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Backbone assignments of ^{1}H, ^{15}N and ^{13}C resonances and secondary structure prediction of MRA1997 from *Mycobacterium tuberculosis* H37Rv

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Abstract MRA1997 is a 76-residue conserved hypothetical protein of Mycobacterium tuberculosis H37Ra, one of the most pathogenic bacterial species and the causative agent of tuberculosis. In this study, the sequence–specific backbone resonance assignment of MRA1997 was performed using NMR spectroscopy. Approximately 88.3% of the total resonances could be unambiguously assigned. By analyzing deviations of the Cα and Cβ chemical shift values, the secondary structure of MRA1997 was calculated. The result revealed that secondary structure of MRA 1997 consists of one α-helix and five β-sheets. Our structural study will be a footstone towards the characterization of the three-dimensional structure of MRA1997.

Keywords MRA1997, a5u406, Mycobacterium tuberculosis, NMR, secondary structure

Introduction

Mycobacterium tuberculosis is one of the most pathogenic species affecting public health around the world. Tuberculosis is an infectious disease caused by bacteria belonging to the Mycobacterium tuberculosis complex. In many cases, the infection remains in an inactive or latent state, but it can progress to active disease which, if left untreated, kills more than 50% of those infected. [1] However, the known therapeutic agents for tuberculosis such as etambutol, isoniazid, pyrazinamide and rifampicin, cause thrombocytopenia, neuropathy and liver damage as adverse effects. [2] Moreover, resistance concern has been arising. [3] Vaccination is limited because of the variable effectiveness of the vaccine against adult pulmonary tuberculosis. [4] In this regard, to overcome these problems, identification of novel drug targets is important, but many of *Mycobacterium tuberculosis* protein remains hypothetical and unknown. MRA1997 is a conserved hypothetical protein of *Mycobacterium tuberculosis* H37Ra. MRA1997 consists of 76 amino acid residues with a molecular weight 8.3 kDa and calculated isoelectric point of 1.49. The present study reports the sequence-specific backbone resonance assignments and secondary structure of hypothetical protein MRA1997 of *Mycobacterium tuberculosis* H37Ra. This structural study will be helpful for a better understanding of the mechanism of pathogenesis and a search for therapeutic targets for tuberculosis.

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Experimental Methods

Sample preparation- Recombinant plasmid for MRA1997 was constructed using pET21a(+) vector system to acquire high quality of target protein. Uniformly ^{15}N- or ^{15}N/^{13}C-labeled MRA1997 was expressed in *Escherichia coli* strain, BL21(DE3) grown in M9 medium supplemented with ^{15}N-NH_4Cl and/or ^{13}C-glucose. Recombinant protein expression was induced by 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) to cells grown for 4 hours at 37°C.

The cell lysate was centrifuged at 18,000 rpm for 1 hour at 4°C. The cleared supernatant was purified by binding to Ni affinity column eluting by imidazole. Further purification was achieved by gel filtration chromatography using Superdex75 column (Pharmacia) which was previously equilibrated with buffer (50mM Tris-HCl, pH 7.5 and 200mM NaCl). The purity of six histidine-tagged MRA1997 was estimated to be over 95% by SDS-PAGE. The purified protein was concentrated to 1mM in 50mM phosphate buffer (pH 6) containing 200mM NaCl to which 10% D_2O was been added.

NMR experiments- NMR experiments were recorded by JEOL ECA 600 MHz NMR spectrometers. All NMR experiments were carried out at 298K. Nearly complete sequential–backbone assignment of the protein was achieved using a series of triple resonance spectra [three-demensional (3D) HNCO, HN(CA)CO, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH]. [5] ^{1}H, ^{15}N and ^{13}C chemical shifts were externally referenced to tetramethylsilane (TSP). [6] All NMR data were processed with NMRpipe[7] and

