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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the C-terminal domain of human lamin A/C

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Biological context

The nuclear envelope of eukaryotic cells is composed of nuclear membranes, nuclear pores and the nuclear lamina, a meshwork of intermediate filaments including lamin proteins and lamin-binding proteins. The nuclear lamina is essential to control nuclear shape, to space nuclear pore complexes and play crucial roles in cell cycle regulation (Stuurman et al., 1998; Gruenbaum et al., 2000). It is also required for chromatin organization, DNA replication, apoptosis and might be involved in transcriptional repression (Cohen et al., 2001; Stuurman et al., 1998). Recently, mutations in the nuclear lamina proteins were shown to cause four different genetic diseases called laminopathies (Bonne et al., 2001; Wilson et al., 2001 and references herein). In particular, the gene LMNA (Lin and Worman, 1993) that encodes A-type lamins is mutated in autosomal-dominant Emery–Dreifuss muscular dystrophy, limb-girdle muscular dystrophy type 1B, dilated cardiomyopathy with conduction defects and Dunningan-type partial lipodystrophy.

Lamins are nuclear-specific type V intermediate filaments that range in size from 60 to 70 kDa. Lamin proteins consist of a small N-terminal head, a large coiled-coil rod domain of 360 residues and a C-terminal tail that includes a globular domain. Mutations related to muscular diseases and cardiomyopathy are scattered throughout the protein sequence, both in the rod and C-terminal domains. Mutations responsible for Dunningan-type partial lipodystrophy are localized in the C-terminal tail. However, as no structural information at the atomic level is available for lamins, the consequences of the missense mutations are poorly understood. We are currently studying the structure of the lamin C-terminal globular domain domain, in order to understand the impact of the mutations linked to the different diseases in this domain. We report here the ^{1}H, ^{13}C and ^{15}N resonance assignment of the C-terminal domain of human lamin A/C (residues 428–547).

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Methods and experiments

Region 411-553 of lamin A/C was expressed in *E. coli* strain BL21 transformed with plasmid pGEX-4T-1 (Amersham Pharmacia Biotech, Inc.) that encodes glutathione-S-transferase (GST) and a thrombin cleavage site 5′ to the multiple cloning site. Expression and purification of the GST-lamin fusion protein were performed according to the manufacturer's instructions, using Glutathione Sepharose 4B (Amersham Pharmacia Biotech, Inc.). The lamin-protein was cleaved using thrombin protease. Uniformly labeled ^{15}N protein was produced in minimum media M9 containing 0.7 g l^{-1} of $(^{15}NH4)_2SO_4$ (Boehringer) as the nitrogen source. Uniformly labeled ^{13}C/^{15}N protein was produced in a rich medium prepared from uniformly labeled ^{13}C/^{15}N *Spirulina maxima* cyanobacteria. The molecular mass of the protein was checked by electrospray ionization mass spectroscopy and its N-terminus was sequenced. The monomeric state of the protein was checked by analytical ultracentrifugation. NMR samples (about 1 mM) were obtained by dissolving the lyophilized protein in 20 mM phosphate buffer pH 6.3 in either 90% H_2O/10% D_2O or in D_2O and contained approximately 2 mM DTT, 1 mM EDTA and 0.1 mM NaN_3. 3-(trimethylsilyl)[2,2,3,3-$^{2}H_4$] propionate was added as a chemical shift reference. All experiments were performed at 30 °C on Bruker DRX-500, DRX-600 or DRX-800 spectrom-

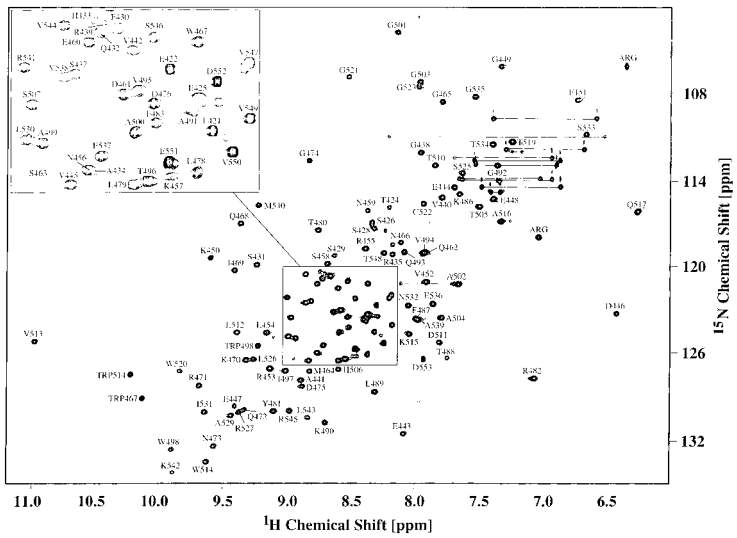

Figure 1. $^{1}H^{N}$-^{15}N HSQC spectrum of the C-terminal globular domain of human lamin A/C. Assignments of the backbone amides and tryptophan imine resonances are indicated. Two connectivities related to the side chain of arginines are labeled as 'Arg'.

eters equipped with triple-resonance probes. Sequential backbone resonance assignments were achieved using data from the following experiments: ^{15}N-HSQC, HNCO, HNCA, HN(CO)CA, HNCACB, CB-CACONH, HNHA and ^{15}N-NOESY-HSQC. Side chain assignments were obtained using HBHACONH, H(CCCO)NH, C(CCCO)NH, HCCH-TOCSY and ^{13}C-HSQC NOESY experiments. A 2D NOESY experiment was also recorded for the assignment of the aromatic side chains of the Tyr, Phe, His and Trp residues. All spectra were processed with the programs Xwinnmr (Bruker) or NMRPipe (Delaglio et al., 1995) and were analyzed using Felix (Molecular Simulations).

Extent of assignments and data deposition

Figure 1 shows the ^{15}N-HSQC spectrum of the C-terminal domain of lamin A/C (residues 411–553). With few exceptions (backbone connectivities of Thr 436, Asn 518 and Thr 528 and side chain connectivities of Asn 518 and Trp 520), all expected $^{1}H^{N}-^{15}N$ connectivities are observed. Residues 411–427 and 548–553 appear flexible as indicated by their backbone chemical shifts and their ^{15}N relaxation properties. Therefore, only the Ser 428-Val 547 region has been considered for the structural study. Backbone and side chain resonances were successfully assigned for residues Ser 428-Val 547, reaching completeness levels of 97% for $^{1}H^{N}$, 96% for ^{15}N, 90% for ^{13}CO and 100% for $^{1}H\alpha$ $^{13}C\alpha$ and $^{13}C\beta$. Side chain assignments are also almost complete. Gaps in the assignment concern the His 433, Arg 453, Trp 467, Trp 498, His 506, Trp 514, Trp 520, Arg 527, Arg 541, and Lys 542 side chains, which could not be totally assigned. For the His 433, Trp 467, Trp 498, His 506 and Trp 514 residues, the missing assignments only correspond to ^{13}C resonances.

The ^{1}H, ^{13}C and ^{15}N chemical shifts of the C-terminal region 428–547 region of human lamin A/C have been deposited in the BioMagResBank (http://bmrb.wisc.edu) under accession number 5224.

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