Journal of Biomolecular NMR, **14:** 185–186, 1999.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N assignments of the neural cell adhesion molecule module-1

Peter Holme Jensen[a,c], Niels Kirk Thomsen[a], Vladislav Soroka[a,b], Vladimir Berezin[b], Elisabeth Bock[b] & Flemming M. Poulsen[a,c,*]

[a]*Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark;* [b]*Protein Laboratory, Institute of Molecular Pathology, Panum Institute, University of Copenhagen, DK-2200 Copenhagen N., Denmark;* [c]*Present address: Institute of Molecular Biology, University of Copenhagen, Øster Farimagsgade 2A, DK-1353 Copenhagen, Denmark*

Received 5 August 1998; Accepted 22 September 1998

Key words: ^{1}H, ^{13}C and ^{15}N assignments, module-1, neural cell adhesion molecule

Biological context

Neural cell adhesion molecule, NCAM, is a cell-surface glycoprotein, which mainly is expressed by neural cells, but it is also found in smaller amounts on the surface of other cells. NCAM is known to play a role in the development of the nervous system and in the processes of learning. The extracellular part of NCAM consists of 7 modules; 5 Ig-like modules and 2 fibronectin type III-like modules. NCAM is expressed in three major isoforms, NCAM-A, NCAM-B and NCAM-C. They differ in their cytoplasmic part, where the two longest forms NCAM-A and NCAM-B both have a transmembrane peptide and an intracellular module. The shortest form, NCAM-C, does not have these features but binds to the cell membrane by a GPI anchor.

Structure determination of the individual modules of NCAM is in progress. It has been shown that the structure of module-1 is an I-set of the immunoglobulin superfamily (Thomsen et al., 1996). The atomic coordinates are available from the Protein Data Bank by accession code 2NCM.

Methods and results

For production of murine NCAM module-1, a cDNA fragment corresponding to residues 20–116 (SWISS-PROT, accession number P13595) was subcloned into a Xho I/Bam HI site of the pHIL-S1 plasmid. The recombinant plasmid, linearized with Nsi I, was used for transformation of a *Pichia pastoris* strain His 4 GS-115 (Invitrogen Co., San Diego, CA). The sequence numbering of NCAM module-1 refers to the expression product, which contains two N-terminal residues from the vector. Three samples of module-1 of NCAM have been studied. They are respectively, unlabelled, ^{15}N labelled, and ^{13}C and ^{15}N double labelled NCAM module-1. They were prepared by growing *Pichia pastoris* in minimal media with ^{15}N labelled ammonium sulphate and ^{13}C labelled methanol/glucose as the sole ^{15}N and ^{13}C sources in the appropriate preparations. The expression media were desalted and subsequently NCAM module-1 was purified by gelfiltration in 20 mM NaCl, pH 6.0 and concentrated to a final concentration of approximately 2 mM in the unlabelled sample, and 1 mM in the samples of ^{15}N and $^{15}N/^{13}C$ labelled protein.

The following NMR spectra were recorded, with the indicated number of acquired complex points in the indicated dimensions, and used for assignment: TOCSY (2048 (t_2,^{1}H) × 512 (t_1,^{1}H)) in H_2O and in D_2O both with τ_m = 70 ms (Braunsweiler and Ernst, 1983); DQFCOSY (2048 (t_2,^{1}H) × 512 (t_1,^{1}H)) in H_2O and in D_2O (Piantini et al., 1982); NOESY (2048 (t_2,^{1}H) × 512 (t_1,^{1}H)) in H_2O and in D_2O with τ_m in the range 50–200 ms (Kumar et al., 1981). The spectral widths of the 2D homonuclear experiments were 7812.5 × 7812.5 Hz. ^{15}N HSQC (1024 (t_2,^{1}H) × 512 (t_1,^{15}N)) (Bodenhausen et al., 1980) using spectral widths of 7812.5 × 2000 Hz. ^{15}N TOCSY-HSQC (1024 (t_3,^{1}H) × 128 (t_2,^{1}H) × 32 (t_1,^{15}N)) with τ_m = 70 ms, and ^{15}N NOESY-HSQC (1024 (t_3,^{1}H) ×

*To whom correspondence should be addressed. E-mail: fmp@crc.dk

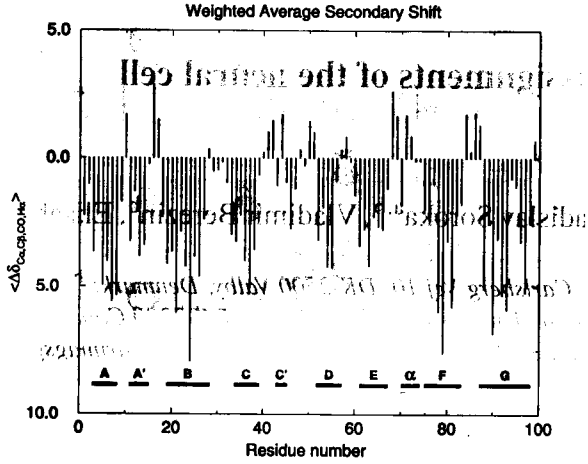

Figure 1. WASS-plot (Weighted Average Secondary Structure) of NCAM module-1 (G. Gippert, personal communication). The diagram shows the average chemical shift deviation from random coil values of Cα, Cβ, CO and Hα for each residue. When the index number is >1, α-helical secondary structure is expected, and if the index number is < −1, β-sheet secondary structure is expected. Thick lines show the actual secondary structure in NCAM module-1 (Thomsen et al., 1996). β-strands are labelled A → G, and the helical turn is labelled α.

