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Letter to the Editor: Sequence-specific ^{1}H, ^{15}N and ^{13}C resonance assignments of the inhibitory prodomain of human furin

Surajit Bhattacharjya, Ping Xu & Feng Ni*
Biomolecular NMR Laboratory, Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montreal, PQ, Canada H4P 2R2

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Biological context

Proteolytic enzymes are often synthesized as inactive zymogens with N-terminal prodomains which are indispensable for the correct folding, stability, intracellular sorting of zymogens and ephemeral regulations of the enzymatic activities through specific inhibitions (Khan and James, 1998). However, despite the immense biological functions of the prodomains, the structural basis of these functions is poorly understood due to the intrinsic conformational flexibilities and a high tendency for aggregation for many of the prodomains in isolation (Hu et al., 1996). Recently, it has been shown that many biologically active proteins are also significantly unfolded, suggesting a growing need for structural characterization of such protein states (Wright and Dyson, 1999).

Mammalian proprotein convertases (PCs) comprise a family of newly discovered endoproteases that are responsible for the post-translational processing of a variety of higher molecular weight precursor proteins (Seidah et al., 1995). This class of enzymes is homologous to the bacterial subtilisins and the yeast prohormone convertase kexin (Seidah et al., 1995). Like other proteases, PCs are also synthesized as proenzymes with N-terminal proregions. Among all PCs the biological functions of the prodomain of furin have been the most extensively investigated. It has been shown that the 81-residue prodomain (Seidah et al., 1995) of furin, or pFurin, is essential for the folding, inhibition and compartmentalization of furin in ex vivo and in vivo systems (Zhong et al., 1999). In this Letter, we report the resonance (^{1}H, ^{15}N, ^{13}C) assignments of the prodomain of human furin as a first step towards elucidating the conformations, dynamics and specific binding of pFurin to the target protease.

*To whom correspondence should be addressed. E-mail: feng.ni@nrc.ca

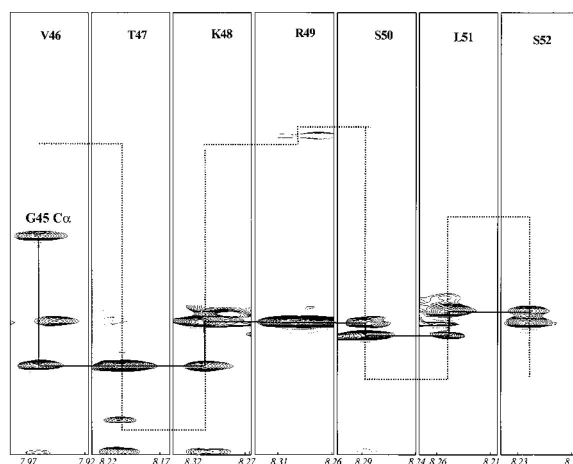

Figure 1. Strips along the ^{13}C and ^{1}H dimensions of the HNCACB spectra, showing sequential connectivities from residues V46 to S52. Lines are drawn sequentially connecting the C^{α} resonances (solid line) and C^{β} resonances (broken line) separately. Negative peaks belonging to C^{β} resonances are shown by light shaded contours.

Methods and results

Recombinant pFurin was over-expressed and purified by use of *E. coli* strain BL21 (DE3). The details of the cloning procedure have been published elsewhere (Zhong et al., 1999). Apart from the 81 residues of the prodomain, the expression vector also contains an extension of a few residues at the N- and C-termini, including a six-residue His-tag for Ni^{2+} affinity purification, making the

final amino acid sequence *MASMTGGQQMGRDP*-(Q1KV—KR81)-*DVAAALE*-H_6. The expressed prodomain, pFurin, with the additional residues is highly active as a selective inhibitor ($IC_{50} = 0.3$ nM) against the enzymatic activity of furin in vitro and ex vivo (Zhong et al., 1999). For the production of uniformly labeled (^{15}N, $^{15}N/^{13}C$ and ^{13}C) pFurin, the expressing bacteria were grown in the M9 minimal medium containing either $(^{15}NH_4)_2SO_4$, $(^{15}NH_4)_2SO_4/^{13}C$-glucose or ^{13}C-glucose. Purified pFurin was extensively dialyzed against a 50 mM sodium-acetate buffer at pH 4.0. Samples for NMR analyses were prepared in a solution of 50 mM sodium-acetate at pH 4.0, containing 10% D_2O and protease inhibitors (phenylmethylsulfonyl fluoride, leuptin and pepstain). The expressed pFurin domain was highly soluble at high concentrations without aggregation, therefore facilitating NMR studies. Triple-resonance experiments (Sattler et al. (1999) and references therein), including HNCACB, CBCA(CO)NH, HNCA, HN(CO)CA, HNCO, HN(CA)CO, H(CCO)NH and ^{15}N-edited 3D NOESY-HSQC, 3D TOCSY-HSQC were performed on a Bruker Avance-800 spectrometer equipped with pulse field gradient accessories at 303 K. The NMR data were processed using NMRPipe/NMRDraw (Delaglio et al., 1995) and analyzed by the use of NMRView (Johnson and Blevins, 1994). All the chemical shifts were indirectly referenced to that of DSS (Wishart et al., 1995).

Extent of assignments and data deposition

Low dispersion of the amide proton resonances (~1 ppm) indicates that the prodomain of human furin is globally unfolded in the aqueous solution of 50 mM sodium acetate at pH 4.0 and 303 K. Resonance assignments of unfolded/partially folded proteins are normally difficult due to the chemical shift degeneracy and broadening of peaks, which is a consequence of the intermediate rates of conformational exchange at ms time scales (Wüthrich, 1994). However, we have achieved almost complete assignments for the backbone and non-aromatic side-chain proton resonances. Most of the backbone (^1H, ^{15}N, $^{13}C^{\alpha}$) and side-chain $^{13}C^{\beta}$ resonances could be assigned by a combination of the HNCACB and CBCA(CO)NH spectra correlating the i and $i - 1$ residue pairs. Figure 1 shows strips along the ^{13}C and ^1H dimensions of the HNCACB spectra demonstrating connectivities encompassing residues V46 to S52. For some relatively broad resonances at the N-terminus, HNCA and HN(CO)CA experiments were helpful for assignments. Assignments for the aliphatic side-chain protons and backbone $C^{\alpha}H$ protons were obtained from the TOCSY-HSQC and NOESY-HSQC or H(CCO)NH experiments. In conclusion, almost complete resonance assignments of pFurin were achieved including some additional tagging residues from the N- and C-termini. Residues I25, Q33, I34, Y38 and Y39 and signals from histidine tag could not be assigned and partial assignments (C^{α}, C' and C^{β}) were made for K25, D37 and H40 residues. The resonance assignments have been deposited in the BioMagResBank (accession number: 4450).

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