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MINI-REVIEW

The central carbohydrate metabolism of the hyperthermophilic crenarchaeote *Thermoproteus tenax*: pathways and insights into their regulation

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Abstract Although the complexity and modifications of the archaeal central carbohydrate metabolism (CCM) are well established, the knowledge about its regulation is rather limited. The facultatively heterotrophic, hyperthermophilic crenarchaeote *Thermoproteus tenax* utilizes a modified version of the reversible Embden-Meyerhof-Parnas (EMP) and the catabolic, branched Entner-Doudoroff (ED) pathway for glucose metabolism. Glucose is completely oxidized to carbon dioxide via the oxidative tricarboxylic acid (TCA) cycle, which is supposedly used in the reductive direction for carbon dioxide fixation under autotrophic growth conditions. Elemental sulfur is used as final electron acceptor. The CCM of *T. tenax* has been well studied on protein level as well as on gene level by performing a focused transcriptional analysis (CCM DNA microarray). In contrast to the classical pathways found in Bacteria and Eucarya allosteric regulation seems to play a minor role, therefore emphasizing the important role of regulation on transcript level in *T. tenax*. Whereas the EMP pathway and the TCA cycle show a highly coordinated regulation on gene level, the catabolic, branched ED pathway reveals no strong regulation. The CCM pathways in *T. tenax* and the current understanding of their regulation are presented.

Keywords Central carbohydrate metabolism · *Thermoproteus tenax* · Archaea · Hyperthermophile · Embden-Meyerhof-Parnas pathway · Entner-Doudoroff pathway · Regulation of metabolism

Abbreviations

CCM	Central carbohydrate metabolism
ED	Entner-Doudoroff
EMP	Embden-Meyerhof-Parnas
TCA	Tricarboxylic acid

Introduction

The increasing availability of archaeal genome sequence information (about 31 euryarchaeal and 17 crenarchaeal genomes; http://archaea.ucsc.edu/) reveals the basis for further insights into characteristic features of the archaeal domain. Bioinformatic and biochemical studies have shown that Archaea represent a chimera of bacterial and eucaryal features, as well as possess specific archaeal characteristics (Koonin and Galperin 2003). Classical biochemical studies together with genome-based reconstruction of archaeal metabolism and comparative genomic analyses revealed a wide diversity in archaeal metabolic pathways, which seems to be often governed by non-orthologous gene displacement (Verhees et al. 2003; Siebers and Schönheit 2005; Van der Oost and Siebers 2007). Focusing on the central carbohydrate metabolism (CCM) different modifications of the classical anabolic and/or catabolic pathways, which are highly conserved in Bacteria and Eucarya were

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identified. In Archaea carbohydrates are metabolized by variants of the classical Embden-Meyerhof-Parnas (EMP) and the Entner-Doudoroff (ED) pathway, which are characterized by a great variety of novel, unusual enzymes, e.g. ADP-dependent kinases and/or glyceraldehyde-3-phosphate ferredoxin oxidoreductases (Ronimus and Morgan 2003; Verhees et al. 2003; Sakuraba et al. 2004; Siebers et al. 2004; Siebers and Schönheit 2005; Van der Oost and Siebers 2007). However, compared to the current knowledge concerning complexity and modifications of archaeal CCM, the information about its regulation is rather scarce.

The anaerobic, facultative heterotrophic crenarchaeote *Thermoproteus tenax* was one of the first hyperthermophilic Archaea described. The *T. tenax* strain Kra1 was originally isolated from a solfatare in Iceland by Zillig et al. (1981). The organism is sulfur-dependent, using elemental sulfur as terminal electron acceptor, and shows optimal growth at 86°C and pH 5.6. Beside chemolithoautotrophic growth on carbon dioxide and hydrogen (CO_2/H_2), *T. tenax* shows chemoorganoheterotrophic growth on various carbohydrates such as glucose, starch, glycogen, glycerate, ethanol, methanol and malate (Zillig et al. 1981; Fischer et al. 1983). Glucose was shown to be completely oxidized to CO_2 (Selig and Schönheit 1994) and no fermentative growth has been observed (Zillig et al. 1981). Less efficient peptidolytic growth on casamino acids has been reported (Zillig et al. 1981). Due to its metabolic versatility, *T. tenax* represents a suitable model organism for studying the regulation of the CCM in response to autotrophic and heterotrophic growth conditions (glycolytic/gluconeogenic switch). The *T. tenax* genome sequence information was used for the reconstruction of the CCM (Siebers et al. 2004) and moreover, biochemical data for many CCM enzymes (15, Table 1) are available. A focused transcriptional analysis using a partial CCM DNA microarray complements the picture of CCM regulation in *T. tenax* (Zaparty et al. 2008). In this review, the current knowledge of the *T. tenax* CCM and its regulation in response to heterotrophic (glucose) and autotrophic (CO_2/H_2) growth conditions will be discussed.

