Journal of the Korean Magnetic Resonance Society **2013**, *17,* 54-58
DOI 10.6564/JKMRS.2013.17.1.054

Backbone ^{1}H, ^{15}N, and ^{13}C Resonances Assignment and Secondary Structure Prediction of SAV0506 from *Staphylococcus aureus*

In Gyun Lee, Ki-Young Lee, Ji-hun Kim , Susanna Chae and Bong-Jin Lee*

[1]Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, San 56-1, Shillim-Dong, Kwanak-Gu, Seoul 151-742, Korea

Received June 1, 2013; Revised June 7, 2013; Accepted June 10, 2013

Abstract SAV0506 is an 87 residue hypothetical protein from *Staphylococcus aureus* strain Mu50 and also predicted to have similar function to ribosome associated heat shock protein, Hsp 15. Hsp15 is thought to be involved in the repair mechanism of erroneously produced 50S ribosome subunit. In this report, we present the sequence specific backbone resonance assignment of SAV0506. About 82.5% of all resonances could be assigned unambiguously. By analyzing deviations of the Cα and Cβ chemical shift values, we could predict the secondary structure of SAV0506. This study is an essential step towards the structural characterization of SAV0506.

Keywords *Staphylococcus aureus*, SAV0506, Hsp15, NMR

Introduction

Staphylococcus aureus is an anaerobic Gram-positive bacterium found in the respiratory tract and skin of a various animals including human. *S. aureus* is the common cause of various types of diseases ranging from relatively mild infections to life threatening illnesses such as sepsis, meningitidis, and pyemia [1]. As a result, *S. aureus* ranks the first among the causative agents of hospital-acquired infections[2]. However, the treatment of *S. aureus* infections has become more difficult recently because of the development of resistance to antibiotics. Only few years after Flemming's discovery of penicillin in 1940s, penicillin resistant *S. aureus* was emerged [3]. This led to the development of new antibiotics such as penicillin-derivative methicillin or non-beta-lactam antibiotics vancomycin, but no longer after, methicillin-resistant *S. aureus*(MRSA) and vancomycin resistant *S. aureus*(VRSA) has emerged [4].Until now, new-generation antibiotics have been developed such as linezolid, doxycycline, rifampin to overcome this kinds of drug resistant bacteria but *S. aureus* continues to evolve with resistance to these new drugs already emerging [5]. In an effort to develop new-generation antibiotics susceptible to multi-drug resistant *S. aureus*, and as a part of our structural genomics effort on pathogenic bacteria[6], we characterized the structural information of hypothetical protein SAV0506 from *S. aureus subsp. aureus* strain Mu50 which is also predicted to have similar function to ribosome associated heatshock protein, Hsp 15. Hsp15 is distinguished from other types of heat shock proteins in that it does not act as a molecular chaperon or protease [7]. Instead, hsp15 binds to the 50S ribosome subunit which carries nascent polypeptide chain. Under heat shock condition, this "loaded" 50S subunit is produced as an erroneous dissociation and hsp15 binds to this 50S particle and thought to be involved in the repair mechanism of such 50S particles [8]. Here, we report the sequence-specific backbone resonance

* Address correspondence to: **Bong-Jin Lee,** Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, San 56-1, Shillim-Dong, Kwanak-Gu, Seoul, 151-742, Korea, Tel: 82-2-880-7869; Fax: 82-2-872-3632; E-mail: lbj@nmr.snu.ac.kr

assignments and the prediction of secondary structure of SAV0506 using TALOS+ and CSI.

Experimental Methods

Sample preparation- The DNA genes coding SAV0506 were amplified by PCR reaction and expressed from the expression vector pCOLDI (Takara Bio.), as a His-tagged fusion protein. To obtain uniformly ^{15}N,^{13}C-labeled protein, transformed E. coli BL21cells were ultivated in minimal (M9) medium supplemented with ^{13}C-D-Glucose and $^{15}NH_4Cl$. Cells were harvested by centrifugation (8,000 rpm), resuspended in 50 mM Tris–HCl pH 8.0, 500 mM NaCl, and lysed by ultrasonication.

