Purification of Antigen 85 Complex of *Mycobacterium tuberculosis*

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[Abstract] Serodiagnosis of tuberculosis using purified native antigens is one of the approaches for the early detection of TB. The Antigen 85 complex consisting of Ag 85A, 85B and 85C are the most abundant antigens in the culture supernatant of mycobacteria. It has also been shown to be the immunodominant (the property of an antigenic determinant that causes it to be responsible for the major immune response in a host) antigens of CFA (culture filtrate antigens – proteins secreted by mycobacteria into the culture medium). This protocol gives the details for purification of the Ag85 complex from CFA and also the method of isolation of the individual components of the complex by column chromatography.

Materials and Reagents

1. NaH_2PO_4
2. EDTA
3. DTT
4. Tris
5. NaCl
6. Glycine
7. Ethylene glycol
8. HCl
9. QAE Sepharose Column Starting buffer (see Recipes)
10. QAE Sepharose Column Elution buffer (see Recipes)
11. Buffer A (see Recipes)
12. Buffer B (see Recipes)
13. Buffer C (see Recipes)

Equipment

1. High Performance Liquid Chromatography (HPLC) system (Millenium V2.00, Waters, USA)

2. SDS-PAGE (Mini-PROTEAN® Tetra Cell, catalog number: 165-8000)
3. Phenyl Sepharose HP column (3.5 x 1 cm) (GE Healthcare, catalog number: 17-1082-01)
4. QAE sepharose column (Amersham Pharmacia - anion exchange chromatography)

Procedure

A. Purification of 30 kDa antigen:

The secreted Antigen 85 complex of mycobacteria consists of three proteins Ag85A, B and C. Among these Antigen 85B otherwise known as 30 kDa antigen is one of the major immunogenic antigen.

The 30 kDa antigen was purified from the whole culture filtrate of *M. tuberculosis* by first passing through QAE column followed by passing the 30 kDa enriched fraction through a Phenyl Sepharose hydrophobic interaction column.

1. Anion exchange chromatography-QAE sepharose (purification of Ag85 complex)
   a. Culture filtrate antigen (CFA) between 10 to 30 mg was prepared as mentioned in the 38 kDa antigen purification protocol (Hyperlink here) was separated into multiple fractions by passing through a prepacked anion exchange column of QAE Sepharose. 0.05 M Tris-HCl buffer (pH 8.1) was used as the starting buffer and 0.05 M Tris- HCl (pH 8.1), with 1.0 M sodium chloride was used as the elution buffer. The separation was carried out in the High Performance Liquid Chromatography (HPLC, Millenium V2.00, Waters, USA) system under standard running conditions. The eluted fractions were analyzed on 12.5% SDS-PAGE which separates proteins based on its molecular weight, was run under standard conditions (120 V, 1.5 A). The Ag85 complex is identified as a 30/31 kDa doublet band on the gel. The fractions containing Antigen 85 complex were pooled and concentrated by Amicon centrifuge filters with the molecular cut off of 3 kDa.
   b. Further purification of the A, B and C components from the antigen 85 complex was done by passing it through the hydrophobic interaction chromatography (HIC) column namely the Phenyl Sepharose HP column.
2. Hydrophobic interaction chromatography-phenyl sepharose (separation of the individual components of the antigen 85 complex)
   a. Purification of the antigen 85 complex into its individual components Ag 85A; 85B and 85C was done by passing it through the Phenyl Sepharose HP column using the Biologic Chromatography system (FPLC, Bio-Rad, USA). Three different buffers were used during the run. The buffers A, B, C used are mentioned under recipes section.
   b. The run conditions were such that the column was subjected to an isocratic flow of Buffer A for 10 min (flow rate 1 ml/min), followed by the injection of the sample (2 ml)

which was dialysed against buffer A at the rate of 0.5 ml/min. Then an isocratic flow of Buffer B for 10 min at the rate of 1 ml/min was used for washing the unbound material.

c. Elution of the individual components of Antigen 85 complex namely Ag85A, B and C were carried out with a linear gradient of Buffer C (from 0 -100%) for 30 min with the flow rate of 1 ml/ min. Subsequently, the protein containing fractions were identified by optical density measured at 280 nm on a spectrophotometer. The fractions containing Ag85B were pooled, concentrated and checked on 12.5% SDS PAGE for purity.

Recipes

1. QAE Sepharose Column Starting buffer
   0.05 M Tris-HCl (pH 8.1)
2. QAE Sepharose Column Elution buffer
   0.05 M Tris-HCl (pH 8.1)
   1 M NaCl
3. Buffer A
   0.01 M NaH_2PO_4
   1 mM EDTA
   1 mM DTT (pH 6.8)
4. Buffer B
   0.01 M Tris
   100 mM Glycine
   1 mM EDTA
   1 mM OTT (pH 8.9)
5. Buffer C
   0.01 M Tris
   100 mM Glycine
   1 mM DTT
   50% (v/v) Ethylene Glycol (pH 8.9)

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References

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