ISOLATION OF MYCOBACTERIUM TUBERCULOSIS 31 kDa ANTIGEN PROTEIN OF DIAGNOSTIC INTEREST FROM CULTURE FILTRATE USING ANTI- ES-31 ANTIBODY BY AFFINITY CHROMATOGRAPHY

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ABSTRACT

Proteins secreted into the culture medium by *Mycobacterium tuberculosis* (M. tb) are shown to be source of antigens of immunodiagnostic interest. An *in vitro* released 31 kDa antigen ESAS-7F isolated from M.tb H₃₇Ra culture filtrate by salt precipitation, SDS-PAGE and cation exchange fast protein liquid chromatography (FPLC) was shown earlier to be a diagnostically important antigen fraction. In this report, we describe the isolation of ESAS-7F antigen using monospecific antibody coupled to sepharose CL-4B column. The percentage recovery of ESAS-7F antigen using affinity chromatography was approximately 8% of the total ES antigen proteins compared to 0.05% obtained by conventional purification steps using salt precipitation, SDS-PAGE and FPLC. Similar seroreactivity was observed by the antigen isolated by both the methods in indirect ELISA. Affinity chromatography helped in an increased recovery of ESAS-7F antigen and obviates the need for time consuming conventional purification steps.

KEY WORDS : Affinity chromatography, ESAS-7F, ELISA

INTRODUCTION

Tuberculosis is an ancient scourge of mankind that continues to be an important health problem worldwide (1). It was until recently perceived as a well controlled disease but reemerged back with vengeance. Immunodiagnosis of tuberculosis using the excretory-secretory culture filtrate antigens of *M. tuberculosis* and polyclonal antibodies have been well documented (2). All these tests have reinforced the belief that antigen and/ or antibody detection can be useful in the serodiagnosis of tuberculosis. Nevertheless, it has been felt for a long time that identification, isolation and use of strain specific antigens would provide the necessary sensitivity as well as specificity (3,4). Mycobacteria contain complex antigenic structures

and a variety of immunogenic components which need purification before application to immunodiagnostic test.

The ESAS-7F (ES-31) antigen isolated by physicochemical methods involving salt precipitation, SDS-PAGE and cation exchange FPLC from culture filtrate of *M. tuberculosis* H₃₇Ra has been earlier shown by us to be an immunodiagnostically useful *in vitro* released 31 kDa excretory-secretory antigen (submitted for publication). We have also identified this *in vitro* released antigen with *in vivo* released circulating antigen isolated from bacteriologically confirmed tuberculosis sera (5).

Though the isolated ES-31 antigen was found to be diagnostically useful, but the isolation procedure is cumbersome and the yield is low. Affinity chromatographic technique helps in the rapid separation compared to time consuming and difficult conventional techniques of purification. Accordingly the present study had two objectives (1) Isolating affinity purified antibodies to specific ES-31 antigen for use

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in constructing the antibody column (2) Isolating ES-31 antigen from affinity purified goat anti ES-31 antibody coupled sepharose-4B column using affinity chromatography.

MATERIALS AND METHODS

Sera

Blood samples were collected from cases of pulmonary tuberculosis and non-tuberculosis disease controls. Normal blood samples were collected from healthy donors without any evidence of tuberculosis infection. Sera were separated and stored at -20°C with Sodium azide as a preservative.

Mycobacterium tuberculosis H₃₇Ra excretory-secretory (*M.tb* ES) protein

Mycobacterium tuberculosis H₃₇Ra excretory-secretory (ES) antigen was prepared by *in vitro* culture of the bacilli in thyroxine supplemented Sauton medium and *M.tb* ES antigen was purified using 50% ammonium sulphate precipitation followed by SDS-PAGE to obtain an active antigen fraction ESAS-7 (5,6). ESAS-7 antigen was further purified using cation-exchange FPLC to isolate a highly seroreactive 31 kDa antigen protein ESAS-7F (submitted for publication).

Affinity purified goat *M.tb* ES-31 antibody

Polyclonal antibodies to *M.tb* ESAS-7 antigen were raised in goat and specific antibodies against ES-31 antigen were isolated from immune sera using ES-31 antigen coupled CNBr-activated sepharose-4B

column (Pharmacia) as described earlier (6).

