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

Henry J. Lamble[a], Alex Theodossis[b], Christine C. Milburn[b], Garry L. Taylor[b], Steven D. Bull[c], David W. Hough[a], Michael J. Danson[a,*]

[a] *Centre for Extremophile Research, Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK*
[b] *Centre for Biomolecular Sciences, University of St. Andrews, North Haugh, St. Andrews, Fife KY16 9ST, UK*
[c] *Department of Chemistry, University of Bath, Bath BA2 7AY, UK*

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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.

*Corresponding author. Fax: +44 0 1225 386779.
E-mail address: M.J.Danson@bath.ac.uk (M.J. Danson).

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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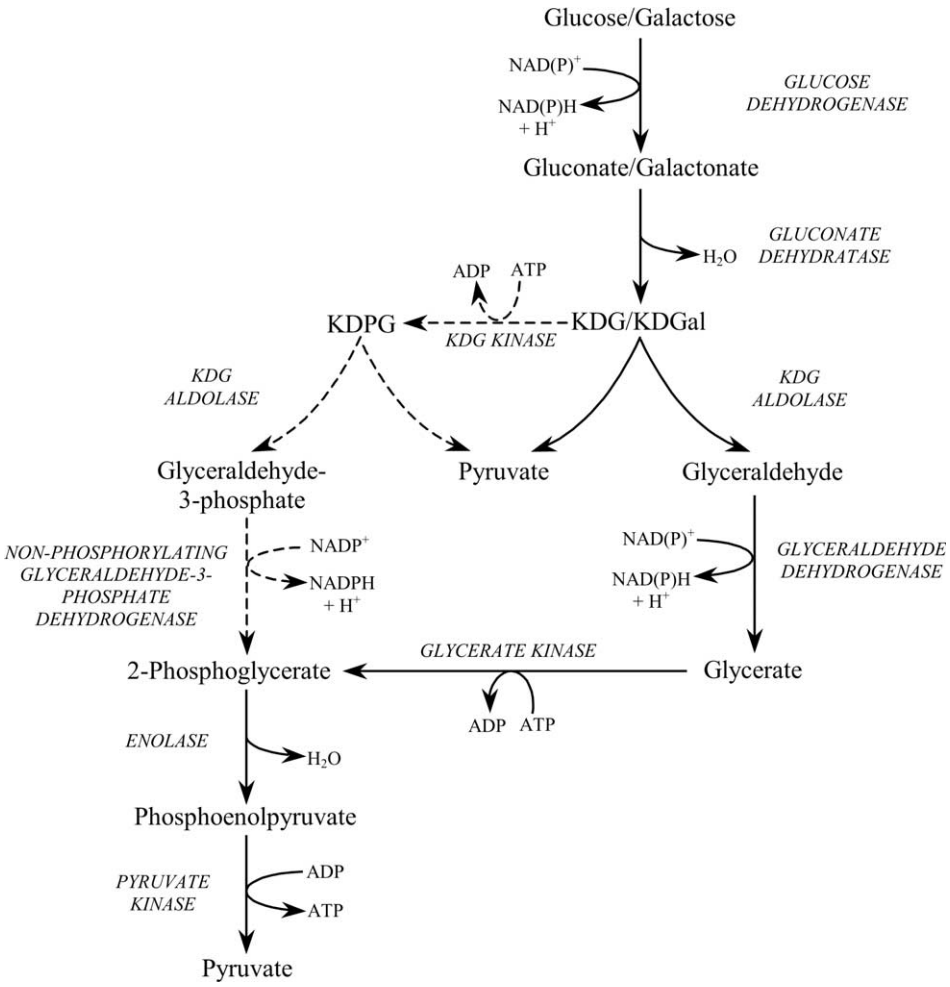

Fig. 1. Entner–Doudoroff metabolism in *Sulfolobus solfataricus*. The non-phosphorylative pathway enzymes catalyse the metabolism of glucose and galactose to pyruvate. The part-phosphorylative pathway (dashed arrows) exists in parallel as an alternative route for glucose metabolism.