The central carbohydrate metabolism of *T. tenax*

Analysis of the sugar metabolism in *T. tenax* revealed the presence of two unusual metabolic pathways, a modified version of the EMP pathway and the branched ED pathway (Siebers and Hensel 1993; Selig et al. 1997; Siebers et al. 1997, 2004; Ahmed et al. 2005), both of which represent modifications of the classical pathways found in Bacteria and Eucarya. In vivo NMR analyses, following the distribution of the ^{13}C label in alanine from cells grown on [1-^{13}C]glucose, indicate that *T. tenax* utilizes both pathways for glucose catabolism simultaneously, although the EMP pathway represents the prominent route (Siebers et al. 1997). Remarkably, *T. tenax* is the only Archaeum known to date that utilizes both pathways in parallel.

Growth studies and the measurement of enzyme activities in crude extract revealed that pyruvate, provided by the different glycolytic routes, is completely oxidized to carbon dioxide under heterotrophic growth conditions via the oxidative tricarboxylic acid cycle (TCA cycle; Selig and Schönheit 1994) with elemental sulfur as terminal electron acceptor (sulfur respiration). The *T. tenax* genome data as well as enzymatic studies in the close relatives *T. neutrophilus* (Beh et al. 1993; Schäfer et al. 1986) and *Pyrobaculum islandicum* (Hu and Holden 2006; Hügler et al. 2003) suggest that CO_2 fixation in *T. tenax* proceeds via the reverse, reductive TCA cycle. Energy is gained from anaerobic H_2 oxidation using sulfur as terminal electron acceptor (Hydrogen/sulfur autotrophy).

Alternative pathways for the fixation of CO_2 seem to be absent in *T. tenax* as deduced from the genome data (Siebers et al. 2004) as well as enzyme measurements in crude extracts (Selig and Schönheit 1994). Three ORFs (TTX_0327, TTX_0328, TTX_0326) with similarity to the small, medium and large subunit of carbon monoxide dehydrogenase (*coxM-coxS-coxL*), key enzyme of the reductive acetyl-CoA (Wood-Ljungdahl) pathway have previously been identified in the *T. tenax* genome (Siebers et al. 2004). However, these genes show significant similarity to the genes encoding the three subunits of the characterized aldehyde oxidoreductase from *Sulfolobus acidocaldarius* (Kardinahl et al. 1999; Jung and Lee 2006).

The EMP pathway of *T. tenax*.

The modified EMP pathway of *T. tenax* operates in both directions and thus, represents the major route for glycolysis as well as gluconeogenesis. Therefore, as in Bacteria and Eucarya, the pathway needs major sites of regulation, which determine the glycolytic/gluconeogenic carbon flux in response to the cellular needs (e.g. autotrophic or heterotrophic growth, glycogen formation or degradation) and to avoid futile cycling. Only few of the EMP enzymes identified in *T. tenax* represent homologs of bacterial and eucaryal enzymes, e.g. the pyrophosphate-dependent phosphofructokinase (PP_i-PFK; Siebers et al. 1998) or the pyruvate kinase (PK; Schramm et al. 2000). Of the 17 identified enzymes of the *T. tenax* EMP pathway, 11 were characterized in detail (Table 1, Fig. 1) and will be discussed in the following.

Hexokinase The first phosphorylation step in the EMP variant of *T. tenax* is characterized by an ATP-dependent hexokinase (ATP-HK; Fig. 1). The enzyme requires Mg^{2+} for activity and exhibits broad substrate specificity being active on glucose, fructose, mannose and 2-deoxyglucose

