Global transformation of erythrocyte properties via engagement of an SH2-like sequence in band 3

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Significance

Src homology 2 (SH2) domains regulate signaling by binding phosphotyrosines. While all known SH2 domains consist of contiguous sequences of ∼100 aa, we describe a highly conserved SH2-like sequence motif located in the second cytoplasmic loop of membrane-spanning domain of erythrocyte membrane band 3. Upon tyrosine phosphorylation of the cytoplasmic domain of band 3, the domain rotates to bind the SH2 signature sequence within the membrane-spanning domain of band 3, promoting significant changes in erythrocyte properties. Because sequence homology searches do not recognize SH2-like sequence motifs, the possibility arises that many other SH2-like structures remain undetected in membrane-spanning proteins where they perform critical steps in kinase-facilitated signaling pathways.

Abstract

Src homology 2 (SH2) domains are composed of weakly conserved sequences of ∼100 aa that bind phosphotyrosines in signaling proteins and thereby mediate intra- and intermolecular protein–protein interactions. In exploring the mechanism whereby tyrosine phosphorylation of the erythrocyte anion transporter, band 3, triggers membrane destabilization, vesiculation, and fragmentation, we discovered a SH2 signature motif positioned between membrane-spanning helices 4 and 5. Evidence that this exposed cytoplasmic sequence contributes to a functional SH2-like domain is provided by observations that: (*i*) it contains the most conserved sequence of SH2 domains, GSFLVR; (*ii*) it binds the tyrosine phosphorylated cytoplasmic domain of band 3 (cdb3-PO_4) with K_d = 14 nM; (*iii*) binding of cdb3-PO_4 to erythrocyte membranes is inhibited both by antibodies against the SH2 signature sequence and dephosphorylation of cdb3-PO_4; (*iv*) label transfer experiments demonstrate the covalent transfer of photoactivatable biotin from isolated cdb3-PO_4 (but not cdb3) to band 3 in erythrocyte membranes; and (*v*) phosphorylation-induced binding of cdb3-PO_4 to the membrane-spanning domain of band 3 in intact cells causes global changes in membrane properties, including (*i*) displacement of a glycolytic enzyme complex from the membrane, (*ii*) inhibition of anion transport, and (*iii*) rupture of the band 3–ankyrin bridge connecting the spectrin-based cytoskeleton to the membrane. Because SH2-like motifs are not retrieved by normal homology searches for SH2 domains, but can be found in many tyrosine kinase-regulated transport proteins using modified search programs, we suggest that related cases of membrane transport proteins containing similar motifs are widespread in nature where they participate in regulation of cell properties.