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Letter to the Editor: Sequence-specific resonance assignments of ICln, an ion channel cloned from epithelial cells

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Biological context

Normal function of organs and cells is thightly linked to the cytoarchitecture, and control of the cell volume is thus vital for the organism. An efficient and widely used strategy for cells to maintain cellular integrity by counteracting swelling is the activation of chloride and potassium channels, leading to a net efflux of salts (acompanied by water). Changes of the chloride permeability of cells are the basis of various human inherited diseases (e.g., cystic fibrosis, Thiele et al., 1998; Pilewski and Frizzell, 1999). There is now ample evidence for the involvement of swelling-dependent chloride channels being differentially activated under hypotonic stress conditions (Jakab et al., 2002). The ICln protein was isolated from a cDNA library originated from Madin-Darby canine kidney (MDCK) cells by using the expression cloning technique (Paulmichl et al., 1992). The expression of the ICln protein in *Xenopus Laevis* oocytes leads to an ionic current, which can be blocked by diverse chloride channel blockers. Using antisense oligonucleotides the ICln protein was identified as an ion channel involved in the volume regulation of cells (Gschwentner et al., 1995). The reconstitution of the ICln protein in lipid bilayers verified the channel nature of the protein (Fürst et al., 2000). Here, we report the sequence specific assignment for a truncated form of ICln comprising residues 1–158 (out of 235).

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Methods and results

Expression culture

For purification purposes an N-terminal His_6-Tag together with a two residue spacer (plus the starting methionine) was attached to ICln. The final ICln construct thus comprised 167 residues. ICln was uniformly labelled with ^{15}N and ^{13}C by growing a 1:100 dilution of an overnight bacterial culture (transformed *E. coli* BL21(DE3) grown in ZB-medium (5 g of NaCl and 10 g of NZ-amine AS (Sigma, Austria) in 1 litre of MilliQ water) in minimal medium (4.8 g of Na_2HPO4, 3 g of KH_2PO4, 0.5 g NaCl and 1 g of $^{15}NH_4Cl$ (Cambridge Isotopes Laboratories, U.S.A.) in 1 litre of MilliQ water) supplemented with 20ml of an 18% (w/v) [^{13}C]-D-Glucose solution (Cambridge Isotopes Laboratories, U.S.A.), 2 ml of 1 M $MgSO_4$, 4 ml of 10 mM $ZnSO_4$, 100 μg ml^{-1} ampicillin at 37 °C in a rotary shaker.

Purification of recombinant ICln

Bacterial pellets of 1 l of culture were resuspended in 30 ml of 25 mM K_2HPO_4, pH 7.0 and lysed in a French pressure cell at 15 000 psi. The lysate was cleared by centrifugation and the supernatant applied on a Ni-NTA agarose column (Qiagen, Germany, 4 ml bed volume) connected to a GradiFrac chromatography (AP biotech, Austria) at 0.5 ml min^{-1} followed by washing with 25 mM K_2HPO_4, 200 mM NaCl, pH 7.0 at 1 ml min^{-1}. Bound protein was eluted with a

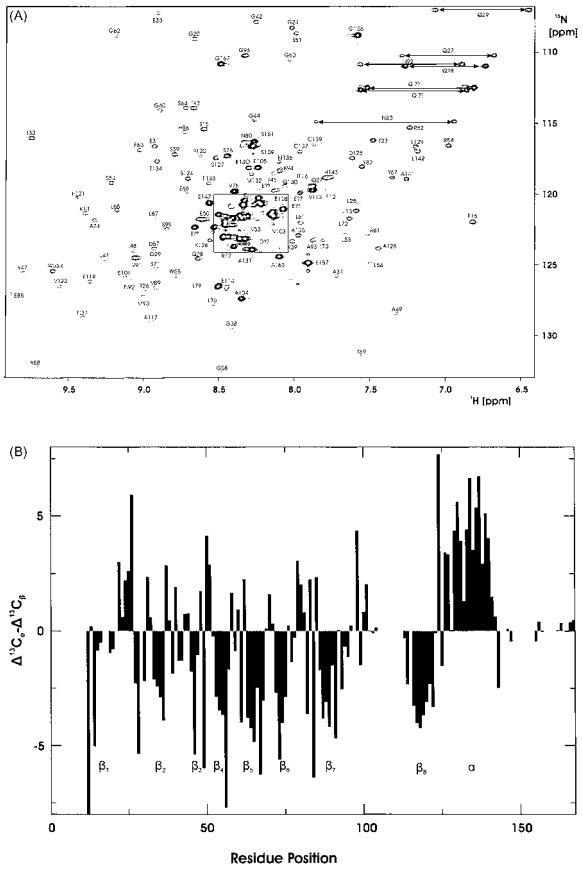

Figure 1. (A) Assigned $^{1}H^{N}$-^{15}N HSQC spectrum of ICln. (B) $\Delta^{13}C^{\alpha}$-$\Delta^{13}C^{\beta}$ secondary chemical shifts are plotted as a function of residue position.

gradient to 200 mM Imidazole-HCl in washing buffer, pH 7.0, ICln containing fractions were concentrated to 1.5 ml by ultrafiltration (Ultrafree 5K NMWL, Millipore, Austria) and applied to a Sephacryl 200 gel filtration column (bed volume 70 ml, AP biotech, Austria) pre-equilibrated with 25 mM K_2HPO_4, 200 mM NaCl, pH 7.0) at a flow rate of 0.5 ml min^{-1}. The final purification step consisted of an anion exchange chromatography step by applying the appropriate fractions of the gel filtration to a Source 30Q column (AP biotech, Austria, bed volume 8 ml), washing with 25 mM K_2HPO_4, 200 mM NaCl, pH 7.0 and eluting ICln with a gradient to 2 M NaCl in washing buffer. ICln containing fractions were pooled, concentrated by ultrafiltration (see above), the salt concentration adjusted to 150 mM NaCl in 25 mM K_2HPO_4, pH 7.0 and used for NMR analysis.

NMR spectra were obtained at 25 °C on Varian INOVA 800 MHz and 500 MHz spectrometer, respectively. The experiments performed included ^{1}H-^{15}N HSQC, 3D HNCO, 3D HN(CA)CO, 3D HNCA, 3D HNCACB, 3D CBCA(CO)NH (Cavanagh et al., 1996). NMR data were processed and analyzed with the NMRPipe (Delaglio et al., 1995) and NMRView (Johnson and Blevin, 1994) software packages, respectively.

Extent of assignments and data deposition

High-quality NMR data for ICln was obtained as shown by the ^{1}H-^{15}N HSQC spectrum collected at 800 MHz in Figure 1A. The boxed region (see also Insert) contains residues located in a flexible linker region connecting two well-defined β-strands, for which only partial sequential assignment was obtained. In total 110 of the non-proline ^{1}H and ^{15}N backbone resonances were assigned. $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and ^{13}CO resonances were assigned for 127 residues. Residues for which no assignment was possible are located either in the flexible linker region (residues 98–116) or at the N-terminus (residues 1 to 11) or C-terminus (144 to 168), respectively. Using the assigned chemical shifts of $^{13}C^{\alpha}$ and $^{13}C^{\beta}$, we have employed the consensus chemical shift index (CSI) (Wishart and Sykes, 1994) to identify the secondary structure of ICln. It consists of 8 β-strands and one α-helix (see Figure 1B) in the C-terminal part of the polypeptide chain. The assignments have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 5736.

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