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

1 × 8 anion exchange chromatography with 0–0.1 M HCl as eluant. The pooled product was adjusted to pH 7.0 using NaOH and was then dried. Salt was removed by selective precipitation of KDPG and KDPGal in 80% (v/v) ethanol. KDPG and KDPGal were analysed by mass spectrometry using an LCT machine (Micromass) and ^{1}H NMR spectroscopy using an Avance 300 machine (Bruker).

2.5. KDG aldolase assay

Recombinant KDG aldolase was prepared and purified as described previously [6]. The cleavage of KDPG and KDPGal was monitored by a continuous assay with *Bacillus stearothermophilus* L-lactate dehydrogenase (Sigma–Aldrich) as coupling enzyme, as described previously [6]. Kinetic parameters were determined by the direct linear method [19].

3. Results

3.1. Cloning and expression of KDG kinase

The gene encoding KDG kinase was identified in the genome of *S. solfataricus* based on homology searches. The gene appears in a cluster along with genes for gluconate dehydratase, KDG aldolase and non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase [11]. No consensus Shine–Dalgarno sequence was found upstream of the start site, although a TATA box promoter element (Box A), TTAAAA, was found 24–29 bp upstream and a polypyrimidine termination sequence, TTTTTCC, was found 16–23 bp downstream of the gene. Based on other genes in *Sulfolobus* species, this suggests that the KDG kinase gene is expressed as a monocistronic transcript, despite its location 9 bp downstream of the KDG aldolase gene [20,21]. The gluconate dehydratase and KDG aldolase genes contain regulatory elements consistent with their expression as the first and second genes of a polycistronic transcript [7]. The possibility to regulate KDG kinase gene transcription independently of the gluconate dehydratase and KDG aldolase genes may be crucial to whether the organism uses the part-phosphorylative or non-phosphorylative pathway, as discussed later.

The KDG kinase gene encodes a 313 amino acid polypeptide with a theoretical molecular weight of 34 875 Da. In this work, the protein was successfully expressed in *E. coli* with a histidine tag on the N-terminus of the protein. This tag facilitated the efficient purification of the protein in a single chromatography step, despite it only comprising a small percentage of total soluble protein (Fig. 2). Mass spectrometric analysis of the purified protein revealed a molecular weight of 37 513 Da, which is consistent with the expected size of KDG kinase with a histidine tag after the N-terminus methionine has been processed by *E. coli*. This analysis provides a valuable confirmation that the protein has been expressed correctly in *E. coli*.

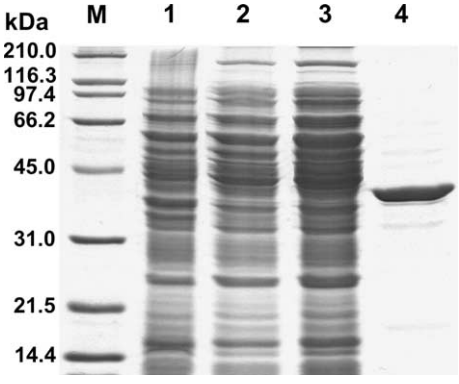

Fig. 2. Purification of recombinant KDG kinase. This SDS–PAGE gel shows samples from throughout the purification of KDG kinase. 1, whole cell sample; 2, soluble cell extract; 3, His-bind chromatography unbound sample; 4, His-bind chromatography eluted sample.

3.2. KDG kinase kinetics

The activity of KDG kinase was confirmed in the current work by coupling the reaction to the reduction of NAD^+ using KDG aldolase and glyceraldehyde-3-phosphate dehydrogenase. The assay gave a precise assessment of kinase activity and was used to determine the kinetic parameters for the phosphorylation of KDG and KDGal (Table 1). Both compounds were good substrates for the enzyme, and were phosphorylated with similar catalytic efficiency, providing good evidence that they are both natural substrates during metabolism of glucose or galactose via the part-phosphorylative pathway. Recombinant KDG kinase was used for the preparative synthesis of KDPG and KDPGal, the purity and identity of which were confirmed by mass spectrometry and ^{1}H NMR spectroscopy, giving data consistent with those reported in the literature [22,23]. These compounds were subsequently used for the kinetic analysis of the KDG aldolase.

