Journal of Biomolecular NMR, 10 (1997) 307–308
KLUWER/ESCOM

J-Bio NMR 483

Assignment of the ^{1}H, ^{15}N and ^{13}C resonances of the calcium-free and calcium-bound forms of the first C_2-domain of synaptotagmin I

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Received 26 June 1997
Accepted 28 July 1997

Keywords: C_2-domain; Synaptotagmin; Neurotransmitter release; Ca^{2+} binding; Triple-resonance spectroscopy; ^{1}H, ^{15}N and ^{13}C assignments

Biological context

Synaptotagmin I is a synaptic vesicle protein that is essential for the fast component of neurotransmitter release (Geppert et al., 1994) and is believed to be the Ca^{2+} sensor in this process (Südhof and Rizo, 1996). The crystal structure of the first C_2-domain of synaptotagmin I (which we will refer to as the C_2A-domain) consists of a β-sandwich formed by two four-stranded β-sheets (Sutton et al., 1995). Using NMR spectroscopy, we have shown that the C_2A-domain binds at least two Ca^{2+} ions through five conserved aspartate residues located on two loops at the tip of the β-sandwich fold (Shao et al., 1996). Initial structural studies indicated that Ca^{2+} binding produces minimal conformational changes in the C_2A-domain (Shao et al., 1996) and analysis of the mode of Ca^{2+}-dependent binding of the C_2A-domain to syntaxin showed that this interaction is driven by a Ca^{2+}-induced change in the electrostatic potential of the C_2A-domain (Shao et al., 1997). In order to conclusively establish whether or not the C_2A-domain undergoes Ca^{2+}-induced conformational changes and to completely elucidate its Ca^{2+}-binding mode, it is necessary to obtain high-resolution structures of the C_2A-domain in its Ca^{2+}-free and Ca^{2+}-saturated forms. As a first step toward these goals, we have assigned the ^{1}H, ^{15}N and ^{13}C resonances of the Ca^{2+}-free and Ca^{2+}-bound C_2A-domain using multidimensional NMR techniques.

Methods and Results

Recombinant C_2A-domain (residues 140–267 of synaptotagmin I) was obtained as previously described (Shao et al., 1996). NMR samples contained 1 mM protein dissolved in 40 mM perdeuterated acetate at pH 5.0, containing 100 mM NaCl and either 0.2 mM EGTA (for the Ca^{2+}-free form) or 30 mM $CaCl_2$ (for the Ca^{2+}-saturated form). H_2O/D_2O 9:1 was used as the solvent. All NMR data were acquired on a Varian Unity-500 spectrometer at 34 °C in the hypercomplex mode. The spectral widths and the number of complex points acquired in the F3, F2 and F1 dimensions for the primary experiments used for resonance assignments, with the number of scans per FID and the total measurement time in parentheses, were the following: HNCO, $7600 \times 1163 \times 1670$ Hz, $512 \times 28 \times 70$ (8 scans, 18 h); HNCACB, $7600 \times 1163 \times 7650$ Hz, $512 \times 28 \times 32$ (32 scans, 36 h); (H)CBCACO(CA)HA, $4000 \times 1670 \times 7650$ Hz, $256 \times 64 \times 54$ (16 scans, 61 h); (H)C(CO)NH-TOCSY, $7600 \times 1163 \times 7650$ Hz, $512 \times 28 \times 64$ (32 scans, 65 h); HCCH-TOCSY, $8000 \times 3222 \times 4600$ Hz, $512 \times 40 \times 110$ (8 scans, 39 h). The mixing times for the (H)C(CO)NH-TOCSY and HCCH-TOCSY experiments were 18 and 14 ms, respectively.

We initially obtained complete assignments of the backbone ^{1}H and ^{15}N resonances of the C_2A-domain (Shao et al., 1996), as well as assignments for 70% of the side-chain protons, using standard 2D homonuclear experiments and 3D ^{1}H-^{15}N TOCSY-HMQC and NOESY-HMQC spectra. From these assignments, it was quite straightforward to assign the ^{13}C resonances and complete the proton assignments using a few triple-resonance experiments. Backbone and C^{β} resonances were assigned using 3D HNCO, HNCACB and (H)CBCACO(CA)HA experiments, and most side chains were assigned from 3D (H)C(CO)NH-TOCSY and HCCH-TOCSY spectra. Pulsed field gradient versions of these experiments, with flip-back pulses and sensitivity enhancement in the ^{15}N-dimensions, were used (Kay, 1993; Kay et al., 1993,1994; Muhandiram and Kay, 1994). To assign the carboxyl carbons of aspartic acid and glutamic acid side chains, as well as the amide carbons of asparagine and glutamine

