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Short communication

Backbone ^{1}H, ^{15}N, and ^{13}C Resonance Assignment and Secondary Structure Prediction of HP0495 from *Helicobacter pylori*

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HP0495 (Swiss-Prot ID; Y495_HELPY) is an 86-residue hypothetical protein from *Helicobacter pylori* strain 26695. The function of HP0495 cannot be identified based on sequence homology, and HP0495 is included in a fairly unique sequence family. Here, we report the sequence-specific backbone resonance assignments of HP0495. About 97% of all the ^{1}HN, ^{15}N, $^{13}C\alpha$, $^{13}C\beta$, and ^{13}CO resonances were assigned unambiguously. We could predict the secondary structure of HP0495, 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 HP0495 consists of two α-helices and four β-strands. This study is a prerequisite for determining the solution structure of HP0495 and investigating the protein-protein interaction between HP0495 and other *Helicobacter pylori* proteins.

Keywords: Backbone assignment, HP0495, NMR, Secondary structure

Introduction

Helicobacter pylori is a gram-negative bacterium, measuring 2 to 4 μm in length and 0.5 to 1 μm 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 (Kusters *et al.*, 1997). The organism has 2 to 6 unipolar, sheathed flagella of approximately 3 μm in length, which often carry a distinctive bulb at the end (O'Toole *et al.*, 2000). *H. pylori* is related with many serious gastric problems, ranging from gastritis to gastric carcinoma or lymphoma (Blaser *et al.*, 1990; Forman *et al.*, 1991; Parsonnet *et al.*, 1994). The genome of *H. pylori* has been fully sequenced for two prototype strains (strain 26695 and strain J99) (Jean-F. Tomb *et al.*, 1997). The *H. pylori* strain 26695 genome includes 1,590 genes, whereas the genome of strain J99 includes only 1,491 genes (Alm *et al.*, 1999; Boneca *et al.*, 2003). 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 HP0495, one of the proteins from *H. pylori* by using NMR. The HP0495 gene of *Helicobacter pylori* encodes a 86-residue hypothetical protein from *Helicobacter pylori* strain 26695 with a molecular weight of 10192.7 Da and a calculated isoelectric point of 8.71. HP0495 is included in a fairly unique sequence family. The result of sequence homology search showed that HP0495 has a restricted sequence homology with unknown proteins from several bacteria, and has not been classified in a protein domain family (Pfam). Here, we report the sequence-specific backbone resonance assignments and predict the secondary structure of HP0495.

Materials and Methods

HP0495 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 in M9 medium. Cells were grown at 37°C until an OD_{600} of 0.6 and then induced with 1 mM IPTG for 4 h. The soluble protein was purified using SP-sepharose column (HiPrep™ 16/10 SP FF; Amersham Biosciences) and Gel filtration (Superdex™ 75 10/300; Amersham Biosciences). The NMR sample was prepared at a concentration of about 1.2 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.

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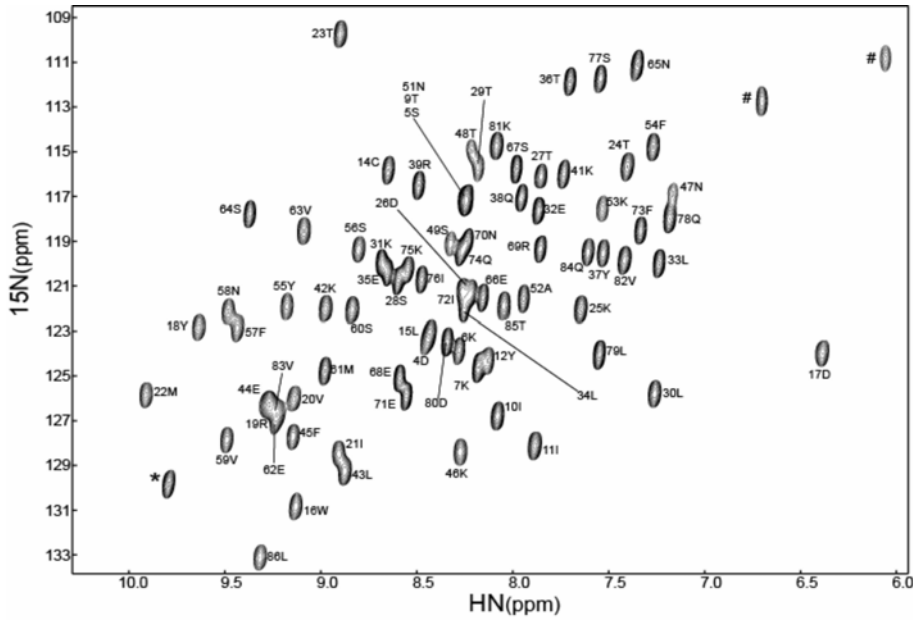

