Journal of the Korean Magnetic Resonance Society **2008**, *12*, 65-73

Backbone ^{1}H, ^{15}N, and ^{13}C Resonance Assignment and Secondary Structure Prediction of HP1298 from *Helicobacter pylori*

WonJe Kim[1], Jongsoo Lim[2], Woosung Son[3], Heechul Ahn[2] and Bong-Jin Lee[1*]

[1]*Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, Korea*
[2]*Advanced Analysis Center, Korea Institute of Science and Technology (KIST) 39-1 Hawolgogdong, Sungbuggu, Seoul 136-791, Korea*
[3]*Present address ; Department of Chemistry and Biochemistry University of California, San Diego 9500 Gilman Drive, NSB #3117 La Jolla, CA 92093*
Received January 24, 2008

Abstract : HP1298 (Swiss-Prot ID ; P65108) is an 72-residue protein from *Helicobacter pylori* strain 26695. The function of HP1298 was identified as Translation initiation factor IF-1 based on sequence homology, and HP1298 is included in IF-1 family. Here, we report the sequence-specific backbone resonance assignments of HP1298. About 97% of all the ^{1}HN, ^{15}N, $^{13}C\alpha$, $^{13}C\beta$, and ^{13}CO resonances could be assigned unambiguously. We could predict the secondary structure of HP1298, by analyzing the deviation of the $^{13}C\alpha$ and $^{13}C\beta$ shemical shifts from their respective random coil values. Secondary structure prediction shows that HP1298 consists of six β-strands. This study is a prerequisite for determining the solution structure of HP1298 and investigating the structure-function relationship of HP1298 . Assigned chemical shift can be used for the study on interaction between HP1298 and other Helicobacter pylori proteins.
Keywords : HP1298; NMR; Backbone assignment; Secondary structure

INTRODUCTION

Helicobacter pylori is a gram-negative bacterium, measuring 2 to 4 ㎛ in length and 0.5 to 1 ㎛ in width. Although usually spiral-shaped, the bacterium can appear as a rod, while coccoid shapes appear after prolonged in vitro culture or an antibiotic treatment[1]. The organism has 2 to 6 unipolar, sheathed flagella of approximately 3 ㎛ in length, which often

* To whom correspondence should be addressed. E-mail : lbj@nmr.snu.ac.kr

carry a distinctive bulb at the end.[2] *H. pylori* is related with many serious gastric problems, ranging from gastritis to gastric carcinoma or lymphoma.[3-5] The genome of *H. pylori* has been fully sequenced for two prototype strains (strain 26695 and strain J99)[6]. The *H. pylori* strain 26695 genome includes 1,590 genes, whereas the genome of strain J99 includes only 1,491 genes.[7,8] About 33 % protein sequences in the whole genome have no homologues in other organisms and whose function and three-dimensional structures have never been identified.

As a part of our structural genomics on *Helicobacter pylori*, we studied the solution structure of HP1298, one of the proteins from *H. pylori* by using NMR. The HP1298 gene of *Helicobacter pylori* encodes a 72-residue hypothetical protein from *Helicobacter pylori* strain 26695 with a molecular weight of 8348 Da and a calculated isoelectric point of 9.46. HP1298 is included in IF-1 family. The result of sequence homology search showed that HP1298 has a S1-like domain. The S1 domain of around 70 amino acids, originally identified in ribosomal protein S1, is found in a large number of RNA-associated proteins . Here, we report the sequence-specific backbone resonance assignments and predict the secondary structure of HP1298.

MATERIALS AND METHODS

HP1298 of *H. pylori* was cloned into the expression vector pET-21a and was expressed in the *Escherichia coli* BL21 (DE3) strain. Uniformly ^{15}N, ^{13}C labeled protein was prepared by growing the cells in the isotope-supplemented M9 medium. Cells were grown at 37℃ until an OD_{600} of 0.6 and then induced with 1 mM IPTG for 6 hr. The soluble protein was purified using Ni^{2+}-agarose column (His bind® Resin ; Novagen Inc. Darmstadt, Germany) and Gel filtration (Superdex™ 75 10/300; Amersham Biosciences). The NMR sample was prepared at a concentration of about 1.0 mM in 90 % H_2O/10 % D_2O containing 50 mM NaH_2PO_4/Na_2HPO_4 (pH 6.0), 150 mM NaCl, 1 mM EDTA, and 1 mM BME.

