Promiscuity in the part-phosphorylative Entner–Doudoroff pathway of the archaeon *Sulfolobus solfataricus*

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Abstract The hyperthermophilic archaeon *Sulfolobus solfataricus* metabolises glucose and galactose by a 'promiscuous' non-phosphorylative variant of the Entner–Doudoroff pathway, in which a series of enzymes have sufficient substrate promiscuity to permit the metabolism of both sugars. Recently, it has been proposed that the part-phosphorylative Entner–Doudoroff pathway occurs in parallel in *S. solfataricus* as an alternative route for glucose metabolism. In this report we demonstrate, by in vitro kinetic studies of D-2-keto-3-deoxygluconate (KDG) kinase and KDG aldolase, that the part-phosphorylative pathway in *S. solfataricus* is also promiscuous for the metabolism of both glucose and galactose.

Keywords: Archaea; *Sulfolobus solfataricus*; Entner–Doudoroff pathway; Metabolic pathway promiscuity; KDG kinase; KDG aldolase

1. Introduction

The hyperthermophilic archaeon *Sulfolobus solfataricus* grows optimally at 80–85 °C and pH 2–4, utilising a wide range of carbon and energy sources [1]. It has become one of the most comprehensively researched model organisms of archaeal sugar metabolism [2]. Central metabolism in this organism involves a modified Entner–Doudoroff pathway [3], production of acetyl-CoA by pyruvate:ferredoxin oxidoreductase [4] and the citric acid cycle coupled to oxidative phosphorylation [5]. The modified Entner–Doudoroff pathway is a non-phosphorylative variant of the classical pathway and proceeds with no net production of ATP.

It has recently been discovered that the non-phosphorylative Entner–Doudoroff pathway in *S. solfataricus* is promiscuous for the metabolism of both glucose and galactose (Fig. 1). Glucose dehydrogenase first catalyses the NAD(P)-dependent oxidation of both glucose and galactose, producing gluconate or galactonate, respectively [6]. Gluconate dehydratase then catalyses the dehydration of gluconate to D-2-keto-3-deoxygluconate (KDG) and galactonate to D-2-keto-3-deoxygalactonate (KDGal) [7]. Both these compounds are cleaved by KDG aldolase to yield pyruvate and glyceraldehyde [6]. Glyceraldehyde dehydrogenase is then thought to oxidise glyceraldehyde to glycerate, which is phosphorylated by glycerate kinase to give 2-phosphoglycerate. A second molecule of pyruvate is produced from this by the actions of enolase and pyruvate kinase. This non-phosphorylative Entner–Doudoroff pathway is also found in *Aspergillus* fungi, although in this case separate enzymes exist for the metabolism of glucose and galactose [8–10]. The discovery of metabolic pathway promiscuity in *S. solfataricus* has been proposed to have physiological and evolutionary significance [6].

Very recently, it has been reported that the part-phosphorylative Entner–Doudoroff pathway exists in parallel in *S. solfataricus* as an alternative pathway for glucose metabolism [11] (Fig. 1), a phenomenon that had previously been reported in the hyperthermophilic archaeon *Thermoproteus tenax* [12]. In this pathway, glucose is converted to KDG via glucose dehydrogenase and gluconate dehydratase, as occurs in the non-phosphorylative pathway. KDG is then phosphorylated by KDG kinase to produce D-2-keto-3-deoxy-6-phosphogluconate (KDPG), which undergoes an aldol cleavage to pyruvate and glyceraldehyde-3-phosphate. This is performed by KDG aldolase, which represents a bifunctional KDG/KDPG aldolase. Glyceraldehyde-3-phosphate is converted by non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase to give 2-phosphoglycerate, which is converted to a second molecule of pyruvate via the actions of enolase and pyruvate kinase. In *S. solfataricus* the genes encoding gluconate dehydratase, KDG aldolase, KDG kinase and glyceraldehyde-3-phosphate dehydrogenase are found in a cluster. The relevant enzyme activities have also been detected in cell extracts of the organism, providing convincing evidence that the part-phosphorylative pathway exists alongside the non-phosphorylative variant [11].

To date it has not been established whether this parallel part-phosphorylative pathway in *S. solfataricus* is specific for glucose or whether it exhibits a similar promiscuity to that observed in the non-phosphorylative variant. This possibility was investigated in the current work by in vitro studies of KDG kinase and KDG aldolase.

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Abbreviations: KDG, D-2-keto-3-deoxygluconate; KDGal, D-2-keto-3-deoxygalactonate; KDPG, D-2-keto-3-deoxy-6-phosphogluconate; KDPGal, D-2-keto-3-deoxy-6-phosphogalactonate