3.3. KDG aldolase kinetics

The KDG aldolase from *S. solfataricus* has been reported to have a dual activity with KDG and KDPG [11]. In the current work, this dual activity was investigated further by kinetic analysis in the cleavage direction. Unexpectedly, it was discovered that the enzyme could also cleave KDPGal, with similar efficiency to KDPG (Table 2). Interestingly, the observed K_m values of the enzyme with the phosphorylated substrates were significantly lower than those for KDG and KDGal [6]. This gives the enzyme a significantly improved catalytic efficiency with the phosphorylated compounds and may have crucial importance for metabolism in the organism, as discussed later.

Table 1
Kinetic parameters of KDG kinase from *S. solfataricus* at 60 °C

Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} mM^{-1})
ATP[a]	2.8 (±0.2)	3.8 (±0.2)	1.4
KDG[b]	3.6 (±0.1)	5.0 (±0.1)	1.4
KDGal[b]	8.1 (±0.2)	5.4 (±0.1)	0.7

The production of KDPG and KDPGal were measured by coupling to NADH formation using KDG aldolase and glyceraldehyde-3-phosphate dehydrogenase, as described in Section 2.
[a]Reactions were carried out in the presence of 20 mM KDG.
[b]Reactions were performed with 10 mM ATP.

Table 2
Kinetic parameters of KDG aldolase from *S. solfataricus* at 60 °C

Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (s^{-1} mM^{-1})
KDPG	0.10 (±0.01)	61.9 (±0.4)	643
KDPGal	0.17 (±0.01)	34.8 (±0.2)	207
KDG[a]	25.7 (±1.2)	28.2 (±1.4)	1.1
KDGal[a]	9.9 (±0.4)	6.8 (±0.2)	0.7

Reactions were performed by coupling the KDG aldolase cleavage to the reduction of pyruvate by *Bacillus stearothermophilus* L-lactate dehydrogenase, as described in Section 2.
[a]These values were reported previously [6].

This feature of the KDG aldolase reaction was not revealed by previous analysis in which the enzyme was assayed only in the condensation direction using only racemic glyceraldehyde-3-phosphate [11]. The phosphorylated compounds KDPG and KDPGal have a higher proportion of open-chain form in solution in comparison to KDG and KDGal [24], where no open-chain form of the sugars can be detected by NMR [25]. This is likely to have implications for the observed kinetic parameters of the aldolase cleavage reaction, which must operate via the open-chain form of the sugar.

4. Discussion

The work reported here provides added significance to the previously described phenomenon of 'metabolic pathway promiscuity' [6,7], and presents it in the light of a recent report on the discovery of the part-phosphorylative Entner–Doudoroff pathway in *S. solfataricus* [11]. In vitro kinetic studies of KDG kinase and KDG aldolase have provided further confirmation that the part-phosphorylative pathway occurs in parallel with the non-phosphorylative pathway in this organism, and also demonstrate that the part-phosphorylative variant is promiscuous for the metabolism of both glucose and galactose. These observations have potential physiological and evolutionary significance, in addition to providing a fascinating insight into the unusual features of central metabolism in this hyperthermophilic archaeon.