The resulting cell lysate was centrifuged at 18,000 rpm for 1 h at 4°C. The cleared supernant was purified by binding to a Ni_2 affinity column and eluting with imidazole. Fractions containing protein was loaded to a superdex75 gel column (GE Healthcare) equilibrated with 20mM Bis-tris pH 6, 100mM Nacl. Purified SAV0506 was concentrated to approximately 1 mM using Amicon Ultra 3kDa MWCO centrifugal filters. NMR samples comprised 1 mM protein in 20 mM Bis-Tris pH 6.0, 100 mM NaCl, 1 mM EDTA, 0.1 mM PMSF and 200 mM deuterated SDS to which 10 % D2O was been added.

NMR experiments- NMR experiments were recorded by JEOL 600 MHz spectrometers. All NMR experiments were carried out at 303 K. The NMR data were processed using NMRpipe[9] and analyzed using NMRView J[10]. Nearly complete sequential backbone assignments of the 103aa N-terminal tagged SAV0506 protein were achieved using through bond 3D HNCACB, CBCA(CO)NH, HNCA,

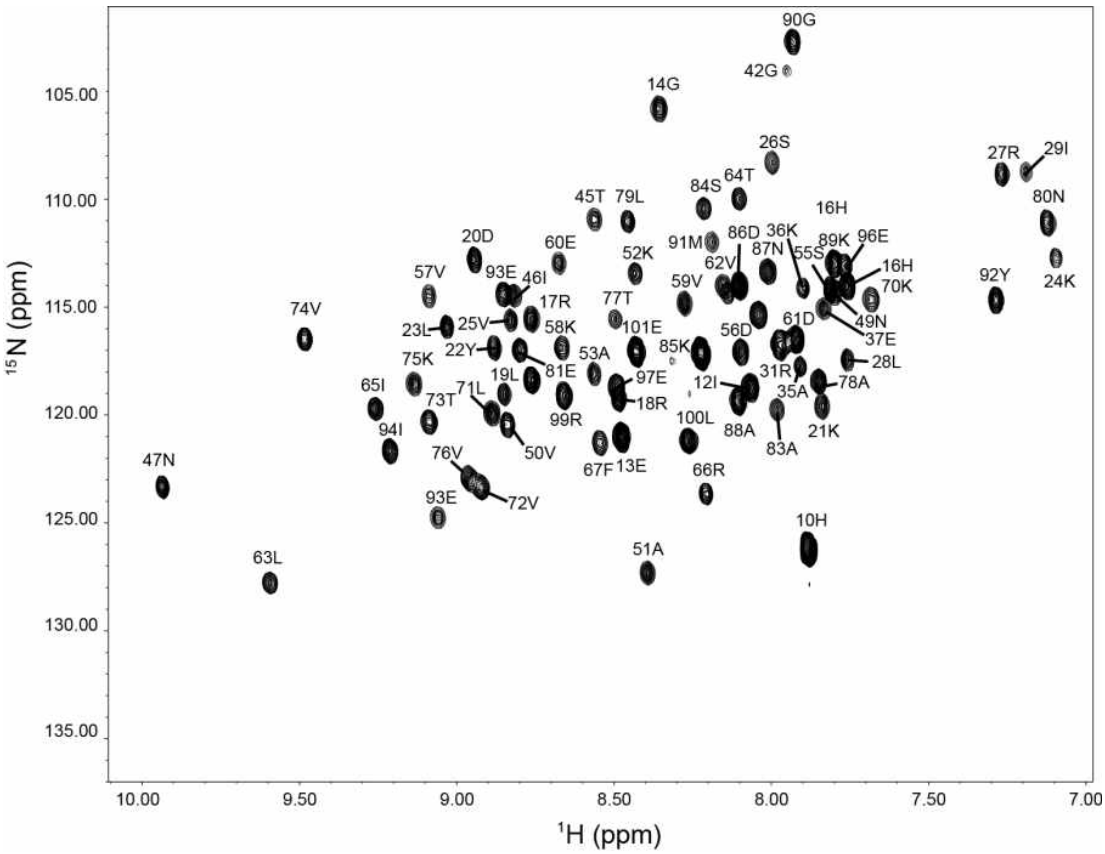

Figure 1. Assigned amide protons and nitrogens of SAV0506

HN(CO)CA, HNCO,HN(CA)CO experiments.

