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Backbone ^{1}H, ^{15}N, and ^{13}C resonance assignments and secondary structure prediction of NifU-like protein, HP1492 from *Helicobacter Pylori*

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Abstract HP1492 is a NifU-like protein of *Helicobacter pylori* (*H. pylori*) and plays a role as a scaffold which transfer Fe-S cluster to Fe-S proteins like Ferredoxin. To understand how to bind to iron ion or iron-sulfur cluster, HP1492 was expressed and purified in *Escherichia coli* (*E. coli*). From the NMR measurement, we could carry out the sequence specific backbone resonance assignment of HP1492. Approximately 91% of all resonances could be assigned unambiguously. By analyzing results of CSI and TALOS from NMR data, we could predict the secondary structure of HP1492, which consists of three α-helices and three β-sheets. This study is an essential step towards the structural characterization of HP1492.

Keywords *Helicobacter pylori*, NifU-like protein, HP1492, NMR

Introduction

H. pylori is a gram-negative and spiral shaped bacteria, which can survive in the extreme acidic pH and is related to gastritis, peptic ulcer, gastric carcinoma or lymphoma.[1-4] There exist some antibiotics used for *H. pylori* infection, but the global prevalence of antibiotics-resistant *H. pylori* has increased over the past few decades.[5-6] In addition, various therapies which recently were used for *H. pylori* infection work against not only *H. pylori* but also other beneficial bacteria, causing side effects.[7] Especially, there are no specific antibiotics for *H. pylori*. Therefore, it is necessary to overcome problems of antibiotic resistance and discover novel antibiotics selective to *H. pylori*. One method of drug discovery is to identify three-dimensional (3D) structure of the biological target related to disease allowing design of lead compounds or drug candidates. As a part of drug discovery and our structural genomics on *H. pylori*, we studied the solution structure of HP1492 using NMR spectroscopy.

HP1492 consists of 89 amino acid residues with a molecular weight of 12.28 kDa and a calculated isoelectric point of 7.81. HP1492 is known as a NifU-like protein which has a role of scaffold transferring Fe-S cluster to Fe-S proteins like Ferredoxin.[8-9] Fe-S proteins are essential in cellular activities such as TCA cycle, heme biosynthesis, iron homeostasis and etc.[10-11] Fe-S clusters which exist in Fe-S proteins are required for critical biochemical pathway like respiration. Assembly of these iron cofactors is a carefully controlled process in cells to avoid toxicity from free iron and sulfide.[12] Therefore, severe defects in Fe-S cluster can decrease the

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growth of cell or cause cell death.[13] In addition, iron plays a vital role in all microorganisms. To obtain iron, they have developed sophisticated mechanisms such as the siderophore system. Especially, various pathogens including *H. pylori* synthesize siderophores to take iron ion from host. Accordingly, inhibition of Fe-S cluster transfer by blocking the Fe-S binding site of HP1492 or absorption of iron from host can be new approach to develop antibiotics for *H. pylori*.

Here, we report the sequence-specific backbone resonance assignments of HP1492. This result will facilitate the structure determination of HP1492. Furthermore, it will make it possible to understand mechanism of transferring Fe-S cluster to Fe-S proteins.

Experimental Methods

Cloning, expression, and purification- The HP1492 gene was cloned into a pCold vector which has NdeI and XhoI cutting site by standard cloning protocols. The recombinant plasmid was transformed into *Escherichia coli* strain BL21 (DE3) and this plasmid was purified and sequenced to confirm the exact gene cloning of HP0315 (Bioneer, Daejeon, KR). This construct contains N terminal $(His)_6$-tag which facilitates the efficiency of purification. *E. coli* containing this plasmid was cultured in M9 minimal media supplemented with 1.0 *g*/*l* [U-^{13}C] glucose and 1.0 *g*/*l* [^{15}N] NH_4Cl for ^{15}N-labeled and both ^{15}N- and ^{13}C-labeled proteins. Bacterial culture was grown at 37 ℃ to an optical density of 0.5-0.6. After 0.5 mM IPTG induction at 15 ℃ for 24 hrs, the expressed protein was purified with a nickel-chelating column following the manufacturer's recommendations. Finally, the buffer containing purified protein was dialyzed by 20 mM Bis-Tris, 300 mM glycine, 10 mM DTT and 1mM EDTA (pH 6.5). The protein was passed over the superdex 75 column of size exclusion chromatography. All steps for the purification of the proteins were carried out at 4 ℃ and the purity of the protein was confirmed by SDS-PAGE.

