Kinetic mechanism of phosphofructokinase-2 from Escherichia coli. A mutant enzyme with a different mechanism

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The kinetic mechanisms of Escherichia coli phosphofructokinase-2 (Pfk-2) and of the mutant enzyme Pfk-2* were investigated. Initial velocity studies showed that both enzymes have a sequential kinetic mechanism, indicating that both substrates must bind to the enzyme before any products are released. For Pfk-2, the product inhibition kinetics was as follows: fructose-1,6-P2 was a competitive inhibitor versus fructose-6-P at two ATP concentrations (0.1 and 0.4 mM), and noncompetitive versus ATP. The other product inhibition patterns, ADP versus either ATP or fructose-6-P were noncompetitive. Dead-end inhibition studies with an ATP analogue, adenylyl imidodiphosphate, showed uncompetitive inhibition when fructose-6-P was the varied substrate. For Pfk-2!a,, the product inhibition studies revealed that ADP was a competitive inhibitor versus ATP at two fructose-6-Pconcentrations (0.05 and 0.5 mM), and noncompetitive versus fructose-6-P. The other product, fructose-1,6-P2, showed noncompetiti