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^{1}H and ^{15}N resonance assignment of the calcium-bound form of the *Nereis diversicolor* sarcoplasmic Ca^{2+}-binding protein

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Biological context

The cellular calcium content and its function as second messenger are usually mediated and controlled by cytoplasmic Ca^{2+}-binding proteins. A large number of these proteins contain several helix-loop-helix ion-binding motifs, also called EF-hands. Important amounts of Ca^{2+}-binding proteins, with a wide structural heterogeneity, were found in the sarcoplasm of invertebrate muscle tissues. They are not able to control enzyme activity but are thought to play a role in the regulation of intracellular divalent cation concentration and fluxes. A member of this class NSCP (*Nereis* Sarcoplasmic Calcium-binding Protein), containing 174 residues, was isolated from the annelid *Nereis diversicolor* (a sandworm) (Cox and Stein, 1981) and its structure was determined by X-ray (Vijay-Kumar and Cook, 1992).

Understanding of the equilibrium and kinetic features of the ion-binding process requires detailed structural information on the apo, partially-liganded and saturated forms of the protein. The present study was undertaken in order to characterize its solution structure and dynamics in various Ca^{2+}/Mg^{2+}-liganded forms. As the first stage, we report here an almost complete proton and nitrogen-15 assignment of the Ca^{2+}-bound form of NSCP in solution.

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Methods and results

Wild-type NSCP was purified according to the method of Cox and Stein (1981). The NSCP open reading frame was amplified by PCR starting from the plasmid pNDner04, cloned into the vector pET22b and transfected into the host *E. coli* strain BL21(DE3)/pDIA17/pHSP234. [^{15}N]-ammonium sulfate was used for nitrogen labeling. In the minimal medium, the labeled NSCP represents 10% of the total soluble proteins.

NMR samples (1.0–1.2 mM) were prepared in deuterated Tris/HCl buffer (50 mM), $CaCl_2$ (1 mM), pH 6.5 in 95% $^{1}H_2O$/5% $^{2}H_2O$ or in 100% $^{2}H_2O$. All NMR spectra were acquired on a Varian Unity-500 spectrometer. Proton chemical shifts in parts per million (ppm) were referenced relative to the water signal that, at 308 K, resonates at 4.68 ppm from the sodium 2,2-dimethyl-2-silapentane sulfonate. ^{15}N reference was set relative to a $^{15}NH_4Cl$ solution (3 M in 1M HCl) in which the nitrogen resonates at 24.93 ppm from the liquid NH_3 (Levy and Lichter, 1979).

Standard methods were used to obtain pure absorption DQF-COSY and phase-sensitive NOESY spectra with mixing times of 100 and 150 ms. TOCSY experiments employed an MLEV-16 pulse sequence for the spin lock and 40–80 ms spin lock times. 2D and 3D heteronuclear experiments (HSQC, NOESY-HMQC and TOCSY-HSQC) used the published pulse sequences (Bodenhausen and Ruben, 1980; Marion et al., 1989; Kay et al., 1989) and were acquired as 128 (t_1)×32(t_2)×512(t_3) complex points. The spectral width was 1500 Hz in the nitrogen dimension,

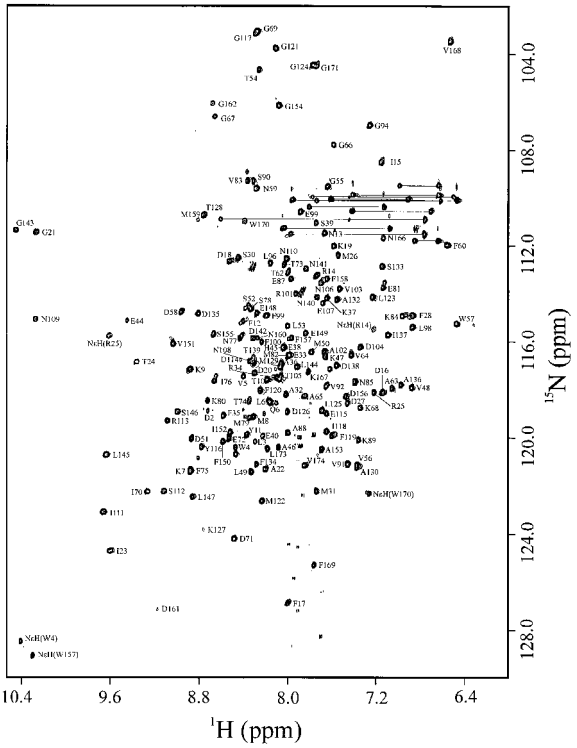

Figure 1. ^{1}H-^{15}N HSQC spectrum of NSCP in 50 mM Tris/HCl buffer pH 6.5, at 308 K. Assignment of cross-peaks is shown using the one-letter code for amino acids. Pairs of peaks connected by a horizontal line represent Asn and Gln side chain NH_2 groups.

3200 Hz in the amide proton dimension and 7000 Hz for the all-proton dimension.

Both wild type and recombinant proteins were used for the assignment procedure. Identification of the majority of the side chain spin systems corresponding to different amino acid types was performed by the analysis of homonuclear 2D spectra (DQF-COSY and TOCSY) recorded in $^{1}H_2O$ and $^{2}H_2O$ on the wild type sample (Wüthrich, 1986).

The sequential assignment was mainly done using the heteronuclear spectra recorded on the ^{15}N-labeled recombinant NSCP. The wide dispersion of amide nitrogen frequencies and the quality of heteronuclear data are illustrated in the ^{1}H–^{15}N HSQC spectrum recorded at 308 K (Figure 1). All the expected main-chain amide peaks and the 12 peak pairs corresponding to the side chain amino groups (1 Gln and 11 Asn) were observed. Whenever possible, the sequential connectivities observed in the heteronuclear spectra were searched in the homonuclear 2D spectra of the wild type protein. We thus obtained the proton assignment for both the wild type and recombinant molecules. Comparison of the two sets of chemical shift values shows a high similarity with significant differences (larger than 0.05 ppm) in several short segments (V5-T10, M79-K84 and M159-G162). The assignment procedure revealed that the N-terminal residue (Ser1) is acetylated in the wild type protein but not in the recombinant variant. This explains the lack of assignment for this residue in the labeled protein and may be the cause of several chemical shift differences between the two samples. Inspection of the NOESY spectra showed that residues from the above-mentioned fragments are in NOE contact with side chain protons of Trp4, in the N-terminal end of the protein. Therefore, a small rearrangement of the Trp4 indole ring could change significantly the ring current shifts undergone by the neighboring residues, accounting for the observed chemical shift differences.

Extent of assignment and data deposition

Spin systems for 171 from 174 residues (about 98%) were partially or completely assigned. Unassigned resonances correspond to a polypeptide fragment situated in an irregular secondary structure region (D163-T165) at the C-terminal site of the sequence. The secondary structure elements (8 α-helices and the 4 short β-strands) predicted from the secondary shifts of α protons and short and medium NOE connectivities are in good agreement with the crystal structure. The chemical shift values of the proton and nitrogen resonances are available as supplementary material and were deposited in BioMagResBank (accession number 4129).

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