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ORIGINAL ARTICLE

Cloning, expression and immunological evaluation of a short fragment from *Rv3391* of *Mycobacterium tuberculosis*

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Abstract Tuberculosis (TB) is still one of the greatest health care problems in the world. In order to identify antigens that may be used in the serodiagnosis of active tuberculosis, a short fragment from *Rv3391* (*fRv3391*) of *Mycobacterium tuberculosis* was cloned and expressed. Its molecular weight and secondary structure elements were identified by mass spectrometry and circular dichroism. And its immunological nature was also evaluated by enzyme-linked immunosorbent assay (ELISA). The fRv3391 was expressed in *Escherichia coli* BL21 (DE3) with the molecular weight of 42.5 kDa and the secondary structure elements 36.2% α-helix, 0.0% β-sheet, 32.6% β-turn, and 31.3% random coil. Evaluation of fRv3391 as an ELISA solid-phase antigen on a set of human sera from well-characterized TB cases and healthy subjects revealed that there was strong serum antibody reactivity to fRv3391 in many human TB patients. Taken together, a short fragment from *Rv3391* of *Mycobacterium tuberculosis* was cloned and expressed and the ELISA results showed that the protein may be useful as an immunodominant antigen for the serodiagnosis of active TB.

Keywords *Rv3391* · *Mycobacterium tuberculosis* · Serodiagnosis · Antigen · Gene expression · Circular dichroism

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Introduction

Tuberculosis (TB) is still one of the greatest health care problems in the world. In 2008, there were an estimated 8.9–9.9 million incident cases and 9.6–13.3 million prevalent cases of TB, and approximately 95% of the cases were found in developing countries (WHO 2009). For effective control of TB, it is critical to identify infected individuals and screen their immediate contacts so that drug treatment can be administered quickly. Thus, early detection of *Mycobacterium tuberculosis* becomes more and more important in the control of tuberculosis both for the clinical treatment of infected individuals and for the identification of exposed individuals (Chakraborty et al. 2009). Other than several diagnostic techniques such as nucleic acid amplification (De Beenhouwer et al. 1995; Somoskovi et al. 2006), and immune reactions based on the cell-mediated immune response (Okkels et al. 2003; Van Pinxteren et al. 2000), interest in developing serodiagnostic methods is increasing

because detection of antibodies is rapid, simple, relatively inexpensive, and does not require a living cell.

During the past few years, advances in molecular techniques, such as whole genome sequencing, cloning, expression and purification of proteins, have accelerated the identification of novel antigens for the serodiagnosis of TB, and a number of novel antigens have been tested individually or in combinations to obtain the desired sensitivity and specificity (Davidow et al. 2005; Hendrickson et al. 2000; Rosenkrands et al. 2008; Silva et al. 2003). Our previous study has reported a novel multi-antigen ELISA with both good sensitivity (61.8%) and specificity (93.0%), which was based on the newly-found antigen Rv3425 and two other well-known immunodominant antigens (Zhang et al. 2009). Thus, in order to identify other antigens that may be useful in the serodiagnosis of active TB, we analyzed the protein sequences of *M. tuberculosis* H_{37}Rv by means of bioinformatics and finally found some with potential diagnostic value including a short fragment from Rv3391 (fRv3391).

In order to get an insight into fRv3391 and prepare enough evidence for the succeeding diagnostic research, a study on cloning and expression of fRv3391 is required. In this study, we successfully expressed the recombinant *M. tuberculosis* H_{37}Rv fRv3391 with a 6× His-tag (His-fRv3391) in *Escherichia coli*. The recombinant fRv3391 protein was purified by nickel-nitrilotriacetic acid (Ni-NTA) His-binding resin affinity chromatography and Sephadex G-75 column chromatography, and then its second structural properties were analyzed through circular dichroism (CD) measurements. We also evaluated the immunological nature of *M. tuberculosis* fRv3391 by ELISA using sera from patients with TB and healthy control subjects.

Materials and methods

All the chemicals were bought from Sigma-Aldrich (St Louis, USA) except when otherwise noted. All polymerases, restriction endonucleases, and ligase were provided by New England Biolabs (Beijing, China). The pET32a vector was purchased from Novagen (Darmstadt, Germany). Bacteria DNA mini kit, gel extraction mini kit, and plasmid mini kit were obtained from Watson Biotechnologies (Shanghai, China).

