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Letter to the Editor: Assignments of the ^{1}H, ^{13}C, and ^{15}N resonances of the 21 kDa Vesl/Homer family protein, Vesl-1S

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Biological context

The proteins within the Vesl/Homer family (∼394 residues) are expressed in the postsynaptic densities and appear to function as adapter proteins involved in the association of postsynaptic components (for review see Xiao et al., 2000). The N-terminal region (∼110 residues) of each Vesl/Homer protein includes an Ena/Vasp Homology 1 (EVH1) domain (30% sequence similarity). The EVH1 domains are highly conserved in the Vesl/Homer proteins, which selectively bind to the proline-rich Vesl/Homer ligand motif, PPXXF, found in the cytoplasmic regions of the group I metabotropic glutamate receptors (mGluR1α and mGluR5: mGluRs), the inositol triphosphate receptors (IP3Rs), the Shank proteins, and potentially the ryanodine receptors (Tu et al., 1998; Kato et al., 1998; Naisbitt et al., 1999). On the other hand, the C-terminal regions of the long form Vesl/Homer proteins contain a leucine-zipper motif sequence responsible for specific self-dimerization via a coiled-coil interaction. Due to these properties, the Vesl/Homer proteins are thought to regulate (i) the clustering of mGluRs in the postsynaptic membrane, (ii) the direct physical linking of synaptic mGluRs to IP3Rs associated with the endoplasmic reticulum, (iii) the linking of the mGluR and NMDA receptor signalling pathways in collaboration with the Shank scaffold proteins, and (iv) the distribution of endoplasmic reticulum associated mGluRs.

Vesl-1S/Homer 1a (186 residues, 21 kDa) is one of the alternative splicing variants of the Vesl/Homer proteins, and its expression is upregulated during long term potentiation in the hippocampus neurons, while the other Vesl/Homer proteins are constitutively expressed in the brain (Brakeman et al., 1997; Kato et al., 1997). Among all of the Vesl/Homer proteins, only Vesl-1S/Homer 1a lacks a leucine zipper motif sequence at the C-terminus, and therefore it cannot self-multimerize. The EVH1 domain of Vesl-1S/Homer1a, however, can bind to the receptors competitively against the other Vesl/Homer proteins. Thus, Vesl-1S/Homer 1a appears to dominant-negatively regulate the protein-protein interactions between the other Vesl/Homer proteins and the receptors in the postsynaptic densities. Consequently, Vesl-1S/Homer 1a is assumed to participate in the formation of synaptic plasticity, and thus in memory and learning.

Recently, the crystal structure of the Homer 1a EVH1 domain (residues 1–120) was resolved and the PPXXF peptide selectivity was elucidated (Beneken et al., 2000). However, the structure of the unique C-terminal region in Vesl-1S/Homer 1a and the topology of full length Vesl-1S/Homer 1a have not been investigated. Here, we report the nearly complete ^{13}Cα, ^{1}Hα, ^{13}Cβ, ^{1}Hβ and ^{13}CO NMR assignments and the secondary structure of Vesl-1S. These data will allow us to determine the solution structure of Vesl-1S and to investigate the structure-function relationships in the native form.

Methods and results

Vesl-1S was expressed in *Escherichia coli* strain BL21(DE3) as an N-terminal fusion to glutathione-S-transferase (GST). The GST-Vesl-1S fusion protein was immobilized on glutathione Sepharose 4B resin (Amersham Pharmacia Biotech Inc.), and the Vesl-

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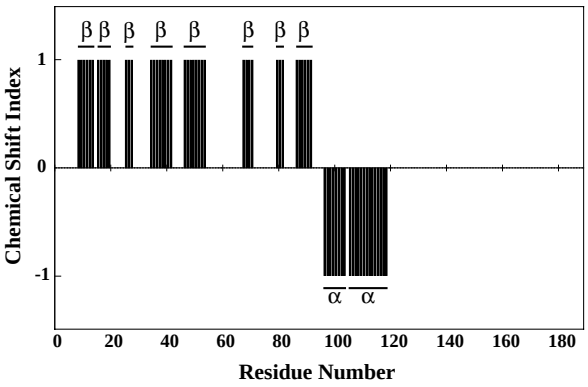