Fig. 1. 2D ^{1}H and ^{15}N HSQC spectrum of HP0495. The each resonance in the spectrum is labeled with the assigned amino acid residues. Three unassigned peaks are Trp sidechain (*) and sidechains of Gln and Asn (#).

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 (Bodenhausen *et al*., 1980; Wittekind *et al*., 1993; Yamazaki *et al*., 1994; Reid *et al*., 1997). 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 (Delaglio *et al*., 1993), and were analyzed using the program NMRView (Johnson *et al*., 1994). The secondary structure was predicted from the chemical shift values using Chemical Shift Index (CSI, Wishart *et al*., 1994) and Torsion Angle Likelihood Obtained from Shift and sequence similarity (TALOS, Gabriel *et al*., 1999).

Results and Discussion

HSQC spectrum of HP0495 showed good peak resolution (Fig. 1). Assignments of HP0495 were achieved nearly completely (Table 1). The backbone amide ($^{1}H_N$ and ^{15}N) resonances were completely assigned except 4 prolines and N-terminal two residues (residues 1 and 3). These two residues (1MET and 3SER) are not observed in a 2D ^{1}H-^{15}N HSQC spectrum, due to the rapid exchange of the amide hydrogens. Therefore, they could not be assigned. Although all ^{13}Cα, ^{13}Cβ resonances were also completely assigned, only 92% of ^{13}CO resonances were assigned, because of overlapping with some peaks. All available ^{15}N, ^{13}C and ^{1}H chemical shifts of backbone as well as side-chain atoms of HP0495 have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number: 15101.

For predicting secondary structure of HP0495, chemical shift difference method between measured values and random-coil values using Cα, Cβ, and (ΔCα-ΔCβ) (Metzler *et al*., 1993) and CSI protocol was used. Correlations have been observed between Cα (Spera & Bax, 1991; Wishart *et al*., 1991; Fairbrother *et al*., 1992) and Cβ (Spera & Bax, 1991) 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 two potentially helical regions, and four or five potentially β-strand regions (because the division of first and second strands are ambiguous). The regions of α-helices and 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 HP0495 (Fig. 1) can be used to detect the protein-protein interaction between HP0495 and other *Helicobacter* proteins.

Table 1. Chemical shifts of ^{1}HN, ^{15}N, ^{13}CO, ^{13}Cα and ^{13}Cβ of HP0495. All chemical shifts were referenced to the frequency of the methyl proton resonance of DSS

