Journal of the Korean Magnetic Resonance Society **2009**, *13*, 108-116

Backbone NMR assignments of a putative secretory protein from *Helicobacter pylori*, using a high-field (900 MHz) NMR

Dae-Won Sim[1], Hee-Chul Ahn[2] and Hyung-Sik Won[1,*]

[1]*Department of Biotechnology, College of Biomedical and Health Science, Konkuk University, Chungju, Chungbuk 380-701, Korea*
[2]*Chemical Analysis Center, Korea Institute of Science and Technology, Seoul 136-791, Korea*

Abstract : The HP0902, a homodimeric 22.1 kDa protein, has been suggested as a putative secretory protein from *Helicobacter pylori*, although the protein possesses no signal peptide for secretion. Since it may be associated with the virulence of the bacterium, NMR study has been initiated in terms of structural genomics. In our previous effort to assign the backbone NMR resonances, using 800 MHz NMR machine at pH 7.8, the resonances from eight of the 99 residues could not be assined due to missing of the signals. In this work, to enhance the extent of assignments, a 900 MHz machine was employed and the sample pH was reduced down to 6.5. Finally, almost all signals, except for those from G9 and S24, could be clearly assigned. The determined secondary structure using the assined chemical shifts indicated that the HP0902 consists of 11 β-strands with no helices. In our database search result, HP0902 was predicted to interact with VacA (Vacuolating cytotoxin A), which is a representative virulence factor secreted from *Helicobacter pylori*. Thus, molecular interaction between HP0902 and VacA would be worthy of investigation, on the basis of the present results of NMR assignments.
Keywords : Chemical Shift Assignments, *Helicobacter pylori*, HP0902, VacA, Virulence Factor

INTRODUCTION

Helicobacter pylori is known to colonize the stomach in approximately half of the human population and to cause severe gastric disorders, including chronic gastritis, peptic ulcers and stomach cancer [1-3]. Due to this importance as a human pathogen, gemomic

* To whom correspondence should be addressed. E-mail : wonhs@kku.ac.kr

sequencing has been completed for three different strains (26695, J99, and HPAG1) of *H. pylori* [4-6]. Subsequently, structural genomics studies are also in progress world-widely on the bacterium, to facilitate the selection of drug targets [7]. HP0902, previously grouped into hypothetical proteins, was one of our structural genomics targets from the *H. pylori* strain 26695. Recently, it has been suggested that the protein may be involved in virulence of *H. pylori* [8, 9].

It would be reasonable to consider the secreted proteins from *H. pylori* as candidates for virulence factor of the bacterium, since they can contribute to gastric inflammation and epithelial damage. For example, the protein VacA (Vacuolating cytotoxin A), a representative toxin from *H. pylori*, is secreted from the bacterium and intoxicates human gastric cells by causing a defect in vesicular trafficking [10, 11]. In the recent *in vitro* study by Kim *et al.* [8] to identify the secreted proteins from *H. pylori*, HP0902 was also identified as one of the 13 released protein from the bacterium. Among the 13 proteins identified, five are functionally unkown and five proteins have no signal peptide for secretion. HP0902 was unique in that it has no signal peptide and it is functionally unkown. HP0902 has been also suggested to be over-expressed in a mutant strain of *H. pylori* lacking the *fdx*A gene, which regulates the resistance to an antibiotics [9]. Based on those reports, HP0902 can be regarded as a good structural genomics target to identify its function and virulence from structural information, and thus we have initiated NMR study of the protein, to first obtain complete NMR assignments.

In the previous biochemical study, we have revealed that the protein is highly thermostable and behaves as a dimer with 22.1 kDa molecular weight [7]. In addition, NMR results have indicated that HP0902 can be structurally grouped into a Cupin superfamily, of which members are diverse in function [12]. Unfortunately, however, the previous backbone NMR assignments, which were conducted on a 800 MHz machine at pH 7.8, were not complete with eight missing residues (M1, H5, G9, F12, L15, S24, G62, and D63), out of the total 99 possible assignments, in the NMR spectra. In this study, we report a database search result on HP0902 together with an extension of NMR assignments, by using 900 MHz NMR macine at pH 6.5.

