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^{1}H and ^{15}N resonance assignment of neural cell adhesion molecule module-2

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Biological context

Neural cell adhesion molecule, NCAM, is a modular protein present mainly on the surface of nerve cells. NCAM and a number of other molecules are responsible for the property of nerve cell adhesion. It is known to play a role in the development of the nervous system and in learning. NCAM is mainly expressed in three major isoforms, NCAM-A, NCAM-B and NCAM-C. They differ in their C-terminal part, where the two longest forms NCAM-A and NCAM-B both have a trans-membrane peptide and an intracellular module. The shortest form, NCAM-C, does not have these features but it binds to the cell membrane by a GPI anchor. The extracellular part of each of the isoforms consists of 7 modules; 5 Ig-like modules and 2 fibronectin type III-like modules.

NCAM mediate cell-cell interactions by homophilic and heterophilic binding mechanisms. A fragment of NCAM containing module-1 has been shown to bind to a fragment of NCAM containing module-2 (Kiselyov et al., 1997). In addition to this module-3 has shown evidence for isologous binding (Rao et al., 1994).

The structures of module-1 (Thomsen et al., 1996) and module-2 (Jensen et al., in preparation) have been determined by NMR spectroscopy. The atomic coordinates of NCAM module-1 are available from the Protein Data Bank by accession code 2NCM.

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Methods and results

The cDNA fragment encoding for Ig module-2 of NCAM (corresponding to residues 100–191) was synthesized by PCR using rat NCAM-C cDNA as a template. The amplified cDNA fragment was subcloned into *Sna*BI/*Avr*II site of pPIC9K plasmid (Invitrogen Corp.). An *E. coli* strain Top 10 F′ (Invitrogen) was used for transformation and the recombinant clones were identified by restriction analysis of the plasmid DNA. The recombinant plasmid was linearized with *Sac*I and used for transformation of *Pichia pastoris* strain *His*4 GS-115 (Invitrogen). Transformation and selection were performed according to the protocol supplied by the manufacturer.

The recombinant Ig module-2 of NCAM was expressed after induction in a 2 liter fermentor (MBR Mini Bioreactor). Subsequently the expression medium was concentrated 10 times by ultra-filtration. Module-2 was purified by gel filtration on Sephadex G-25 (Pharmacia) followed by ion exchange chromatography on HiTrap SP 5 ml column (Pharmacia). The yields were 10–15 mg per liter of expression medium. The authenticity of recombinant module-2 was confirmed by amino acid sequencing and mass spectrometry. In the N-terminal the original residues Lys^{100} and Leu^{101} were replaced with Tyr^{100} and Val^{101} due to cloning site considerations.

The NMR-samples used for the complete assignment of ^{1}H and ^{15}N resonances were: (a) unlabeled NCAM module-2 in H_2O, ~1 mM, (b) unlabelled NCAM module-2 in D_2O, ~1 mM, and (c) ^{15}N-labeled NCAM module-2 in H_2O, ~1 mM. In all

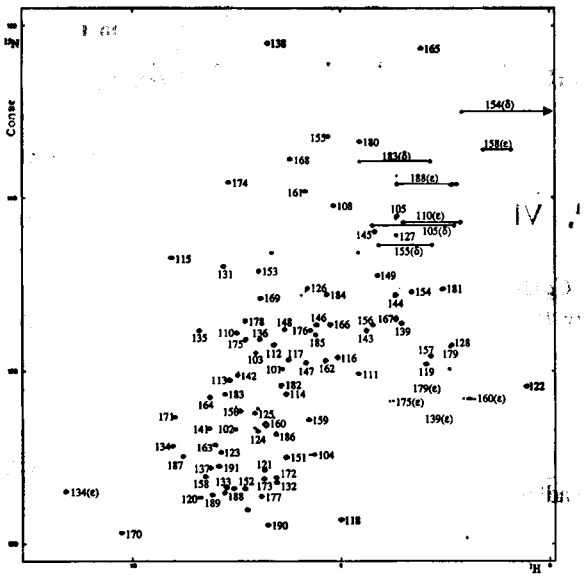

Figure 1. ^{15}N HSQC spectrum of neural cell adhesion molecule module-2. The signals are labeled according to the residue number in the full NCAM sequence (100–191). Residue numbers 100, 106 and 140 are not observed. The proline residues are 107, 109, 129 and 130.

cases the buffer was 50 mM NaCl, 20 mM potassium phosphate pH 6.0. 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 using τ_m = 70 ms (Brausweiler 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)) respectively, in H_2O and in D_2O with τ_m = 100 ms or 200 ms (Kumar et al., 1981); ^{15}N HSQC (1024 (t_2,^{1}H) × 512 (t_1,^{15}N)) (Bodenhausen et al., 1980); ^{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) × 128 (t_2,^{1}H) × 32 (t_1,^{15}N)) with τ_m = 100 ms (Zhang et al., 1994). The NMR experiments were performed on a Bruker AMX-600 MHz spectrometer and on a Varian Unity Inova 750 MHz spectrometer. All spectra were recorded at 298 K. The assignment of the ^1H and ^{15}N resonance lines from these spectra were performed using the computer program PRONTO (Kjær et al., 1994). The ^{15}N HSQC spectrum of NCAM module-2 is shown in Figure 1.

Extent of assignments and data deposition

The assignments of the ^1H and ^{15}N chemical shifts of NCAM module-2 have been deposited in the BioMagResBank database (accession number: 4143).

NMR signals that could not be observed include all signals of Ala^{106}, Pro^{129}, Pro^{130} and Asp^{140}. Of the remaining 88 residues, 70 were completely assigned and 18 partially assigned. For the remaining residues all expected ^{15}NH backbone cross peaks were assigned and all ^{15}NH side chain cross peaks of Asn, Gln, and Arg were assigned except $^{15}N^{\epsilon}H$ of Arg^{148} and Arg^{171}. For 34 residues the dihedral angle χ^1 was determined. This led to stereospecific assignments of 15 pairs of H^{β}s in methylene groups and the H^{γ}s of the methyl groups of seven valines. The remaining χ^1 angles were determined for two threonines and ten isoleucines. These measurements were based on the DQFCOSY and NOESY spectra.

^{15}N TOCSY-HSQC and ^{15}N NOESY-HSQC spectra were used for the sequential assignment.

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