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Cloning, Expression, and Characterization of the First Archaeal ATP-Dependent Glucokinase from Aerobic Hyperthermophilic Archaeon *Aeropyrum pernix*

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The gene encoding the ATP-dependent glucokinase of hyperthermophilic archaeon *Aeropyrum pernix* was identified, cloned, and functionally expressed in *Escherichia coli*. The deduced amino acid sequence showed 40% identity to that of the putative glucokinase from hyperthermophilic archaeon *Pyrobacurum aerophilum*. The purified recombinant enzyme was a monomer with a molecular mass of 35 kDa. The enzyme retained its full activity on heating at 70°C for 10 min and retained 65% of the activity after 10-min incubation at 100°C. The enzyme exclusively catalyzed the phosphorylation of D-glucose using ATP as a phosphoryl donor. ITP was accepted in addition to ATP. The rate dependence with both glucose and ATP followed Michaelis-Menten kinetics, with apparent K_m values of 0.054 and 0.50 mM, respectively. The enzyme activity required divalent cations; Mg^{2+}, which was most effective, could partially be replaced by Mn^{2+} or Ca^{2+}. Phylogenetic analysis revealed that the glucokinase from *A. pernix* does not belong to the clusters of enzymes found in bacteria and eukarya. This is the first description of the characteristics of an ATP-dependent glucokinase from an archaeon.

Key words: *Aeropyrum pernix*, glucokinase, hexokinase, hyperthermophile, archaea sugar kinase.

ATP-dependent glucokinase (ATP-GK, EC 2.7.1.2) is one of the key enzymes in sugar degradation via the Embden-Meyerhof pathway in bacteria and eukarya, and catalyzes the irreversible phosphorylation of glucose to glucose-6-phosphate. The genes encoding the ATP-GKs from a variety of bacteria and eukarya have been cloned and sequenced. In a phylogenetic hexokinase family tree constructed by comparing the sequences of 60 sugar kinases, Bork *et al*. (*1*) observed that glucokinases appear to form three clusters; (i) mammalian glucokinases; (ii) yeast glucokinases, which are grouped with yeast hexokinases; and (iii) bacterial glucokinases. They stated that a divergent evolutionary relationship between these glucokinases was unlikely, and rather that evolutionary convergence to glucose specificity must have occurred independently in mammals, yeast and bacteria. The hyperthermophilic archaea are relatively deeply branched archaea and are considered to be phylogenetically ancient organisms. Therefore, structural analysis of glucokinases from these organisms may provide abundant information for phylogenetic analysis of glucokinases. A novel sugar kinase, ADP-dependent (AMP-forming) glucokinase (ADP-GK), has been found in hyperthermophilic archaeon *Pyrococcus furiosus* (*2*). Recently, we discovered the presence of ADP-dependent glucokinase/phosphofructokinase (ADP-GK/PFK), a novel bifunctional enzyme, in hyperthermophilic archaeon *Methanococcus jannaschii* (*3*). These enzymes require ADP as a phosphoryl group donor instead of ATP, and are proposed to be involved in a modified Embden-Meyerhof pathway in the organisms. We cloned and sequenced the gene encoding the ADP-GKs from *P. furiosus* and *Thermococcus litoralis*, a near relative of *P. furiosus* (*4*). About 59% amino acid sequence identity was observed between the two enzymes. The ADP-GK/PFK from *M. jannaschii* showed 29% identity with *P. furiosus* ATP-GK (*3*). However, they did not show similarity with any ATP-dependent kinases that have been reported so far. This indicates that these ADP-dependent kinases belong to a novel kinase family. On the other hand, an archaeal ATP-GK has not been described to date, although sequence information of such an enzyme may contribute significantly to phylogenetic analysis of glucokinase evolution. Recently, Kawarabayasi *et al*. (*5*) determined the complete sequence of the genome of an aerobic hyperthermophilic archaeon, *Aeropyrum pernix* K1, and they assigned APE2091 as the gene encoding a putative glucokinase. We performed cloning and expression of APE2091 in *Escherichia coli*. However, no functional products were obtained (data not shown). We reanalyzed the sequence based on the amino

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acid sequence similarity with other glucokinases, and estimated that GTG 169-bp downstream from the 5′-terminal of the predicted open reading frame is more preferable as an initial codon.