Serum samples and patients

A total of serum samples (n=106) from HIV-seronegative individuals were studied. They were all collected from Linyi Chest Hospital, Shandong, China. Fifty-six serum samples were patients (age range, 10–70 years) with active pulmonary TB. Of the 56 active pulmonary TB patients, 30 cases were smear-positive for acid-fast bacilli (AFB) and culture positive, and 26 cases were smear-negative culture positive. All the patients had not yet started anti-tuberculosis chemotherapy when the serum samples were taken. In this study, active TB patients were diagnosed by the isolation and identification of *M. tuberculosis*, as well as clinical and radiological findings. Mycobacterial isolates were obtained as Lowenstein-Jensen cultures, and were identified to the species level by biochemical procedures (Metchock et al. 1999), and further confirmed by genotyping based on the 16 S-23 S rRNA gene internal transcribed spacer sequence (Zhang et al. 2007). Fifty serum samples were obtained from healthy control subjects (age range, 16–52 years), they had not TB previously, and had negative chest X-rays and negative sputum culture results for *M. tuberculosis*. Thirty-one of these had previously been vaccinated with *Mycobacterium bovis* BCG, 21 were PPD positive, and 29 were PPD negative at the time the serum samples were taken. Sera were stored at −20°C.

Bacterial strains and culture conditions

E. coli strains DH5α and BL21 (DE3) were propagated at 37°C in Luria Bertani (LB) medium or on LB agar plate containing 25 μg/ml kanamycin. *M. tuberculosis* H_{37}Rv strain supplied by Henan Chest Hospital was cultivated at 37°C in Middlebrook 7 H9 medium supplemented with 2% glycerol and 10% (v/v) oleic acid–albumin–dextrose complex.

Bioinformatics

The antigenicity prediction of Rv3391 protein sequences was achieved using both methods of Jameson and Wolf (1988) (DNASTAR, Madison, Wisconsin, USA) and Kolaskar and Tongaonkar (1990) (http://tools.immuneepitope.org/tools/bcell/iedb_input). The selected sequence of *M. tuberculosis* H_{37}Rv was also compared to the corresponding sequences of other mycobacterium and non-mycobacterium species with ClUSTALX, such as *M. tuberculosis* CDC1551, *M. tuberculosis* F11, *M. tuberculosis* KZN 1435, *M. bovis*, *Mycobacterium ulcerans*, *Mycobacterium avium*, *Mycobacterium smegmatis*, *Mycobacterium abscessus*, *Mycobacterium gilvum* and *Mycobacterium vanvaalenii*.

Construction of the fusion expression plasmid pET32a-*fRv3391*

The gene fragment of Rv3391 (GenBank identifier (GI): 15610527) was obtained by PCR with genomic DNA of *M. tuberculosis* H_{37}Rv strains as template. The sequences of primers were as follows: forward primer: 5′-CATG CCATGG (*Nco* I) GGTTTATCGGGCGCCACG-3′; reverse

primer: 5′-AGCTCGAG (*Xho* I) CTAGGCGTGCATGA GCGCCACC-3′. The amplicon comprising *fRv3391* was cloned at the *Nco* I and *Xho* I sites of the cloning vector pET32a. The conditions for the PCR were 1 cycle at 94°C for 5 min, followed by 30 cycles at 94°C for 45 s, 62°C for 45 s, 72°C for 45 s and then 72°C for 10 min, using the forward primer and the reverse primer.

Both the plasmid pET32a and the PCR product were digested with *Xho* I and *Nco* I restriction endonucleases, purified with gel extraction mini kit, and then ligated together using T4 DNA ligase overnight at 16°C. Then the recombinant plasmid, named pET32a-*fRv3391*, was transformed into *E. coli* strain DH5α. The strain carrying the recombinant plasmid was selected and the sequence of the target gene was verified by DNA sequencing (Sangon Sequencing Facility, Shanghai, China).

Expression and purification of His-fRv3391

After being propagated in *E. coli* strain DH5α, the pET32a-*fRv3391* plasmid was extracted and then transformed into *E. coli* strain BL21 (DE3) for His-fRv3391 protein expression. Transformed cells were cultivated at 37°C in LB medium (containing 25 μg/ml kanamycin) until the optical density at 600 nm (OD_{600}) reached 0.4–0.5. Expression of His-Rv3391 protein was induced by addition of 1.0 mM isopropyl-d-thiogalactopyranoside and incubation was continued for another 2 h at 37°C. The induced BL21 cells were collected by centrifugation at 10,000 *g* for 20 min at 4°C. And the molecular weight of His-Rv3391 was preliminarily estimated by Coomassie brilliant blue R250-stained sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS–PAGE) compared with the molecular weight standards.

