Phosphorylation of the dimeric cytoplasmic domain of the phytosulfokine receptor, PSKR1

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Abstract

Phytosulfokines (PSKs) are plant peptide hormones that co-regulate plant growth, differentiation and defense responses. PSKs signal through a plasma membrane localized leucine-rich repeat receptor-like kinase (phytosulfokine receptor 1, PSKR1) that also contains a functional cytosolic guanylate cyclase with its cyclase catalytic center embedded within the kinase domain. To functionally characterize this novel type of overlapping dual catalytic function, we investigated the phosphorylation of PSKR1 in vitro. Tandem mass spectrometry of the cytoplasmic domain of PSKR1 (PSKR1cd) revealed at least 11 phosphorylation sites (8 serines, 2 threonines and 1 tyrosine) within the PSKR1cd. Phosphomimetic mutations of three serine residues (Ser686, Ser696 and Ser698) in tandem at the juxta-membrane position resulted in enhanced kinase activity in the on-mutant that was suppressed in the off-mutant, but both mutations reduced guanylate cyclase activity. Both the on and off phosphomimetic mutations of the phosphotyrosine (Tyr888) residue in the activation loop suppressed kinase activity, while neither mutation affected guanylate cyclase activity. Size exclusion and analytical ultracentrifugation analysis of the PSKR1cd suggest that it is reversibly dimeric in solution, which was further confirmed by biflourescence complementation. Taken together, these data suggest that in this novel type of receptor domain architecture, specific phosphorylation and dimerization are possibly essential mechanisms for ligand-mediated catalysis and signaling.