NMR experiments and secondary structure prediction of HP1492- All NMR experiments were measured at 298 K on Bruker AVANCE 500 MHz and JEOL ECA 600 MHz NMR spectrometers. ^{15}N-labelled sample was used to measure by Two-dimensional (2D) ^{15}N-HSQC and ^{15}N-HSQC TROSY. The 3D NMR spectra recorded on ^{15}N/^{13}C-labelled sample were a series of triple resonance spectra like HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HN(CA)CO and HNCO to allow sequence specific backbone and side-chain assignments.[14] Proton chemical shift was externally referenced to 2, 2-dimethylsilapeentane-sulfonic acid (DSS), and ^{15}N and ^{13}C chemical shifts were referenced to DSS indirectly.[15] All NMR data were processed by the NMRPipe/nmrDraw[16] and analyzed by the NMRView program.[17] The sequence-specific resonance assignment was carried out using standard procedures.[18-20] The secondary structure of HP1492 was predicted from the values of CSI program.[21] The backbone dihedral torsion angles were calculated using TALOS program which uses a combination of ^{15}N, ^{13}Cα, ^{13}Cβ, ^{1}Hα and ^{13}CO chemical shift of adjacent residues.

Results and Discussion

A 2D ^{1}H-^{15}N HSQC spectrum of HP1492 with assignments is shown in Figure 1. It is not easy to discriminate 3 successive Sers from 53Ser to 55Ser. In addition, we couldn't assign 11Lys and 36 Val because of spectral overlap or signal missing. About 90% of ^{1}HN and 91% of ^{15}N of the backbone amides of HP1492 (excluding the two Pro residues), and 92% of Cα, 87% of Cβ and 91% of CO of HP1492 could be assigned. Chemical shifts of ^{1}HN, ^{15}N, Cα, Cβ and CO are presented in Table 1.

Secondary structure information was acquired on the basis of the chemical shifts, which is a good means for molecular conformation, backbone dihedral angle, hydrogen bond interactions, backbone dynamics and so on. To estimate the residue-specific secondary structure of HP1492, CSI and TALOS programs were applied. The results of CSI and TALOS programs are

presented in Figure 2. CSI characterize deviation of chemical shifts of certain nuclei in amino acid relative to their random coil values. Generally, the values '1' and '-1' in the CSI indicate, respectively, β-strand and α-helical tendencies, while the other values (zero) indicate neither a β–strand nor a helical tendency. Backbone dihedral angles (φ, ψ) information using chemical shift values can be obtained from TALOS.

As a result, the secondary structure prediction of HP1492 is composed of three α-helices and three β-sheets. These correspond to Residues 6Asp-25Leu (α1), 30Asn-33Val (β1), 42Tyr-46Glu (β2), 57Ile-71Ile (α2), 76Glu-79Cys (β3), and 83Ala-87Asp (α3).