The bacterial pellet collected from 1,000 ml culture was resuspended at 50 ml binding buffer (300 mM NaCl, 50 mM NaH_2PO_4, and 10 mM imidazole, pH 8.0). The cells were then broken by sonication on ice at the intensity of 5 s burst at 500 W with a 5 s cooling period between each burst. The lysate was centrifuged at 12,000 *g* for 60 min at 4°C to discard the bacteria debris and insoluble materials. The supernatant containing soluble cellular materials was gently collected and used for subsequent purification. His-fRv3391 protein was purified by Ni-NTA His-binding resin affinity chromatography according to the manufacturer's protocol (Bio-Rad). Briefly, the column containing Ni-NTA affinity resin was pre-equilibrated with binding buffer. Then, the supernatant was loaded into the column and washed with buffers containing 50, 100, and 250 mM imidazole, consecutively, and the target protein was eluted with buffer containing 250 mM imidazole. The homogeneity of purified protein was preliminarily analyzed by SDS–PAGE. Then, the fractions containing His-fRv3391 antigen were pooled and concentrated to approximately 5 ml by centrifugation at 5,000 *g* at 4°C (Millipore, Bedford, USA) and loaded on a 15-ml Sephadex G-75 column (XK 1/20) equilibrated in PBS. The column was developed with PBS at a flow rate of 0.2 ml/min. The elution was monitored at 280 nm and 2.0-ml fractions were collected. The fractions were analyzed by SDS–PAGE and fractions containing His-fRv3391 were pooled. At last, the purified His-Rv3391 protein was concentrated by centrifugation at 5,000 *g* for 2 times at 4°C using the Millipore-Amicon-Ultra column (Millipore) and lyophilized. Protein concentration was determined by the Bradford method (Bradford 1976) using bovine serum albumin (BSA) as the protein standard.

Mass spectrometry and circular dichroism measurement

To determine the accurate molecular weight of the recombinant His-fRv3391, the His-fRv3391 protein sample was analyzed by electronspray ionization time-of-flight (ESI-TOF) on an Agilent TOF mass spectrometer 6220 (Angilent, USA). Data were collected separately in positive and negative ion electrospray mode in full scan mode from 300 to 2,000 m/z. The capillary cottage was 4,000 V, and the tragmentor voltage was 120 V, with a scan rate of 0.10 scan/s; nebulizer pressure was 20 psig, with drying gas at 6 L/min. The parameters were identical for positive, except for the polarity.

To analyze the secondary structure elements of His-fRv3391, far-UV CD spectroscopic studies were deployed using a JASCO J-715 spectropolarimeter (Jasco, Tokyo, Japan) installed with RTE bath/circulator (NESLAB RTE-111; NESLAB, Tokyo, Japan). The tested His-fRv3391 protein samples with the concentration 120 μg/ml (in water, pH 7.0) were prepared by incubating for 10 min at temperature 20°C before use. After an N_2 purge for about 30 min, the spectra were recorded in the wavelength range from 190 to 250 nm. The shown spectra were the average of five scans at the temperature, and the reported results were smoothed and corrected by buffer control. In this study, the secondary structure parameters were assessed by the PROSEC program (Yang et al. 1986).

ELISA assay and statistical analysis

The optimal concentration of His-fRv3391, dilution of sera and dilution of labeled antihuman-IgG (rabbit) conjugate for ELISA were determined according to the phalanx titration principle. Combinations which gave the highest signal–noise ratios (S/N) were determined as optimum. And the signal–noise ratios were determined by the ratio of the OD values of the positive control sera to those of the negative control sera at each combination. Polystyrene flat-

bottom microtiter plates (Costar, USA) were coated with the concentration of 2.0 μg/ml His-fRv3391 diluted in 0.05 M carbonate-bicarbonate buffer (pH 9.6). The plates were incubated overnight at 4°C and washed three times with washing buffer (PBST) containing 0.05% Tween-20. After blocking the plates for 2 h at 37°C with 3% BSA in PBST (pH 7.4), the plates were washed again three times and 100 μl of serum (diluted 1:100 in buffer PBST, pH 7.4) were added to each well. All samples were tested in duplicate. The plates were incubated for 1 h at 37°C, washed three times with washing buffer, and filled with 100 μl of a 1:5,000 dilution of anti-human IgG-horseradish peroxidase (Sigma, Poole, UK). Then, the plates were incubated for 1 h at 37°C in a water bath and washed again five times, followed by addition of 100 μl of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution (0.04% TMB, 0.04% urea-peroxide in 0.1 M sodium acetate-citric acid buffer, pH 4.0). After 10-min incubation in the dark at room temperature, the reaction was stopped by the addition of 50 μl of 2 M H_2SO_4 to each well, and the OD_{450} was measured with a microtiter plate reader (Bio-kinetics reader; Bio-tech Instruments, Winooski, USA).

