Structure and Dynamics of Ca^{2+}-Binding Domain 1 of the Na^{+}/Ca^{2+} Exchanger in the Presence and Absence of Ca^{2+}

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Introduction

Calcium ions (Ca^{2+}) regulate many different cellular functions. In the heart, for example, an influx of Ca^{2+} into the cytosol leads to muscle contraction. Defects in Ca^{2+} cycling result in smaller and slower transients that are characteristic of heart failure. The major exporter of Ca^{2+} across the plasma membrane of cardiomyocytes is the Na^{+}/Ca^{2+} exchanger (NCX). The activity of the exchanger is regulated by a large intracellular loop that contains two Ca^{2+} binding domains, CBD1 and CBD2. CBD1 binds Ca^{2+} with much higher affinity than CBD2 and is considered to be the primary Ca^{2+} sensor.

Experimental

The effect of Ca^{2+} on the structure and dynamics of CBD1 has been characterized by NMR spectroscopy using chemical shifts, residual dipolar couplings, and spin relaxation.[1] These experiments were performed on a Bruker 800 MHz spectrometer equipped with a TCI cryoprobe at the National High Magnetic Field Laboratory.

Results and Discussion

Residues for which Ca^{2+} binding elicits a substantial change in chemical environment are indicated by large values of the amide chemical shift change $\Delta\delta_{NH,i}$. In Figure 1, the $\Delta\delta_{NH,i}$ values are plotted as a function of residue number. The largest $\Delta\delta_{NH,i}$ values cluster in four regions of the protein sequence, each of which contributes one or more Ca^{2+}-binding ligands.

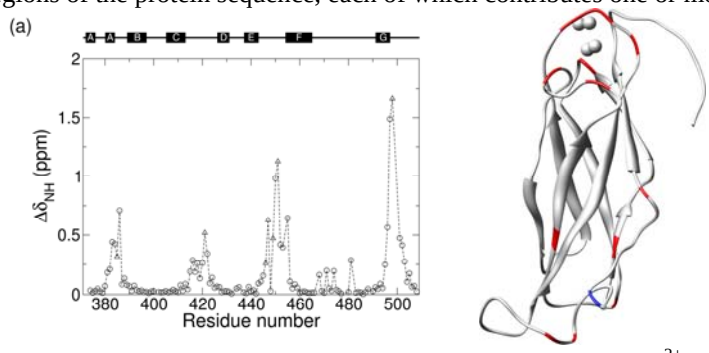

Figure 1: (Left) Plot of $\Delta\delta_{NH,i}$ as a function of residue number. Residues that contribute a Ca^{2+}-binding ligand are represented by triangles. (Right) Structural context for the S^2 changes that accompany Ca^{2+} binding. Residues whose S^2 value is higher (lower) in the Ca^{2+} bound state are represented in red (blue). Ca^{2+} ions are represented as spheres.

Residues that exhibit a substantial conformational change are also sites of altered dynamics. Ca^{2+} binding is associated with an increase in the NMR order parameter S^2 for many binding site residues (Figure 1). Future studies of a construct containing both CBD1 and CBD2 will determine how the changes identified here are transmitted to other parts of the molecule.

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References

[1] Johnson, E., Bruschweiler-Li, L., Showalter, S.A., Vuister, G.W., Zhang, F. & Brüschweiler, R. Submitted.