Results and Discussion

Excluding the Met residue at N-terminus, 82.5% of all ^{1}HN, ^{15}N, ^{13}CO, $^{13}C_{\alpha}$ and C_{β} could be unambiguously assigned. 2D-[^{1}H-^{15}N] HSQC spectrum with assignments is shown in Fig. 1. Chemical shifts of ^{1}HN, ^{15}N, ^{13}CO, $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ are presented in Table.1. The assignment of remaining resonances was not possible due to spectral overlap and ambiguity. To estimate the secondary structure of SAV0506, prediction methods PSIPRED[11]and TALOS+ [12] were applied and predictions are displayed for comparison in Fig. 2 (a),(b), respectively. These estimates were also compared to the predictions based on differences between the experimental and random coil $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ [13]; [14] in Fig. 2 (c), (d). The secondary structure prediction obtained from NMR data is composed of seven beta strands and two alpha helices. This NMR data based prediction is very well comparable to the sequence based prediction method except for the C-terminal residues which is predicted to beta strand rather than alpha helix in sequence based method. These assignments including HSQC spectrum can be used to obtain information on the drug screening, or protein-protein interaction.

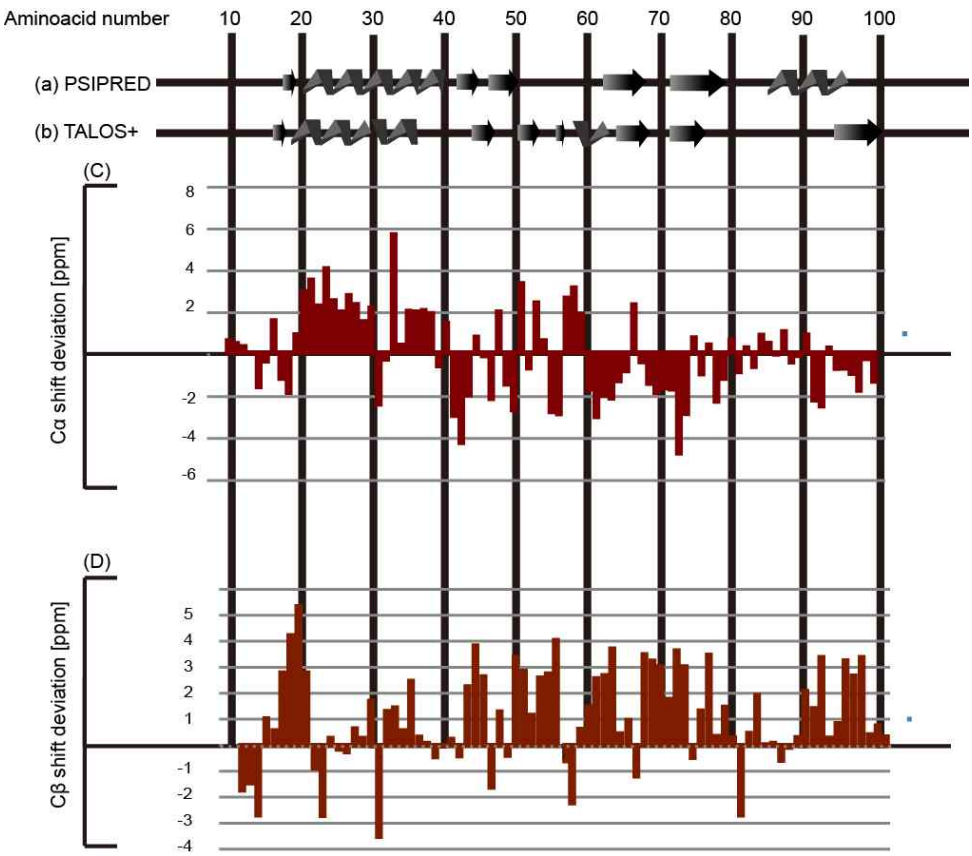

Figure. 2 Comparison of the secondary structure with experimentally observed and predicted structures using: PSIPRED (a), TALOS + (b) and analysis of the experimental $^{13}C_{\alpha}$(c) and $^{13}C_{\beta}$ (d) chemical shift deviations from random coil values.

Table 1. Chemical shifts of ^{1}HN, ^{15}N, ^{13}CO, ^{13}Cα, and $^{13}C_{\beta}$ of SAV0506. All chemical shifts were referenced to the frequency of the methyl proton resonances of DSS.