The ELISA results were analyzed by using cut-off value equal to the mean OD plus two standard deviations (SD) from the healthy control group. The mean OD value and standard deviation were calculated using the Statistics Package for Social Science 13.0 (SPSS, Chicago, IL, USA). The evaluation of positive sera for ELISA was based on a positive score derived from the OD values above the cut-off point. Sensitivity was defined as the percentage of individuals in the true-positive group who showed OD values higher than the cut-off value and specificity was defined as the percentage of individuals in the true-negative group who showed ELISA values that were lower than the cut-off value.

Results and discussion

Computer analysis and antigen selection

Both the Jameson–Wolf and Kolaskar–Tongaonkar methods are well used in protein antigenic prediction (Amela et al. 2007; Da Silva et al. 2009; Frikha-Gargouri et al. 2008; Sharma et al. 2009; Sollner et al. 2008). The output of the former algorithm, the antigenic index, is used to create a linear surface contour profile of the protein, and it offers a reliable means of predicting potential antigenic determinants (Jameson and Wolf 1988). On the other hand, application of the latter to a large number of proteins has shown that the method can predict antigenic determinants with about 75% accuracy which is better than most of the other known methods (Kolaskar and Tongaonkar 1990). As shown in Fig. 1, the entire prediction of antigenic propensity for the protein Rv3391 was provided. The fragment of Rv3391 ($aa_{10\text{-}226}$) were selected since it had a high antigenic propensity from prediction by both methods. Then, the sequence of fRv3391 was analyzed to identify whether it was specific to *M. tuberculosis* by comparing the publicly available mycobacterial databases with ClustalX. The result indicated multiple indels (gaps resulting in insertions or deletions) between the *M. tuberculosis* complex and environmental mycobacteria that are reflected in the amino acid sequence alignments (Fig. 2). Sequences analysis also indicated that it has no homogy with other species (data not shown). Therefore, fRv3391 may be a promising candidate antigen for the serodiagnosis of TB.

Expression and purification of His-fRv3391

The short fragment from *M. tuberculosis* H_{37}Rv *Rv3391* gene was cloned into the pET32a vector resulting in the

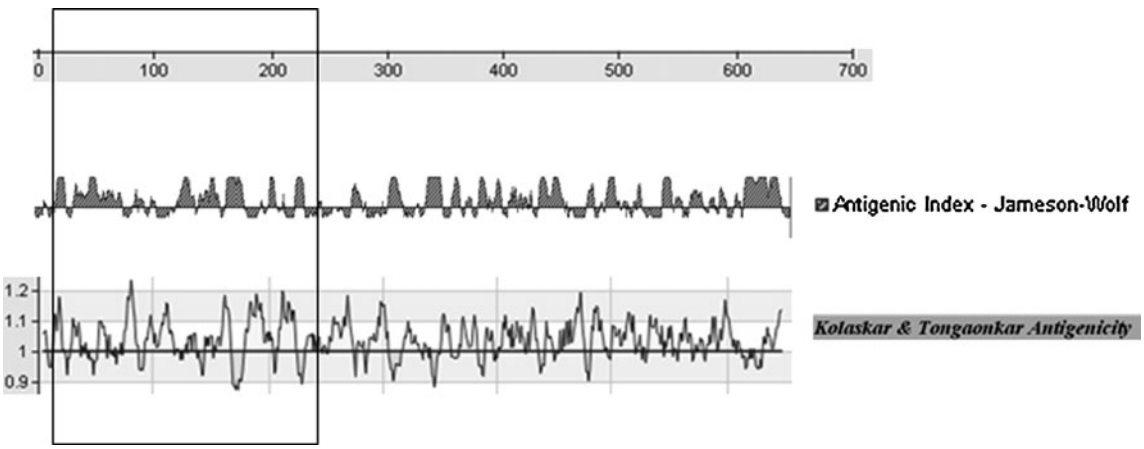

Fig. 1 Comparative Jameson–Wolf and Kolaskar–Tongaonkar antigenic profiling in the prediction of antigenic propensity for the protein Rv3391. Antigenic domains are indicated