We here describe the cloning and heterologous expression of the newly predicted open reading frame for the glucokinase from *A. pernix*. Characterization of the produced enzyme revealed the presence of the first ATP-GK in a hyperthermophilic archaeon.

MATERIALS AND METHODS

Materials—*E. coli* strains JM109 and BL21(DE3) codon plus RIL were obtained from Toyobo (Osaka) and Stratagene (La Jolla, CA), respectively. Plasmid DNA pET11a was obtained from Novagen (Madison, WI). Glucose-6-phosphate dehydrogenase from yeast was purchased from Wako Pure Chemical Industries (Osaka). All other chemicals were of reagent grade.

Cloning and Expression of the Gene Encoding ATP-GK—Plasmid DNA (pUAPGK, positions 1,319,821–1,321,500 in the entire genome of *A. pernix*, has been inserted into the *Hin*cII site of pUC118) containing APE2091 was prepared from shotgun clone AS2GR0170 as described previously (*5*). The following set of oligonucleotide primers was used to amplify the ATP-GK gene fragment by PCR: the first primer (5′-GTGTTGCCATATGGCGGAGGTTGTTGCT-3′) introduced a unique *Nde*I restriction site overlapping the newly predicted 5′ initiation codon (GTG has been changed to ATG), and the other (5′-TACTCCTGGATCCCTAGAAGATTGGGAGGT-3′) a unique *Bam*HI restriction site proximal to the 3′ end of the termination codon. Plasmid DNA pUAPGK was used as a template. The amplified 1.0 kb fragment was digested with *Nde*I and *Bam*HI, and then ligated with expression vector pET11a linearized with *Nde*I and *Bam*HI to generate pEAPGK. *E. coli* strain BL21(DE3) codon plus RIL was transformed with pEAPGK. The transformants were cultivated at 37°C in 200 ml of a medium, comprising 2.4 g of tryptone, 4.8 g of yeast extract, 1 ml of glycerol, 2.5 g of K_2HPO_4, 0.76 g of KH_2PO_4, and 10 mg of ampicillin, until the optical density at 600 nm reached 0.6. Induction was carried out by the addition of 1.0 mM isopropyl-β-D-thiogalactopyranoside to the medium and then the cultivation was continued for 3 h.

Purification of Recombinant ATP-GK—Cells were harvested by centrifugation, suspended in 50 mM Tris/HCl buffer (pH 7.5) containing 0.1% (v/v) 2-mercaptoethanol and 0.2 M Na_2SO_4, and then disrupted by ultrasonication. The crude extract was heated at 80°C for 10 min and the denatured protein was removed by centrifugation (12,000 ×*g* for 10 min). The supernatant was dialyzed against 50 mM Tris/HCl buffer (pH 7.5), and then applied to a Uno-Q column (1.5 × 6.8 cm) (Bio-Rad, Hercules, CA) equilibrated with the same buffer. After washing with 50 ml of the same buffer, the enzyme was eluted with a 100 ml linear gradient of 0–0.5 M NaCl. The active fractions were pooled, dialyzed against 50 mM Tris/HCl buffer (pH 7.5), and then used as the purified enzyme preparation.

Assaying of the Enzyme—ATP-GK activity was assayed spectrophotometrically by measuring the formation of NADPH in a coupled assay with glucose-6-phosphate dehydrogenase from yeast. The reaction system comprised, unless specified otherwise, 50 mM Tris/HCl, pH 8.0, 10 mM glucose, 2.5 mM ATP, 10 mM $MgCl_2{\cdot}6H_2O$, 1 unit/ml glucose-6-phosphate dehydrogenase, 1 mM NADP, and the enzyme preparation, in a total volume of 1.0 ml. The reaction was started by the addition of ATP. The absorbance of NADPH was followed at 340 nm (ε_M= 6.22 mM^{-1} cm^{-1}). One unit of enzyme activity was defined as the amount of enzyme required to convert 1 μmol of glucose to glucose-6-phosphate per min at 50°C. Protein was determined by the method of Bradford (*6*), with bovine serum albumin as the standard.

