

High-Resolution Crystal Structures and Active Site Properties of the Catalytic Domain of Human Protein Kinase Associated with Apoptosis and Tumor Suppression

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Abstract

We have determined x-ray crystal structures of the catalytic domain of death-associated protein kinase (DAPK), the first described member of a novel family of pro-apoptotic and tumor suppressive serine/threonine kinases. X-ray analysis was performed to a maximum resolution of 1.5 Å, the highest for any protein kinase structure reported to date. The catalytic domain was found to be in the active “closed” conformation, and its flexibility was assessed in two different crystal forms. The geometry of the active site was studied in the apo-form, in the complex with AMPPnP, and in a ternary complex consisting of kinase, AMPPnP, and either Mg²⁺ or Mn²⁺. The resolution of the x-ray data revealed a previously unreported water-mediated stabilization of the interaction between the universally conserved active site lysine and the β- and γ-phosphates of ATP, as well as conformational changes at the active site upon ion binding. Comparison between these structures and nucleotide triphosphate complexes of several other kinases, including phosphorylase kinase and cyclic AMP-dependent kinase, reveals unique features of the DAPK catalytic domain. Among them is a highly ordered basic loop in the N-terminal domain that may participate in catalysis.

Methods and Materials

Orthorhombic apo form and binary and ternary complexes. Crystals were shock-frozen in mother liquor supplemented with 25% glycerol. Diffraction data were collected at 100 K on the 5-ID beamline of the DND-CAT at the Advanced Photon Source (APS). Single-wavelength anomalous diffraction data were collected at a wavelength of 1.00928 Å using a single Hg-DAPK crystal. The maximum resolutions of x-ray data for native and derivative crystals were 1.5 Å and 1.8 Å, respectively. All data were integrated and merged in the DENZO/SCALEPACK program suite. Data collection with crystals of the ternary complex between DAPK, AMPPnP, and manganese were performed on the 17-ID beamline

of the IMCA-CAT at the APS at a wavelength of 1.8923 Å in order to maximize the anomalous contribution of manganese. *Cubic apo form.* A crystal was shock-frozen in mother liquor and diffraction data were collected on the 5-ID beamline of the DND-CAT at the APS to a maximum resolution of 3.5 Å.

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Reference

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