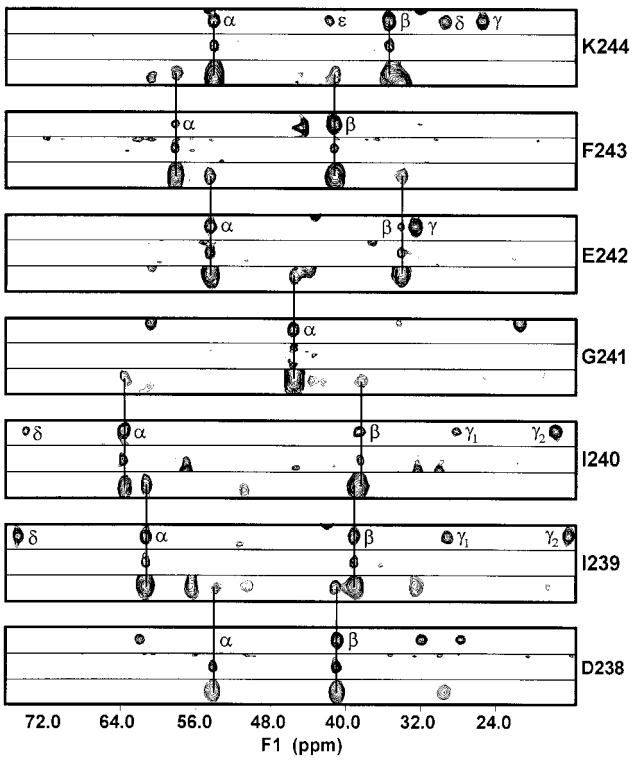

Fig. 1. Composite of (F1,F3) strips from triple-resonance experiments illustrating the sequential assignment of residues Asp^{238}–Lys^{244} of the C_2A-domain of synaptotagmin I. Strips corresponding to each residue from HNCACB (bottom), (H)CBCACO(CA)HA (middle) and (H)C(CO)NH-TOCSY (top) spectra are shown. The strips were taken at the backbone ^{15}N and ^{13}CO chemical shifts (F2 dimension) of the same residue for the HNCACB and (H)CBCACO(CA)HA spectra, respectively, and at the backbone ^{15}N chemical shift (F2 dimension) of the following residue in the sequence for the (H)C(CO)NH-TOCSY spectrum. The positions of the cross peaks along the F1 dimension in the latter spectrum have been labeled with the Greek letter associated to the corresponding carbon. Note that the cross peaks involving C^{δ} carbons of isoleucine residues are folded in the F1 dimension. Vertical lines correlate the cross peaks involving the C^{α} and C^{β} resonances of a given residue in the different strips.

side chains, we acquired additional (H)CBCACO(CA)HA experiments where all aliphatic ^{13}C pulses were centered at 43 ppm and the τ_d delay of the $^{13}C \rightarrow {}^{1}H$ INEPT transfer was set to 0.9 ms to allow observation of methylene protons. All these experiments yielded very high quality data. Virtually every single cross peak that can be predicted from the final assignments is observed in the HNCO, HNCACB and (H)CBCACO(CA)HA spectra. Strips of the HNCACB, (H)CBCACO(CA)HA and (H)C(CO)NH-TOCSY spectra illustrating the assignment of residues 238–244 of the C_2A-domain are shown in Fig. 1. Assignment of the aromatic side-chain resonances, which are not observed in the triple-resonance experiments described above, was accomplished using homonuclear 2D DQF-COSY, TOCSY and NOESY data, in combination with ^{1}H-^{13}C HSQC spectra and 3D HCCH-TOCSY experiments acquired with the carbon carrier in the center of the aromatic region.

Extent of assignments and data deposition

We have assigned all the protonated ^{15}N resonances from the backbone and from the asparagine, glutamine and tryptophan side chains, and 99.0% of the ^{13}C resonances from the protonated and the carbonyl carbons, for both the Ca^{2+}-free and the Ca^{2+}-saturated C_2A-domain; 99.4% and 99.2% of the nonlabile ^{1}H resonances have been assigned for the Ca^{2+}-free and Ca^{2+}-bound forms, respectively. The assignments have been deposited in the BioMagResBank database (accession numbers 4039 and 4041).

A comparison of the ^{1}H, ^{15}N and ^{13}C chemical shift changes caused by Ca^{2+} binding to the C_2A-domain leads to similar conclusions to those drawn previously from the Ca^{2+}-induced changes in ^{1}H and ^{15}N amide chemical shifts (Shao et al., 1996). As expected, large chemical shift changes were observed in some of the nuclei of the side chains of the five aspartate residues that form the bipartite Ca^{2+}-binding model that we proposed (Shao et al., 1996). However, large chemical shift changes for the C^{β} of Ser^{178}, Ser^{235} and His^{237}, together with a detailed examination of the crystal structure in this region, suggest that one or two additional Ca^{2+}-binding sites may exist. Clarification of this point will be very important to understand the mechanism of synaptic vesicle exocytosis since binding of multiple Ca^{2+} ions by the C_2A-domain of synaptotagmin I would imply that this domain by itself can account for the high Ca^{2+} cooperativity observed in neurotransmitter release.

Acknowledgements

We are very grateful to Lewis Kay for providing pulse sequences for all triple-resonance experiments, and to Lawrence McIntosh and Lewis Kay for assistance in setting up these experiments. This work was supported by a grant from the William Randolph Hearst Foundation and the United Cerebral Palsy Research and Educational Foundation, and by a grant from the NIH (R29-NS-33731).

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