Polyacrylamide Gel Electrophoresis—SDS–polyacrylamide gel electrophoresis (PAGE, 12% acrylamide slab gel, 1 mm thick) was performed by the procedure of Laemmli (*7*). The protein band was stained with Coomassie Brilliant Blue R 250.

Molecular Mass Determination—The molecular mass of the purified enzyme was determined by analytical gel filtration on Superose 6 (Amersham Bioscience, USA) pre-equilibrated with 50 mM Tris/HCl buffer (pH 7.5) containing 0.15 M NaCl. Bovine thyroid thyroglobulin (670 kDa), horse spleen ferritin (440 kDa), rabbit muscle aldolase (158 kDa), bovine serum albumin (67 kDa), and bovine pancreas ribonuclease A (13.7 kDa) in Gel Filtration Calibration Kits (Amersham Biosciences) were used as molecular mass standards. The subunit molecular mass of the purified enzyme was determined by SDS-PAGE. The marker proteins (NEW ENGLAND BioLabs) used were as follows: fusion of *E. coli* maltose-binding protein and β-galactosidase (175 kDa), fusion of *E. coli* maltose-binding protein and paramyosin (83 kDa), bovine liver glutamate dehydrogenase (62 kDa), rabbit muscle aldolase (47.5 kDa), rabbit muscle triosephosphate isomerase (32.5 kDa), bovine milk β-lactoglobulin A (25kDa), and chicken egg white lysozyme (16.5 kDa).

Thermal Stability, pH Optimum and Kinetic Parameters—Thermostability was determined using the diluted enzyme (1.8 mg/ml pure enzyme was diluted 100 times with 50 mM Tris/HCl buffer, pH 7.5). The optimal pH of the enzyme was determined by performing the standard assay at 50°C with acetate buffer (50 mM), Bis/Tris buffer (50 mM), Tris/HCl buffer (50 mM), and glycine-NaOH buffer (50 mM) for the pH ranges of 4.0–6.0, 6.0–7.0, 7.0–8.5, and 8.5–10.0, respectively. The Michaelis constants were determined from Lineweaver-Burk plots (*8*) of data obtained from the initial rate of glucose phosphorylation at 50°C.

Substrate Specificity—The reactivity of D-fructose, D-mannose, D-galactose, 2-deoxy-D-glucose, D-sorbitol, D-myoinositol, D-mannitol, sucrose, and D-1,5-anhydroglucitol as substrates for ATP-GK was examined by measuring the formation of ADP from ATP by HPLC. The reaction mixture comprised 50 mM Tris/HCl, pH 8.0, 10 mM each substrate, 2.5 mM ATP, 10 mM $MgCl_2{\cdot}6H_2O$, and 100 μl of enzyme preparation, in a total volume of 0.5 ml. After incubation for 10 min at 50°C, the reaction was stopped by cooling on ice. After 5 min, each solution was passed through a cellulose acetate filter (pore size 0.2 mm. ADVANTEC, Tokyo). An aliquot (20 μl) of each filtrate was applied to a column (7.6 mm × 25 cm) of Asahipak GS320HQ (Asahi Chemical Industry, Shizuoka), NaH_2PO_4 (200 mM, pH 5.0) being used as the mobile

Table 1. **Purification of the recombinant ATP-GK.**

	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification (fold)
Crude extract	99.6	419	4.2	100	1
Heat treatment	10.5	278	24.6	66.3	5.9
Uno-Q	1.8	87	47.2	20.8	11.2

phase at the flow rate of 1.0 ml/min. The effluent from the column was monitored with a UV detector at the wavelength of 260 nm. ATP, ADP, and AMP were separated on the column at retention times of about 10.2, 11.6, and 15.5 min, respectively. For detection of the specificity for the phosphoryl group donor, GDP, CDP, UDP, IDP, ADP, GTP, CTP, ITP, and AMP (each 2 mM) were used instead of ATP in the standard assay mixture described under "Assaying of the Enzyme". The divalent cation requirement was examined by the addition of 10 mM $MgCl_2$, $CoCl_2$, $NiCl_2$, $MnCl_2$, $CaCl_2$, or $CuCl_2$ to the standard assay mixture.