Residue	HN	N	CO	CA	CB	Residue	HN	N	CO	CA	CB
1MET	ND	ND	ND	ND	ND	52LYS	8.4215	113.4734	174.0795	51.4552	40.8803
2ASN	ND	ND	ND	ND	ND	53ALA	8.5544	118.0866	174.5627	53.293	31.93
3HIS	ND	ND	ND	ND	ND	54GLY	7.8977	131.7429	172.744	41.7481	
4LYS	ND	ND	ND	ND	ND	55SER	7.8049	114.1447	169.4647	58.1646	28.5727
5VAL	ND	ND	ND	ND	ND	56ASP	8.114	117.0422	173.4985	52.1734	24.9543
6HIS	ND	ND	ND	ND	ND	57VAL	9.0866	114.4249	170.4449	57.6604	38.9058
7HIS	ND	ND	ND	ND	ND	58LYS	8.6574	116.8422	172.7261	51.2634	30.6646
8HIS			173.5478	53.8716	27.7522	59VAL	8.2611	114.8248	174.2977	63.0939	41.9544
9HIS	8.2121	117.1894	172.3407	53.7348	28.0257	60GLU	8.6712	112.9483	173.5659	57.2906	68.2647
10HIS	7.8959	126.2662	179.8565	53.586	26.795	61ASP	7.9152	116.5256	172.4531	53.434	38.6918
11HIS	8.2866	119.1103	172.1643	53.2676		62VAL	8.1511	114.0715	174.0417	58.7388	28.2306
12ILE	8.0746	118.6983	173.3081	58.4502	36.0065	63LEU	9.5863	127.7914	174.172	50.1217	37.7425
13GLU	8.4791	121.0716	174.2513	53.7781	27.7445	64THR	8.0953	110.0519	170.8123	58.5356	26.3936
14GLY	8.3452	105.8625	171.2029			65ILE	9.2503	119.7322	171.6835	57.9142	33.2734
15ARG	8.0423	123.5468	171.138	55.3193	33.7082	66ARG	8.1992	123.6973	172.3817	52.4414	42.6226
16HIS	7.7689	114.0475	171.8138	52.0531	35.6231	67PHE	8.5376	121.3326	173.1109	54.514	32.2036
17MET	8.7554	115.5567	171.1421	52.1829	30.5622	68GLY	8.7006	109.242	172.1696	44.7772	
18ARG	8.4862	119.2919	175.623	54.6535	38.4954	69GLN			172.3749	53.2474	32.8213
19LEU	8.8435	119.0578	174.9064	56.1068	35.5718	70LYS	7.6739	114.6555	170.8539	52.7191	32.8093
20ASP	8.9382	112.8419	175.971	55.0571	30.0542	71LEU	8.8787	119.9586	173.546	51.2742	28.7095
21LYS	7.8445	119.6343	175.15	56.4251	36.0183	72VAL	8.9123	123.3708	171.3555	58.8036	66.9043
22TYR	8.8761	116.8973	176.6021	60.1066	39.1198	73THR	9.0753	120.3633	172.1405	58.8416	19.9575
23LEU	9.0282	115.958	174.4845	55.6738	30.4151	74VAL	9.4743	116.4431	170.0742	55.6862	39.7264
24LYS	7.0978	112.7545	177.6431	56.1454	29.4565	75LYS	9.1254	118.5315	173.5182	51.2767	37.9483
25VAL	8.8207	115.6768	175.0361	63.2167	61.8813	76VAL	8.9627	122.8977	174.6339	61.2008	27.4751
26SER	7.9872	108.2806	172.1569	58.0886	24.2664	77THR	8.4911	115.565	172.5312	59.5616	26.795
27ARG	7.2605	108.9017	172.9219	55.2761	40.6839	78ALA	7.8436	118.4585	171.8024	50.3592	16.9489
28LEU	7.7521	117.43	175.2659	55.3193	36.4247	79LEU	8.4349	111.0033	172.6908	50.867	62.0976
29ILE	7.1737	108.7247	172.8791	57.6289	30.3505	80ASN	7.1184	111.108	171.4691	49.8513	29.8035
30LYS			173.5499	53.8716	30.2456	81GLU	8.7817	116.9962	173.534	54.7847	38.3586
31ARG	7.9979	116.7727		59.4237	28.1002	82HIS			172.3842	52.3673	35.8966
32ARG	8.4275	118.5931	173.6251			83ALA	8.0039	119.757	175.0171	50.2267	16.4019
33THR					39.4611	84SER	8.2126	110.4939	172.2928	55.1166	30.077
34LEU			174.5627	53.5519	16.0276	85LYS	8.345	117.4724	174.1433	55.0266	32.3568
35ALA	7.9065	117.7677	176.3597	51.976	29.7416	86ASP	8.0866	114.0631	173.6251	52.0366	37.5983
36LYS	7.8912	114.1395	174.3626	56.1377	27.4163	87ASN	7.9958	113.3326	172.7853	51.0075	30.5622
37GLU	7.8176	115.0589	174.4064	56.2109		88ALA	8.0985	119.4016	175.1096	50.9998	35.265
38VAL	ND	ND	ND	ND	ND	89LYS	7.7872	112.9743	174.1667	53.7348	35.8205
39SER					37.8718	90GLY	7.928	102.7584	172.1757	42.3351	
40ASP			175.1733	53.4613		91MET	8.1674	111.9951	174.7486	54.9339	29.8508
41GLU	7.8689	112.9017	173.9885	53.5578		92TYR	7.2847	114.7364	170.8199	53.8147	31.1628
42GLY	7.9358	104.0545	172.4503	43.8931		93GLU	8.8395	114.4758	173.4688	51.637	28.193
43ARG					69.4076	94ILE	9.209	121.7761	173.5078	60.313	40.1296
44ILE			173.0781	57.0849	37.6185	95ILE	9.0459	124.6809	173.5628	59.3292	27.5087
45THR	8.5543	110.9543	171.1207	56.2862	34.8633	96GLU	7.7657	113.0719	170.5479	53.4363	
46ILE	8.811	114.4707	173.95	58.0549	37.7658	97GLU	8.4865	118.6749	171.9061	53.1878	
47ASN	9.9303	123.3631	172.3749	51.8319	28.7841	98ARG	8.751	118.3356	171.8997	51.9781	
48GLY	8.5797	134.144	170.8115	42.3252		99ARG	8.6505	119.0822	173.4459	53.4923	
49ASN	7.7936	114.3366		48.894	32.6349	100LEU	8.2507	121.1504	173.8851	51.8168	
50VAL	8.8356	120.475	172.6448	62.4318		101GLU	8.4137	116.9594		53.6849	
51ALA	8.3501	127.6349	173.1563	48.4803	61.3376	102GLU	ND	ND	ND	ND	ND
						103ALA	ND	ND	ND	ND	ND