All NMR measurements were performed at 308 K on Bruker Avance 600 spectrometer equipped with cryo probe. Backbone assignments were performed with the HNCA, HN(CO)CA, HNCACB, HN(CO)CACB, and HNCO. Side-chain resonances were

assigned with HCCH-TOCSY, 3D ^{15}N-TOCSY-HSQC, and CCCONH TOCSY.[9-12] Slowly exchanging amide proton and ring proton resonances were assigned by dissolving the protein in D2O and acquiring a 2D-NOESY. Chemical shifts were referenced to 2, 2-dimethylsilapentane-sulfonic acid (DSS) externally.

All spectra were processed using the nmrPipe/nmrDraw software[13], and were analyzed using the program NMRView[14]. The secondary structure was predicted from the chemical shift values using Chemical Shift Index (CSI)[15] and Torsion Angle Likelihood Obtained from Shift and sequence similarity (TALOS)[16].

RESULTS AND DISCUSSION

HSQC spectrum of HP1298 showed good peak resolution (Fig. 1.). Assignments of HP1298 were achieved nearly completely (Table 1). The backbone amide ($^{1}H_N$ and ^{15}N) resonances were completely assigned except 2 prolines. Although all $^{13}C\alpha$, $^{13}C\beta$ resonances were also completely assigned, only 95 % of ^{13}CO resonances were assigned, because of overlapping with some peaks.

For predicting secondary structure of HP1298, chemical shift difference method between measured values and random-coil values using Cα, Cβ, and (ΔCα - ΔCβ)[17] and CSI protocol was used. Correlations have been observed between Cα[8-20] and Cβ[18] chemical shifts and the local backbone conformation for a number of proteins. Backbone dihedral angles (ψ) are predicted using TALOS methods from chemical shifts. Comparing relative random coil chemical shifts, Cα resonances tend to shift upfield in β–sheets and extended strands, and they tend to shift downfield in helices. The opposite trend holds for the Cβ resonances. Because the Cα and Cβ secondary shifts are of similar magnitude and opposite sign for both helices and sheets, subtraction of the Cα and Cβ secondary shifts (ΔCα - ΔCβ) enhances the correlation between the secondary structural elements and the secondary shifts. As shown in Fig. 2., examination of {ΔCα – ΔCβ} plot indicates the presence of six potentially β-strand regions .The regions of β-strands correspond well to the CSI and TALOS predictions. Because all backbone amide ($^{1}H_N$ and ^{15}N) resonances were assigned, HSQC spectrum of HP1298 (Fig. 1.) can be used to detect the protein-protein interaction between HP1298 and other *Helicobacter* proteins

Table 1. Chemical shifts of ^{1}HN, ^{15}N, ^{13}CO, ^{13}Cα and ^{13}Cβ of HP1298. All chemical shifts were referenced to the frequency of the methyl proton resonance of DSS.

