Journal of Biomolecular NMR, **23:** 255–256, 2002.
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Letter to the Editor: Assignment of ^{1}H, ^{13}C and ^{15}N resonances of human Ca^{2+}-S100B in complex with the TRTK-12 peptide

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Received 21 May 2002; Accepted 25 June 2002

Key words: calcium-binding protein, human S100B, resonance assignments, TRTK-12

Biological context

Changes in intracellular calcium levels control many cellular processes including muscle contraction, cell growth and metabolism. One group of proteins that respond to fluctuations in intracellular calcium concentrations is the EF-hand family of calcium-binding proteins. The S100 proteins are EF-hand proteins that exist as homo- or heterodimers where each monomer contains two EF-hand calcium-binding loops connected by a flexible linker region (Schafer and Heizmann, 1996). S100B, a homodimer of 91-residue S100β monomers, has been implicated as a binding partner for more than 20 target proteins including cellular architecture proteins and kinase substrates (for review see Donato, 1999). Apo- and calcium-bound structures of S100B have revealed that calcium binding to this protein results in the exposure of several previously buried hydrophobic residues in the C-terminus and linker region between the 2 EF-hands providing a site for target protein interactions (Smith and Shaw, 1998).

Previously, an S100B binding sequence [(R/K)(L/I)(XWXXIL)] was identified using a random bacteriophage library (Ivanenkov et al., 1995). TRTK-12 is a synthetic 12-residue peptide derived from this sequence that has been shown to interact with S100B in a calcium-dependent manner ($K_d < 1\ \mu M$) (Barber et al., 1999). This peptide has been shown to compete for Ca^{2+}-S100B binding with other proteins including CapZ-α (Ivanenkov et al., 1995). To date, a single three-dimensional structure exists of Ca^{2+}-S100B bound to a target (Rustandi et al., 2000). Here we report the NMR assignments of Ca^{2+}-S100B in complex with the TRTK-12 peptide as progress towards the three-dimensional structure.

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Methods and experiments

Human S100B protein was expressed in *E. coli* (strain N99) and purified as previously described (Smith et al., 1996). M9 minimal media containing 1 g/l 99% $^{15}NH_4Cl$ or 1 g/l 99% $^{15}NH_4Cl$ and 2 g/l ^{13}C-glucose was used to prepare uniformly ^{13}C- and ^{15}N-labelled S100B. Uniformly $^{2}H/^{15}N$ labelled S100B was prepared in M9 minimal media containing 99% D_2O. The unlabelled TRTK-12 peptide (Ac-TRTKIDWNKILS-NH_2) was synthesized by the Queen's Peptide Synthesis Lab (Queen's University, Kingston, Canada). Purity was confirmed using reversed-phase HPLC and mass spectrometry.

NMR experiments were acquired at 35 °C on Varian 500 and 600 MHz spectrometers with pulsed field gradient triple resonance probes. NMR samples contained 1 mM S100β monomer, 1.2 mM TRTK-12 peptide, 4 mM $CaCl_2$ and 5 mM DTT in 90% H_2O/10% D_2O (v/v) at pH 7.05.

Sequential S100B backbone resonance assignments were made using HNCACB, CBCA(CO)NH, HNCO and ^{15}N-HSQC experiments (Bax and Grzesiek, 1993). Sidechain assignments were made using ^{15}N-edited TOCSY, HCCH-TOCSY, HC(CO)NH and C(CO)NH experiments. Uniformly labelled $^{15}N,^{2}H$ S100B and two-dimensional NMR experiments were used to determine TRTK-12 assignments. ^{1}H resonances were identified from a watergateTOCSY, and ^{13}C resonances were obtained from a natural abundance ^{13}C-HSQC. Spectra were processed and ana-

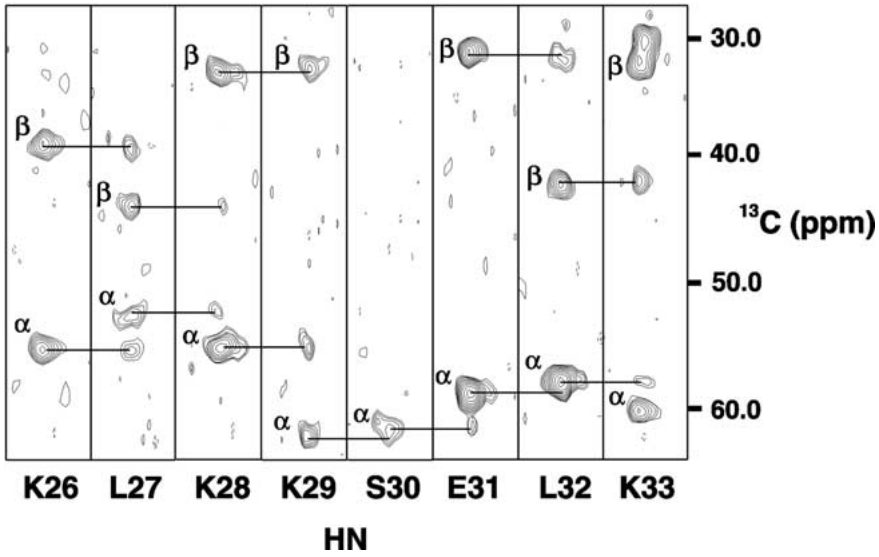

Figure 1. Strip plots extracted from the HNCACB spectrum of Ca^{2+}-S100B bound to TRTK-12. Strips represent F3-F1 (^{1}H-^{13}C) planes of the 3D HNCACB spectrum at F2 (^{15}N) frequencies of residues K26-K33 in the N-terminal calcium-binding loop and N-terminus of helix II of S100B. Strong intraresidue CA and CB correlations (labelled α and β, respectively) and weaker sequential correlations to the preceding residue are observed.

lyzed on a Silicon Graphics Indigo workstation using NMRPipe, NMRDraw (Delaglio et al., 1995), Pipp and Stapp (Garrett et al., 1991) programs.

Extent of assignments and data deposition

A region of the 500 MHz HNCACB spectrum showing residues K26-K33 of Ca^{2+}-S100B bound to TRTK-12 is shown in Figure 1. Backbone $^{1}H^{N}$ and ^{15}N resonances have been assigned for 89 of 91 residues in S100B with the exception of G22 and D23. The extent of S100B assignments in the complex is: 100% of $^{13}C\alpha$, 100% of $^{1}H\alpha$, 88% of ^{13}CO, 85% of sidechain ^{13}C and 91% of sidechain ^{1}H. All backbone amide protons for TRTK-12 were identified and 100% of $^{1}H\alpha$ and sidechain ^{1}H were assigned. Chemical shifts of all assigned nuclei have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu), entry 5377.

Acknowledgements

The authors thank Kathryn Barber for technical support. We also thank Frank Delaglio and Dan Garrett (NIH) for NMRPipe and Pipp and Lewis Kay (University of Toronto) for providing all pulse sequences. This research was supported by operating and maintenance grants from the Canadian Institute of Health Research (GSS) and graduate studentships to KAM from the Alzheimer Society of London and Middlesex and the Natural Sciences and Engineering Research Council. Funding for 500 and 600 MHz NMR spectrometers was made possible through grants from the Canada Foundation for Innovation, the Medical Research Council of Canada, the Academic Development Fund of The University of Western Ontario and generous gifts from R. Samuel McLaughlin Foundation and London Life Insurance Co. of Canada.

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