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Review

Biochemistry and regulatory functions of bacterial glucose kinases

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ABSTRACT

Glucokinases (Glks) are enzymes widely distributed in all three domains of life. They are located at the beginning of the glycolytic pathway and are responsible for the glucose phosphorylation from various phosphate group donors such as ATP, ADP and polyphosphate. So far, there are eight crystallized Glks, and at least one belongs to each of the three reported Glk families. Structural studies have elucidated the mechanism for Glk action and multimerization. Cloning, overexpression and biochemical characterization have demonstrated the wide diversity of these enzymes. As reported for various microorganisms, in addition to their catalytic activity, some Glks, possessing ROK (Repressor Orf Kinases) motifs, also display a regulatory role. This function has been associated to the mechanisms of carbon catabolite regulation, morphological differentiation and antibiotic production. The present review covers the classification, detailed tertiary structure, mechanism of action, biochemical characterization and some regulatory aspects of bacterial Glks.

Introduction

Glucokinases (Glks) are responsible of glucose phosphorylation. The enzyme product, glucose 6-phosphate, can be subsequently metabolized to various metabolic fates. Glks are widely distributed in all three domains of life. According to the Pfam (Protein family database, [11] Glks are classified based on their primary structure within the sugar kinase/HSP70/actin (CL0108) superfamily, which contains 29 members. It includes the actin family, HSP70 molecular chaperones, the ROK family (Repressor Orf Kinases), hexokinases 1 and 2, the FGGY family (comprising glycerol kinase and related carbohydrate kinases such as L-fucolokinase and xylulokinase), the propionate/acetate kinase family, and others (http://pfam.xfam.org/clan/CL0108). The Glks transfer a phosphoryl group to the alpha-carbon of glucose, using various phosphate group donors such as ATP (EC: 2.7.1.1 and EC: 2.7.1.2), ADP (EC: 2.7.1.147) and polyphosphate (EC: 2.7.1.63) (Kyoto Encyclopedia of Genes and Genomes). In eukaryotes, the enzymes that perform glucose phosphorylation are usually hexokinases [28]. Most prokaryotes use the phosphoenolpyruvate-dependent phosphotransferase system (PTS) which couples carbohydrate transport to its concomitant phosphorylation [72]. However, even when bacteria predominantly use PTS, they also possess active Glks [64,56,27,35]). Moreover, in addition to their catalytic activity, some bacterial Glks have shown some degree of regulatory capacity [1,56,35]. The current classification of Glks is based on data from primary, secondary and tertiary structure [28]. Structural studies have established the catalytic mechanism of these enzymes [39] and the mechanism for multimerization (dimer formation) [44]. Cloning of Glk genes has enabled their overexpression for further purification and in many cases, their biochemical characterization [78,77,63]. In spite of their importance for the bacterial metabolism, this enzyme has not properly reviewed yet. The aim of the proposed review is to cover several issues about bacterial Glks, such as classification, structure, mechanism of action, biochemical characterization and some aspects on their function as regulatory proteins.

Source and diversity

Glucokinases are present both in prokaryotes and eukaryotes. Glucose phosphorylation in eukaryotes is mostly achieved by ATP-dependent kinases, called hexokinases (HK), which show broad substrate specificity for hexoses. In contrast to HK, bacterial Glks usually show high specificity for glucose. Kawai et al. [28] classified the Glks based on their primary, secondary and tertiary protein structure. Thus, Glks from eukaryotes or prokaryotes were broadly classified into two distinct non-homologous families, hexokinase (HK) and ribokinase (RK). The RK family comprises

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ADP-dependent Glks from euryarchaeota and eukaryotes (mammals). In the HK family, Glks are subgrouped into three small clusters, HK, A and B. The HK group consists entirely of HKs from eukaryotic origin. Group A is composed by ATP-dependent Glks from Gram-negative bacteria, cyanobacteria, and amitochondriate protists. Finally, group B includes HKs from crenarchaeota, Glks from Gram-positive bacteria, and some bacterial polyphosphate glucokinases. It is noteworthy to mention that group B also includes glucokinases that belong to the ROK family of proteins.

Lunin et al. [39], classified microbial Glks based on their primary amino acid structure, thus Glks can be divided into three distinct families: (i) Glks from archaea, (ii) ATP-Glks without a ROK motif and, (iii) ATP-Glks belonging to the ROK family.

1. The first family (Pfam: PF04587), involves ADP-dependent Glks (ADP-Glks) from archaea and some higher eukaryotes (Fig. 1). Nowadays, approximately 200 sequences belonging to this family are compiled in the Pfam database [53]. These enzymes are involved in a modified Embden–Meyerhof pathway in archaea requiring ADP as the phosphoryl group donor, instead of ATP [62,18]. In euryarchaeota, two types of glucose-phosphorylating enzymes have been reported: (i) the ADP-Glks from the hyperthermophilic euryarchaea *Pyrococcus furiosus* [29,30,69], *Thermococcus litoralis* [30] and *Archaeoglobus fulgidus* strain 7324 [33]; and (ii) ATP-dependent glucose-phosphorylating enzymes (treated later in this section). Additionally, a bifunctional ADP-Glk/phos phofructokinase has been described in *Methanococcus jannaschii* [58]. A considerable number of ADP-dependent Glks from eukaryotes are reported in the Pfam database, followed by archaea and bacteria (Fig. 1). Likewise, eleven of such Glks were reported in firmicutes, three in actinobacteria, two in proteobacteria and only one in spirochaetia.