RESULTS AND DISCUSSION

Identification of the Hypothetical Gene Encoding ATP-GK in A. pernix—The complete sequence of the genome of *A. pernix* has been reported by Kawarabayasi *et al*. (*5*). APE2091 (1,131 bp, positions 1,321,210–1,320,080 in the entire genome) has been assigned as the gene encoding a putative glucokinase (a protein of 376 amino acids with a molecular weight of 40,549). The estimated molecular weight is obviously larger than those (29–34 k) of bacterial glucokinases and the putative glucokinase of hyperthrmophilic archaeon *Pyrobacurum aerophilum*. We performed cloning and expression of APE2091 in *Escherichia coli*. However, no functional products were obtained (data not shown). The criterion used for assignment of the potential coding region in the *A. pernix* genomic sequence was the detection of sense codons starting with ATG or GTG (*5*). The sequence of APE2091 was reanalyzed with the same criterion. On the basis of the amino acid sequence similarity with other glucokinases such as the putative *Pb. aerophilum* glucokinase (GenBank accession number NP_560732), it was revealed that GTG 169-bp downstream from the 5′-terminal of the predicted open reading frame was more preferable as an initial codon. The newly predicted gene (963 bp, positions 1,321,042–1,320,080 in the entire genome) is estimated to code a protein of 320 amino acids with a molecular weight of 33,875.

Purification of the Recombinant Enzyme—E. coli BL21(DE3) codon plus RIL cells transformed with pEAPGK produced hyperthermostable ATP-GK, whose activity was not lost on incubation at 80°C for 10 min. Table 1 shows a summary of the purification of the recombinant enzyme from an extract of *E. coli* cells. The enzyme was purified about 11-fold with a 21% recovery by heat treatment and successive Uno-Q anion-exchange chromatography. The purified enzyme was found to be homogeneous on SDS-PAGE (Fig. 1). About 1.8 mg of the purified enzyme was obtained from 200 ml of *E. coli* culture. The specific activity of the final preparation was estimated to be 47.2 units/mg. The activity of ATP-GK in a cell extract of *A. pernix* has been observed to be around 0.01 units/mg (Mitani *et al*., unpublished result). Thus, this expression system is quite useful for obtaining a pure enzyme.

Characteristics of the A. pernix ATP-GK—The optimum pH of the *A. pernix* ATP-GK reaction was around pH 8.0. The enzyme retained its full activity on heating at 70°C for 10 min, and retained 65% of the activity after 10-min incubation at 100°C. The most thermostable ATP-GK previously reported is the enzyme from *Bacillus stearo-*

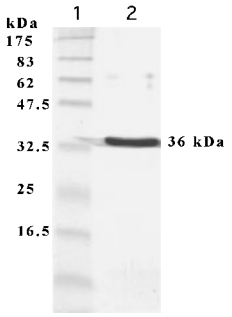

Fig. 1. **SDS-PAGE of the purified recombinant ATP-GK.** Lanes: 1, molecular mass standards; 2, *A. pernix* ATP-GK.

Table 2. **Specificity for phosphoryl group donors and cation dependence of *A. pernix* ATP-GK.**

Phospholylgroup donor*	Relative activity (%)	Divalent cation**	Relative activity (%)
ATP	100	Mg^{2+}	100
ITP	36	Mn^{2+}	36
		Ca^{2+}	12
		Cu^{2+}	5

*The following phospholyl group donors were inert under the used conditions: CTP, GTP, ADP, GDP, CDP, UDP, and AMP. **Co^{2+} and Ni^{2+} were inert under the used conditions.