A	AA	H	N	CA	CB	CO	#	AA	H	N	CA	CB	CO
1	M		ND	56.377	ND	ND	37	S	8.502	121.629	58.285	64.62	176.342
2	A	9.119	104.114	51.783	19.503	ND	38	G	9.204	114.642	47.142		175.38
3	R	8.636	121.22	56.78	30.949	174.064	39	K	8.243	120.937	52.799	32.882	175.985
4	D	8.397	121.044	54.263	41.034	175.959	40	M	7.639	119.314	57.024	33.062	178.22
5	D	8.184	120.556	54.263	41.068	175.621	41	R	8.018	121.162	58.186	30.152	177.338
6	V	7.748	118.231	61.371	34.198	175.445	42	M	7.917	118.264	56.389	32.131	177.808
7	I	8.617	125.743	60.44	40.3	175.376	43	H	7.744	117.778	55.651	29.608	176.019
8	E	8.336	125.738	54.779	32.401	174.575	44	Y	7.865	119.514	58.683	37.376	173.997
9	V	8.855	120.506	59.791	36.168	176.174	45	I	7.465	122.818	60.667	38.856	175.114
10	D	8.038	121.101	52.751	43.729	172.795	46	R	8.082	126.878	55.501	31.096	174.623
11	G	8.743	104.335	46.104		176.479	47	I	8.013	126.512	59.903	37.65	175.182
12	K	8.388	120.546	53.791	36.305	170.617	48	A	8.903	132.238	49.685	22.887	173.796
13	V	9.099	127.549	64.673	31.94	176.082	49	L	8.192	119.574	57.054	41.505	176.618
14	I	8.968	123.674	61.679	40.677	177.169	50	G	8.862	114.097	45.085		178.247
15	E	7.675	121.896	57.875	34.6	175.748	51	D	7.975	122.052	55.406	41.326	174.145
16	A	9.105	131.668	51.608	20.246	173.565	52	R	8.885	122.993	54.839	31.128	175.099
17	L	8.15	126.662	ND	41.466	175.195	53	V	9.112	118.224	58.102	36.092	175.991
18	P			63.551	32.177		54	K	8.489	121.682	55.313	36.135	173.859
19	N	8.419	117.516	54.476	36.779	176.012	55	L	9.486	122.939	53.607	45.735	175.331
20	A	8.42	119.499	53.262	17.059	175.086	56	E	8.693	118.402	53.942	32.7	174.838
21	T	8.331	109.252	60.722	71.344	175.171	57	L	8.989	125.363	53.946	44.108	176.843
22	F	9.124	120.331	56.873	43.723	172.967	58	T	8.521	117.723	ND	69.499	176.531
23	K	8.904	122.939	56.359	34.737	175.269	59	P			64.195	31.874	
24	V	9.159	127.312	60.351	34.738	175.522	60	Y	7.649	115	58.016	37.173	176.711
25	E	9.457	129.21	54.905	33.553	173.262	61	S	7.706	116.489	57.324	64.257	175.478
26	L	9.139	129.677	54.463	41.437	176.622	62	L	8.516	124.285	55.91	43.967	174.193
27	D	9.105	122.379	57.435	39.846	178.705	63	D	8.439	117.076	54.246	41.614	176.653
28	N	7.581	116.199	52.665	37.058	176.699	64	K	7.789	120.994	55.341	35.581	175.738
29	K	8.16	111.551	58.184	29.221	175.957	65	G	8.127	107.438	45.852		175.983
30	H	8.054	120.135	56.148	29.265	176.355	66	R	8.476	120.094	53.942	33.513	173.204
31	V	8.305	124.352	61.387	33.61	173.586	67	I	9.019	125.521	62.542	39.971	175.439
32	V	9.158	125.647	59.924	35.249	175.513	68	T	9.085	118.012	61.559	69.841	175.991
33	L	8.45	129.411	54.688	43.15	174.716	69	F	7.608	122.897	58.658	43.289	175.286
34	C	9.57	122.374	57.735	33.213	177.644	70	R	7.487	126.185	ND	32.009	172.036
35	R	7.682	119.955	54.334	33.343	174.043	71	Y	ND	ND	59.191	39.063	ND
36	I	9.215	121.33	61.965	38.876	175.992	72	K	8.585	123.394	56.218	33.37	ND

