Insights into the phosphoryl transfer catalyzed by cAMP-dependent protein kinase

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Protein kinases are involved in a number of cell signaling pathways. They catalyze phosphorylation of proteins and regulate the majority of cellular processes (such as growth, differentiation, lipid metabolism, regulation of sugar, nucleic acid synthesis, etc.). Chemically, protein kinases covalently transfer the gamma-phosphate group of a nucleoside triphosphate (e.g. ATP) to a hydroxyl group of a Ser, Thr or Tyr residue of substrate protein or peptide. The reaction involves moving hydrogen atoms between the enzyme, substrate and nucleoside. The unanswered question is whether the proton transfer from the Ser residue happens before the phosphoryl transfer using the general acid-base catalyst, Asp166, or after the reaction went through the transition state by directly protonating the phosphate group. To address this key question about the phosphoryl transfer, we determined a number of X-ray structures of ternary complexes of catalytic subunit of cAMP-dependent protein kinase (PKAc) with various substrates, nucleotides and cofactors. Importantly, we were able to trap and mimic the initial (Michaelis complex) and final (product complex) stages of the reaction. The results demonstrate that Mg²⁺, Ca²⁺, Sr²⁺, and Ba²⁺ metal ions bind to the active site and facilitate the reaction to produce ADP and a phosphorylated peptide. The study also revealed that metal-free PKAc can facilitate the phosphoryl transfer reaction; a result that was confirmed with single turnover enzyme kinetics measurements. Comparison of the product and the pseudo-Michaelis complex structures, in conjunction with molecular dynamics simulations, reveals conformational, coordination, and hydrogen bonding changes that help further our understanding of the mechanism, roles of metals, and active site residues involved in PKAc activity.

Keywords: protein kinase, catalytic mechanism, X-ray crystallography