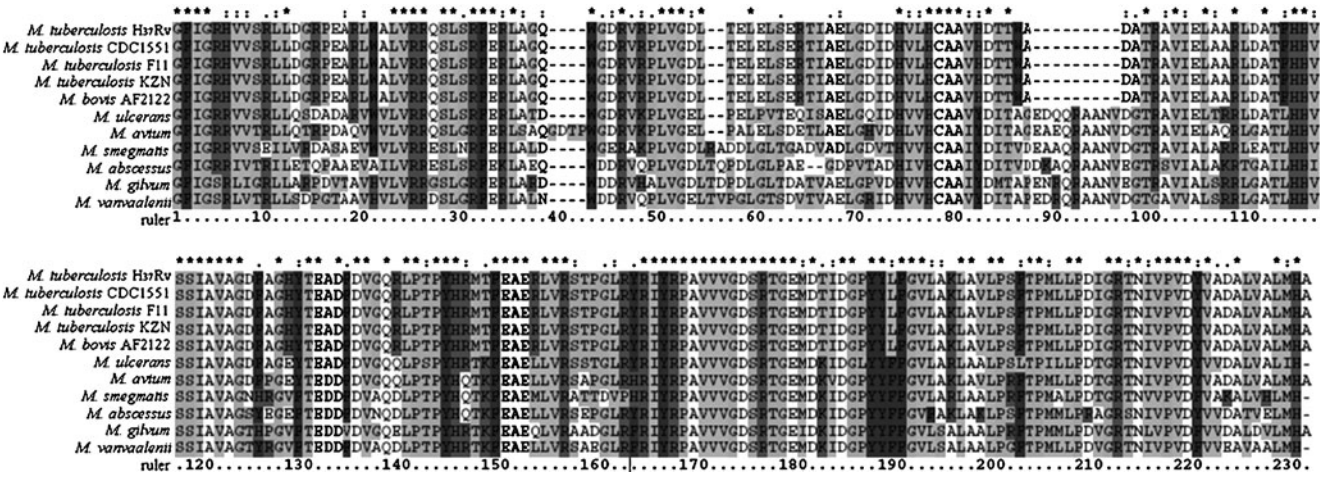

Fig. 2 Amino acid alignments of fRv3391 from different mycobacterium species. Identical amino acids are indicated with *gray shading*

expression of recombinant fRv3391 in fusion with the His-tag (His-fRv3391). To obtain maximum His-fRv3391 protein that is soluble, the expression conditions were optimized by a series of trials and the highest percentage of soluble protein was obtained when the expression of His-fRv3391 protein was induced by 1.0 mM IPTG at 37°C for 2 h. Under the optimal condition, about 35% of the His-fRv3391 was present in soluble fraction analyzed by SDS–PAGE with Coomassie brilliant blue staining (Fig. 3).

It is well known that His-tags are competent tools for purifying recombinant proteins from crude extract, and the immobilized metal affinity chromatography is the commonest method employed to purify recombinant proteins containing a short His tag (Arnau et al. 2006; Loughran et al. 2006). Considering this, Ni-NTA His-binding resin affinity chromatography was employed to purify the soluble recombinant fRv3391 (with His-tag) (Fig. 4a). After washing with buffers containing 50 mM, and 100 mM imidazole successively, the protein was eluted with 250 mM imidazole. SDS–PAGE result (Fig. 4a, lane 1) showed that the His-fRv3391 protein fraction was eluted with 250 mM imidazole, and the band migrated between the 47.5 and 32.5 kDa protein standards, thus the molecular weight was estimated to be 42.5 kDa. The yield of the recombinant protein was assessed as 65 mg from 50 ml crude supernatant (Table 1). We assumed that the other materials were possibly DNA and/or lipids which hydrophobically interacted with the fusion protein and eluted together from the nickel column. In order to obtain homogenous His-fRv3391, we tried several purification protocols (data not shown) and finally confirmed that this recombinant protein could be further purified by Sephadax G-75 column chromatography (Fig. 4b, lane 2), then it was concentrated by ultrafiltration, and about 11.1 and 9.75 mg of the His-fRv3391 protein was obtained, respectively (Table 1).

Molecular weight determination and secondary structure analysis

The accurate molecular weight of the recombinant protein indicated by ESI-MS was 42,510.72 Da (Fig. 5), which was close to the theoretical value, thus providing evidence for both the identity and purity of the recombinant protein.

