Journal of Biomolecular NMR **28:** 87–88, 2004.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments for the N-cadherin prodomain

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Received 7 May 2003; Accepted 11 June 2003

Key words: cell-cell adhesion, N-cadherin, NMR resonance assignment, prodomain

Biological context

Cadherins are a large family of membrane-associated glycoproteins that mediate Ca^{2+}-dependent cell-cell adhesion in vertebrates and invertebrates. Differential expression of cadherins is important for the development of tissues and organs and for tissue integrity. Consequently, cadherins are involved in cellular adhesion processes ranging from embryogenesis to the formation of neural circuits in the CNS (Tepass et al., 2000). Cadherins also play dominant roles in pathological conditions where cell adhesion is altered or impaired. For example, loss of, or a switch in cadherin subtype expression has been attributed to tumor malignancy in many cancers (Cavallaro et al., 2002). The cadherin superfamily can be subdivided into six subfamilies; classical (type I) cadherins, type II cadherins, desmocollins, desmogleins, protocadherins, and cadherin-related proteins (Nollet et al., 2000). Classical cadherins consist of five extracellular cadherins domains, a single transmembrane helix and a highly conserved cytosolic domain. These cadherins have mainly homophilic interaction activity (Koch et al., 1999), thus providing cells with homotypic adhesion (Takeichi, 1988). Classical cadherins are synthesized on rough ER as precursor proteins with large prosequences prior to their N-terminal cadherin domains. These prosequences have to be cleaved off to activate cadherins for adhesion (Ozawa, 2002; Ozawa and Kemler, 1990). To understand the structural basis for activation of classical cadherins by prosequence removal, we use heteronuclear multidimensional NMR techniques to determine the three-dimensional structure of a prototypic cadherin prosequence from N-cadherin. During these studies it became apparent that the N-cadherin prosequence constitutes a folded protein domain and was therefore termed N-cadherin prodomain (NPro). Here, we report the sequence-specific backbone and side-chain ^{1}H, ^{13}C and ^{15}N resonance assignments of NPro.

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Methods and results

The N-cadherin prodomain construct (NPro) encoding the entire prosequence of N-cadherin without the signal peptide (residues Glu24 to Arg159) was subcloned into a pET-19b expression vector (Novagen) and overexpressed in *E. coli* BL21(DE3) cells at 25 °C. Uniformly ^{15}N- and ^{15}N/^{13}C-labelled NPro samples were prepared by growing bacteria in minimal medium containing $^{15}NH_4Cl$ (1 g/l) with or without $^{13}C_6$-glucose (2 g/l). Uniformly ^{15}N/^{13}C-labelled and fractionally deuterated protein samples were prepared by growing bacteria in 75% $^{2}H_2O$. Soluble proteins were purified by Ni^{2+}-NTA affinity chromatography (Qiagen), followed by extensive dialysis and gel filtration chromatography (Superose 12/20, Amersham). Protein samples were concentrated (typically 0.8–1 mM) and dialyzed. The final NMR protein samples were in 50 mM sodium phosphate buffer (pH 6.0), containing 0.05% sodium azide (w/v), in $H_2O/^{2}H_2O$ (9/1) or in $^{2}H_2O$. Purity and correct size of NMR samples was judged by SDS-PAGE, mass spectrometry, and N-terminal sequencing (Protein Core Facility, Columbia University, New York). All NMR experiments were conducted at 35 °C on a 500 MHz or 600 MHz Bruker DRX NMR spectrometer equipped with four RF channels and a triple-resonance probe with triple-axis pulsed field gradients. The NMR spectra were processed with NMRPipe/NMRDraw (Delaglio et al., 1995) and analyzed by using NMRView (Johnson and Blevins, 1994). The backbone resonances of NPro were sequentially assigned using deuterium-decoupled 3D triple resonance spectra of HNCACB, and HN(CO)CACB, recorded on uniformly ^{15}N/^{13}C-labelled and fractionally deuterated protein samples (Sattler et al., 1999; Yamazaki et al., 1994). To aid backbone assignment ^{15}N-HSQC spectra of protein samples prepared from media with selectively labeled amino acids (^{15}N-Leu, -Val, -Glu, or -Phe) were also recorded. The side-chain ^{13}C atoms were assigned using a 3D (H)C(CO)NH-TOCSY experiment (Sattler et al., 1999) recorded on the ^{2}H(75%)/^{13}C/^{15}N-labeled sample. Side chain