EXPERIMENTAL

The isotope-enriched [^{15}N, ^{13}C]HP0902 protein with N-terminal His-tag was produced using the pET-15b vector and the *E. coli* BL21(DE3)pLysS strain, as described previously [7]. After purification by a nickel-affinity chromatography, the tagged histidines were cleaved by thrombin, producing the protein with three additional residues (GSH-) still attached to the N-terminus. Then, the protein was further purified by removing the thrombin and other impurities, via the sequential application of nickel-affinity, anion-exchange, and size-exclusion chromatography. For NMR, the purified solution was buffer-exchanged with a 25 mM sodium phosphate buffer at pH 6.5 containing 100 mM NaCl, 2 mM DTT, and 1 mM EDTA. Finally, the solution was concentrated to the final concentration of approximately 1.5 mM and D_2O was added into the solution up to 7%(v/v), for the lock signal in NMR experiments. The 2D-[^{1}H-^{15}N]HSQC and the TROSY-type of 3D-HNCACB and 3D-HN(CO)CACB spectra were acquired at 313 K on a Varian VNMRS 900 spectrometer. All NMR spectra were processed using the NMRPipe/NMRDraw software [15], and analyzed with NMRView software [16]. Sequential assignments were achieved by verifying and linking of peak clusters [7, 17] as described previously. Secondary structure was determined by combining the CSI and TALOS analysis with the assigned chemical shifts that were referenced to DSS, as described previously [7].

RESULTS AND DISCUSSION

We first prediced the protein-protein interactions of HP0902 in *H. pylori*, using two different tools of bioinformatics accessible through websites [13,14] : PIM*Rider* (http://pim.hybrigenics.com) and *hp*-DPI (http://dpi.nhri.org.tw/hp/). Both the two databases commonly indicated that HP0902 would interact with HP0588 (a ferrodoxin-like protein), HP1409 (unknown), and VacA (HP0887). Among them, as noted in the Introduction, VacA is a representative toxin secreted from *H. pylori*. Thus, if HP0902 indeed interacts with VacA, the protein without signal peptide for secretion could be also released from the bacterium, as observed by Kim *et al.* [8]. In addition, it might be possible that HP0902 could

act as a virulence factor or a co-factor for VacA. To clearify all these possibility, the molecular interaction between HP0902 and VacA would be worthy of investigation. NMR provides the most efficient tool to screen and investigate molecular interactions at the level of atomic structure. For this, the most fundamental information would be the backbone NMR chemical shifts. In this respect, since our previous approach was not successful to assign all the backbone NMR signals, the assignments were performed again in this work under different condition.

The missing of some resonances in the previous study using 800 MHz machine at pH 7.8 was attributable to a severe peak broadening, probably due to a chemical exchange at an intermediate time scale. Thus, in the present work, we used a higher magnetic field (900 MHz) to overecome the line broadening and an acidic (pH 6.5) buffer condition to alter the exchanging rate of amide protons. Fig. 1. shows 2D-[^{1}H, ^{15}N]HSQC spectrum where all of the backbone amide resonances detected was completely assigned. As indicated in the Experimental section, our recombinant protein possesses the N-terminal, additional three-residue sequence (GSH-), which did not show any signal in the NMR spectra, as in the previous study. However, as illustrated in Fig. 2 with sequential $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ connectivities in the HNCACB spectrum, the H5 and L15 resonances that had not been observed previously from 800 MHz spectrum could be clearly assigned. Likewise, the residues M1, F12, G62 and D63, which had been also missing in the previous study, showed detectable signals. Consequently, among the eight residues that were previously not detected, six could be additionally assigned in the present work (Fig. 1). Although the amide signals from G9 and S24 were not detected yet, their $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ resonances could be assigned by the inter-residue ^{13}C correlation in the triple resonance spectra. Consequently, the CSI and TALOS analysis could be completely applied to the HP0902 full sequence, mainly using the ^{13}C chemical shifts (Fig. 3). The determined secondary structure using the new chemical shifts set was not different from the previously determined one, since the ^{13}C chemical shifts were not significantly perturbed under the changed buffer condition. The previously undetected residues were located at the loop regions connecting the β-strands. Thus, as concluded previously, HP0902 consists of 11 β-strands and would belong to an all-β topology of cupin superfamily, of which members share a β-barrel fold.