Table 3. **Comparison of the specificities for phosphoryl group acceptors.**

Phosphoryl group acceptor (10 mM)**	Relative activity		
	A. pernix ATP-GK (%)	*B. stearothermophilus* ATP-GK (%)*	Yeast ATP-HK (%)*
D-Glucose	100	100	100
D-Mannose	0	5	83
D-Galactose	0	2	24
D-Fructose	0	0	260
2-Deoxy-D-glucose	0	0	135
D-Glucosamine	0	0	76
D-1,5-Anhydroglucitol	0	2	2

*Koga *et al*., 2000 (*4*) **The following phospholyl group acceptors were inert under the used conditions: D-sorbitol, D-myoinositol, D-mannitol, and sucrose.

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A. pernix          1 M-AEVVAVDVGATWVRMAIVRGGVI---EA-IKRERNPGTEEGLERVLQ-GLAEGL-GID    53
Pb. aerophilum     1 MTLY-LGIDVGATWTR-AI---L-IDENLQVFK---------RL-K-IRTGVNP-LADVA    42
                           ****** * **     *       *          *      *    *

A. pernix         54 RGRVE-----KVGAASIGPLDLRRGYIVGSPNIKS---HIVRLSTILKRLFPKSKVAIAN   105
Pb. aerophilum    43 VA-VEKWSFDSIGVGSIGPMDLRSGRVVNSPNSPSRQFPLVEP---LKK-FGKPVV-VAN    96
                        **       *  **** *** *  * ***  *     *     **  * *  *  **

A. pernix        106 DAVAAAWGEYLLG-RLAGTP-DLGYITMSTGV-GGGFVVGGRLLLGSR-GNAHEVGHIVV   161
Pb. aerophilum    97 DCVAAVWGEYVFKHR----VENLVYVTLSTGVGIGAI-VNGTLLLG-KDGNAHELGHAVI   150
                     * *** ****    *       * * * ****  *   * * ****   ***** ** *

A. pernix        162 DMGWEGG----RCGCGGTGHWEA-IAG----GRWIPRTSSVLARGWRGPETSLYRAALEG   212
Pb. aerophilum   151 -----DFRSSRQCGCGGFGHFEAYV-GGANIPK-----------------W-FQE-LTG   184
                         ***** ** **   *                              * *

A. pernix        213 R-VGS-AREV----FEAAAVGDD----FALHVIDY--IARASAAGIASVKAAYDVDAVI-   259
Pb. aerophilum   185 EALNDAA-EVFKRYRD----G-DFKARQ--F-IDLWLDALA--AGIATVIAAYDPE-LLI   232
                        * **          * *          **    * *  **** * ****

A. pernix        260 IGGS-VYLNN----R-RMLRPLIERHLAA----YAPFSSRIEVV-DASFGDNEGVMG---   305
Pb. aerophilum   233 IGGSIG-LNNWDIISRD-L-P-I-R-LKKYLGVR-PP--A---ITQASFGDDEVAIGAAA   280
                     ****   ***        * * * * *        *          ***** *   *

A. pernix        306 -AYAIAYRNPEDLPIF----                                        320
Pb. aerophilum   281 LA----YRVPESLKKFGYPR                                        296
                      *    ** ** *  *
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Fig. 2. **Alignment of the amino acid sequences of the *A. pernix* ATP-GK and putative glucokinase of *Pb. aerophilum*.** Asterisks represent conserved residues in the two enzymes.