Table 1 Characterized enzymes of the EMP and the ED pathway of *T. tenax*

ORF ID	Gene information[a]	Enzyme	Classification[b]	Kinetics (K_m, V_{max}, k_{cat}/K_m), assay temperature (°C)	Effector studies (−) inhibitor (+) activator	References
EMP pathway						
TTX_0060	*hxk* *orfX-hxk*	ATP-dependent hexokinase (HK)	AJ510140 1940 Pf00480 2.7.1.1	Glucose: K_m 0.058 mM, V_{max} 14.8 U/mg, k_{cat}/K_m 8,179 mM^{-1} min^{-1} Fructose: K_m 0.734 mM, V_{max} 31.6 U/mg, k_{cat}/K_m 1,389 mM^{-1} min^{-1} Mannose: K_m 0.043 mM, V_{max} 5.7 U/mg, k_{cat}/K_m 4,276 mM^{-1} min^{-1} 2-deoxyglucose: K_m 0.187 mM, V_{max} 25.6 U/mg, k_{cat}/K_m 4,416 mM^{-1} min^{-1} ATP: K_m 0.29 mM ADP: K_m ND 50°C		Dörr et al. (2003)
TTX_0980	*pgi*	Glucose-6-phosphate isomerase (PGI)	AJ621272 0166 PF01380 5.3.1.9	G6P: K_m 2mM, V_{max} 170 U/mg F6P: K_m 0.17 mM, V_{max} 44.5 U/mg 50°C		Siebers et al. (2004)
TTX_1277	*pfp* *fba-pfp* H	PP_i-dependent phosphofructokinase (PP_i-PFK)	Y14655 0205 PF00365 2.7.1.90	PP_i: K_m 0.023 mM, V_{max} 2.7 U/mg F6P: K_m 0.053 mM, V_{max} 2.9 U/mg P_i: K_m 1.43 mM, V_{max} 2.1 U/mg $F1,6P_2$: K_m 0.033 mM, V_{max} 2 U/mg 50°C		Siebers et al. (1998)
TTX_1278	*fba* *fba-pfp* H	Fructose-1,6-bisphosphate aldolase (FBPA), archaeal-type class I	AJ310483 1830 PF01791 4.1.2.13	F1,6P: K_m 9 µM, V_{max} 0.23 U/mg, k_{cat}/K_m 734.4 $mM^{-1}min^{-1}$ F1P: K_m 4.48 mM, V_{max} 0.3 U/mg, k_{cat}/K_m 1.89 $mM^{-1}min^{-1}$ 50°C		Siebers et al. (2001) Lorentzen et al. (2003*, 2005*)
TTX_0494	*tpi* *acn-tpi* H	Triosephosphate isomerase (TIM)	AJ515539 0149 PF00121 5.3.1.1	DHAP: K_m 0.9 mM, V_{max} 2000 U/mg GAP: K_m 0.2 mM, V_{max} 9500 U/mg 70°C		Walden et al. (2004*)
TTX_1169	*gapN*	Non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN)	Y10625 1012 PF00171 1.2.1.9	GAP: K_m 20 µM, V_{max} 36 U/mg (70°C) 1,3BPG: K_m ND (45°C) NAD^+: K_m 3.1 mM (70°), V_{max} 36 U/mg (45°C) $NADP^+$: K_m 20 mM, V_{max} 14 U/mg (45°C)	(−) K_D NADPH 0.3 µM K_D $NADP^+$ 1 µM K_D ATP 3 mM (+) K_D G1P 1 µM K_D AMP 0.14 mM K_D F6P 0.2 mM K_D ADP 0.25 mM 70°C	Brunner et al. (1998, 2001) Lorentzen et al. (2004*)
TTX_1534	*gap* *pgk-gap* A	Phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Y10126 0057 PF00044, PF02800 1.2.1.13	GAP: K_m 200 µM, V_{max} 2 U/mg 1,3BPG: K_m 10 µM, V_{max} 10 U/mg 45°C		Brunner et al. (2001)