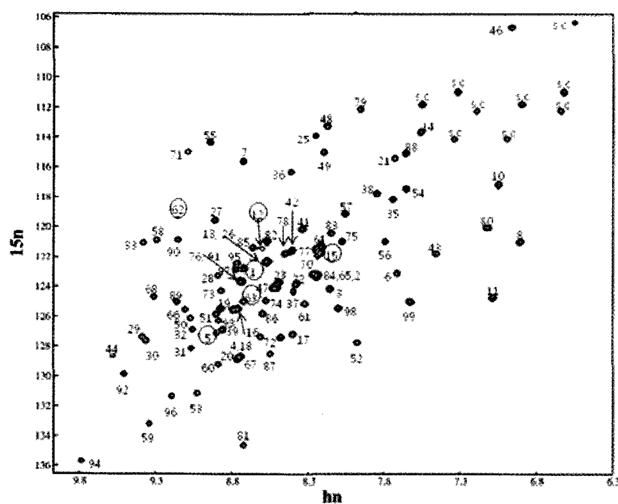

Fig. 1. NMR assignments of the [^{13}C, ^{15}N]HP0902 at pH 6.5 on the 900 MHz 2D-[^{1}H, ^{15}N]HSQC spectrum. Side-chain signals are marked with "s.c". The other peaks from backbone amides are marked with the corresponding residue numbers (refer to Fig. 3. for the amino acid sequence). The signals that had not been detected previously at pH 7.8 on a 800 MHz spectrum are indicated by circles.

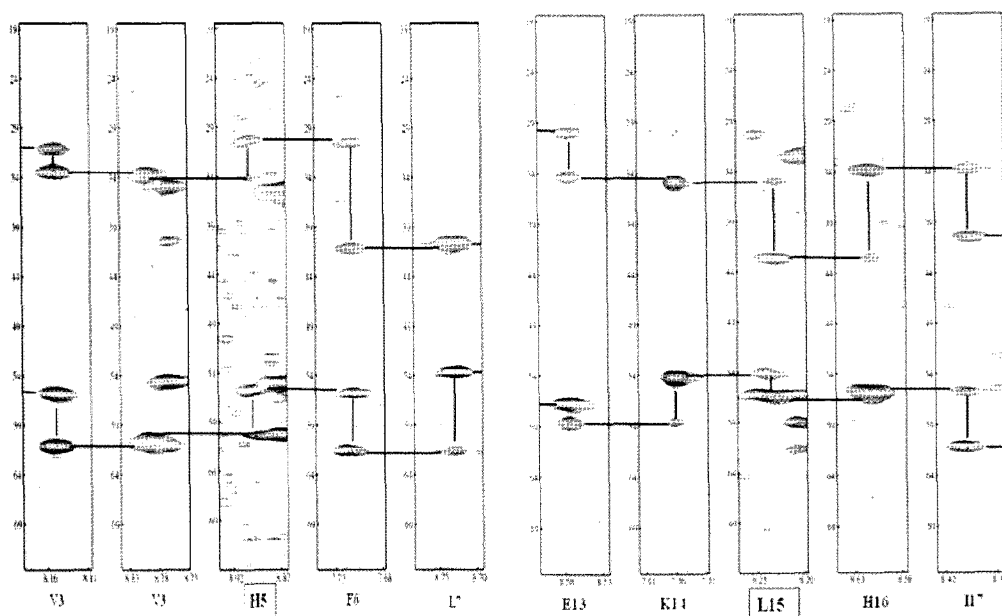

Fig. 2. Strip plots of 900 MHz HNCACB spectrrum showing sequential $^{13}C^{\alpha}$ (black, positive signals) and $^{13}C^{\beta}$ (red, negative signals) connectivities in the V3~L7 and E13~I17 regions. The resonances from residues H5 and L15 had not been observed in the previous study using 800 MHz NMR.

