Mode of Substrate Binding for Ketohexokinase across Isozymes and Species Implies an Induced-Fit mechanism

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Ketohexokinase (KHK) is an adenosine triphosphate (ATP)-dependent enzyme that catalyzes the first reaction in fructose metabolism. The liver isozyme, KHK-C, has become a target for pharmacological development against fatty liver and metabolic syndrome. Recent evidence has implied that the more ubiquitous isozyme, KHK-A, functions as a protein kinase after it is phosphorylated by AMP-dependent protein kinase, an activity absent in KHK-C. This property leads to downstream effects ameliorating reactive-oxygen species (ROS) and the expression of genes required for cell adhesion, without which metastasis is promoted. The KHK isozymes differ by alternative splicing of exon 3, which encodes 45 out of 298 amino acids. Both isozymes exist as homodimers interlocked with a β-clasp domain. The structure of mouse KHK-A in its unliganded form was determined using macromolecular X-ray crystallography and refined to 1.85 Å resolution. The structure revealed that mKHK-A could indeed adopt a different conformation than the human KHK-A structures when substrates are bound, contrary to the previous proposal (Trinh et al., 2009). Furthermore, the conformational change observed in the mouse KHK-A structure is conserved with that of human KHK-A. When compared to other unliganded KHK-A structures, mouse KHK-A structure adopts a β-clasp conformation similar to other unliganded KHK-A structures, whereas KHK-C unliganded structures adopt a wider range of rotational/hinge motion of the β-clasp. Adenosine kinase and ribokinase, which belong to the same PfkB family of carbohydrate kinases, exhibit a similar mode of conformational change upon ribose/nucleoside binding, which has been proposed as an induced-fit mechanism for ATP binding. This suggests that the KHK isozymes across different species operate in a similar mechanism of induced-fit binding during catalysis.