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Letter to the Editor: Sequence-specific resonance assignment of the carboxyl terminal domain of Connexin43

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Biological context

The gap junction family of integral membrane proteins enables the direct cytoplasmic exchange of ions and small molecules (<1 kDa), including second messengers. Gap junctions are involved in a diverse array of cellular processes including cellular differentiation and development, metabolic homeostasis, and in excitable tissue, electrical coupling. They are formed by the apposition of connexons from adjacent cells, where each connexon is formed of six connexin proteins. Connexins are four transmembrane domain proteins with intracellular N- and C-terminal regions. More than twenty different mammalian connexins exist with the major divergence occurring in the cytoplasmic loop (CL) and carboxyl terminal (CT) domains. The subject of this paper, Cx43, is the most widely expressed gap junction protein and is essential for normal cardiac development and function.

Recently, the idea of gap junctions being formed solely of the connexin proteins has been replaced by the concept that connexons may be centerpieces of a macromolecular complex or 'Nexus' (Spray et al., 1999). Integral tight junction, tight junction-associated, cytoskeletal, adhedrens junctional complex and tyrosine kinase proteins bind to and/or modify the CT of Cx43, consistent with a more active role for gap junctions in cellular functions (Duffy et al., 2002a). A 7.5 Å resolution structure of a recombinant Cx43 cardiac gap junction channel (Unger et al., 1999) has been solved; however, Cx43 was truncated to remove most of the CT. Such truncated constructs form functional channels, but pH sensitivity and interactions with the 'Nexus' proteins are altered. Separate co-expression of the CT domain partially restores pH sensitivity (Morley et al., 1996), and recombinant CT domain binds to the Nexus proteins (Duffy et al., 2002b).

The hypothesis that acidification-induced uncoupling results from the intramolecular interaction between the CT domain and a separate region of the protein affiliated with the pore (Morley et al., 1996), was supported by our studies demonstrating pH dependent binding between the CT domain and a region of CL (L2) (Duffy et al., 2002c). Binding of L2 induced no significant chemical shift changes in ^{15}N labeled CT, suggesting that the CT structure necessary for recognizing and binding CL is pre-formed. To understand the structural bases of connexin regulation, we are studying the Cx43CT in the two protonation states for which binding (pH 5.8) or no binding (pH 7.3) to L2 loop are observed. Here we report the sequence-specific assignments of the loop-binding Cx43CT conformation at pH 5.8. These assignments will be generally useful for mapping the binding sites for all of the Nexus proteins.

Methods and experiments

NMR samples (1.5 mM) of purified isotopically labeled (^{15}N or ^{15}N^{13}C) recombinant rat Cx43CT were prepared in 300 μl of phosphate buffered saline at pH 5.8 (Duffy et al., 2002b). pH and sample stability were monitored by comparing ^{1}H^{15}N-HSQC spectra before and after each 3D NMR experiment.

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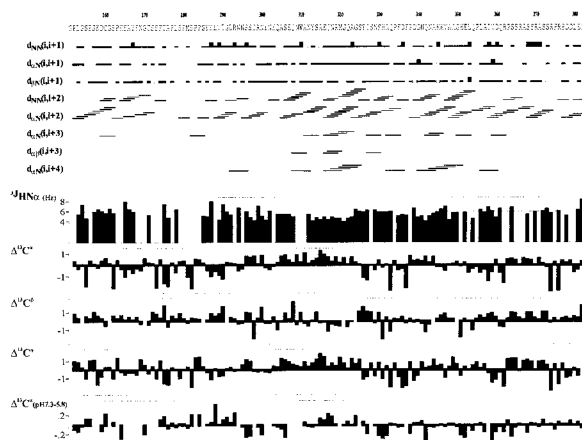

Figure 1. Backbone NOEs, $^{3J}HN_{\alpha}$ coupling constants, and secondary shifts of $^{13}C'$, $^{13}C^{\alpha}$, and $^{13}C^{\beta}$ for the low pH form of Cx43CT. A comparison of backbone chemical shifts at pH 7.3 with those at pH 5.8 is also shown. Blank values for the pH comparison indicate cross-peaks missing (pH 7.3) because of chemical exchange. Open boxes indicate spectral overlap.

NMR data were acquired at 7 °C using a Bruker DRX-600 spectrometer, processed using NMR-Pipe/NMRDraw (Delaglio et al., 1995), and analyzed using NMRView (Johnson and Blevins, 1994). Backbone assignments were obtained using the following 3D experiments: CBCANH, CBCA(CO)NH, HNCO, HN(CO)CA, and HCACO. Side chain chemical shifts were obtained from 3D HCCH-TOCSY, ^{15}N-TOCSY-HSQC, ^{15}N-NOESY-HSQC, and ^{13}C-NOESY-HSQC experiments. $^{3}J_{HN\alpha}$ coupling constants were determined from an HNHA experiment (for all 3D experiments, see Cavanagh et al., 1996).

Secondary structural elements were identified on the basis of chemical shifts, $^{3}J_{HN\alpha}$ coupling constants, and medium range NOEs (Figure 1). Analysis of the chemical shift deviations from random coil for $^{13}C'$, $^{13}C^{\alpha}$, and $^{13}C^{\beta}$ predicts one predominant region of α-helical structure (A311-S325), and possibly a second helical region (D339-K345). This was supported by the small $^{3}J_{HN\alpha}$ coupling constants (<6 Hz) along the sequence and by the NOE pattern. Comparison of the $^{13}C^{\alpha}$ chemical shifts with those of the protein at pH 7.3 suggests possible structural alterations with a gain of helical characteristics around L288 and a loss around Q342. Additional regions of altered structure were evident at about E316 and N329. Although the chemical shift differences are small (∼0.2 ppm), they may become more pronounced upon binding of the Cx43CT structure to the 'Nexus' proteins.

Extent of assignments and data deposition

All ^{1}H, ^{15}N, and ^{13}C backbone resonances except Gly1 and most side chain resonances of Cx43CT at pH 5.8 were assigned and deposited in the BioMagResBank database, accession number 5431.

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