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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N assignments of the tandem WW domains of human MAGI-1/BAP-1

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Biological context

WW domains are small modules composed of approximately 30 amino acid residues (Sudol and Hunter, 2000). WW domains contain two highly conserved tryptophan residues and mediate protein-protein interactions by recognizing proline-containing sequences. They are found in cytoskeletal and intracellular signaling proteins such as YAP65, Pin1, dystrophin and Nedd4. WW domains are classified at least into four major groups with regard to their binding specificity. Group I WW domains bind to the PY motif, which contains Pro-Pro-Xaa-Tyr sequence.

MAGI-1 is found in various tissues and is localized in the tight junction of epithelial cells (Ide et al., 1999). MAGI-1, -2, and -3 belong to MAGUK family, which are cytoplasmic scaffold proteins that support the plasma membrane of cells. MAGUKs generally have a few PDZ domains and one SH3 domain whereas MAGIs have five PDZ domains and one or two WW domains. MAGI-1 binds to β-catenin or α-actinin4 through a PDZ domain and to β-dystroglycan through the first WW domain (Patrie et al., 2002; Pirozzi et al., 1997). MAGI-1 binds synaptopodin in glomerular podocytes, which suggests that MAGI-1 may play a role in actin cytoskeleton dynamics within polarized epithelial cells (Patrie et al., 2002).

In the second WW domain of MAGI-1, the second Trp is replaced by Tyr. This Trp is important for the specific recognition of PY motif by Group I WW domains (Kato et al., 2002). However, the second WW domain of MAGI-1 is capable of binding to the PY motif in synaptopodin without the second Trp (Patrie et al., 2002). Conformational analysis would elucidate the underlying mechanism that exploits Tyr residue instead of Trp for the specific recognition of the PY motif in synaptopodin.

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Methods and results

The cDNA encoding the tandem WW domains of human MAGI-1 (Ala295 through Ala394) was amplified with PCR and inserted into pET-21b (Novagen). Naturally occurring cysteine residues at 333 and 344 were replaced by serine. *E. coli* BL21(DE3) (Novagen) harboring the above-mentioned plasmid was cultivated at 37 °C in the M9 medium supplemented with 1x basal medium eagle vitamin (Gibco), thiamine and trace elements (Cai et al., 1998). The protein was purified from the soluble fraction of *E. coli* cell lysate using Phenyl Sepharose and Superdex 75 (Amersham). ^{15}N-labeled and ^{15}N/^{13}C-labeled proteins were prepared using the modified M9 medium containing 1 g/l ^{15}N NH_4Cl and/or 2 g/l ^{13}C glucose. The samples for NMR experiments contained 2.5 mM protein in 90% $^{1}H_2O$/10% $^{2}H_2O$ or 100% $^{2}H_2O$ with 10 mM Na-Pi buffer (pH 6.8, not corrected for isotope effects), 100 mM NaCl, 0.05 mM DSS, 0.05% NaN_3 and 0.1 mM *p*-ABSF. Microtubes (5-mm outer dia-

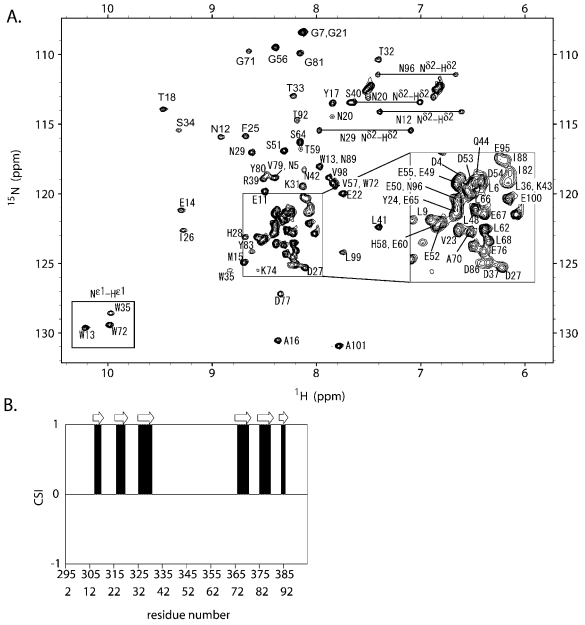

Figure 1. (A) Representative strips from the CBCA(CO)NH and HNCACB spectra showing connectivities for residues F318 to W328. (B) Plot of the consensus Chemical Shift Index (CSI) ($^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, and $^{13}C'$) of MAGI-1 WW domains, calculated using the program CSI. Indices of -1 and 1 indicate helical structure and β-strand structure, respectively. Expected secondary structural elements are shown as arrows for β-strands.

meter, Shigemi, Tokyo) were used for recording NMR spectra.

All NMR spectra were acquired at 37 °C on a Varian Unity INOVA 500 spectrometer equipped with a Narolac z-axis gradient probe. NMR data were processed and analyzed using NMRPipe (Delaglio et al., 1995) and SPARKY3 (Goddard, T.D. and Kneller, D.G., University of California, San Francisco). ^{1}H chemical shifts were referenced to internal DSS. ^{13}C and ^{15}N chemical shifts were referenced indirectly. The backbone resonance assignments were obtained using ^{1}H-^{15}N HSQC, HN(CO)CA, HNCA, CBCA(CO)NH, HNCACB, HNCO and (HCA)CO(CA)NH experiments. The aliphatic side-chain assignments were obtained using C(CO)NH, H(CCO)NH, HCCH-TOCSY and HCCH-COSY experiments. Secondary structure was predicted using the program CSI (Wishart and Sykes, 1994).

Extent of assignments and data deposition

Backbone assignment was carried out with the sequential assignment procedure. Signals of backbone amides (^{15}N and $^{1}H^{N}$) were unambiguously assigned for 92 residues out of 93 (99%) except for Lys339 in the loop region, which is due to severe overlap (Figure 1A). The tandem WW domain region of MAGI-1 has seven proline residues so that the full-length of our product protein is 101 residue-long including the artificially added N-terminal methionine residue. In addition, resonances of other backbone atoms (98% of $^{13}C^{\alpha}$, 98% of $^{13}C^{\beta}$, 95% of $^{13}C'$ and 90% of $^{1}H^{\alpha}$) as well as those of aliphatic (97% of ^{13}C, 40% of ^{15}N and 85% of ^{1}H) and aromatic (78% of ^{1}H) side-chain atoms were assigned. We predicted the secondary structure of the tandem WW domains using CSI (Figure 1B). The result indicates that each WW domain contains three β-strands like other WW domains (Kato et al., 2002), while the linker between the WW domains contains no secondary structure. In contrast, tandem WW domains of Prp40 are connected by an α-helical linker, which defines the relative orientation of these domains (Wiesner et al., 2002). Conformational analysis and dynamics study of the tandem WW domains of MAGI-I in the presence and absence of its ligand would show how each WW domain and the linker act in ligand recognition.

The ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 6086.

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