Residue	HN	N	CO	CA	CB	Residue	HN	N	CO	CA	CB
1MET	ND	ND	ND	ND	ND	44GLU	9.2	126.2	175.29	54.89	34.38
2PRO			ND	62.64	32.48	45PHE	9.1	127.6	174.82	57.98	39.49
3SER	ND	ND	174.09	58.65	64.03	46LYS	8.21	128.32	174.89	54.89	32.5
4ASP	8.4	123.44	ND	54.37	41.51	47ASN	7.07	116.69	174.23	53.63	40.28
5SER	8.19	117.2	174.63	58.72	63.97	48THR	8.17	114.98	174.67	61.11	70.65
6LYS	8.23	123.78	177.05	56.33	32.97	49SER	8.27	119.15	175.51	58.36	64.58
7LYS	8.13	124.59	ND	54.31	32.81	50LYS	8.67	123.85	ND	58.36	32.09
8PRO			176.87	63.27	32.38	51ASN	8.21	116.99	174.56	53.1	38.23
9THR	8.2	117.22	174.1	62.43	70.06	52ALA	7.88	121.37	176.3	53.04	18.35
10ILE	8.04	126.89	174.76	60.86	39.01	53LYS	7.44	117.32	175.38	56.8	33.34
11ILE	7.83	128.12	174.44	60.09	38.92	54PHE	7.2	114.66	174.36	54.83	41.66
12TYR	8.09	124.32	ND	56.63	40.19	55TYR	9.12	121.86	174.22	57.7	41.66
13PRO			175.54	62.74	36.19	56SER	8.75	119.3	172.93	57.62	65.07
14CYS	8.57	115.64	172.54	55.86	31.18	57PHE	9.37	122.7	175.03	56.68	43.46
15LEU	8.38	122.93	175.35	55.55	42.05	58ASN	9.42	121.98	174.65	52.81	42.73
16TRP	9.06	130.77	173.08	56.61	33.81	59VAL	9.41	127.72	174.85	60.83	35.2
17ASP	6.3	123.81	175.17	52.68	43.16	60SER	8.76	121.9	174.71	56.58	65.22
18TYR	9.57	122.69	174.92	57.27	40.67	61MET	8.9	124.65	173.25	55.79	37.9
19ARG	9.23	126.24	175.01	55.11	30.95	62GLU	9.18	126.97	175.48	57.24	30.88
20VAL	9.07	126	174.53	61.72	35.42	63VAL	9.02	118.31	177.1	59.35	35.37
21ILE	8.84	128.35	176.48	60.44	38.4	64SER	9.29	117.64	173.54	61.54	64.64
22MET	9.85	125.69	177.15	54.73	40.24	65ASN	7.27	110.89	173.66	51.64	38.88
23THR	8.83	109.54	173.72	61.8	68.56	66GLU	8.08	121.45	177.42	59.52	30.25
24THR	7.32	115.61	169.79	58.48	69.72	67SER	7.92	115.59	177.53	62.06	62.3
25LYS	7.59	121.96	176.02	55.6	32.72	68GLU	8.53	125.02	177.67	59.66	31.09
26ASP	8.16	121.17	176.69	53.89	40.77	69ARG	7.79	119.25	180.56	58.69	30
27THR	7.79	116.06	175.82	61.26	69.11	70ASN	8.17	118.94	176.94	55.39	37.93
28SER	8.52	120.6	176.74	63.53	62.38	71GLU	8.48	125.83	178.61	59.87	30.29
29THR	8.13	115.53	176.66	65.52	68.45	72ILE	8.19	121.14	177.46	66.41	38.2
30LEU	7.19	125.83	177.38	57.49	42.18	73PHE	7.25	118.37	178.07	62.25	40.25
31LYS	8.62	119.86	177.66	60.98	32.74	74GLN	8.19	119.3	178.92	58.9	28.52
32GLU	7.78	117.46	179.05	59.5	29.8	75LYS	8.48	120.17	180.26	60	33.01
33LEU	7.14	119.88	180.11	57.93	41.64	76ILE	8.4	120.66	177.63	66.17	38.21
34LEU	8.19	121.99	180.69	58.07	39.65	77SER	7.46	111.61	174.8	61.38	63.66
35GLU	8.59	120.31	179.85	59.33	29.84	78GLN	7.1	117.88	176.55	55.68	29.48
36THR	7.62	111.68	176.05	65.04	69.87	79LEU	7.47	124.01	178.51	55.56	41.65
37TYR	7.45	119.44	175.38	61.34	37.36	80ASP	8.28	123.42	177.31	56.87	41.51
38GLN	7.87	117	174.08	56.89	27.19	81LYS	8.05	114.61	175.38	56.98	31.23
39ARG	8.44	116.3	ND	53.87	30.68	82VAL	7.33	119.64	176.73	63.39	32.2
40PRO			175.69	63.4	32.52	83VAL	9.16	126.4	176.07	62.49	33.5
41PHE	7.68	115.89	173.72	56.05	42.16	84GLN	7.53	119.47	173.23	56.2	32.9
42LYS	8.88	121.88	174.74	55.05	35.57	85THR	7.97	121.88	172.37	61.94	70.24
43LEU	8.83	129.28	175.5	54.51	44.56	86LEU	9.25	132.96	ND	56.95	45.68