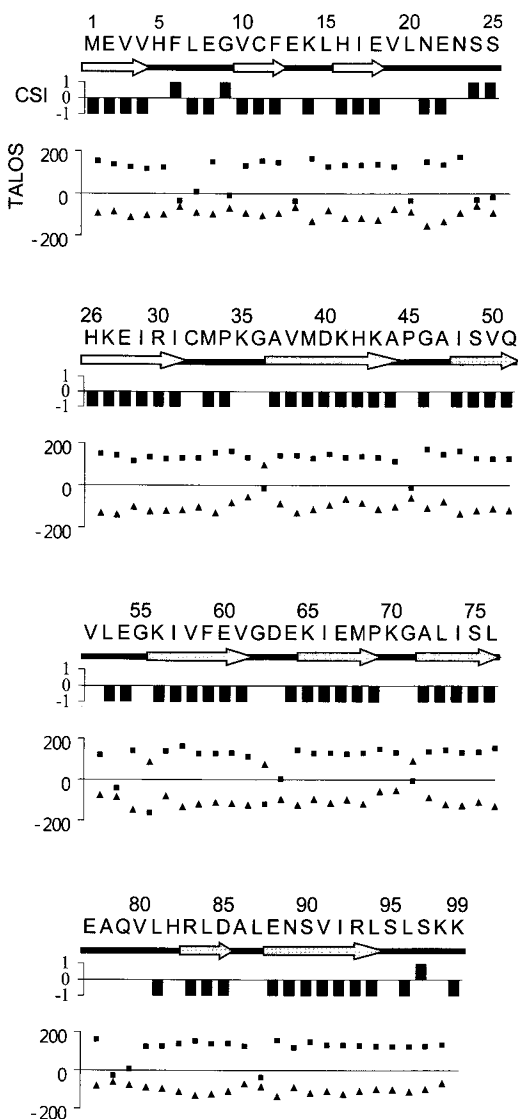

Fig. 3. Secondary structure determination of HP0902. The CSI results are depicted as a consensus-CSI ("1" for α-helix and "-1" for β-strand tendency). The TALOS results show the predicted backbone dihedral ϕ (triangles) and φ (circles) angles with the standard deviation (error bars). The secondary structure element was defined at the region where three or more consecutive residues evidence the same secondary structure preference seen in both the CSI and TALOS results. The determined secondary structure is displayed with the arrows for β-strands along the sequence.

CONCLUDING REMARKS

Virulence factors of *H. pylori* can be direct targets for new drug developments to treat or prevent gastric diseases. HP0902 can be regarded as a new candidate for virulence factor of *H. pylori*, since the protein was suggested to be secreted from the bacterium and to be related to antibiotic resistance. In order to identify the protein as a virulence factor, the predicted interaction with VacA would be worthy of examination, as well as its inflammation activity against human gastric cells. This study provided near complete backbone NMR assignments of HP0902. Thus, it is expected that the molecular interaction between HP0902 and VacA could be investigated in detail on the basis of the present results. In addition, in terms of structural genomics, the present chemical shifts and secondary structure information will eventually provide the most fundamental and critical data for 3D structure determination and functional studies, which are in progress in our laboratory.

Acknowledgment

This paper was supported by Konkuk University. We thank to the staff at NMR facility in KIST, for assisting the NMR measurements.