thermophilus. This enzyme is stable up to 60°C, but loses the activity at 75°C (*9*). Thus, the *A. pernix* ATP-GK is probably the most thermostable ATP-GK among the enzymes from many other organisms described to date. For D-glucose phosphorylation, ITP was accepted as a phosphoryl group donor in addition to ATP (Table 2). GDP, CDP, UDP, IDP, ADP, GTP, CTP, and AMP were inert. The enzyme required a divalent cation for the activity. $MgCl_2$ was most effective, and was able to replace $MnCl_2$ or $CaCl_2$ to some extent (Table 2). The ability of the enzyme to catalyze the phosphorylation of various sugars was examined. The enzyme showed strict specificity for D-glucose (Table 3). The following substrates were inert: D-fructose, D-mannose, D-galactose, 2-deoxy-D-glucose, D-sorbitol, D-myoinositol, D-mannitol, sucrose, and D-1,5-anhydroglucitol. In general, the substrate specificity of eukaryotic glucokinases or hexokinases for sugars is relatively low. For example, yeast hexokinase utilizes D-mannose, D-fructose and 2-deoxy-D-glucose as phosphoryl group acceptors as well as D-glucose (Table 3) (*4*). The *A. pernix* enzyme does not act on these sugars. On the other hand, the glucokinases from bacteria exhibit high specificity for D-glucose, as in the case of the *B. stearothermophilus* enzyme (Table 3) (*4*). In this respect, the enzyme is similar to the bacterial glucokinases. The *A. pernix* ATP-GK showed typical Michaelis-Menten kinetics. The K_m values for D-glucose and ATP at 50°C were calculated to be 0.054 and 0.50 mM, respectively. Eukaryotic hexokinases exhibit high affinity for glucose (K_m = 20–130 μM), whereas bacterial and eukaryotic glucokinases exhibit relatively low affinity for glucose (K_m = 5–8 mM) (*10*). In this regard, the *A. pernix* enzyme is similar to the eukaryotic hexokinases. The molecular mass of

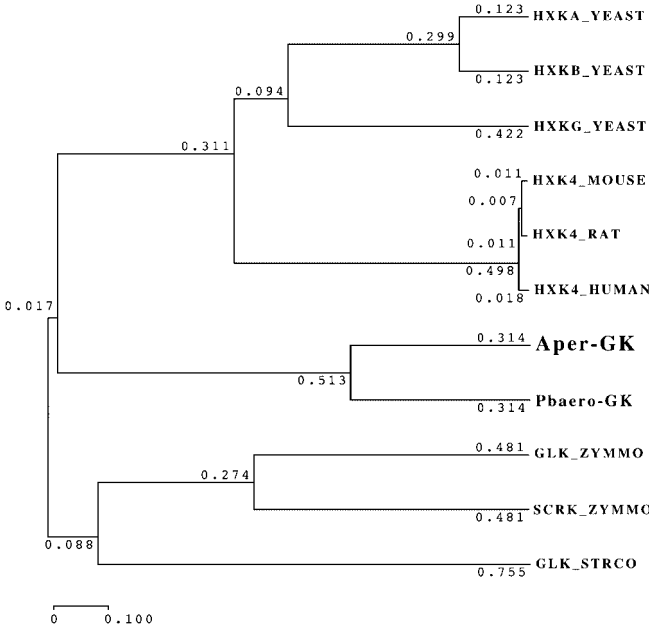

Fig. 3. **Phylogenetic tree of ATP-GKs.** The tree was constructed by the UPGMA method using GENETYX-SV/RC10.1 software (Software Development, Tokyo). The branch lengths in the tree are drawn to scale and indicated by numbers. HXKA_YEAST and HXKB_YEAST, hexokinases A and B from *Saccharomyces cerevisiae*, respectively. HXKG_YEAST, glucokinase from *S. cerevisiae*. HXK4_HUMAN, HXK4_RAT, and HXK4_MOUSE, hexokinases D from man, rat and mouse, respectively. GLK_ZYMMO and SCRK_ZYMMO, glucokinase and fructokinase from *Zymomonas mobilis*, respectively. GLK_STRCO, glucokinase from *Streptomyces coelicolor*. Aper-GK, ATP-GK from *Aeropyrum pernix*. Pbaero-GK, putative glucokinase of *Pyrobaculum aerophilum*.

the *A. pernix* ATP-GK was estimated to be about 35 kDa on gel filtration. From the results of SDS-PAGE, the subunit molecular mass of the enzyme was determined to be about 36 kDa (Fig. 1). This indicates that the native enzyme has a monomeric structure. The glucokinases from bacteria are usually homodimers with a subunit size of 24–35 kDa (*9*, *11*). Eukaryotic hexokinases exhibit a dimeric structure with subunits of 50 kDa (*12*, *13*), whereas eukaryotic glucokinases exhibit a monomeric structure (50 kDa) (*12*, *14*). Therefore, a monomeric structure with a size of about 35 kDa is one of the remarkable characteristics of the *A. pernix* enzyme.