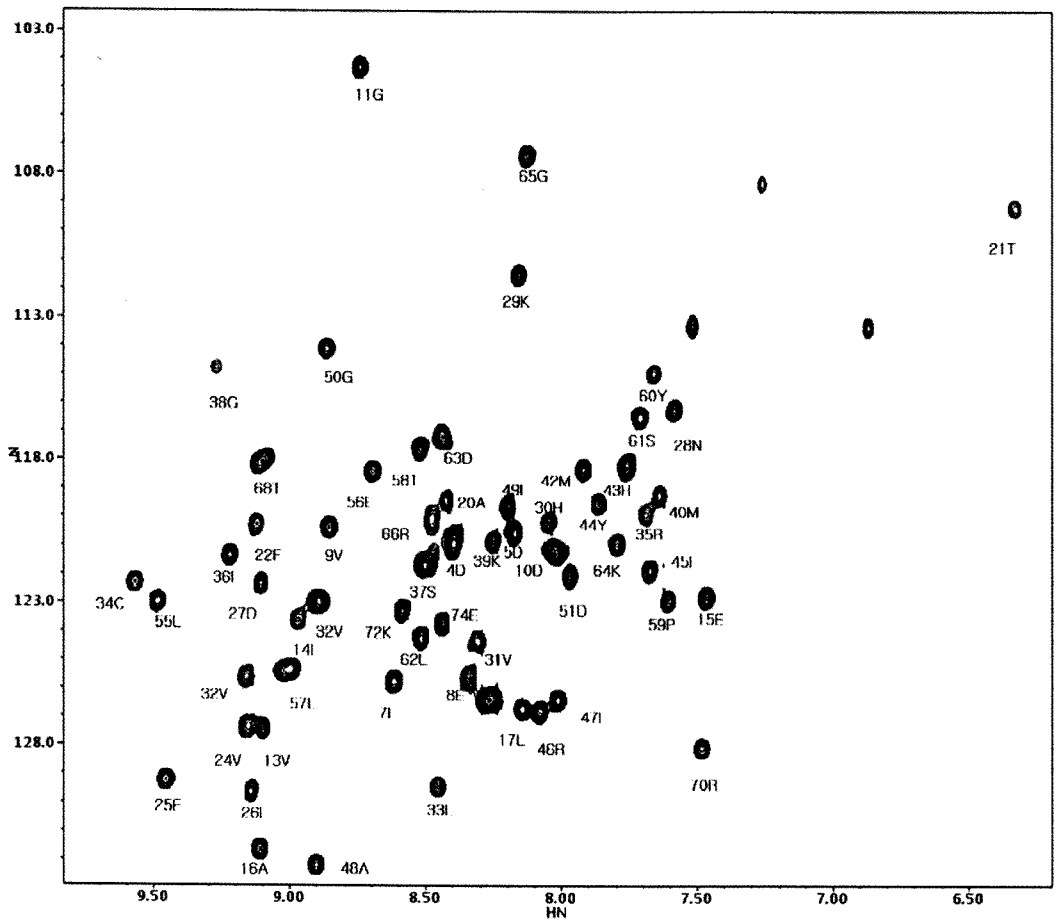

Fig. 1. 2D ^{1}H and ^{15}N HSQC spectrum of HP1298. The each resonance in the spectrum is labeled with the assigned amino acid residues. Unassigned peaks are Trp sidechain and sidechains of Gln and Asn