REFERENCES

1. Ferreira, A. C., Isomoto, H., Moriyama, M., Fujioka, T., Machado, J. C., Yamaoka, Y. *Helicobacter.* **1**, 28-34 (2008)
2. Bartnik, W. *Pol. Arch. Med. Wewn.* **118**, 426 (2008)
3. Covacci, A., Telford, J. L., del Giudice, G., Parsonnet, J., Rappuoli, R. *Science.* **284**, 1328-1333 (1999)
4. Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S., Dougherty, B. A., Nelson, K., Quackenbush, J., Zhou, L., Kirkness, E. F., Peterson, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H. G., Glodek, A., McKenney, K., Fitzegerald, L. M., Lee, N., Adams, M. D.,

Hickey, E. K., Berg, D. E., Gocayne, J. D., Utterback, T. R., Peterson, J. D., Kelley, J. M., Cotton, M. D., Weidman, J. M., Fujii, C., Bowman, C., Watthey, L., Wallin, E., Hayes, W. S., Borodovsky, M., Karp, P. D., Smith, H. O., Fraser, C. M., Venter, J. C. *Nature.* **388**, 539-547 (1997)

5. Alm, R. A., Ling, L. S., Moir, D. T., King, B. L., Brown, E. D., Doig, P. C., Smith, D. R., Noonan, B., Guild, B. C., deJonge, B. L., Carmel, G., Tummino, P. J., Caruso, A., Uria-Nickelsen, M., Mills, D. M., Ives, C., Gibson, R., Merberg, D., Mills, S. D., Jiang, Q., Taylor, D. E., Vovis, G. F., Trust, T. J. *Nature.* **397**, 176-180 (1999)
6. Oh, J. D., Kling-Bäckhed, H., Giannakis, M., Xu, J., Fulton, R. S., Fulton, L. A., Cordum, H. S., Wang, C., Elliott, G., Edwards, J., Mardis, E. R., Engstrand, L. G., Gordon, J. I. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 9999-10004 (2006)
7. Sim, D.-W., Lee, Y.-S., Kim, J.-H., Seo, M.-D., Lee, B.-J., Won, H.-S. *BMB reports* **42**, 387-392 (2009)
8. Kim, N. Y., Weeks, D. L., Shin, J. M., David, R. S., Young, M. K., Sachs, H. *J. Bacteriol*, **184**, 6155-6162 (2002)
9. Nam, W. H.; Lee, S. M.; Kim, E. S.; Kim, J. H.; Jeong, J. Y. *J. Life Sci.* **2007**, 17, 723.
10. Gangwer, K.A., Mushrush, D.J., Stauff, D.L., Spiller, B., McClain, M.S., Cover, T.L. *Proc. Natl. Acad. Sci. U.S.A* **104**, 16293-16298 (2007)
11. Cover, T.L., Blanke, S.R. *Nat. Rev. Microbiol.* **3**, 320-332 (2005)
12. Dunwell, J. M., Purvis, A., Khuri, S. *Phytochemistry.* **65**, 7-17 (2004)
13. Rain, J.-C., Selig, L., De Reuse, H., Battaglia, V., Reverdy, C., Simon, S., Lenzen, G., Petel, F., Wojcik, J., Schächter, V., Chemama, Y., Labigne, A., Legrain, P. *Nature.* **409**, 211-215 (2001)
14. Lin, C.-Y., Chen, C.-L., Cho, C.-S., Wang, L.-M., Chang, C.-M., Chen, P.-Y., Lo, C.-Z., Hsiung, C.A. *Bioinformatics.* **21**, 1288-1290 (2005)
15. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., Bax, A. *J. Biomol. NMR.* **6**, 277-293 (1995)
16. Johnson, B. A. *Methods Mol. Biol.* **278**, 313-352 (2004)
17. Won, H.-S.; Yamazaki, T., Lee, T.-W., Jee, J.-G., Yoon, M.-K., Park, S.-H., Otomo, T., Aiba, H., Kyogoku, Y., Lee, B.-J. *J. Biomol. NMR.* **16**, 79-80 (2000)