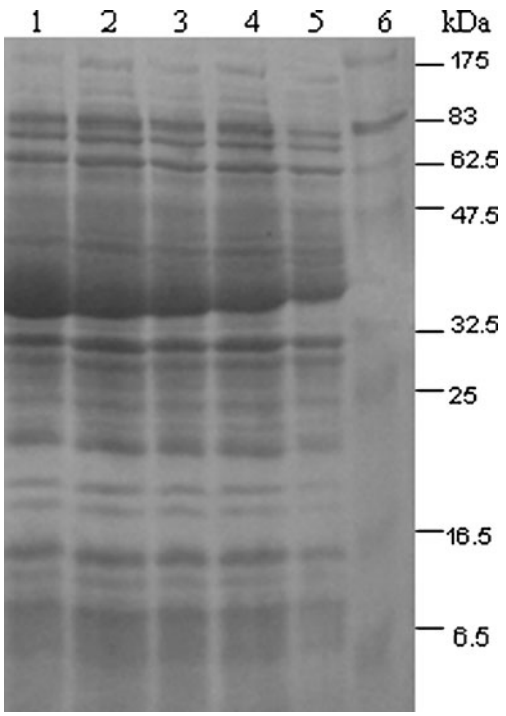

Fig. 3 SDS–PAGE analysis of His-fRv3391 expression. The recombinant protein was expressed in strain BL21 (DE3) of *E. coli*. *Lane 1*, the induced BL21 cells (37°C, 1.0 mM IPTG, 2 h); *lane 2* the induced BL21 cells (37°C, 0.5 mM IPTG, 6 h); *lane 3* the induced BL21 cells (37°C, 0.5 mM IPTG, 4 h); *lane 4* the induced BL21 cells (37°C, 0.5 mM IPTG, 2 h); *lane 5* the non-induced BL21 cells; *lane 6* molecular weight markers (P7708; New England Biolabs, USA). Proteins were visualized with Coomassi brilliant blue staining

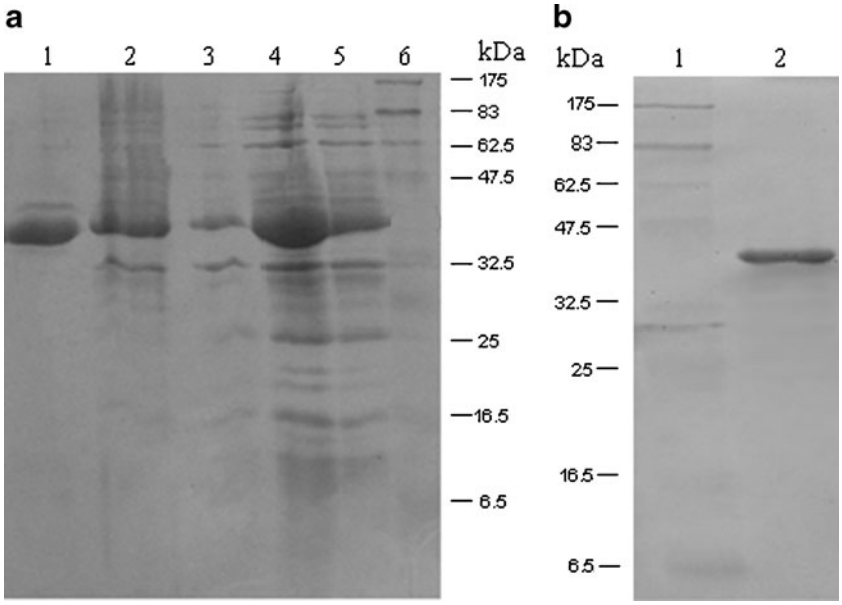

Fig. 4 SDS–PAGE analysis of His-fRv3391 purification. The recombinant protein was purified to homogeneity using the His-bind column protein purification kit (**a**) and Sephadex G-75 column chromatography (**b**). **a** *Lane 1* fraction washed with 250 mM imidazole; *lane 2* fraction washed with 100 mM imidazole; *lane 3* fraction washed with 50 mM imidazole; *lane 4* crude supernatant fraction before addition to the Ni-NTA column; *lane 5* the non-induced BL21 cells; *lane 6* molecular weight markers (P7708; New England Biolabs, USA). **b** *Lane 1* molecular weight markers (P7708; New England Biolabs); *lane 2* purified His-fRv3391 after Sephadex G-75 column chromatography. Proteins were visualized with Coomassi brilliant blue staining

His-fRv3391 protein was also subjected to far-UV CD measurements to obtain its secondary structure. The CD spectrum of the purified His-fRv3391 protein showed a maximum CD (mdeg) value at 196 nm and a minimum at 219 nm at 20°C (Fig. 6). The percentages of calculated secondary structure elements of the His-fRv3391 were 36.2% α-helix, 0.0% β-sheet, 32.6% β-turn, and 31.3% random coil, which is partly in accordance with the results of the prediction of its second structure (http://www.cbs.dtu.dk/services/NetSurfP/).