Table 1 continued

ORF ID	Gene information[a]	Enzyme	Classification[b]	Kinetics (K_m, V_{max}, k_{cat}/K_m), assay temperature (°C)	Effector studies (−) inhibitor (+) activator	References
TTX_1891	*pyk* H	Pyruvate kinase (PK)	AF065890 0469 PF00224 2.7.1.40	PEP: K_m 3 mM, V_{max} 45 U/mg Pyruvate: K_m ND ADP: K_m 0.7 mM 50°C		Schramm et al. (2000)
TTX_0910	*pps* A	Phosphoenolpyruvate synthetase (PEPS)	AJ515537 0574 PF01326, PF00391, PF02896 2.7.9.2	Pyruvate: K_m 0.4 mM; V_{max} 0.45 U/mg k_{cat}/K_m 102.5 $mM^{-1}min^{-1}$ PEP: K_m ND ATP: K_m 1 mM 70°C	(−) K_i 2-OG 0.6 mM K_i AMP 0.5 mM K_i ADP 2.6 mM 70°C	Tjaden et al. (2006)
TTX_0683	*ppdk*	Pyruvate,phosphate dikinase (PPDK)	AJ515538 0574 PF01326, PF00391, PF02896 2.7.9.1	Pyruvate: K_m 0.8 mM, V_{max} 1.1, k_{cat}/K_m 141.3 $mM^{-1}min^{-1}$ ATP: K_m 8 mM,k_{cat}/K_m 14.13 $mM^{-1}min^{-1}$ P_i: Km 3.5 mM, k_{cat}/K_m 32.29 $mM^{-1}min^{-1}$ 70°C PEP: K_m 0.5 mM, V_{max} 1.3 U/mg, k_{cat}/K_m 266 $mM^{-1}min^{-1}$ AMP:K_m 20 µM, k_{cat}/K_m 6650$mM^{-1}min^{-1}$ PP_i: K_m 80 µM, k_{cat}/K_m 1662 $mM^{-1}min^{-1}$ 55°C	(−) K_i (ATP) 75 µM 55°C	Tjaden et al. (2006)
ED pathway						
TTX_0329	*gdh*	Glucose dehydrogenase (GDH)	AJ621346 1063 PF08240, PF00107 1.1.1.47	Glucose: K_m 0.3 mM, V_{max} 40 U/mg Xylose: K_m 8 mM, V_{max} 60 U/mg $NADP^+$: K_m 0.7 mM NAD^+: K_m 86 mM 70°C		Siebers et al. (1997)
TTX_1156	*gad* ED *cluster* (*gad, kdgA-kdgK-gaa*)	Gluconate dehydratase (GAD)	AJ621281 4948 PF02746, PF01188 4.2.1.39	Activity confirmed (with gluconate) 70°C		Ahmed et al. (2005)
TTX_1156a	*kdgA* *kdgA-kdgK-gaa*	2-keto-3-deoxy-(phospho)gluconate aldolase (KD(P)GA)	AJ621282 0329 PF00701 4.1.2.-	Activity confirmed (with GA and pyruvate as well as GAP and pyruvate) 70°C		Ahmed et al. (2005) Pauluhn et al. (2008*)
TTX_1157	*kdgA-kdgK-gaa*	2-keto-3-deoxy-gluconate kinase (KDGK)	AJ621283 0524 PF00294 2.7.1.45	KDG: K_m 0.2 mM, V_{max} 43.3 U/mg 70°C		Ahmed et al. (2005)

Table 1 continued

ORF ID	Gene information[a]	Enzyme	Classification[b]	Kinetics (K_m, V_{max}, k_{cat}/K_m), assay temperature (°C)	Effector studies (−) inhibitor (+) activator	References
TTX_0788	*garK*	Glycerate kinase (GK)	AJ621354	Glycerate: K_m 0.02 mM, V_{max} 5.1 U/mg, k_{cat}/K_m 8913 $mM^{-1}min^{-1}$	(−) 0.1 mM ADP	Kehrer et al. (2007)
	garK-ilvD/edd		2379 PF05161	ATP: K_m 0.03 mM, V_{max} 4.41 U/mg,	70°C	
	A		2.7.1.31	k_{cat}/K_m 7187 $mM^{-1}min^{-1}$ 70°C		

Gene information (ORF ID, gene organization, regulation on gene level) as well as available protein information (classification, kinetic parameters and effectors [inhibitors (−), activators (+)] are given

For abbreviations see Fig. 1. *F1P* fructose 1-phosphate, *ND* not detectable

* Crystal structure

[a] Gene information: gene name, organization, up-regulation of the genes under autotrophic (A)/heterotrophic (H) growth conditions is indicated

[b] Enzyme classification: Accession number (EMBL)/COG/Pfam/EC number

(Table 1; Dörr et al. 2003). Interestingly, in contrast to Bacteria and Eucarya, the enzyme shows no or if at all only very low regulatory properties. Inhibition was observed only in the presence of high concentrations of glucose 6-phosphate (>8 mM), UDP, AMP, P_i (>10 mM) and ADP, PP_i (>1 mM) leaving doubts about the physiological significance. Phylogenetic studies indicate that the enzyme is a member of the *r*epressor protein, *o*pen reading frame, sugar kinase (ROK) family (PF00480), which harbors different archaeal and bacterial sugar kinases, mostly of unknown substrate specificity, and transcriptional regulators, e.g. XylR, NagC (Dörr et al. 2003; Hansen and Schönheit 2003). Transcript analyses revealed that the encoding gene (*hxk*) is co-transcribed with a small open reading frame (*orfX*, OrfX) coding for an 11 kDa protein of unknown function (Dörr et al. 2003). Both genes show no regulation on transcript level in response to a change of the carbon source (Zaparty et al. 2008). However, whereas the monocistronic *hxk* transcript was observed under heterotrophic growth conditions, only the bicistronic *orfX-hxk* transcript was detected under autotrophic growth conditions. This finding suggests that regulation via RNA processing might be involved. It has been speculated that the monocistronic transcript under heterotrophic growth conditions, where the active enzyme is required, represents the active transcript (Dörr et al. 2003).