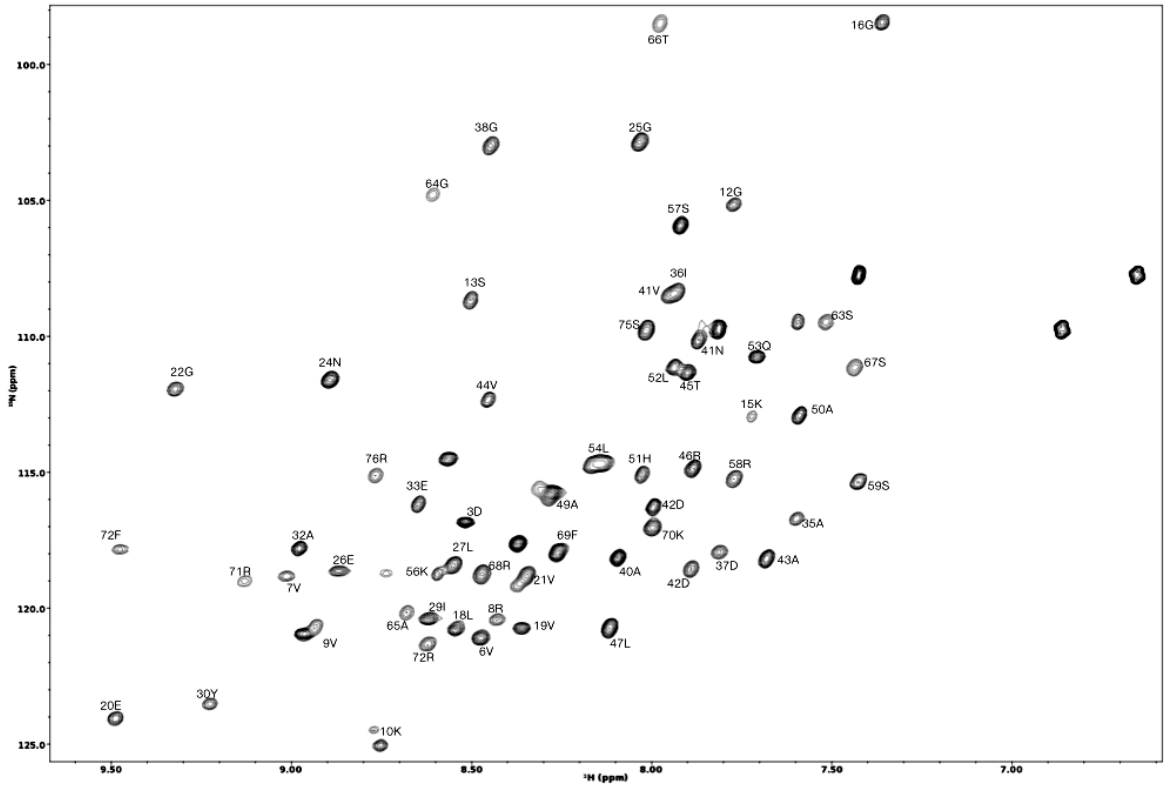

Figure 1. 2D ^{1}H /^{15}N HSQC spectrum of MRA1997. Each of the backbone NH resonances on the spectrum is labeled with the assigned amino acid residues.

analyzed by NMRviewJ. [8] The backbone dihedral torsion angles were calculated using the TALOS program which uses a combination of ^{1}H, ^{15}N, $^{13}C\alpha$, $^{13}C\beta$ and ^{13}CO chemical shifts of adjacent residues. [9-11]

Results and Discussion

A 2D $^{1}H/^{15}N$ HSQC spectrum of MRA1997 is shown with the assigned amino acid residues (Figure 1). Approximately 88.3% chemical shifts of ^{1}HN, ^{15}N, ^{13}CO, $^{13}C\alpha$ and $^{13}C\beta$ were assigned. All the assigned chemical shift values are presented in Table 1. The assignment of remaining resonances was not possible due to spectral overlap and ambiguity.

In general, the chemical shift values are sensitive to molecular conformation, backbone dihedral angle, hydrogen bond interactions, backbone dynamics, ring-flip rates, etc. Residue-specific secondary structures of MRA1997 were estimated on the basis of the assigned chemical shifts applied to the TALOS program. Figure 2 shows the values of the backbone dihedral angles phi(φ) and psi(ψ) against the protein residues. The secondary structure of MRA 1997 is composed of one α-helix and five β-strands; the β-strands correspond to residues 3-10(β1), 17-21(β2), 25-30(β3), 59-63(β4), 71-76(β5) while the α-helix correspond to residues 39-51(α1).