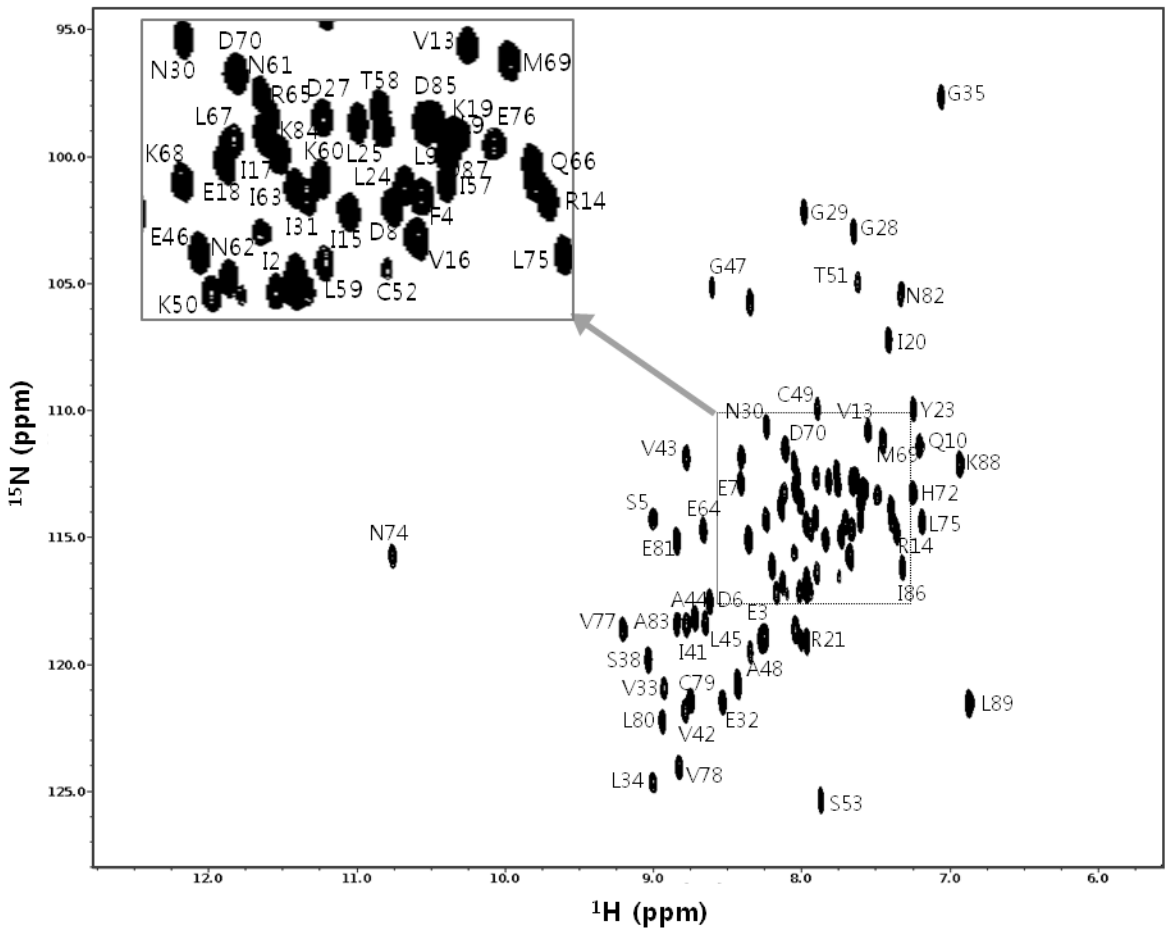

Figure 1. 2D ^{1}H and ^{15}N HSQC of HP1492. The each resonance in the spectrum is labeled with the assigned amino acid residues.

Table 1. Chemical shifts of ^{1}HN, ^{15}N, ^{13}Cα, ^{13}Cβ, and ^{13}CO of HP1492. All chemical shifts were referenced to the frequency of the methyl proton resonance of DSS.

Residue	HN	N	Cα	Cβ	CO	Residue	HN	N	Cα	Cβ	CO
1MET		116.109	53.688	30.087	173.593	46GLU	8.206	116.141	52.706	30.334	173.558
2ILE	7.976	116.756	59.510	36.025	172.936	47GLY	8.613	105.164	42.159		172.087
3GLU	8.260	118.928	53.918	27.997	172.667	48ALA	8.353	119.506	51.155	16.064	175.628
4PHE	7.672	114.653	54.027	38.856	172.905	49CYS	7.903	109.928	56.219	25.015	172.447
5SER	9.013	114.255	54.638	62.255	171.819	50LYS	8.102	117.222	55.133	30.133	174.491
6ASP	8.630	117.516	55.103	37.490	176.139	51THR	7.629	104.957	59.396	66.682	171.709
7GLU	8.419	112.871	56.646	26.651	176.321	52CYS	7.758	116.491	59.933		172.593
8ASP	7.742	114.930	53.532	38.331	174.936	53SER	7.856	121.968	54.488		176.381
9LEU	7.578	113.049	56.327	41.203	174.715	54SER					
10GLN	7.217	111.422	57.817	25.405	175.069	55SER					
11LYS						56LYS			57.547	29.428	175.100
12PHE			63.700	28.580	176.372	57ILE	7.613	114.306	61.054	35.080	175.016
13VAL	7.559	110.788	63.892		174.121	58THR	7.770	112.436	63.754	66.064	173.739
14ARG	7.398	114.328	57.479	26.670	176.165	59LEU	7.905	116.374	55.651	38.992	175.452
15ILE	7.844	115.021	62.141	35.203	176.130	60LYS	7.914	114.190	58.023	29.126	175.219
16VAL	7.681	115.746	64.440	29.094	175.371	61ASN	8.056	112.032	53.381	35.457	175.407
17ILE	8.015	113.583	61.698	33.421	175.026	62VAL	8.136	116.764	64.112	29.306	176.182
18GLU	8.141	113.795	56.488	26.343	176.234	63ILE	7.977	114.431	62.962	35.476	174.556
19LYS	7.596	113.095	56.395	29.848	175.792	64GLU	8.670	114.684	58.500	28.714	174.909
20ILE	7.421	107.188	59.452	36.062	173.963	65ARG	8.034	112.607	56.892	27.235	175.778
21ARG	7.972	119.082	59.437	25.616	171.237	66GLN	7.404	113.813	55.911	25.690	175.639
22PRO			63.359	28.324	175.714	67LEU	8.123	113.232	55.778	39.267	176.324
23TYR	7.256	109.919	56.826	35.404	174.918	68LYS	8.249	114.294	55.106	31.344	176.009
23LEU	7.708	114.391	53.757	39.908	176.345	69MET	7.448	111.147	55.082	30.600	175.334
25LEU	7.825	112.793	52.276	38.059	176.354	70ASP	8.116	111.480	53.412	39.391	174.704
26LYS	7.334	116.171	56.165	29.481	174.331	71ILE	7.767	112.901	60.501	36.170	172.064
27ASP	7.912	112.641	51.513	38.821	173.147	72HIS	7.263	113.224	53.569	30.762	
28GLY	7.657	102.914	42.248		171.174	73PRO			61.730	29.838	174.594
29GLY	7.991	102.193	41.763		169.612	74ASN	10.761	115.706	50.152	35.522	173.052
30ASN	8.245	110.633	49.924	39.191	170.457	75LEU	7.193	114.349	53.202	40.242	173.102
31ILE	7.945	114.669	57.238	38.883	170.816	76GLU	7.499	113.366	51.655	32.040	171.907
32GLU	8.540	121.459	51.757	29.889	172.873	77VAL	9.214	118.613	58.031	31.260	171.621
33VAL	8.937	120.919	60.575	28.384	172.724	78VAL	8.832	124.000	58.523	31.438	172.039
34LEU	9.008	124.655	52.448	38.876	174.036	79CYS	8.755	121.416	54.613	24.797	172.005
35GLY	7.066	97.623				80LEU	8.948	122.192	50.519	41.349	174.380
36VAL						81GLU	8.853	115.128	55.775	27.985	173.991
37LYS			58.23	32.109	172.415	82ASN	7.340	105.421	49.406	37.548	172.133
38SER	9.042	119.831			173.959	83ALA	8.851	118.433	52.276	16.258	176.678
39MET	10.526	122.532				83LYS	8.051	113.079	55.713	28.869	176.265
40LYS			52.131	31.433	171.331	85GLU	7.667	112.804	55.764	27.298	175.797
41ILE	8.785	118.437	57.339	36.365	171.308	86PHE	7.369	114.722	57.333	36.796	173.525
42TYR	8.790	121.788	54.483	37.201	173.399	87ASP	7.604	113.530	53.218	38.030	173.678
43VAL	8.785	111.871	55.496	32.997	170.375	88LYS	6.947	112.094	53.240	30.304	173.274
44ALA	8.728	118.155	48.241	18.491	173.674	89LEU	6.881	121.510	54.224	39.931	173.273
45LEU	8.659	118.354	50.853	41.550	172.894						