128 (t_2,^{1}H) × 32 (t_1,^{15}N)) with τ_m = 100 ms (Zhang et al., 1994), using spectral widths of 7812.5 × 7812.5 × 2500 Hz for both experiments. HNCO (1024 (t_3,^{1}H) × 64 (t_2,^{13}C) × 28 (t_1,^{15}N)) (Kay et al., 1990); HNCA (1024 (t_3,^{1}H) × 48 (t_2,^{13}C) × 24 (t_1,^{15}N)) (Kay et al., 1990); HNCOCA (1024 (t_3,^{1}H) × 48 (t_2,^{13}C) × 24 (t_1,^{15}N)) (Grzesiek and Bax, 1992), using the same spectral widths of 7812.5 × 6250 × 2500 Hz for all 3 experiments. And last, HCCH-TOCSY (1024 (t_3,^{1}H) × 128 (t_2,^{1}H) × 32 (t_1,^{13}C)) (Bax et al., 1990), using spectral widths of 6097 × 5555 × 3333 Hz. The NMR experiments were performed on a Bruker AMX-600 MHz spectrometer at 298 K. The complete assignment of the ^{1}H, ^{13}C and ^{15}N resonance lines from these spectra was performed using the computer program PRONTO (Kjær et al., 1994). A WASS-plot (Weighted Average Secondary Structure) of NCAM module-1 is shown in Figure 1 (G. Gippert, personal communication). The plot shows the average chemical shift deviation from random coil values of Cα, Cβ, CO and Hα for each residue. As expected it predicts a β-sheet structure for module-1.

Extent of assignments and data deposition

Here we report the ^{1}H, ^{13}C and ^{15}N chemical shifts of resonances of NCAM module-1. The assignments have been deposited in the BioMagResBank database (accession number: 4162).

For 83 of the 99 residues the ^{1}H, ^{13}C and ^{15}N NMR signals were fully assigned. For 16 residues, including the aromatic residues, partial assignment was obtained. All ^{13}C resonances have been assigned, except for the aromatic residues where only the C^α and C^β resonance lines were observed. All the expected ^{15}NH backbone cross peaks were assigned, and all ^{15}NH side chain cross peaks of Asn, Gln, and Arg were assigned except $N^{\delta 2}H$ of Asn^{57}. For 38 residues the dihedral angle χ^1 was determined. This led to stereospecific assignments of 20 pairs of H^βs in methylene groups and the H^γs of the methyl groups of seven valines. The remaining χ^1 angles were determined for four threonines and seven isoleucines. The stereospecific assignments were obtained from coupling constant measurements in a NOESY spectrum of the ^{15}N labeled NCAM module-1 in combination with coupling constant measurements obtained from NOESY and DQFCOSY spectra. ^{15}N TOCSY-HSQC, ^{15}N NOESY-HSQC, HNCA and HNCOCA spectra were used for sequential assignment.

Acknowledgements

The research project was supported by the Danish Biotechnological Research and Development Programme. This is a contribution from the Danish Instrument Center for NMR spectroscopy of Biological Macromolecules.

References

Bax, A., Clore, G.M. and Gronenborn, A.M. (1990) *J. Magn. Reson.*, **88**, 425–431.

Bodenhausen, G. and Ruben, D.J. (1980) *Chem. Phys. Lett.*, **69**, 185–189.

Braunsweiler, L. and Ernst, R.R. (1983) *J. Magn. Reson.*, **53**, 521–528.

Grzesiek, S. and Bax, A. (1992) *J. Magn. Reson.*, **96**, 432–440.

Kay, L.E., Ikura, M., Tschudin, R. and Bax, A. (1990) *J. Magn. Reson.*, **89**, 496–514.

Kjær, M., Andersen, M.K. and Poulsen, F.M. (1994) *Methods Enzymol.*, **239**, 288–307.

Kumar, A., Wagner, G., Ernst, R.R. and Wüthrich, K. (1981) *J. Am. Chem. Soc.*, **103**, 3654–3658.

Piantini, U., Sørensen, O.W. and Ernst, R.R. (1982) *J. Am. Chem. Soc.*, **104**, 6800–6801.

Thomsen, N.K., Soroka, V., Jensen, P.H., Berezin, V., Kiselyov, V.V., Bock, E. and Poulsen, F.M. (1996) *Nat. Struct. Biol.*, **3**, 581–585.

Zhang, O., Kay, L.E., Olivier, J.P. and Forman-Kay, J.D. (1994) *J. Biomol. NMR*, **4**, 845–858.