**FOR THE RECORD**

# Glucoamylase-like domains in the α- and β-subunits of phosphorylase kinase

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**Abstract**

Phosphorylase kinase is a four-subunit enzyme involved in the regulation of glycogen breakdown. The traditional textbook view is that only the γ subunit has enzymatic activity, whereas the other three subunits have a regulatory role. Evidence from homology searches and sequence alignments, however, shows that the α- and β-subunits possess amino-terminal glucoamylase-like domains and suggests that they might possess a previously overlooked amylase activity. If true, this would have important implications for the understanding, diagnosis, and management of glycogen storage diseases. There is thus a clear need to test this hypothesis through enzymatic assays and structural studies.

**Keywords:** Phosphorylase kinase; glucoamylase; glycogen; glycogen storage disease; PSI-BLAST; *Escherichia coli*; ETT2

Phosphorylase kinase (PhK) is a $Ca^{2+}$-dependent enzyme involved in the regulation of glycogenolysis (Brushia and Walsh 1999). The first protein kinase to be purified, it remains one of the largest and most complex enzymes known, consisting of four subunits (Brushia and Walsh 1999). The traditional "textbook" view (Alberts et al. 1994; Brushia and Walsh 1999) is that only one of these, the γ subunit, possesses enzymatic activity, which is allosterically controlled by the other three regulatory subunits in response to changes in intracellular calcium (mediated by the δ subunit, calmodulin) and cAMP levels (mediated by A-kinase phosphorylation of the α and β subunits). Activation of the kinase activity of the γ subunit leads to phosphorylation of a serine residue in glycogen phosphorylase, triggering activation of this enzyme and the release of glucose residues from glycogen. This view does not, however, explain why PhK should bind directly to glycogen (Andreeva et al. 1999, 2001). Nor is it clear why the α and β subunits (which have distinct but homologous sequences) need to be quite so large to fulfill their regulatory roles (each >1000 residues, forming 81% of the mass of the PhK holoenzyme), nor why far more PhK is present in skeletal muscle than is ever needed for its regulatory function (Brushia and Walsh 1999). Recent studies have located regulatory and protein-binding functions and sites for post-translational modifications toward the carboxyl terminus of these subunits (Brushia and Walsh 1999; Nadeau et al. 1999; Andreeva et al. 2002; Rice et al. 2002), but there are no reports on the structure or function of the amino-terminal regions of either regulatory subunit.

BLASTP searches with a protein (Ecs3736) encoded within the ETT2 pathogenicity island from *Escherichia coli* O157 (Hayashi et al. 2001; Miyazaki et al. 2002) revealed near-full-length homology between this protein and a stretch of >200 residues close to the amino terminus of the α and β domains of PhK from several eukaryotes. This suggested that this stretch of sequence within the PhK α and β subunits might represent a distinct structural and functional domain. A more sophisticated bioinformatics analysis was therefore carried out in the hope of determining the function of this domain. The results of this analysis cast doubt on the completeness of the current textbook view of PhK.

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## Results

Homology searches and genome alignments on the coliBASE Web site (http://colibase.bham.ac.uk/) revealed that Esc3736 from *E. coli* O157 Sakai represented a pseudogene derived from a longer gene intact in the unfinished genome sequence of *E. coli* O42, which I have designated *pkgA*, for "phosphorylase-kinase-like glucoamylase." The 550-amino-acid PkgA sequence consists of the Ecs3737 sequence followed by a single arginine followed by the Ecs3736 sequence. This PkgA sequence was used to initiate a PSI-BLAST search (Altschul et al. 1997; Altschul and Koonin 1998).

By iteration five, PkgA, was linked with significance (expect value $<10^{-15}$) not just to the amino-terminal domains of PhK α- and β-subunits from a wide range of eukaryotes (from *Caenorhabditis elegans* to humans), but also to dozens of glucoamylases, including two of known structure (data not shown). A similar PSI-BLAST search using the human PhK α-subunit sequence as the starting sequence also reported significant similarity (*e* value $10^{-27}$) to glucoamylases of known structure within five iterations, whereas a search starting with a glucoamylase of known structure (1GAI) reported significant similarity (*e* value $10^{-23}$) to PhK subunits within four iterations (data not shown). A multiple alignment of the sequences of two human PhK α subunit isoforms, a human PhK β subunit, two glucoamylases of known structure, and PkgA shows numerous conserved patches, which correspond to the known active site residues and other critical conserved structural features of the glucoamylase structures (Coutinho and Reilly 1997; Fig. 1).