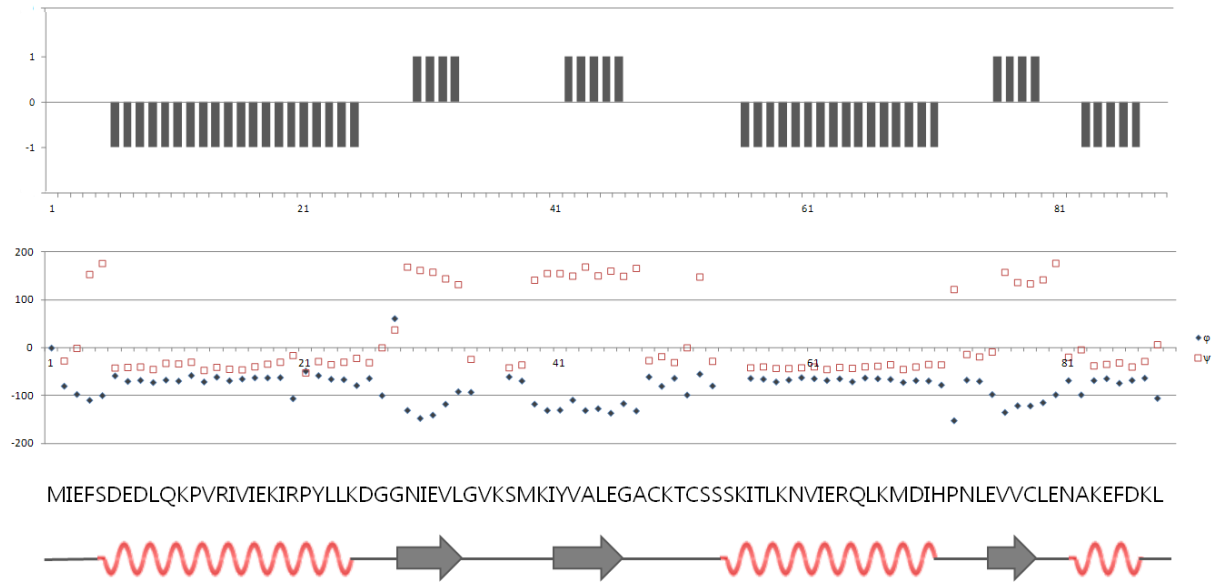

Figure 2. Summary of the secondary structure of HP1492. In the consensus CSI, the values '1' represents the β-strand tendency, while '-1' represents the α-helical tendency. Backbone dihedral angels (phi, psi) were calculated using TALOS. Open and filled rectangles indicate the phi (φ) and psi (ψ) angle, respectively.

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