Phosphoglucose/phosphomannose isomerase The isomerization of glucose 6-phosphate (G6P) to fructose 6-phosphate (F6P) is catalyzed by a bifunctional phosphoglucose/phosphomannose isomerase (PGI/PMI; Fig. 1). As reported previously for the *Pyrobaculum aerophilum* enzyme (Hansen et al. 2004a, b), unlike all known PGIs, the *T. tenax* enzyme shows similar catalytic efficiency on both substrates (G6P, mannose 6-phosphate) and converts them to F6P. The *T. tenax* enzyme has been characterized in respect to G6P conversion (Siebers et al. 2004; Table 1) and belongs to a new family within the PGI superfamily (Hansen et al. 2004a, b). Transcriptional analysis revealed that the encoding *pgi* gene is not regulated in response to autotrophic versus heterotrophic growth (Zaparty et al. 2008).

Phosphofructokinase The phosphorylation of F6P to fructose 1,6-bisphosphate ($F1,6P_2$) is catalyzed by a reversible pyrophosphate (PP_i)-dependent phosphofructokinase (PP_i-PFK; Siebers et al. 1998; Figs. 1, 2). Kinetic characterization revealed a slight preference for the phosphorylating direction (Table 1) and strict dependence on Mg^{2+} ions. The enzyme is the only archaeal PP_i-dependent PFK described so far and homologs have only been identified in a few archaeal genomes, e.g. *Caldivirga maquilingensis* (DOE Joint Genome Institute/UCSC, 2007). The *T. tenax* enzyme shows no regulation by known allosteric modulators of classical ATP-dependent PFKs (e.g. ATP, ADP) and various metabolites (fructose 2,6-