*ND; not detected
**unit; ppm

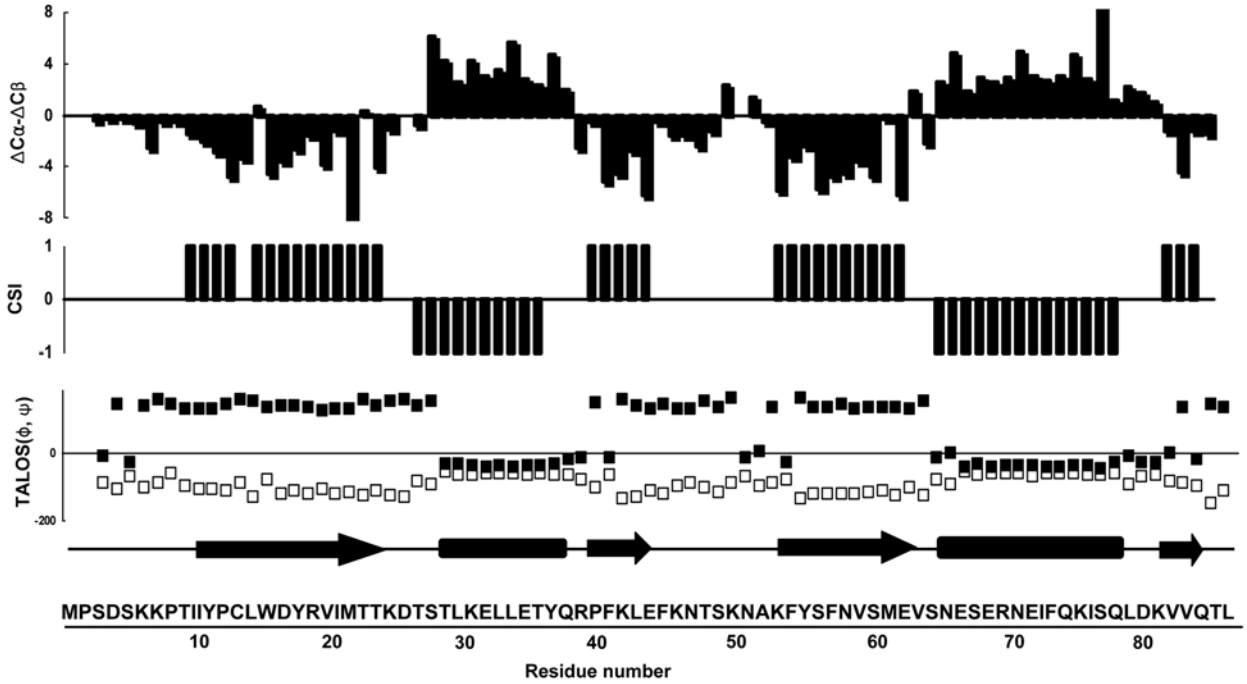

Fig. 2. Summary of backcone resonance assignment of HP0495. Delta values (ΔCα-ΔCβ) of backbone carbon to random coil chemical shifts were plotted. In the consensus CSI, the values '1' represents the β-strand tendency, while '-1' represents the opposite pattern (α-helical tendency). Backbone dihedral angles (phi, psi) were calculated using TALOS, open and filled rectangles indicated the phi (ϕ) and psi (ψ) angle, respectively. Predicted secondary structure elements of HP0495 were indicated by rectangles for α-helices and arrows for β-strands.

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