Amino Acid Sequence Alignment and Pylogenetic Analysis—On amino acid sequence alignment, the identity between the *A. pernix* ATP-GK and the putative glucokinase of *Pb. aerophilum* was found to be 40% (Fig. 2). They did not show any similarity with the ADP-GKs from *P. furiosus* and *T. litoralis.* In a phylogenetic hexokinase family tree constructed by comparing the sequences of 60 sugar kinases, Bork *et al*. (*1*) observed that glucokinases can be divided into the following three clusters; (i) mammalian glucokinases; (ii) yeast glucokinases, which are grouped with yeast hexokinases rather than with mammalian glucokinases; and (iii) bacterial glucokinases from *Zymomonas mobilis* and *Streptomyces coelicolor*, which are grouped with the *Zymomonas* fructokinase. They concluded that a divergent evolutionary relationship between these glucokinases was unlikely. Rather, they argued that evolutionary convergence to glucose specificity must have occurred independently in mammals, yeast, and bacteria (*1*). We constructed a phylogenetic tree based on amino acid sequence alignment of the glucokinases that were reported by Bork *et al*., and the *A. pernix* ATP-GK and putative glucokinase of *Pb. aerophilum.* The following sequences were available: hexokinase A (HXKA_YEAST, SWISSPROT accession number P04806) and hexokinase B (HXKB_YEAST, SWISSPROT accession number P04807) from *Saccharomyces cerevisiae*, glucokinase from *S. cerevisiae* (HXKG_YEAST, SWISSPROT accession number P17709), hexokinases D from man (HXK4_HUMAN, SWISSPROT accession number P35557), rat (HXK4_RAT, SWISSPROT accession number P17712), and mouse (HXK4_MOUSE, SWISSPROT accession number P52792), glucokinase (GLK_ZYMMO, SWISSPROT accession number P21908) and fructokinase (SCRK_ZYMMO, SWISSPROT accession number Q03417) from *Z. mobilis*, glucokinase from *S. coelicolor* (GLK_STRCO, SWISSPROT accession number P40184), and the putative glucokinase of *Pb. aerophilum* (GenBank accession number NP_560732). A phylogenetic tree produced by the UPGMA method is shown in Fig. 3. As shown by Bork *et al*. (*1*), the glucokinases from mammals, yeast, and bacteria appear to form three clusters. On the other hand, the *A. pernix* ATP-GK was clustered with the putative glucokinase from *Pb. aerophilum,* and was separate from the above three clusters of glucokinases. This suggests that glucokinases should be classified into four major classes: *i.e*. glucokinases from mammals, yeast, bacteria, and archaea.

In the present study, the gene encoding the ATP-GK of *A. pernix* was identified, cloned, and functionally expressed in *E. coli.* On the basis of the results of genome analysis of *A. pernix*, the APE0012 gene has been predicted to code a putative sugar kinase of the phosphofructokinase B family (*5*). Hansen and Schoenheit (*15*) expressed the gene in *E. coli* and revealed that the produced enzyme exhibits ATP-dependent phosphofructokinase activity. The genes of fructose-bisphosphate aldolase (APE0011) (*16*), triose-phosphate isomerase (APE1538), phosphoglycerate kinase (APE0173), phosphoglycerate mutase (APE1616), and pyruvate kinase (APE0489) have been assigned in the *A. pernix* genome (*5*). The putative aldehyde dehydrogenase coded by gene APE1786 shows high identity (52%) to glyceraldehyde-3-phosphate dehydrogenase from hyperthermophilic archaeon *Thermoproteus tenax* (*17*). These observations and our results strongly suggest that the conventional type of Embden-Meyerhof pathway is utilized in cells of *A. pernix*, although it has been reported that the organism could not utilize a sugar as a growth substrate (*18*). This shows that the Embden-Meyerhof pathway of aerobic crenarchaeote *A. pernix* is totally different from the modified Embden-Meyerhof pathway observed in anaerobic euryarchaeota such as *P. furiosus* and *T. litoralis* (*19*). Our next project is on the physiological and evolutionary significance of such diversity in central metabolism in hyperthermophilic archaea.

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