KINASE-INTERACTING FHA DOMAIN OF KINASE ASSOCIATED PROTEIN PHOSPHATASE: PHOSPHOPEPTIDE INTERACTIONS AND DYNAMICS

Zhaofeng Ding

Dr. Steven R. Van Doren, Dissertation Supervisor

ABSTRACT

FHA domains are phosphoThr recognition modules found in diverse signaling proteins. Kinase-associated protein phosphatase (KAPP) from Arabidopsis employs its FHA domain in its negative regulation of some receptor-like kinase (RLK) signaling pathways. The interactions between the kinase-interacting FHA (KI-FHA) domain of KAPP and RLK kinase domains have been investigated. Three phosphoThr peptides of KAPP-binding RLKs were found by isothermal titration calorimetry (ITC) and NMR to bind KI-FHA, with K_d values of 8 to 30 μM. Thermodynamics study revealed that their affinities were driven by favorable enthalpy and the hydrophobic effect. Mutagenesis of these three threonine sites suggests Thr546 in the C-lobe of BAK1 kinase domain to be a principal site of KI-FHA binding. BRI1 kinase domain interacts with the same 3/4, 4/5, 6/7, 8/9, and 10/11 recognition loops of KI-FHA as do phosphoThr peptides.

The backbone mobility of KI-FHA, free and bound to pThr868CLV1 peptide has also been investigated using ^{15}N NMR relaxation at 500 MHz and 600 MHz. Binding of the peptide seems to reduce nsec-scale fluctuations of KI-FHA globally. In the psec to nsec timescale, KI-FHA residues that are critical for phosphopeptide recognitions are rigid. Peptide binding rigidifies KI-FHA at the binding site and remote sites across the β-sandwich. Peptide binding increases flexibility around the periphery of the binding site, perhaps relieving strain from the peptide association.