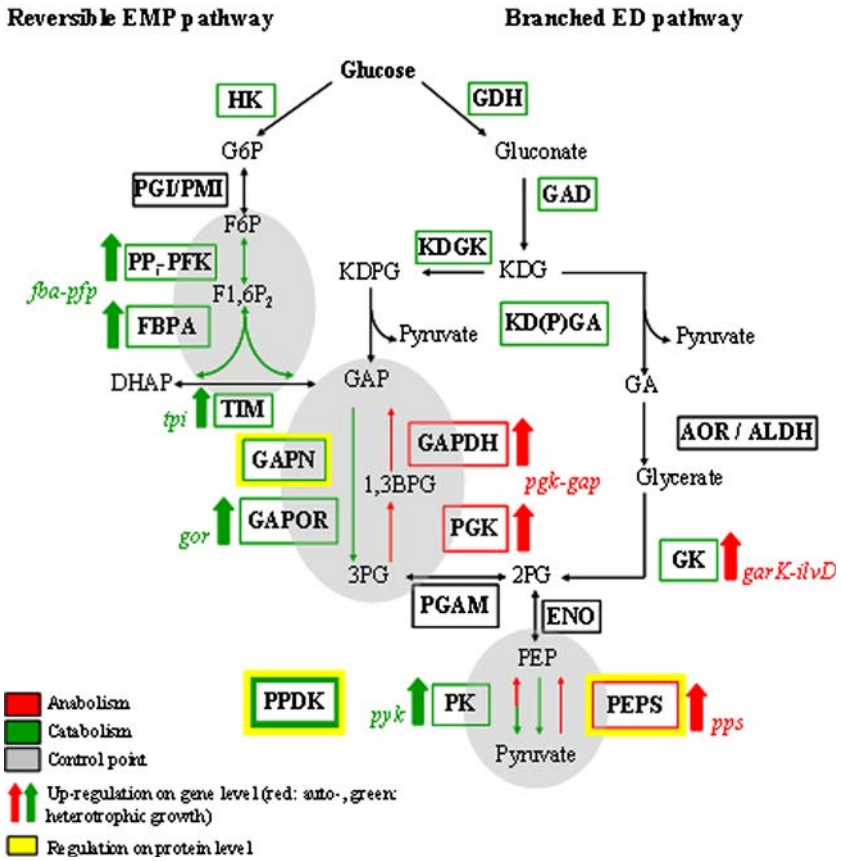
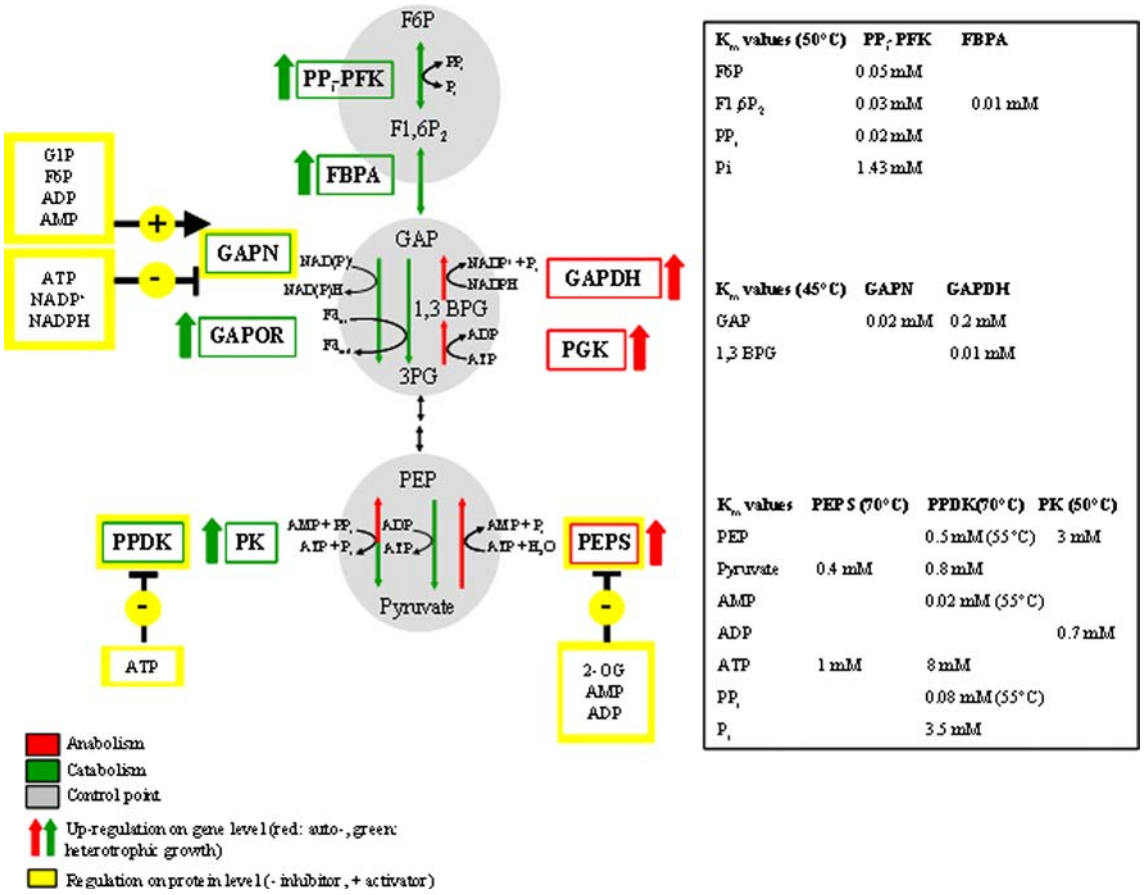


Fig. 1 The reversible EMP and the branched ED pathway of *T. tenax*. Engaged enzymes, their discussed physiological function based on enzymatic analyses [*green box* (catabolism), *red box* (anabolism)], regulation on protein level (*yellow box*) as well as regulation on gene level (*green/red arrows*) are given. Furthermore, the three regulation sites of the *T. tenax* EMP variant are indicated (*gray shading*). Abbreviations enzymes: *AOR/ALDH* ferredoxin-dependent aldehyde oxidoreductase/aldehyde dehydrogenase, *ENO* enolase, *FBPA* fructose-bisphosphate aldolase, *FBPase* fructosebis-phosphatase, *GAD* gluconate dehydratase, *GAPDH* classical phosphorylating, non-allosteric glyceraldehyde-3-phosphate dehydrogenase, *GAPN* non-phosphorylating, allosteric GAPDH, *GAPOR* non-phosphorylating glyceraldehyde-3-phosphate oxidoreductase, *GDH* glucose dehydrogenase, *GK* glycerate kinase, *HK* hexokinase, *KDGK* KDG kinase, *KD(P)GA* bifunctional KD(P)G aldolase, *PEPS* phosphoenolpyruvate synthetase, *PGAM* phosphoglycerate mutase, *PGI/PMI* phosphoglucose/phosphomannose isomerase, *PGK* phosphoglycerate kinase, *PK* pyruvate kinase, *PPDK* pyruvate,phosphate dikinase, PP_i*-PFK* pyrophosphate-dependent phosphofructokinase, *TIM* triosephosphate isomerase; Abbreviations intermediates: *1,3 BPG* 1,3-bisphosphoglycerate, *2PG* 2-phosphoglycerate, *3PG* 3-phosphoglycerate, *DHAP* dihydroxyacetone phosphate, $F1,6P_2$ fructose 1,6-bisphosphate, *F6P* fructose 6-phosphate, *G6P* glucose 6-phosphate, *GA(P)* glyceraldehyde(-3-phosphate), *KD(P)G* 2-keto-3-deoxy-(6-phosphate)-gluconate, *PEP* phosphoenolpyruvate, PP_i pyrophosphate