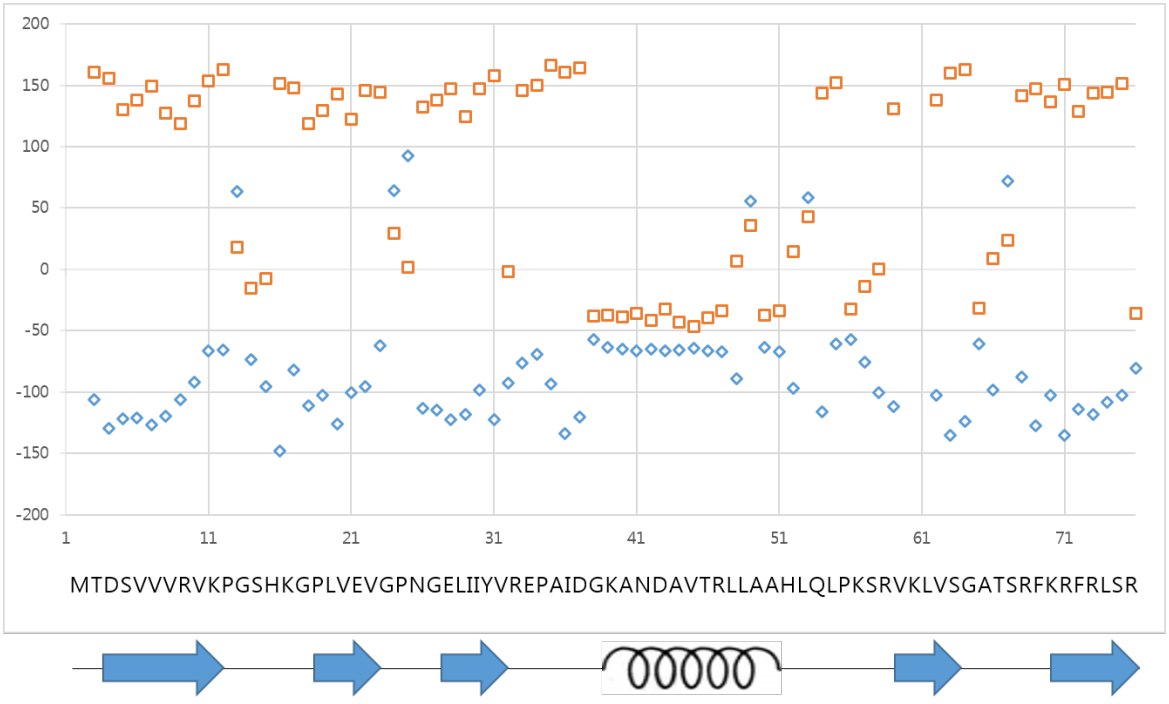

Figure 2. The secondary structure of MRA1997. Backbone dihedral angels, phi(φ) and psi(ψ), were calculated using the TALOS program.

Table 1. Chemical shifts of ^{1}HN, ^{15}N, ^{13}CO, ^{13}Cα and ^{13}Cβ of MRA1997. All the chemical shifts were referenced to the frequency of the TMS.

Residue	HN	Cα	Cβ	CO	Residue	HN	Cα	Cβ	CO
1 MET					39 LYS		56.081	29.295	176.155
2 THR	8.157	59.389	67.387	172.000	40 ALA	8.106	52.187	15.810	177.096
3 ASP	8.463	51.375	39.031	172.614	41 ASN	7.882	54.555	35.624	176.061
4 SER	7.943	54.458		170.768	42 ASP	7.901	55.168	38.342	175.096
5 VAL		57.889	30.235	170.786	43 ALA	7.693	53.031	15.557	177.611
6 VAL	8.483	58.676	30.501	172.511	44 VAL	8.465	64.283	29.013	174.148
7 VAL	9.022	56.586	32.656	172.252	45 THR	7.912	65.225	66.059	172.889
8 ARG	8.443	51.528	29.440	171.972	46 ARG	7.897	57.500	27.545	176.767
9 VAL	8.949	58.505	30.823	172.679	47 LEU	8.008	55.069		176.863
10 LYS	8.779	49.620	29.561	170.534	48 LEU		55.005	39.000	170.829
11 PRO		58.818	29.905	174.660	49 ALA	8.287	53.422	14.451	176.260
12 GLY	7.783	43.793		172.509	50 ALA	7.598	52.273	15.348	178.537
13 SER	8.510	61.894	54.812	174.002	51 HIS	8.037	57.559	28.548	174.509
14 HIS					52 LEU	7.950	51.838	39.449	172.576
15 LYS	7.688	53.128			53 GLN	7.710			
16 GLY	7.303	42.008		168.210	54 LEU	8.179	48.685	42.025	
17 PRO		58.806	43.384		55 PRO		59.360	29.325	175.484
18 LEU	8.553	53.178	43.311	171.032	56 LYS	8.605	57.250	29.094	174.813
19 VAL	8.342	58.956	30.078		57 SER	7.930	57.207	60.037	173.136
20 GLU	9.499	51.572	32.009	171.046	58 ARG	7.782	53.114	27.787	171.234
21 VAL	8.357	59.287	29.194	174.661	59 VAL	7.430	59.184	30.123	171.232
22 GLY	9.334	41.766		170.309	60 LYS				
23 PRO		61.610	29.280	174.851	61 LEU				
24 ASN	8.902	50.251	35.873	173.180	62 VAL		59.512	30.907	173.229
25 GLY	8.045	43.002		171.486	63 SER	7.530	55.692	62.297	170.743
26 GLU	8.802	53.594	27.328	172.522	64 GLY	8.652	43.317		170.765
27 LEU	8.560	51.743	40.814	172.988	65 ALA	8.697	53.255	15.741	176.451
28 ILE		57.487	36.888	172.221	66 THR	8.006			
29 ILE	8.623	57.541	36.618	171.437	67 SER	7.448	61.085	61.007	171.582
30 TYR	9.240	54.011	36.155	173.183	68 ARG	8.514	53.505	28.345	173.505
31 VAL	7.962	56.125	31.857	172.387	69 PHE	8.268	52.560	39.651	174.345
32 ARG	8.987	54.936	27.815	175.252	70 LYS	8.010	52.468	34.311	171.455
33 GLU	8.679	53.826	25.706	173.564	71 ARG	9.140	52.187	30.432	171.917
34 PRO		61.048	29.246	173.288	72 PHE	9.486	53.467	40.189	172.190
35 ALA	7.605	49.068	18.063	173.974	73 ARG	8.622			
36 ILE	7.939	56.928	35.567	171.479	74 LEU		51.625	39.060	172.305
37 ASP	7.818	49.458	39.682	173.868	75 SER	8.018	54.483	63.163	170.770
38 GLY	8.458	44.457		174.389	76 ARG	8.766	57.742	32.874	

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