The crystal structures of the ADP-Glks from *T. litoralis* [25], *P. furiosus* [26] and *Pyrococcus horikoshii* [68] have been solved. These crystal configurations have shown that structures from group I, are similar to those of ATP-dependent kinases, such as the *Escherichia coli* ribokinase and human adenosine kinase. Interestingly, eukaryotic HKs that are homologous to ADP-Glks, have been identified in several eukaryotic genome sequences with an identity from 12–17% [25,59,60,74]. The only mammalian recombinant ADP-Glk described until now, has been cloned, expressed and characterized from mouse (*Mus musculus*) [55]. However, the physiological relevance of this kinase for higher eukaryotes is still unknown.

2. The second family (Pfam: PF02685) comprises ATP-Glks that do not contain the ROK motif. The Pfam database currently contains more than 1500 full or partial protein sequences belonging to this family (Fig. 2). Most of the members of this family are from bacterial origin, 40 from eukaryotes and 1 from archaea. The main bacterial members belong to Proteobacteria and Cyanobacteria (COG0837).

With regard to crystal structures from the members of this family, we observe similarity between the *E. coli* Glk (EcGlk) to that from *Saccharomyces cerevisiae* hexokinase B and to the human brain hexokinase I [39].

3. The third family (ATP-Glks belonging to the ROK family) PFAM (PF00480), essentially comprises ATP-Glks from eukarya, archaea and bacteria (primarily Gram-positive) containing a ROK motif (Fig. 3).

Sugar kinases that are classified as members of the ROK family have been found in many bacterial species and constitute the largest family of bacterial Glks with approximately 3600 members in the Pfam database. From these members, most are present in firmicutes, followed by proteobacteria and actinobacteria (Fig. 3).

Even when a vast number of ROK members are from prokaryotic origin, there are proteins with ROK domains in all branches of life [6]. Currently, approximately 5000 ROK-family proteins have been identified so far, mostly in prokaryotes, but family members are found in all kingdoms of life [65].

The ROK domain has been reported in a large number of proteins, including kinases, repressors, and many proteins with unknown functions. ROK family members can be identified by two signature sequences (known as ROK boxes) located at the central region of the protein. Signature sequences 1 and 2, consist of 28 amino acid residues with a conserved EXGH motif and 14 residues with a conserved Zn-binding motif (CXCGXXGCXE), respectively. Broadly, a general distinction between repressors and kinases can be made based on the N-terminal sequence. Kinases contain an ATP binding motif and repressors contain a helix-turn-helix DNA binding motif [42].

Despite the overwhelming number of ROK family protein sequences, only the crystal structure of the ATP-Glk from *Streptomyces griseus* (SgGlkA) has been determined and constitutes the first example in this group [44].

In some actinobacterial species, polyphosphate-dependent glucokinases have also been identified (polyP-Glks). These Glks uses polyphosphate (polyP) as the phosphoryl donor, as well as ATP. Inorganic polyP is an energy- and phosphorus-rich biopolymer that is present in a variety of organisms. The energy contained in the phosphodiester bonds of polyP is thermodynamically equivalent to the energy obtained from ATP and can be utilized directly and indirectly for phosphorylation of cellular molecules [46]. PolyP can be found in organisms that represent species from each domain in nature: Eukarya, Archaea and Bacteria. Among other functions in prokaryotes, polyP and its associated enzymes play a crucial role in basic metabolism and stress responses [76].

Even when the polyP polymers have been found practically in all species, polyP-Glks have been found only in a few bacteria [54]. In addition, a bifunctional Glk (polyP/ATP-Glk), which utilizes polyP or ATP as phosphoryl donor to phosphorylate glucose, was first reported in *Mycobacterium phlei* [66] and then in many other

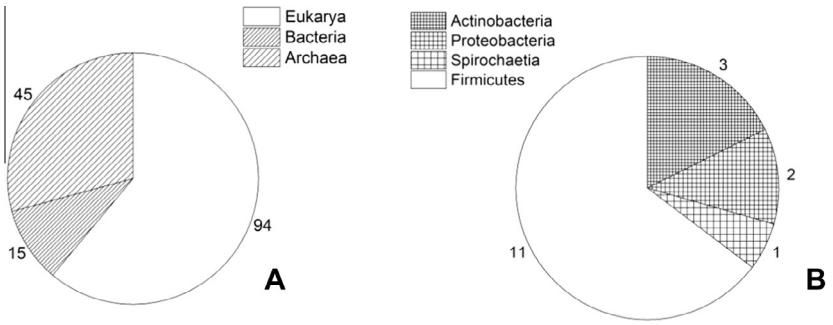

Fig. 1. Distribution of ADP-dependent Glks (ADP-Glks) (Pfam: PF04587) in the three domains of life (A) and in the genera of bacteria (B). The presence of ADP-Glks were searched in the Pfam database, thus numbers in the pie charts indicate how many proteins belonging to PF04587 are reported.