bisphosphate, phosphoenolpyruvate (PEP), citrate, glucose, pyruvate) tested. Phylogenetic analyses revealed that the enzyme is distantly related to ATP-dependent phosphofructokinases and belongs to the phosphofructokinase (PFK A) family (PF00365; Siebers et al. 1998).

Fructose-1,6-bisphosphate aldolase The archaeal-type class I fructose-1,6-bisphosphate aldolase (aFBPA) catalyzes the reversibe cleavage of $F1,6P_2$ forming dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP; Fig. 1 and Fig. 2). Although the enzyme uses the same reaction mechanism as classical Schiff base class I enzymes, the aFBPA shows no obvious sequence similarity to either classical class I or metal-dependent class II FBPA, which are predominantly found in higher Eucarya and Bacteria as well as in few unicellular Eucarya. The enzyme of *T. tenax* was characterized in respect to its catabolic reaction (aldol cleavage). Beside $F1,6P_2$ the enzyme also exhibits activity with fructose-1-phosphate (F1P), albeit with a significantly reduced catalytic efficiency than for $F1,6P_2$, suggesting that $F1,6P_2$ is the physiological substrate (Table 1). A slight (2.2-fold) activation of FBPA was observed by saturating concentrations of citrate (10 mM). Phylogenetic analyses identified orthologs in most archaeal genomes (Siebers et al. 2001), thus representing the true archaeal FBPA, as predicted previously (Galperin et al. 2000). Solved crystal structures of the *T. tenax* enzyme, as well as of active site mutants, revealed some new insights in the evolution of the



K_m values (50°C)	PP_i-PFK	FBPA
F6P	0.05 mM	
F1,6P_2	0.03 mM	0.01 mM
PP_i	0.02 mM	
Pi	1.43 mM	

K_m values (45°C)	GAPN	GAPDH
GAP	0.02 mM	0.2 mM
1,3 BPG		0.01 mM

K_m values	PEPS (70°C)	PPDK (70°C)	PK (50°C)
PEP		0.5 mM (55°C)	3 mM
Pyruvate	0.4 mM	0.8 mM	
AMP		0.02 mM (55°C)	
ADP			0.7 mM
ATP	1 mM	8 mM	
PP_i		0.08 mM (55°C)	
P_i		3.5 mM	

Fig. 2 The three regulation sites of the *T. tenax* EMP pathway at the level of F6P/F1,6P_2, GAP and PEP/pyruvate conversion. The color code is given as in Fig. 2. In addition, the cosubstrates and the effectors are depicted [*yellow box*; effectors: activator (+), inhibitor (−)]. The K_m values for the different enzymes (*black box*, right-hand side) are shown. Abbreviations: see Fig. 1; *2-OG* 2-oxoglutarate, *G1P* glucose 1-phosphate

$(\alpha\beta)_8$ triosephosphate isomerase (TIM) barrel family and the general reaction mechanism of Schiff base forming FBPAs (Lorentzen et al. 2003, 2005).

The *T. tenax* PP_i-dependent PFK and FBPA encoding genes are organized in an operon (*fba-pfp* operon) and an increased transcript abundance in glucose grown cells in correlation with increased enzyme activities (about tenfold) point to higher catabolic fluxes under hetrotrophic growth conditions compared to autotrophic growth (Siebers et al. 2001; Zaparty et al. 2008). Therefore, the conversion of F6P and F1,6P_2 represents the first site of regulation in the EMP modification of *T. tenax*, which is exclusively performed on gene level (Fig. 2). Interestingly, in addition to the reversible PP_i-dependent PFK, a homolog of the unidirectional archaeal-type fructose-1,6-bisphosphatase (FBPase, class V; AM884389) was identified in the complete *T. tenax* genome sequence. The homolog from *Thermococcus kodakaraensis* (TK2164; 61% sequence identity; Rashid et al. 2002) has been characterized recently, and a mutational approach confirmed its function as the true FBPase in Archaea (Sato et al. 2004). In addition, a gene coding for a homolog of a FBPase class IV/myo-inositol-1(or 4)-monophosphatase (FBPase/IMPase; AM920546) with 30% sequence identity to the characterized enzyme from *Methanocaldococcus jannaschii* (MJ0109, Stec et al. 2000) was found in the *T. tenax* genome. So far no biochemical information is available for both candidates, but an additional function in gluconeogenesis, beside the reversible PP_i-PFK, cannot be excluded. The *T. tenax* CCM DNA microarray revealed no regulation on gene level for the archaeal-type FBPase encoding gene (*fbpA*) (Zaparty et al. 2008). This finding is in contrast to the gene encoding the archaeal-type FBPase V from *T. kodakaraensis* (Rashid et al. 2002; Sato et al. 2004). Therefore, ongoing enzymatic studies have to be awaited in order to unravel the role of the two FBPases, beside the reversible PP_i-PFK, in *T. tenax* gluconeogenesis.

