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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N assignments for the II–III loop region of the skeletal dyhydropyridine receptor

Yanfang Cui[a], Yamuna Karunasekara[a], Peta J. Harvey[a], Philip G. Board[a], Angela F. Dulhunty[a] & Marco G. Casarotto[a,*]
[a]*Division of Molecular Bioscience, John Curtin School of Medical Research, Australian National University, 334, Canberra, ACT 2601, Australia*

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Biological context

The α1 subunit of the dihydropyridine receptor (DHPR) is a protein of approximately 250 kDa and is located in the transverse (t)-tubule membrane of skeletal muscle, in close apposition to the terminal cisternae of the sarcoplasmic reticulum (SR) and ryanodine receptor (RyR) Ca^{2+} release channels. It acts as a surface membrane voltage-activated Ca^{2+} channel and voltage sensor for Ca^{2+} release. It is activated when the membrane is depolarised as a part of the excitation-contraction coupling machinery. During this process, a conformational change in the loop region connecting the second and third membrane segments (II–III loop) is thought to take place which subsequently triggers calcium release from SR stores via the RyR. Numerous studies have suggested that in skeletal muscle the II–III loop forms a physical interaction with the foot region of the RyR and any structural change in the II–III loop structure is thought to be transmitted to the RyR via this interaction (Dulhunty et al., 2002). While it is not entirely clear whether the II–III loop region alone is responsible for EC coupling, it has been noted that the full II–III loop and its fragments are capable of interacting with the RyR and eliciting calcium release (Dulhunty et al., 1999).

*To whom correspondence should be addressed. E-mail: Marco.casarotto@anu.edu.au

Previous structural studies have focused on active fragments of the II–III loop (Casarotto et al., 2000, 2004). As part of the study to elucidate the basis of the molecular interaction between the 14.1 kDa DHPR II–III loop region we report the near complete ^{1}H, ^{13}C and ^{15}N NMR assignments and secondary structure of the II–III loop region of the skeletal DHPR. The assignment of the DHPR II–III loop presented here is the initial step towards determining the structure of the full II–III loop region and the location of its binding site with the RyR.

Methods and experiments

The 391 bp cDNA fragments encoding the skeletal DHPR II–III loop (126 amino acids) were amplified by PCR and cloned in frame down stream of a poly-histidine tagged ubiquitin sequence in the plasmid pHUE. The normal and mutated constructs were checked by sequencing to exclude amplification errors. The plasmid was transferred into *E. coil* BL21 and expression of fusion protein induced by addition of 0.1 mM isopropyl-β-D-thiogalactopyranoside to the culture media. The His-tagged protein was purified by chromatography on Ni-agarose (Catanzariti et al., 2004). Ubiquitin was removed from the N-terminal end of the II–III loop by digestion with a His-tagged ubiquitin-dependent protease (Catanzariti et al., 2004). The ubiquitin protease and cleaved ubiqu-

itin were removed by re-chromatography on Ni-agarose. The recombinant II–III loop, without additional residues, was further purified by preparative electrophoresis under native conditions using a Bio Rad model 491 prep cell. The sample was eluted in 25 mM Tris, 192 mM glycine pH 8.3. Uniformly $^{13}C/^{15}N$ labelled protein was produced by growing the expression strain in M9 minimal media with $^{13}C_6$-glucose and $^{15}NH_4C1$ as the sole carbon and nitrogen sources, respectively. The selectively ^{15}N glutamic acid labelled sample was grown using the same strain in a medium containing a mixture of unlabelled amino acids to which the ^{15}N glutamic acid was added.

All NMR data were acquired at 5 °C with samples containing 1.5 mM $^{13}C/^{15}N$ labelled II–III loop in 50 mM phosphate/200 mM KCl buffer at pH 6.5, 90% H_20/10% D_2O. NMR experiments were recorded at 5 °C on a Varian Inova 600 MHz spectrometer equipped with a pentaprobe and a Bruker Avance 800 MHz spectrometer . All ^1H dimensions are referenced to the H_2O signal relative to DSS and ^{13}C and ^{15}N dimensions are referenced indirectly. Spectra were processed using Felix 2002 (Accelrys Inc) and analyzed with Sparky (T.D. Goddard and D.G. Koeller, Sparky 3.0, University of California, San Francisco).

The assignments of the ^1H, ^{13}C and ^{15}N were based on the following experiments: CACB (CO)NH, CACBNH, HNCO, HNHA, HCCH TOCSY, CC(CO)NH, HC(CO)NH, ^{15}N-edited TOCSY and NOESY (Clore and Gronenborn, 1998; Ferentz and Wagner, 2000). HACAN (Kanelis et al., 2000) combined with (HB)CACB (CO)N(CA)HA were used to track the sequential connection of the proline-rich region (Residues 91–95: PRPRP). The overlap of K68 with R124 was resolved by using a modified sample that consisted of the II–III loop lacking the last C-terminal l2 residues. Because of the glutamic acid rich nature of this protein (19 residues) as well as the severe spectral overlap, the sample was selectively labelled with ^{15}N glutamic acid in order to resolve the assignment ambiguity for several residues (K102, K122, V106, R23, K104, E61, V57).

Extent of assignments and data deposition

All ^1H and ^{15}N backbone resonances were assigned except for the first two residues (Figure 1 in Supplementary material). The backbone resonance assignments for $^1H^N$, ^{15}N, $^{13}C_\alpha$, $^1H_\alpha$ and CO atoms are essentially complete (~98%) and assignments for the C_β and H_β atoms are ~95% complete. Assignments for other side chain atoms (^1H, ^{13}C) extending beyond the β position are ~85% complete.

The identification of the secondary structure unit of the DHPR II–III loop was obtained on the basis of HN, HA and HB NOE data as well as $^3J_{HNHA}$ coupling constants, and chemical shifts indices (Wüthrich, 1986; Wishart and Sykes, 1994) from HA, CA, CO, CB. The II–III loop secondary structure is mainly composed of six helical regions: residues 7–22, 33–37, 58–61, 79–83, 101–104, 109–113. Other regions of the II–III loop contain a mixture of turns and random coil structure. Amide backbone exchange experiments and the absence of long range NOEs indicate that the solution structure of the DHPR II–III loop displays characteristics associated with a molten globule-type state. Such a structure may be important in the loops ability to interact with the RyR as well as its ability to quickly alter its conformation upon DHPR depolarisation. ^1H, ^{13}C and ^{15}N chemical shifts for the skeletal DHPR II–III loop have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under the accession number 6431.f

Supplementary material to this paper is available in electronic format at http://dx.doi.org/10.1007/s10858-005-3038-8.

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