S. solfataricus KDG kinase has been successfully produced in recombinant form and the kinetics of its phosphorylation of KDG and KDGal have been investigated. A recent report of the structure of KDG kinase from *Thermus thermophilus*, which has 35% amino acid identity to the *S. solfataricus* enzyme, provides some insight into the structure of the *S. solfataricus* KDG kinase [14]. The enzyme is a member of the PfkB family of carbohydrate kinases, which includes ribokinase, adenosine kinase and 6-phosphofructokinase [26]. In these enzymes an aspartate residue is predicted to play a critical role in the transfer of the γ-phosphate of ATP to a substrate hydroxyl group, and this aspartate is conserved in *S. solfataricus* KDG kinase (D258). There is a close degree of structural and mechanistic homology between members of the PfkB family, although a variety of different multimeric assemblies are found [14]. The precise nature of the interactions of the *S. solfataricus* enzyme with KDG, KDGal and ATP, and its subunit assembly, will await a full biochemical and structural investigation of the enzyme.

The kinetics of the KDG aldolase-catalysed cleavage of KDPG and KDPGal reported herein provides strong evidence that both compounds are natural substrates of the enzyme. Previously, activity with phosphorylated substrates had only been demonstrated by the condensation of pyruvate and racemic glyceraldehyde-3-phosphate, which does not provide absolute proof of the physiologically relevant activity [11]. From an evolutionary perspective, the discovery that the KDG aldolase from *S. solfataricus* has favourable KDPG aldolase activity was unexpected. Previously, all known KDPG aldolases have been found to be only distantly related to the *N*-acetylneuraminic acid aldolase subfamily, of which the *S. solfataricus* KDG aldolase is a member [27].

S. solfataricus KDG aldolase provides a powerful model for the structural basis of substrate promiscuity, both in terms of its lack of stereocontrol at the C4 position [6] and in its dual activity with phosphorylated and non-phosphorylated compounds. In previous work the high-resolution crystal structure of the enzyme has been determined, in addition to structures of the enzyme with pyruvate, KDG and KDGal bound in the active site as Schiff-base intermediates [28]. This work led to rationalisation of the enzyme's promiscuous substrate recognition, and provided an insight into its mechanism. In the future, attempts will be made to resolve the structure of enzyme-substrate complexes with KDPG and KDPGal. This should help to explain the enzyme's activity with phosphorylated compounds, and the lack of stereocontrol it displays with these substrates.

It has previously been demonstrated that enzymic activities of both the non-phosphorylative and part-phosphorylative Entner–Doudoroff pathways are present in cell extracts of *S. solfataricus* [11]. This 'pathway parallelism' has unknown physiological significance and raises interesting questions about the regulation of the two pathways in the organism. It is clear from the comparison of the catalytic efficiency of KDG aldolase with KDPG ($k_{cat}/K_m = 643\ mM^{-1}\ s^{-1}$) and KDG ($k_{cat}/K_m = 1.1\ mM^{-1}\ s^{-1}$) that cleavage of the phosphorylated substrate would be significantly favoured in a situation where both compounds are present. It therefore seems that if the KDG kinase is present and active then central metabolism will occur via the part-phosphorylative route. However, the KDG kinase gene sequence has consensus promoter and termination sequences consistent with its production as a single transcript. This is unexpected, given the presence of the gene in an Entner–Doudoroff gene cluster, and may imply that transcription of this enzyme gene is regulated independently of the other genes of the pathway. Under certain conditions it is possible that the gene is also transcribed as a polycistronic transcript, along with the gluconate dehydratase and KDG aldolase genes. In addition to possible transcript level control, the local concentration of ATP will have a critical effect on whether KDG and KDGal are phosphorylated, or cleaved directly by KDG aldolase. The K_m of the kinase for ATP of 2.8 mM is high compared to the likely intracellular concentration, and it is possible that the non-phosphorylative pathway would be favoured under starvation conditions, which could produce pyruvate to allow the citric acid cycle to function without ATP input. Further investigation is required to establish the relative contribution of the two pathways to glycolytic flux, and how they are regulated.