## Discussion

Glucoamylase (1,4-α-D-glucan glucohydrolase, EC 3.2.1.3, GA) releases β-D-glucose from the nonreducing ends of glycogen and starch (Sauer et al. 2000). Three nested hypotheses can be put forward to explain the presence of GA domains in the α- and β-subunits of PhK—an enzyme that not only binds glycogen, but is intimately involved in regulating glycogen catabolism:

*Hypothesis 1* The least controversial hypothesis is that the amino-terminal domains in the PhK α- and β-subunits share a common ancestor and similar fold with GA.

*Hypothesis 2* These domains may bind to glycogen but lack glycogenolytic enzymatic activity (note the lack of the WXRD motif in the human PhK α subunit in Figure 1).

*Hypothesis 3* The final, most compelling hypothesis is, however, that either one or both of these subunits possess a previously overlooked amylase activity, binding to and then releasing β-D-glucose from the nonreducing ends of glycogen within muscle or liver cells.

If hypothesis 3 were true, this would overturn the current textbook view of PhK as a purely regulatory enzyme, and establish the PhK/GA protein complex as a multifunctional enzyme with catabolic and regulatory functions. If hypothesis 2 or 3 were true, this would have important practical implications in the understanding, diagnosis, and management of glycogen storage diseases where mutations map to the PhK α and β subunits (Burwinkel et al. 1997; Hendrickx et al. 1999), as these underlying mutations might have a direct, measurable effect on glycogen-binding or glucoamylase activity rather than acting solely through altered allosteric effects on the kinase activity of the γ subunit.

These findings also shed light on the potential function of the bacterial protein PkgA. As with the PhK sububits, the homology between PkgA and glucoamylases can be used to predict a role in glycogen metabolism. This role, however, may not played out in the bacterial cell. PkgA is encoded by a gene at one end of the ETT2 pathogenicity island. This island encodes a bacterial type-III secretion system of unknown function, termed ETT2 (for *E. coli* type-III secretion system 2). By analogy with other type-III secretion systems (for review, see Hueck 1998), it is anticipated that ETT2 will under certain as yet unknown circumstances, translocate so-called "effector" proteins from the bacterial cytoplasm directly into eukaryotic cells, where they then subvert host-cell functions. PkgA is a highly plausible candidate as an ETT2 effector because (1) it is located within the ETT2 pathogenicity island, yet not part of the secretion apparatus itself; (2) it shows close similarity to eukaryotic PhK regulatory subunits, but has no homologs among closely related bacteria, suggesting recruitment of host cell components into the type-III secretion system effector repertoire (a recognized theme in type-III secretion; Stebbins and Galan 2001). Therefore, it is tempting to speculate that PkgA acts as a type-III effector that targets glycogen metabolism within a target eukaryotic cell.

In conclusion, there is now a clear need for those with the interest and experimental expertise to test the hypotheses presented here through binding and enzymatic assays, through structural studies and through other cell biology approaches.

## Materials and methods

PSI-BLAST searches (Altschul et al. 1997; Altschul and Koonin 1998) of the VGE-PEPT database were performed on the Virulogenome Web site (http://www.vge.ac.uk [This database combines the NCBI's NR database with a database of protein sequences predicted from unfinished genome sequences.]). Default PSI-BLAST values were used, except that the cutoff for inclusion in each iteration was set at 0.05 and composition-based statistics and the filter were turned off.

