

Crystal structure of human keto-hexokinase and its complexes with fructose and a nucleotide analogue.

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Introduction

Essential fructosuria is a benign inborn error of metabolism. It is a condition which is believed to result from deficiency of hepatic fructokinase (ketohexokinase, KHK). KHK catalyses the phosphorylation of the ketose sugar fructose, to yield fructose-1-phosphate. Alternative splicing of the KHK gene generates a "central", predominantly hepatic isoform ketohexokinase-C (KHK-C) and a more widely distributed ketohexokinase-A (KHK-A). Both KHK isoforms are active. KHK-A has much poorer affinity for fructose than KHK-C but is considerably more thermostable. Mutations that cause essential fructosuria consequently result in significant loss of KHK-C activity but not of KHK-A. Affected individuals therefore probably have a selective deficiency of hepatic KHK, with peripheral KHK-A being preserved. Here, we report the structure of the human KHK-C and its comparison with the previously solved structure of human KHK-A in complex with fructose and an ATP analogue.

Crystallographic Studies

KHK-C was crystallised using vapour diffusion techniques. Data to a resolution of 2.9 Å was collected using synchrotron radiation at Daresbury Laboratory at a temperature of 100 K. The KHK-C structure was determined by the method of molecular replacement using the structure of KHK-A as the starting model. Model building and refinement was accomplished using the programs O and CNS, respectively.

Structures of ketohexokinase-C and ketohexokinase-A

There are two dimers of KHK-C in the asymmetric unit. Each KHK-C subunit consists of 298 residues. The alternative splicing of the gene results in a difference of a single region between the two isoforms of the protein (Fig. 1). Like the structure of KHK-A the subunit of KHK-C has two distinct secondary structural elements; a central α/β fold and a 4-stranded β -sheet. A notable difference between the two isoform structures is the orientation of the two subunits forming the KHK-C dimer when compared to that for KHK-A. When one of the subunits of KHK-C is superimposed onto the corresponding one from KHK-A, the central α/β fold of the molecules fit well. However the relative orientation of the second subunits is different (Fig. 1).

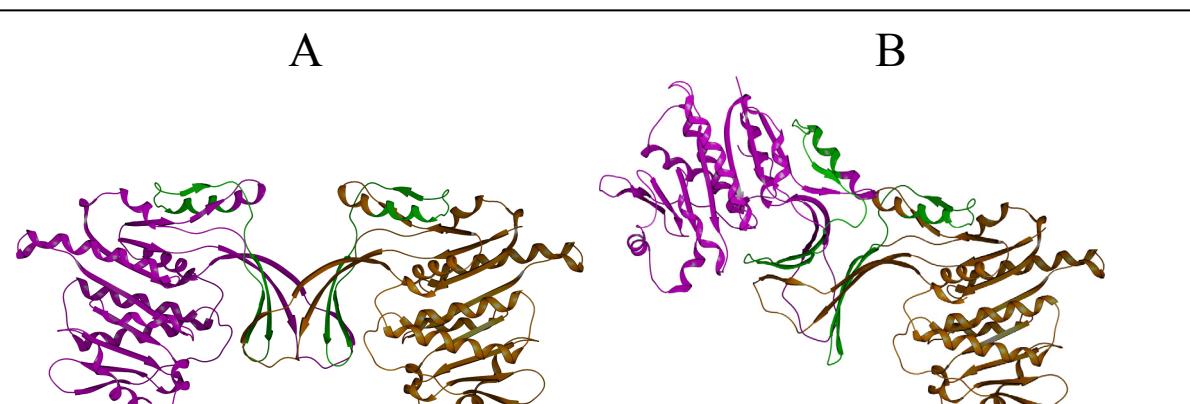


Fig. 1. Ribbon diagram of KHK-A dimer (A) and KHK-C dimer (B). For both structures the gold subunit is in the same orientation. The alternative splicing of the KHK gene resulting in a difference of a single region between the two isoforms of the protein (residues 72 to 115) is shown here in green.

The overall structure of KHK-C is similar to KHK-A. There is one active site per KHK monomer and this is located between the central α/β fold and the 4 stranded β -sheet forming the dimer interface (Fig. 2). For the structure of the KHK-A complex electron density is observed for both the fructose molecule and the nucleotide ATP analogue. Superposition of the two different isoforms monomer subunits revealed the residues forming the binding site to be conserved. The fructose molecule and the ATP analogue from the structure of KHK-A can be modelled into the binding cleft of KHK-C.



Fig. 2. Structure of the ketohexokinase-A monomer binding to a fructose molecule and an ATP analogue, AMP-PNP

Collaborators

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Funding

This work was funded by the Wellcome Trust.