Reconstitution and spectroscopic analysis of caveolin-1 residues 62–178 reveals that proline 110 governs its structure and solvent exposure

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Title:

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Abstract:

Caveolin-1 is a membrane protein that possesses an unusual topology where both N- and C- termini are cytoplasmic as a result of a membrane-embedded turn. In particular, proline 110 has been postulated to be the linchpin of this unusual motif. Using a caveolin-1 construct (residues 62-178) reconstituted into dodecylphosphocholine micelles with and without a cholesterol mimic, the changes that occurred upon P110A mutation were probed. Using far UV circular dichroism spectroscopy it was shown that cholesterol attenuated the helicity of caveolin-1, and that mutation of P110 to alanine caused a significant increase in the α-helicity of the protein. Near UV circular dichroism spectroscopy showed significant changes in structure and/or environment upon mutation that again were modulated by the presence of cholesterol. Stern-Volmer quenching and λ_{max} analysis of tryptophan residues showed that the proline mutation caused W85 to become more exposed, W98 and W115 to become less exposed, and W128 showed no change. This finding provided evidence that regions proximal and far away from the proline are buried differentially upon its mutation and therefore this residue is strongly tied to maintaining the hydrophobic coverage along the caveolin-1 sequence. In the presence of cholesterol, the accessibilities of the two tryptophan residues that proceeded position 110 were altered much more significantly upon P110A mutation than the two tryptophans aft P110. Overall this work provides strong evidence that proline 110 is critical for maintaining both the structure and hydrophobic coverage of caveolin-1 and that cholesterol also plays a significant role in modulating these parameters.

Keywords:

caveolin-1; fluorescence spectroscopy; circular dichroism spectroscopy; topology.

Abbreviations:

DPC, dodecylphosphocholine; CD, circular dichroism; NMR, nuclear magnetic resonance; Cholesterol-PEG600, CPEG; HFIP, 1,1,1,3,3,3-hexafluoroisopropanol; PEG, polyethylene glycol.