0Refrigirator (Krispcold, India)

2. Centrifuge tube(Sigma G 3-18K)

Procedure

TCA/Acetone/PEG-6000 method for plasma protein purification:

1. 100µl plasma was taken in a tube and 50% TCA/Acetone was added to it and incubated for overnight.

2. Then the tube was centrifuged at 12000 RCF for 10 minutes and the supernatant was discarded.

3. Then the 1ml of chilled acetone was added and kept it for 10 minutes at -20^0C

4. Then the tube was centrifuged at above same speed and the supernatant was discarded.

5. Poly ethylene glycol -6000(48%) stock solution was prepared.

6. Various concentrations of 4% to 32% PEG -6000 was added to the samples and kept it for 1 hour in ice. Then the tubes were centrifuged at 12000 RPM for 10 minutes and the supernatant were discarded.

7. The pellets of each tube were washed with acetone twice at same speed. Then air dried the pellet and 150µl rehydration buffer was added in each tube.

8. Then 3K Amicon ultra -4 device was centrifuged at 4000g for 2hrs to remove the salt impurities in the samples and make to narrow volume up to 50µl.Then the samples were measured using the Bradford assay.

9. Then the protein sample was resolved in 10% SDS PAGE and 16% PEG -6000 was found to be better in plasma protein purification.

Troubleshooting

During the protein purification from human plasma samples, the pipetting should be in proper and care to remove the polyethylene glycol-6000 after centrifugation, otherwise the pellet will be disturbed.

Time Taken

The total time taken to complete this protocol would be 16 hours.

Anticipated Results

The polyethylene glycol interpenetrates in to the molecule and formed the gel like solution around the molecule of interest. Different molecular weight of the polyethylene glycol was used to purify different kilodalton of protein molecules. Different concentration (4% to 32%) of polyethylene glycol -6000 was used to purify the proteins from HIV-1 infected human plasma samples. Then the 16% polyethylene glycol-6000 was found to better for purification of HIV-1 infected human plasma samples as it was resolved in 10% SDS PAGE. This protocol is useful for human plasma samples.

References

1. Beck, H. C., Overgaard, M. &Melholt Rasmussen, L. Plasma proteomics to identify biomarkers - Application to cardiovascular diseases. *Transl. Proteomics***7,** 40–48 (2015).

2. MousaviHosseini, K., Pourmokhtar, M., HabibiRoudkenar, M. &Shahabi, M. Human Plasma Derived Drugs Separation by Fractionation of Plasmawith Polyethylene Glycol. *Iran. J. Biotechnol.***12,** 82–85 (2014).

3. Cleland, J. L. *et al.* Polyethylene glycol enhanced protein refolding. *Bio/Technology***10,** 1013–1019 (1992).

4. Sun, L. *et al.*A simple and rapid procedure for purification of haptoglobin from human plasma fraction IV.*Artif.Cells, Blood Substitutes, Biotechnol.***39,** 79–86 (2011).

5. Turecek, P. L., Bossard, M. J., Schoetens, F. &Ivens, I. A. PEGylation of Biopharmaceuticals: A Review of Chemistry and Nonclinical Safety Information of Approved Drugs. *J. Pharm. Sci.***105,** 460–475 (2016).

6. Liu, Z. *et al.* Enhanced detection of low-abundance human plasma proteins by integrating polyethylene glycol fractionation and immunoaffinity depletion. *PLoS One***11,** 1–17 (2016).

Acknowledgements

Ethics approval: This study of HIV drug resistance was approved by the institutional ethics committee.

All biosafty measures for handling HIV samples were followed as per the guidelines laid by Department of AIDS Control, India (http://naco.gov.in/operationalguidelines).

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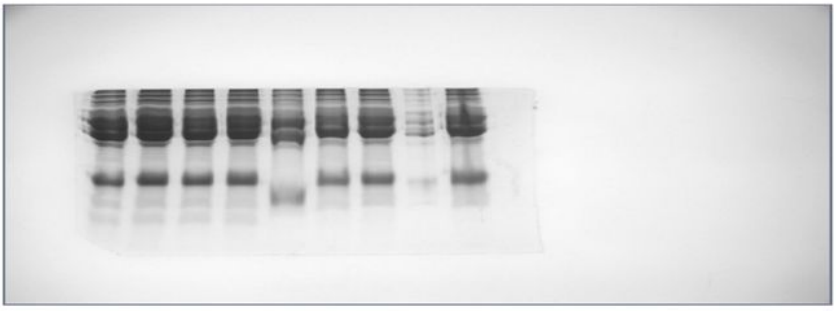
Figures

Figure-1



(10% SDS PAGE, Lane-1 4% PEG, Lane-2 8% PEG,Lane-3 12% PEG, Lane-4 16% PEG, Lane-5 20% PEG, Lane-6, 24% PEG, Lane-7, 28% PEG. Lane -8, 32% PEG, Lane-9, 16% PEG).

Figure 1

(10% SDS PAGE, Lane-1 4% PEG, Lane-2 8% PEG,Lane-3 12% PEG, Lane-4 16% PEG, Lane-5 20% PEG, Lane-6, 24% PEG, Lane-7, 28% PEG. Lane -8, 32% PEG, Lane-9, 16% PEG).

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