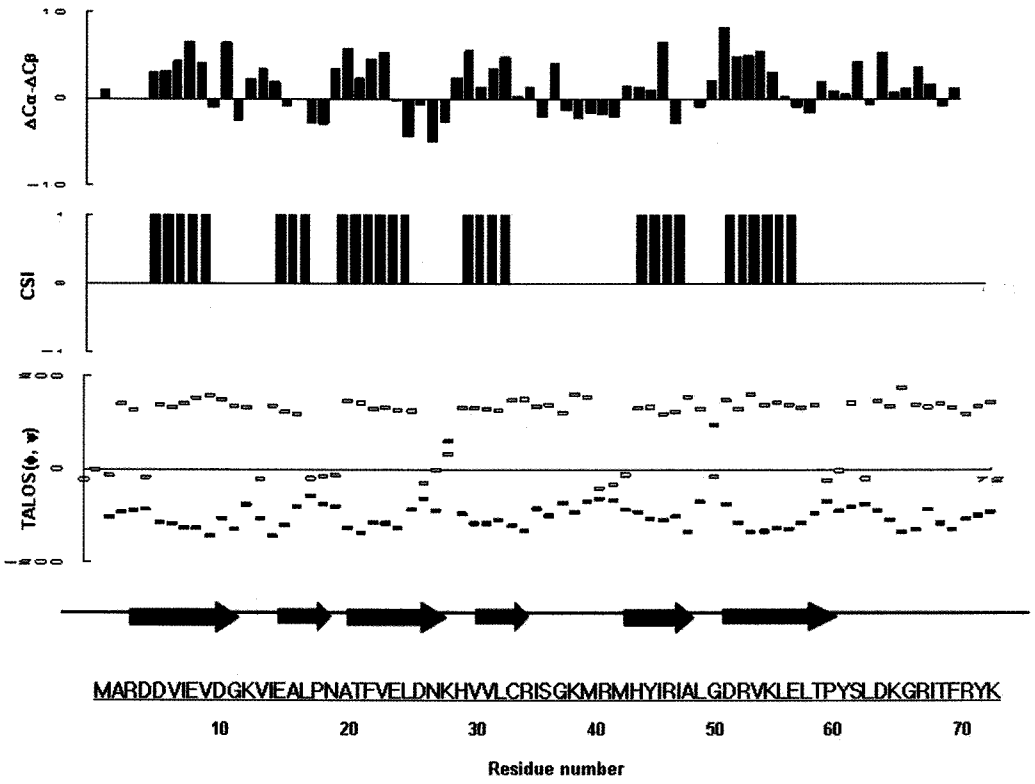

Fig. 2. Summary of backcone resonance assignment of HP1298. Delta values (ΔCα – ΔCβ) of backbone carbon to random coil chemical shifts were plotted. In the consensus CSI, the values '1' represents the β-strand tendency, while '-1' represents the opposite pattern (α-helical tendency). Backbone dihedral angles (phi, psi) were calculated using TALOS. Open and filled rectangles indicated the phi (Φ) and psi (Ψ) angle, respectively. HP1298 is mostly composed with β-strand.

Acknowledgement

This work is financially supported by the Korea Science and Engineering Foundation(KOSEF) grant funded by the Korea government(MOST) (Innovative Drug Research Center for Metabolic and Inflammatory Disease) and 2007 BK21 project for Medicine, Dentistry, and Pharmacy. We thank NCIRF and KBSI for using NMR machines.

REFERENCES

1. J. G. Kusters, M. M. Gerrits, J. A. Van Strijp, and C. M. Vandenbroucke-Grauls, Coccoid forms of *Helicobacter pylori* are the morphologic manifestation of cell death, *Infect. Immun.* **65**, 3672-3679 (1997).
2. P. W. O'Toole, M. C. Lane, and S. Porwollil, *Helicobacter pylori* motility, *Microbes Infect.* **2**, 1207-1214 (2000).
3. M. J. Blaser, *Helicobacter pylori* and the pathogenesis of gastroduodenal inflammation, *J. Infect. Dis.* **161**, 626–633 (1990).
4. D. Forman, D. G. Newell, F. Fullerton, J. W. Yarnell, A. R. Stacey, N. Wald, and F. Sitas, Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation, *Br. Med. J.* **302**, 1302–1305 (1991).
5. J. Parsonnet, S. Hansen, and L. Rodriguez, et al. *Helicobacter pylori* infection and gastric lymphoma, *N. Engl. Med.* **330**, 1267-1271 (1994).
6. F. T. Jean, W. Owen, R. K. Anthony, A. C. Rebecca, G. S. Granger, D. F. Robert, A. K. Karen, P. K. Hans, G. Steven, A. D. Brian, N. Karen, Q. John, Z. Lixin, F. K. Ewen, G. Anna, M. Keith, M. F. Lisa, L. Norman, D. A. Mark, K. H. Erin, E. B. Douglas, M. W. Jeanine, F. Claire, B. Cheryl, W. Larry, W. Erik, S. H. William, B. Mark, D. K. Peter, O. S, Hamilton, M. F. Claire, and V. Craig, The complete genome sequence of the gastric pathogen *Helicobacter pylori, Nature* **388**:7, 539-547 (1997).
7. R. A. Alm, L. S. King, D. T. Moir, B. L. King, E. D. Brown, P. C. Doig, D. R. Smith, B. Noonan, B. C. Guild, B. L. deJonge, G. Carmel, P. J. Tummino, A. Caruso, M. Uria-Nickelsen, D. M. Mills, C. Ives, R. Gibson, D. Merberg, S. D. Mills, Q. Jiang, D. E. Taylor, G. F. Vovis, and T. J. Trust, Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen, *Helicobacter pylori. Nature* **397**, 176-180 (1999).
8. I. G. Boneca, H. de Reuse, J. C. Epinat, M. Pupin, A. Labigne, and I. Moszer, A revised annotation and comparative analysis of *Helicobacter pylori* genomes, *Nucleic Acids Res.* **31**, 1704-1714 (2003).
9. G. Bodenhausen, and D. J. Ruben, Heteronuclear 2D correlation spectra with double in-phase transfer steps, *Chem. Phys. Letters* **69**, 185-189 (1980).

