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Phosphorylation- and Nucleotide-Binding- Induced Changes to the Stability and Hydrogen Exchange Patterns of JNK1β1 Provide Insight into Its Mechanisms of Activation

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Abstract

Many studies have characterized how changes to the stability and internal motions of a protein during activationcan contribute to their catalytic function, even when structural changes cannot be observed. Here, unfoldingstudies and hydrogen–deuteriumexchange (HX)mass spectrometrywere used to investigate the changes to thestability and conformation/conformational dynamics of JNK1β1 induced by phosphorylative activation.Equivalent studies were also employed to determine the effects of nucleotide binding on both inactive andactive JNK1β1 using the ATP analogue, 5'-adenylyl imidodiphosphate (AMP-PNP). JNK1β1 phosphorylationalters HX in regions involved in catalysis and substrate binding, changes that can be ascribed to functionalmodifications in either structure and/or backbone flexibility. Increased HX in the hinge between the N- andC-terminal domains implied that it acquires enhanced flexibility upon phosphorylation that may be a prerequisite for interdomain closure. In combination with the finding that nucleotide binding destabilizes the kinase; thepatterns of solvent protection by AMP-PNP were consistent with a novel mode of nucleotide binding to the C-terminal domain of a destabilized and open domain conformation of inactive JNK1β1. Solvent protection by AMP-PNP of both N- and C-terminal domains in active JNK1β1 revealed that the domains close around nucleotide upon phosphorylation, concomitantly stabilizing the kinase. This suggests that phosphorylation activates JNK1β1 in part by increasing hinge flexibility to facilitate interdomain closure and the creation of a functional active site. By uncovering the complex interplay that occurs between nucleotide binding and phosphorylation, we present new insight into the unique mechanisms by which JNK1β1 is regulated.