It is clear from the current report that regardless of whether *S. solfataricus* uses the part-phosphorylative or the non-phosphorylative Entner–Doudoroff pathway, then the same enzymes can be used for the metabolism of glucose and its C4 epimer galactose. This situation is unusual, and in other microorganisms separate pathways of specific enzymes exist for the metabolism of the two sugars. The existence of a promiscuous central metabolic pathway in *S. solfataricus* may indicate a primitive evolutionary feature in this hyperthermophilic archaeon, or may be an adaptation to survival in its extreme environment [6]. The presence of parallel Entner–Doudoroff pathways in *S. solfataricus*, both of which are promiscuous for the metabolism of glucose and galactose, is a remarkable example of the unusual features and versatility of central carbohydrate metabolism in hyperthermophilic archaea.

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References

[1] Grogan, D.W. (1989) Phenotypic characterization of the archaebacterial genus *Sulfolobus* – comparison of 5 wild-type strains. J. Bacteriol. 171, 6710–6719.
[2] Verhees, C.H., Kengen, S.W.M., Tuininga, J.E., Schut, G.J., Adams, M.W.W., de Vos, W.M. and van der Oost, J. (2003) The unique features of glycolytic pathways in Archaea. Biochem. J. 375, 231–246.
[3] De Rosa, M., Gambaccorta, A., Nicolaus, B., Giardina, P., Poerio, E. and Buonocore, V. (1984) Glucose metabolism in the extreme thermoacidophilic archaebacterium *Sulfolobus solfataricus*. Biochem. J. 224, 407–414.
[4] Kerscher, L., Nowitzki, S. and Oesterhelt, D. (1982) Thermoacidophilic archaebacteria contain bacterial-type ferredoxins acting as electron-acceptors of 2-oxoacid-ferredoxin oxidoreductases. Eur. J. Biochem. 128, 223–230.
[5] Danson, M.J., Black, S.C., Woodland, D.L. and Wood, P.A. (1985) Citric acid cycle enzymes of the archaebacteria – citrate synthase and succinate thiokinase. FEBS Lett. 179, 120–124.
[6] Lamble, H.J., Heyer, N.I., Bull, S.D., Hough, D.W. and Danson, M.J. (2003) Metabolic pathway promiscuity in the archaeon *Sulfolobus solfataricus* revealed by studies on glucose dehydrogenase and 2-keto-3-deoxygluconate aldolase. J. Biol. Chem. 278, 34066–34072.
[7] Lamble, H.J., Milburn, C.C., Taylor, G.L., Hough, D.W. and Danson, M.J. (2004) Gluconate dehydratase from the promiscuous Entner–Doudoroff pathway in *Sulfolobus solfataricus*. FEBS Lett. 576, 133–136.
[8] Elzainy, T.A., Hassan, M.M. and Allam, A.M. (1973) New pathway for nonphosphorylated degradation of gluconate by *Aspergillus niger*. J. Bacteriol. 114, 457–459.
[9] Elshafei, A.M. (1989) Degradation of some sugars and sugar acids by the nonphosphorylated D-gluconate pathway in *Aspergillus ustus*. Acta Biotechnol. 9, 485–489.
[10] Elshafei, A.M. and Abdel-Fatah, O.M. (2001) Evidence for a non-phosphorylated route of galactose breakdown in cell-free extracts of *Aspergillus niger*. Enzyme Microb. Technol. 29, 76–83.
[11] Ahmed, H., Ettema, T.J.G., Tjaden, B., Geerling, A.C.M., van der Oost, J. and Siebers, B. (2005) The semi-phosphorylative Entner–Doudoroff pathway in hyperthermophilic archaea: a re-evaluation. Biochem. J. 390, 529–540.