Figure 1. The consensus Chemical Shift Index (CSI) for Vesl-1S, calculated from the ^{1}Hα, ^{13}Cα, ^{13}Cβ, and ^{13}CO chemical shifts using the CSI computer program (Wishart and Sykes, 1994). Indices of +1, 0, and −1 indicate α-helix, random coil, and β-strand structure, respectively. The secondary structural elements are indicated (α: α-helix and β: β-strand).

1S was cleaved by PreScission Protease (Amersham Pharmacia Biotech Inc.) and eluted. The cleaved Vesl-1S was further purified by Resource Q (Amersham Pharmacia Biotech Inc.) and Superdex 75pg (Amersham Pharmacia Biotech Inc.) column chromatography. The final purified protein has two additional residues, Gly and Pro, at the N-terminus, due to the PreScission protease recognition site. The uniformly ^{15}N/^{13}C enriched protein was produced in M9 minimal medium with $^{13}C_6$-glucose and $^{15}NH_4Cl$ as the sole carbon and nitrogen sources. For NMR spectroscopy, 1.0–1.2 mM protein was prepared in 50 mM sodium phosphate buffer (pH 6.5) containing 0.1 M NaCl, 10% D_2O, and 1 mM DSS.

NMR spectra were acquired on Bruker Avance-500 and Avance-600 spectrometers equipped with pulse field gradient accessories at 303 K. The sequence specific assignment was obtained from triple resonance 3D NMR spectroscopy, HNCACB, CBCA(CO)NNH, HNCA, HN(CO)CA, HNCO, HN(CA)CO, HCA(CO)NNH, and HBHA(CBCACO)-NNH (Sattler et al., 1999). ^{1}H chemical shifts were referenced to DSS at 0.00 ppm, and ^{13}C and ^{15}N chemical shifts were calculated from the ^{1}H frequency. All spectra were processed using Azara v2.0 (provided by Wayne Boucher and the Department of Biochemistry, University of Cambridge) and were analysed using ANSIG v3.3 (Kraulis et al., 1994) on a Silicon Graphics workstation O2.

Using the ^{1}Hα, ^{13}Cα, ^{13}Cβ, and ^{13}CO chemical shift values, it was predicted by the CSI method (Wishart and Sykes, 1994) that Vesl-1S contains two α-helices and eight β-strands (Figure 1). These secondary structural elements are similar to those of the crystal structure of the Homer 1a EVH1 domain, suggesting that in an aqueous solution, the N-terminal region of Vesl-1S probably assumes an EVH1 fold, while the C-terminal region would not assume a typical secondary structure. Note that one β-strand, corresponding to β4, is missing, and that the α-helical region was remarkably extended by six residues to the C-terminus in the predicted solution structure.

Extent of assignments and data deposition

Since the Vesl-1S reported here includes two additional residues at the N-terminus, the assignments include these two residues, Gly1 and Pro2. On the ^{1}H-^{15}N HSQC spectrum, the ^{1}H and ^{15}N resonance assignments of the backbone amides for 165 out of 176 non-Pro residues have been achieved (94% completed). The unassigned residues were Lys31, His32, Ser42, Lys75, Ser82, Ser93, Ser94, Glu95, Glu121, and Arg178. The extents of the assignments are: 97% of ^{13}Cα, 90% of ^{1}Hα, 96% of ^{13}Cβ, 76% of ^{1}Hβ, and 93% of ^{13}CO resonances.

The assigned ^{1}H, ^{15}N, and ^{13}C chemical shifts of Vesl-1S have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu/) under accession number 4766.

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