ELISA assays for IgG antibodies against His-fRv3391

The serum IgG antibody responses to the His-fRv3391 were measured by ELISA in patients with smear-positive, smear-negative TB, and in healthy controls. Of the 30 smear-positive TB patients, serum samples from 46.7% of patients had antibodies to His-fRv3391. In the cohort of 26 smear-negative TB patients, 11.5% cases possessed anti-His-fRv3391 antibodies. With respect to the controls, only three samples from healthy subjects reacted against His-fRv3391, and the specificity estimate for the expressed protein was up to 94.0% (47/50) (Table 2).

Recently, based on the theory of antibody profiles of TB, much attention has been focused on screening multiple antigens or multi-epitopes specific for *M. tuberculosis* to develop novel serodiagnostic tools for TB (Khan et al. 2008; Lee et al. 2008; Shen et al. 2009; Tong et al. 2005). Lee et al (2008) reported that, although the sensitivity and specificity of MTB12 antigen were similar to those of other antigens at 53.0% and 95.4%, the sensitivity increased to 73.0% when the combination of MTB12 and 38 kDa

Table 1 Purification of recombinant His-fRv3391

Steps	Protein concentration (mg/ml)	Volume (ml)	Protein (mg)	Yield (%)
Crude supernatant	1.3	50	65	100
Ni-NTA column	2.1	9	18.8	28.9
Sephadex G-75 chromatography	1.1	10	11.0	16.9
Concentration	3.9	2.5	9.75	15.0

Table 2 Serological reactivity of His-fRv3391 in TB patients and healthy control subjects

	n	OD_{450}	No. of samples positive by ELISA[a] (%)
TB total	56	0.416±0.297	17 (30.3%)
Smear-positive TB	30	0.537±0.338	14 (46.7%)
Smear-negative TB	26	0.277±0.152	3 (11.5%)
Healthy control subjects	50	0.165±0.055	3 (6.0%)

[a] Positive values were greater than the mean OD+2SD of the healthy sera

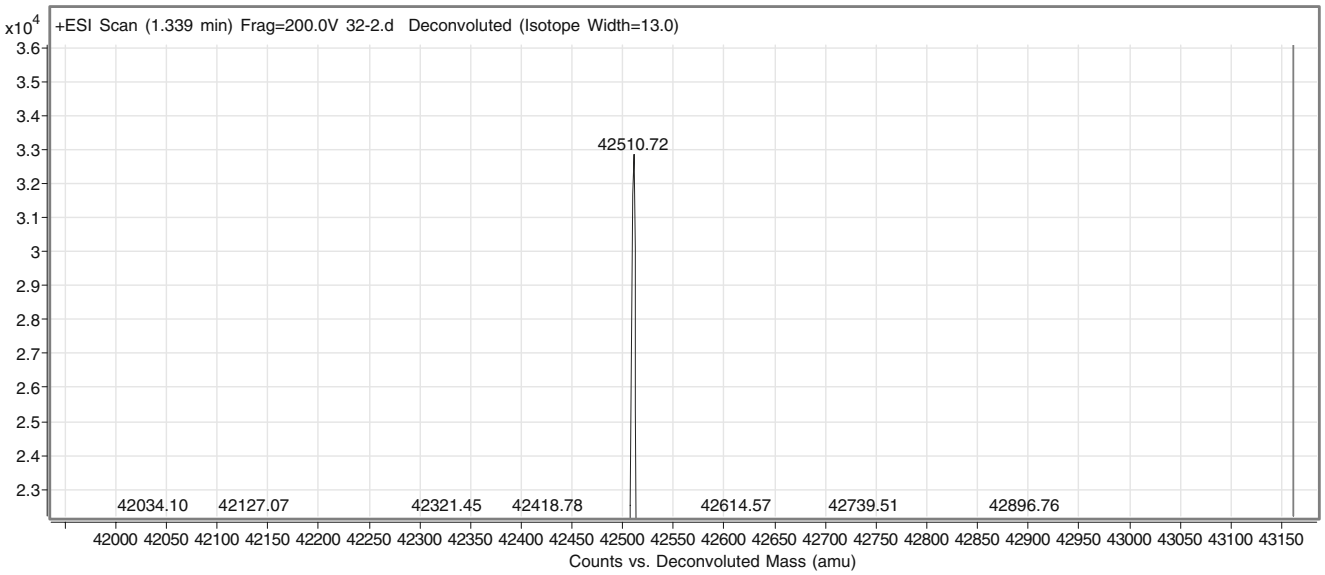

Fig. 5 MS analysis was performed with 6220 Accurate-Mess TOF LC/MS (Agilent, USA). The purified recombinant protein His-fRv3391 only showed a major peak at ~42.5 kDa