The sequences of two human PhK α subunit isoforms (SwissProt entries KPB1_HUMAN and KPB2_HUMAN) and one human PhK β subunit (KPBB_HUMAN and the two glucoamylases of known structure (PDB entries 1GAI and 1AYX) were retrieved

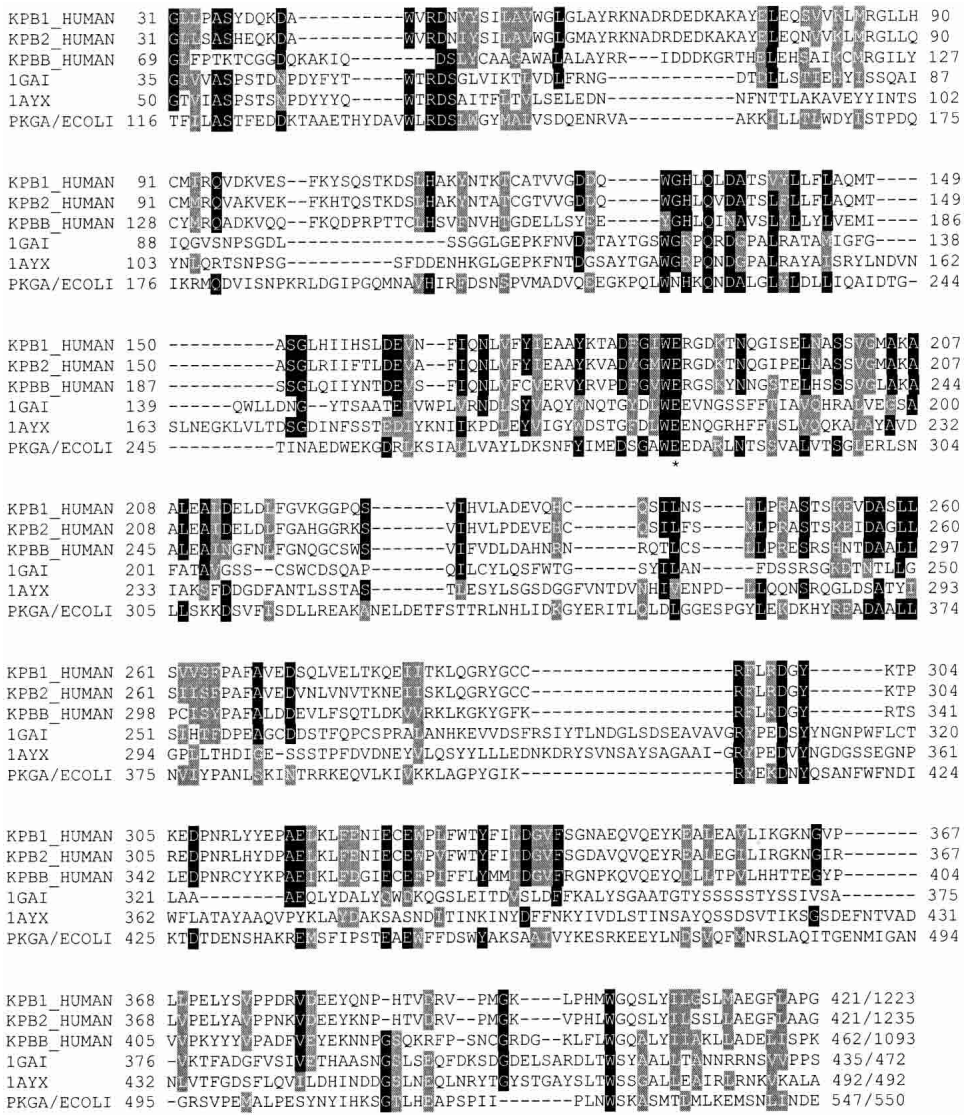

**Figure 1.** Multiple alignment of two isoforms of human phK α subunit (SwissProt entries KPB1_HUMAN and KPB2_HUMAN) and one human PhK β subunit (KPBB_HUMAN), two glucoamylases of known structure (PDB entries 1GAI and 1AYX), and PkgA from *E. coli* O42. The catalytic acid residue in glucoamylase is highlighted with an asterisk. Figures show residue positions at *start* and at *end* of each row. Final figures show overall length of protein.

from the NCBI's NR database and aligned with PkgA using CLUSTALW (http://www.ebi.ac.uk/clustalw/), followed by manual editing of the alignment using Jalview.

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