10. M. Wittekind, and L. Mueller, HNCACB, a highsensitivity 3D NMR experiment to correlate amide proton and nitrogen resonances with the α-carbon and β-carbon resonances in proteins, *J. Magn. Reson. ser.* **B 101**, 214-217 (1993).
11. T. Yamazaki, W. Lee, M. Revington, D. L. Mattiello, F. W. Dahlquist, C. H. Arrowsmith, and L. E. Kay, An HNCA pulse scheme for the backbone assignment of ^{15}N, ^{13}C, ^{2}H labeled proteins: application to a 37-kDa trp repressor-DNA complex, *J. Am. Chem. Soc.* **116**, 6464-6465 (1994).
12. D.G. Reid, "Protein NMR Techniques." Humana Press, New Jersey, 1997.
13. F. Delaglio, S. Grzesiek, G. W. Vuister, G. Zhu, J. Pfeifer, and A. Bax, NMRPipe: a multidimensional spectral processing system based on UNIX pipes, *J. Biomol. NMR* **6**, 277-293 (1995)
14. B. A. Johnson, and R. A. Blevins, NMRView: A computer program for the visualization and analysis of NMR data, *J. Biomol. NMR* **4**, 603-614 (1994).
15. D. S. Wishart, and B. D. Sykes, The ^{13}C chemical-shift index: a simple method for the identification of protein secondary structure using ^{13}C chemical-shift data, *J. Biomol. NMR.* **4**, 171-180 (1994).
16. C. Gabriel, D. Frank, and A. Bax, Protein backbone angle restraints from searching a database for chemical shift and sequence homology, *J. Biomol. NMR* **13**, 289-302 (1995).
17. W. J. Metzler, K. L. Constantine, M. S. Friedrichs, A. J. Bell, E. G. Ernst, T. B. Lavoie, and L. Muller, Characterization of the three-dimensional solution structure of human profiling: ^{1}H,^{13}C, and ^{15}N NMR assignments and global folding pattern, *Biochemistry* **32**, 6201-6211 (1993).
18. S. Spera, and A. Bax, Empirical correlation between protein backbone conformation and C.alpha. and C.beta. ^{13}C nuclear magnetic resonance chemical shifts, *J. Am. Chem. Soc.* **113**, 5490-5492 (1991).
19. D. S. Wishart, B. D. Sykes, and F. M. Richards, Relationship between nuclear magnetic resonance chemical shift and protein secondary structure, *J. Mol. Biol.* **222**, 311-333 (1991).
20. W. J. Fairbrother, A. G. Palmer, M. Rance, J. Reizer, M. H. Saier, and P. E. Wright, Assignment of the aliphatic proton and carbon-13 resonances of the Bacillus subtilis

glucose permease IIA domain using double- and triple-resonance heteronuclear three-dimensional NMR spectroscopy, *Biochemistry* **31**, 4413-4425 (1992).