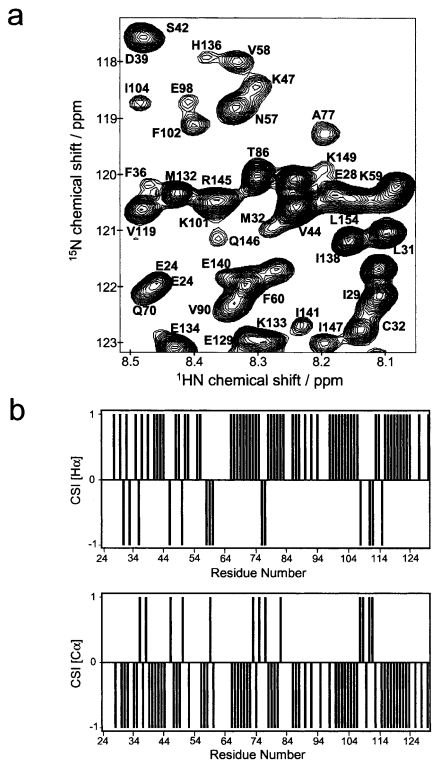

Figure 1. NMR spectral analysis of the N-cadherin prodomain. (**a**) A central region of 2D ^{1}H-^{15}N HSQC spectrum of the protein collected at pH 6.0 and 37 °C, showing amide resonances of selected residues. The assignments are annotated by the resonance peaks. (**b**) Chemical shift index plot for the backbone Cα and Hα atoms as a function of protein chain length.

^{1}H resonances were assigned using a 3D HCCH-TOCSY spectrum (Sattler et al., 1999) using a fully protonated ^{13}C/^{15}N-labeled sample in $^{2}H_2O$, and confirmed with a 3D ^{15}N-edited TOCSY-HSQC experiment. The side chain ^{1}H and ^{13}C resonances for aromatic residues were assigned using a combination of 2D ^{1}H NOESY and TOCSY in addition to CT-^{13}C HSQC and 3D HCCH-TOCSY recorded in the aromatic carbon region.

Extent of assignments and data deposition

Apart from the unstructured C-terminal region (residues 131–159), backbone assignment of ^{1}HN, ^{15}N, ^{13}Cα, and ^{13}Cβ atoms for over 95% of the residues was obtained. Assignment for the C-terminal region proved difficult due to largely too poor chemical shift dispersion and line broadening in the NMR spectra. Since this region also lacked discernable long-range NOEs, it was deemed unstructured. Figure 1A shows a region of the 2D ^{1}H-^{15}N HSQC spectrum for NPro at pH 6.0 and 30 °C. Excluding the unstructured C-terminal region, the side chain ^{1}H and ^{13}C resonance assignments were obtained for about 90% of the residues. Deviations of the ^{13}Cα and ^{1}Hα chemical shifts from random coil values (Wishart et al., 1995), and characteristic sequential and medium range NOEs patterns indicate that the structured region of NPro consists entirely of β-strands and loops. A table of the ^{1}H, ^{13}C and ^{15}N chemical shift assignments of NPro has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 7736.

Acknowledgements

We thank Dr M.A. Gawinowicz for performing mass spectrometry analysis and N-terminal sequencing. This work was supported by grants from the National Institute of Health to M.-M.Z. (CA80938) and to D.R.C. (NS041687), and a New York State Spinal Cord Injury Trust Award to D.R.C. (CO16883). A.W.K. is a recipient of a Swiss National Science Foundation Postdoctoral Fellowship and A.F. is a recipient of a Wellcome Trust Postdoctoral Fellowship.

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