antigen was measured. Our previous study also reported that, compared with the sensitivity estimates provided by the individual antigens (Rv3425: 31.7%; 38 kDa: 20.6%; LAM: 35.3%), the total sensitivity achieved by a combination of Rv3425, 38 kDa and LAM antigens increased to 61.8% based on the newly-found antigen Rv3425 (Zhang et al. 2009). Thus, in order to identify other antigens that may be useful in the serodiagnosis of active TB, we analyzed the protein sequences of *M. tuberculosis* $H_{37}Rv$ by means of bioinformatics and finally found some with potential diagnostic value including a short fragment from Rv3391 (fRv3391).

Rv3391 (ArcA1) was first discovered through genome sequencing of *M. tuberculosis* $H_{37}Rv$ (Camus et al. 2002; Cole et al. 1998). One of the large clusters detected in *M. tuberculosis* is unique since it consists of genes that encode metabolic enzymes, such as acrA1, which is involved in the biosynthesis of mycolic acids (Yuan et al. 1995). It is well known that the mycolic acids are unique, 60–90 carbons long, branched α-alkyl, β-hydroxy fatty acids, which form an outer waxy lipid layer around the mycobacteria (Barry et al. 1998). The early research (Julian et al. 2002; Lopez-Marin et al. 2003) reported the diagnostic value for tuberculosis using glycolipid antigens such as cord factor, DAT, SL-1 and TAT, which had a common trehalose core but different structures of mycolic acids, so we are more interested in such glycolipid antigens and those corresponding metabolic enzymes in the serodiagnosis of TB.

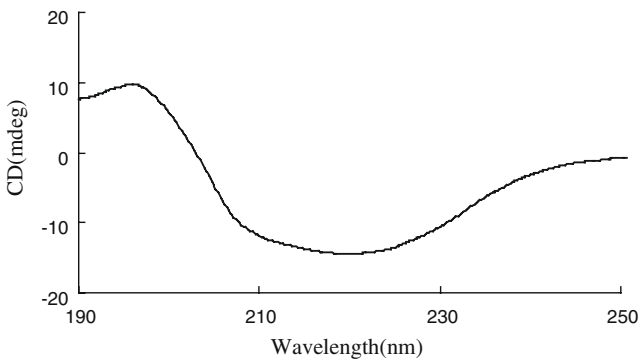

Fig. 6 CD spectrum of the purified His-fRv3391. The secondary structure elements of 36.2% α-helix, 0.0% β-sheet, 32.6% β-turn, and 31.3% random coil at 20°C

In the present study, we expressed the recombinant protein fRv3391 with 6× his-tag in *E. coli* BL21 (DE3) and evaluate its immunological nature by ELISA. The ELISA results revealed that His-fRv3391 is a potential immunodominant antigen for the serodiagnosis of active TB. In the 30 smear-positive TB patients, fRv3391 antigen provided a sensitivity of 46.7% (14/30), much higher than that of in the 26 smear-negative TB patients (11.5%, 3/26). And the total detection sensitivity of His-fRv3391 antigen with 56 active TB patients was 30.3% (17/50), with an overall specificity of 94.0% (47/50). The ELISA results of the present study demonstrated the immunodominant nature of recombinant fRv3391 protein. Furthermore, the fRv3391 protein is worthy of further investigation together with other immunodominant antigens such as Rv3425 and 38 kDa in the serodiagnosis of TB.

Conclusion

In this study, a short fragment of the *M. tuberculosis* $H_{37}Rv$ *Rv3391* gene (*fRv3391*) was first successfully expressed in

E. coli BL21 (DE3). The studies of mass spectrometry and circular dichroism were performed in identifying the recombinant protein His-fRv3391 with the molecular mass 42510 Da and the secondary structure elements 36.2% α-helix, 0.0% β-sheet, 32.6% β-turn, and 31.3% random coil. The ELISA results demonstrated its immunodominant nature based on its reactivity with sera obtained from patients with active TB and negligible reactivity with sera obtained from healthy controls. Furthermore, it is worthy of further investigation with other immunodominant antigens for the development of rapid diagnostic tests for TB.

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Ethical approval Not required.

Conflict of interest The authors declare that they have no conflict of interest.

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