[12] Siebers, B., Tjaden, B., Michalke, K., Dörr, C., Ahmed, H., Zaparty, M., Gordon, P., Sensen, C.W., Zibat, A., Klenk, H.-P., Schuster, S.C. and Hensel, R. (2004) Reconstruction of the central carbohydrate metabolism of *Thermoproteus tenax* by use of genomic and biochemical data. J. Bacteriol. 186, 2179–2194.
[13] She, Q. et al. (2001) The complete genome of the crenarchaeon *Sulfolobus solfataricus* P2. Proc. Natl. Acad. Sci. USA 98, 7835–7840.
[14] Ohshima, N., Inagaki, E., Yasuike, K., Takio, K. and Tahirov, T.H. (2004) Structure of *Thermus thermophilus* 2-keto-3-deoxygluconate kinase: evidence for recognition of an open chain substrate. J. Mol. Biol. 340, 477–489.
[15] Bradford, M.M. (1976) Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding. Anal. Biochem. 72, 248–254.
[16] Laemmli, U.K. (1970) Cleavage of structural proteins during assembly of head of bacteriophage T4. Nature 227, 680–685.
[17] Sambrook, J. and Russell, D.W. (2001) Molecular Cloning: A Laboratory Manual, 3rd ed, CSHL, New York.
[18] Tipton, K.F. (2002) in: Enzyme Assays: A Practical Approach (Eisenthal, R. and Danson, M.J., Eds.), 2nd ed, pp. 32–37, Oxford University Press, Oxford.
[19] Eisenthal, R. and Cornish-Bowden, A. (1974) Direct linear plot – new graphical procedure for estimating enzyme kinetic-parameters. Biochem. J. 139, 715–720.
[20] Tolstrup, N., Sensen, C.W., Garrett, R.A. and Clausen, G. (2000) Two different and highly organized mechanisms of translation initiation in the archaeon *Sulfolobus solfataricus*. Extremophiles 4, 175–179.
[21] Reiter, W.-D., Palm, P. and Zillig, W. (1988) Transcription termination in the archaebacterium *Sulfolobus* – signal structures and linkage to transcription initiation. Nucl. Acids Res. 16, 2445–2459.
[22] Knappmann, B.R., El-Nawawy, M.A., Schlegel, H.G. and Kula, M-R. (1993) Microbial synthesis of 3-deoxy-d-erythro-hex-2-ulosonic acid 6-phosphate. Carbohyd. Res. 242, 153–160.
[23] Cotterill, I.C., Henderson, D.P., Shelton, M.C. and Toone, E.J. (1998) The synthetic utility of KDPGal aldolase. J. Mol. Catal. B 5, 103–111.
[24] Shelton, M.C., Cotterill, I.C., Novak, S.T.A., Poonawala, R.M., Sudarshan, S. and Toone, E.J. (1996) 2-keto-3-deoxy-6-phosphogluconate aldolases as catalysts for stereocontrolled carbon-carbon bond formation. J. Am. Chem. Soc. 118, 2117–2125.
[25] Plantier-Royon, R., Cardona, F., Anker, D., Condemine, G., Nasser, W. and Robertbaudouy, J. (1991) New synthesis of 3-deoxy-D-erythro-2-hexulosonic acid (KDG) from D-glucono-1,5-lactone – synthesis and NMR-study on *O*-methylated derivatives of KDG. J. Carbohyd. Chem. 10, 787–811.
[26] Bork, P., Sander, C. and Valencia, A. (1993) Convergent evolution of similar enzymatic function on different protein folds – the hexokinase, ribokinase, and galactokinase families of sugar kinases. Protein Sci. 2, 31–40.
[27] Lawrence, M.C., Barbosa, J.A.R.G., Smith, B.J., Hall, N.E., Pilling, P.A., Ooi, H.C. and Marcuccio, S.M. (1997) Structure and mechanism of a sub-family of enzymes related to *N*-acetylneuraminate lyase. J. Mol. Biol. 266, 381–399.
[28] Theodossis, A., Walden, H., Westwick, E.J., Connaris, H., Lamble, H.J., Hough, D.W., Danson, M.J. and Taylor, G.L. (2004) The structural basis for substrate promiscuity in 2-keto-3-deoxygluconate aldolase from the Entner–Doudoroff pathway in *Sulfolobus solfataricus*. J. Biol. Chem. 279, 43886–43892.