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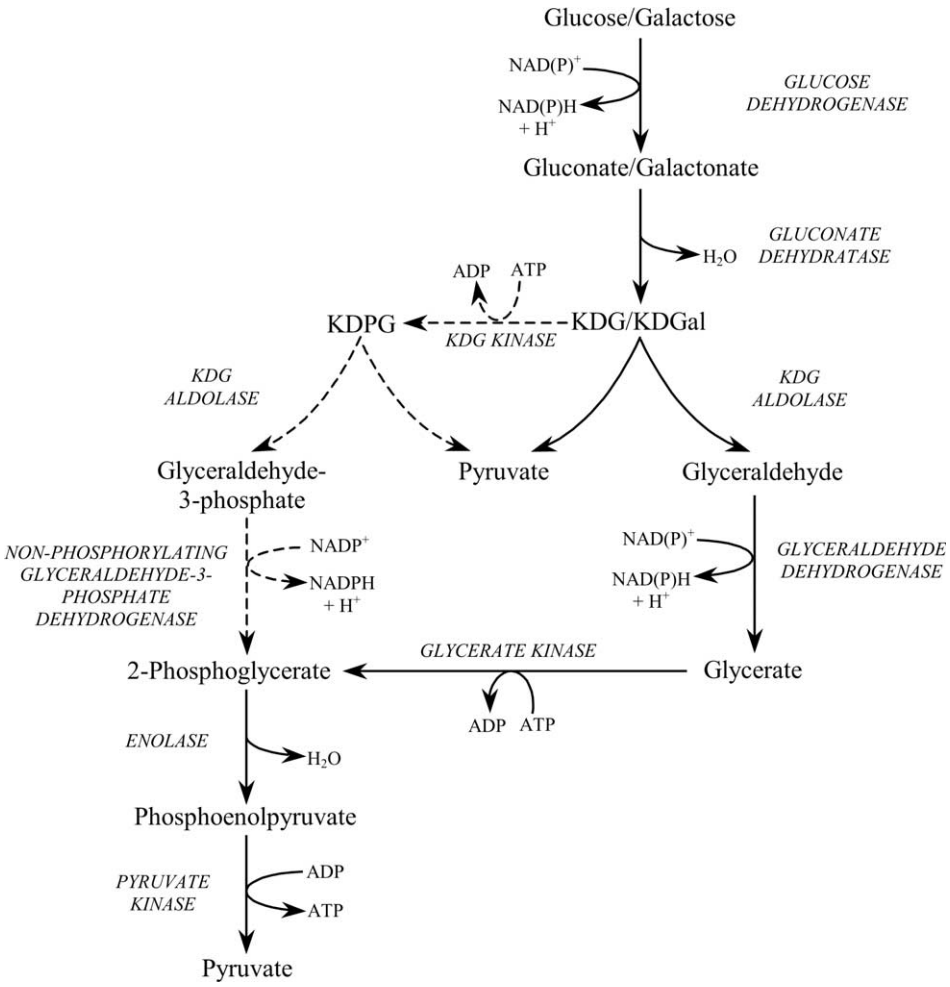

Fig. 1. Entner–Doudoroff metabolism in *Sulfolobus solfataricus*. The non-phosphorylative pathway enzymes catalyse the metabolism of glucose and galactose to pyruvate. The part-phosphorylative pathway (dashed arrows) exists in parallel as an alternative route for glucose metabolism.

2. Materials and methods

2.1. Cloning of the KDG kinase gene

The gene encoding KDG kinase (gi:13816631) was located in the published genome sequence of *S. solfataricus* [13] by homology searches [14]. It was amplified from a genomic extract by PCR with a forward primer designed to introduce an *Nde*I site (5′-CATATGGTTGATG-TAATAGCTTTGGGAGAGCC-3′) and a reverse primer designed to incorporate an *Xho*I site (5′-CACTGATGTTTTCTCGAGAATATA-TATTCATAAATGG-3′). The amplified gene was cloned into the *Nde*I and *Xho*I sites of the expression vector pET-19b (Novagen), which incorporates a histidine tag (MGHHHHHHHHHHSSGHIDDDD-KH) on the N-terminus of the protein.

2.2. Expression and purification of recombinant KDG kinase

The expression vector pET-19b containing the KDG kinase gene was used to transform *Escherichia coli* BL21(DE3) (Novagen). Cells were grown in LB medium at 37 °C for 20 h without induction and were then harvested by centrifugation. An extract was prepared by resuspending the cells in 50 mM Tris/HCl (pH 8.0) containing 5 mM $MgCl_2$ and passing them twice through a cell disruptor (One-shot model, Constant Systems) at 200 MPa, followed by three 30 s bursts of sonication using a 150-W Ultrasonic Disintegrator (MSE Scientific Instruments). Debris was removed by centrifugation at $20000 \times g$ for 30 min. KDG kinase was purified from the supernatant by His-bind resin chromatography, following the recommended protocol (Novagen). The eluted protein was dialysed overnight into 50 mM HEPES/KOH (pH 7.5) containing 5 mM $MgCl_2$. The protein was analysed by mass spectrometry using a TofSpec-2E machine (Micromass). Protein concentrations were determined by the method of Bradford [15] using a calibration curve constructed with bovine serum albumin. SDS–PAGE analysis was performed with a 12% (w/v) gel [16], following standard protocols [17].

2.3. KDG kinase assay

KDG and KDGal were synthesised using *S. solfataricus* gluconate dehydratase [7] and purified and characterised as described previously [6]. KDG kinase assays were performed in 100 μl of 50 mM HEPES/KOH (pH 7.5 at 60 °C) containing 5 mM $MgCl_2$, 10 mM ATP, 0–25 mM KDG or KDGal and 5 μl of KDG kinase. Reactions were heated at 60 °C for 10 min before being transferred to ice. 0.9 ml of a development solution was then added, containing 50 mM sodium pyrophosphate (pH 8.5), 5 mM EDTA, 10 mM sodium arsenate, 100 mM KCl, 10 mM L-cysteine, 1 mM NAD, excess *S. solfataricus* KDG aldolase and excess rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (Roche). The reactions were incubated at 35 °C for 30 min before their absorbance was measured at 340 nm. All appropriate controls were performed to ensure the requirements for coupled enzymatic analysis were met [18] and kinetic parameters were determined by the direct linear method [19].

2.4. Synthesis of KDPG and D-2-keto-3-deoxy-6-phosphogalactonate (KDPGal)

Biotransformations were performed with 100 mg KDG or KDGal and 350 mg ATP in 50 ml of 50 mM HEPES/KOH (pH 7.0 at 50 °C) containing 5 mM $MgCl_2$. One mg recombinant *S. solfataricus* KDG kinase was added and the reactions were incubated at 50 °C for 20 h with shaking. After this time, products were purified by DOWEX