Affinity purified *M.tb* ES-31 antigen protein

Affinity purified *M.tb* ES-31 antigen protein was isolated from *M.tb* crude culture ES antigen by affinity chromatography using affinity purified anti-ES-31 antibody coupled sepharose-4B column. Briefly 4 mg of affinity purified anti-ES-31 antibody was coupled to sepharose-4B column as described earlier (6) and *M.tb* culture filtrate ES antigen (4mg) was applied to the column and washed with 0.01 M PBS. Bound antigen (ES-31) was eluted with glycine-HCl buffer (pH 2.5) and neutralized with Tris-HCl buffer (PH 8.6). Antigenic activity in the recovered ES-31 protein was tested by indirect ELISA.

RESULTS

0.32 mg (8%) of ES-31 antigen protein was recovered from culture filtrate containing 4 mg ES antigen proteins, by affinity chromatography using affinity purified goat anti- ES-31 antibody coupled column. In the comparative yield study of ES-31 antigen, the percentage recovery of ES-31 antigen using salt precipitation, SDS-PAGE and FPLC was only 0.05% (Table 1). ES-31 antigen purified by either process was useful to detect tuberculous antibodies in 9 of 10 bacteriologically confirmed (S+C+) pulmonary tuberculosis cases by stick indirect ELISA (Table 2).

DISCUSSION

Even after the development of highly specific

Table 1. Amount of *M.tb* ES-31 antigen protein isolated from affinity purified goat anti-ES-31 antibody coupled Sepharose-4B using affinity chromatography and physicochemical methods using salt precipitation, SDS-PAGE and cation exchange FPLC.

Source (Protein applied to the column)	Total protein applied (mg)	Protein recovery (mg) of ES-31 antigen protein		% recovery of ES-31 antigen	
		Salt precipitation, SDS-PAGE & FPLC	Affinity Chromatography	Salt precipitation, SDS-PAGE & FPLC	Affinity Chromatography
ES	4	.002	.320	.05	8

Values represented are mean $\pm$ SD of two sets of experiments.

Table 2. Comparative seroreactivity of ES-31 antigen purified by SDS-PAGE followed by FPLC and affinity chromatography using stick indirect penicillinase ELISA

Sera group	Total no. screened	No. (%) showing positive reaction *with ES-31 antigen isolated by	
		Salt precipitation, SDS-PAGE & FPLC	Affinity Chromatography
Pulmonary TB (S+C+)	10	9 (90)	9 (90)
Healthy control	12	1 (8)	1 (8)
Disease control	12	1 (8)	1 (8)
PUO	02	1	1
Pleural effusion	04	-	-
Chronic bronchitis	02	-	-
Pneumonia	04	-	-

* The sera dilution at 1:600 was taken as the threshold level for positivity.

immunodiagnostic assay, their widespread clinical use is hampered by non-availability of immunologic reagents that relatively are simpler to prepare and could be available in ample amounts. Affinity chromatographic procedures have been used by several workers to obtain semipurified or purified antigens from the complex antigenic mixtures.

Earlier studies from our laboratory reported a 31 kDa glycoprotein antigen (ESAS-7F) of immunodiagnostic potential isolated from *M.tb* H₃₇Ra culture filtrate using SDS-PAGE and FPLC fractionation (submitted for publication). Though *M.tb* ESAS-7F was found to be promising in the diagnosis of pulmonary tuberculosis, the yield of this purified protein using the conventional techniques were low. Hence the present study was aimed at isolation of affinity purified antibodies against ES-31 antigen and purification of ES-31 antigen by affinity chromatography on ES-31 antibody coupled sepharose-4B column.

Using affinity chromatography on an affinity purified goat anti ES-31 antibody coupled sepharose-4B column and culture filtrate antigen (ES) as source material, gave a higher percentage recovery (8%) of

ES-31 antigen compared to the recovery (0.05%) by physiochemical methods involving salt precipitation, SDS-PAGE and cation-exchange FPLC. In indirect ELISA, ES-31 antigen isolated by affinity chromatography showed similar reactivity with tuberculosis sera when compared to that of ES-31 antigen isolated by salt precipitation, SDS-PAGE and cation-exchange FPLC (Table 2).

Thus in the present study affinity chromatography helped in an increased recovery of ES-31 antigen and obviated the need for time consuming multi-step physiochemical purification.

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