*ND; Not Detected **Unit; ppm

Acknowledgements

This work was supported by National Research Foundation of Korea Grants 20110001207 and 2012R1A2A1A01003569 funded by the Korean Government (MEST);Korea Health Technology R&D Project, Ministry for Health and Welfare, Republic of Korea Grants A092006; 2012 BK21 Project for Medicine, Dentistry, and Pharmacy.

References

1. J. Schipsema, *Phyrochem. Anal.* **21**, 14. (2009).
2. H. K. Kim, Y. H. Choi, R. Verpoorte, *Nat. Protoc.* **5**, 536. (2010).
3. H. K. Kim, Y. H. Choi, R. Verpoorte, *Trends. Biotechnol.* **29**, 267. (2011).
4. B. Rasmussen, O. Cloarec, H. Tang, D. Staerk, J. W. Jaroszewski, *Planta Med.* **72**, 556. (2006).
5. H. K. Kim, Saifullah; S. Khan, E. G. Wilson, S. D. P. Kricun, A. Meissner, S. Goraler, A. M. Deelder, Y. H. Choi, R. Verpoorte, *Phytochemistry* **71**, 773. (2010).
6. E. Barros, S. Lezar, M. J. Anttonen, J. P. van Dijk, R. M.;Rohlig, E. J. Kok, K.-H. Engel, *Plant Biotechnol. J.* **8**, 436. (2010).
7. Y. H. Choi, Y.-W. Chin, Y. G. Kim, *Arch. Pharm. Res.* **34**, 1843. (2011).
8. Y. Jung, Y.-S. Jung, G.-S. Hwang, *J. Korean Magn. Reson. Soc.* **15**, 90. (2011).J. Kim, J. Park, S.-S. Park, G.-S. Hwang, *J. Korean Magn. Reson. Soc.* **16**, 54. (2012).