Evolutionary history of glucokinase/phosphofructokinase activity in the ADP-dependent sugar kinases family.

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Several archaea of the *Euryarchaeota* present a uniquely modified Embden–Meyerhof pathway that involves only four of the classical enzymes present in the canonical pathway. One of the most striking features of this modified glycolysis is the phosphorylation of glucose and fructose 6-phosphate (fructose-6P) by ADP as the phosphoryl donor, instead of ATP. These ADP-dependent kinases are homologous to each other and they show no sequence similarity to any of the hitherto known ATP-dependent enzymes. It was therefore proposed that they were part of a new family of kinases and has been classified into the ribokinase superfamily, given the structural similarity with these proteins.

In the archaea domain, the ADP-dependent sugar kinases have been found in the genera *Pyrococcus, Thermococcus, Methanosarcina, Methanosaeta, Methanococcoides, Methanococcus, Archaeoglobus* and *Metanocaldococcus*. To date, experimentally characterized enzymes are mainly from the *Termococcales* group; the glucokinase (ADP-GK) from *Termococcus litoralis* and *Pyrococcus furiosus* are highly specific for glucose. Also, ADP-dependent phosphofructokinases (ADP-PFK) from *Pyrococcus horikoshii, Thermococcus zilligii, Pyrococcus furiosus* and *Archeaoglobus fulgidus*, are highly specific for fructose-6P. All other proteins classified as ADP-dependent glucokinases or phosphofructokinases have been assigned only by sequence similarity.

One of the most interesting features in this family is the presence of a dual-function or non-specific enzyme called ADP-dependent glucokinase/phosphofructokinase (ADP-GK/ PFK) present in the archaeon *Methanocaldococcus jannaschii*. The ability to catalyze the transfer of phosphate to glucose and fructose-6P led to the proposition that this nonspecific enzyme was an ancestral state for the whole family which, after a gene duplication event, generates the two specificities known today. Subsequently, phylogenetic analysis showed that the ADP-GK/PFK enzyme does not represent an ancestral form and, since this enzyme is found inside the phosphofructokinase group, rather it would be an ADP-PFK capable to phosphorylate glucose. Although it was discard that this bi-functional enzyme is an ancestral state, to date it is not known when this trait appear or if the enzyme from *M. jannaschii* is the only enzyme in this group with this characteristic.
In this work we reconstructed the phylogeny of this family and the evolutionary history of the use of substrates through the estimation of ancestral states of discrete traits based on likelihood. We inferred, synthesized and expressed the gene for the last common ancestor of phosphofructokinases from the *Thermococcales* and *Methanococcales* groups (ancMT) and the PFK ancestor of the *Thermococcales* group (ancT) and then, we compared the substrate specificity of them with the estimation of ancestral states of discrete traits. The results indicate that the enzymes from *Metanococcales* are not specific phosphofructokinases as are believed to date. These enzymes are able to phosphorylated both glucose and fructose-6P whereas only the enzymes from the *Termococcales* group are specific for fructose-6P. We built homology models of the ancestral proteins structures and use them to performed ligand-docking experiments, which allow us to identify key residues for fructose-6P specialization in the *Termococcales* group.

References