Triosephosphate isomerase The isomerization of DHAP to GAP is catalyzed by TIM (Fig. 1). The enzyme of *T. tenax* was characterized (Table 1) and revealed according to its oligomeric structure an unusual equilibrium between inactive dimers and active tetramers in

solution (Walden et al. 2004). Strikingly, the equilibrium was shifted in the direction of the active tetramer state by the specific interaction with glycerol-1-phosphate dehydrogenase (GLPDH; AM884390) of *T. tenax*, catalyzing the formation of glycerol 1-phosphate from DHAP. A possible physiological function in metabolic thermoadaptation by avoiding thermolabile intermediates, e.g. DHAP (half-life of 79.4 min at 60°C) has been discussed (Walden et al. 2004). The structure of the *T. tenax* TIM was determined and revealed some new, general insights into structural adaptations at high temperature (Walden et al. 2004).

The encoding gene (*tpi*) constitutes an operon with the *acn* gene encoding aconitase. This clustering points to a preferred functional relationship of both reversible pathways—the EMP and the TCA of *T. tenax* (Tjaden 2003). Higher transcript levels for the *tpi-acn* operon were observed under heterotrophic growth conditions (Tjaden 2003; Zaparty et al. 2008).

Glyceraldehyde 3-phosphate conversion The conversion of GAP was shown to play a major role in the regulation of catabolic and anabolic carbon fluxes in *T. tenax* (Hensel et al. 1987; Brunner et al. 1998, 2001; Siebers et al. 2004; Zaparty et al. 2008) and therefore represents the second site of regulation in the EMP variant (Fig. 2).

Classical GAP dehydrogenase The classical, phosphorylating $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of *T. tenax* catalyzes the reversible conversion of 1,3 bisphosphoglycerate (1,3BPG) to GAP (Fig. 1). The enzyme follows classical Michaelis-Menten kinetics with a 20-fold higher affinity toward 1,3BPG than to GAP and an about fivefold higher specific activity in the reductive direction (Table 1, Fig. 2) indicating an anabolic function (Brunner et al. 2001). The GAPDH encoding gene (*gap*) is organized in an operon with the *pgk* gene encoding phosphoglycerate kinase (PGK). Transcriptional analyses revealed a significant (about fourfold) induction of the *pgk-gap* operon under autotrophic growth conditions (Brunner et al. 2001; Zaparty et al. 2008), which is also confirmed on protein level by three to fourfold higher enzyme activities of GAPDH and PGK in crude extracts of autotrophically grown cells (Brunner et al. 2001). Therefore enzymatic as well as transcriptional data clearly support the anabolic role of GAPDH in *T. tenax*.

Non-phosphorylating GAP dehydrogenase The glycolytic conversion of GAP in *T. tenax* is performed by the unidirectional non-phosphorylating $NAD(P)^+$-dependent GAPDH (GAPN; Fig. 1; Hensel et al. 1987, Brunner et al. 1998, 2001, Lorentzen et al. 2004). The direct oxidation of GAP to 3-phosphoglycerate is not coupled to substrate-level phosphorylation, resulting in a reduced energy yield compared to the classical enzyme-couple GAPDH/PGK (Fig. 1). The enzyme shows a much higher affinity (tenfold) for GAP than the anabolic GAPDH clearly underlining its glycolytic function (Table 1, Fig. 2).

The catabolic GAPN exhibits allosteric properties: Its activity is enhanced by glucose 1-phosphate (G1P), F6P, ADP and AMP, whereas activity is inhibited in the presence of ATP, $NADP^+$ and NADPH (Table 1, Fig. 2; Brunner et al. 1998). Thus, the GAPN is regulated by the energy charge of the cell, early intermediates of the EMP pathway and of glycogen metabolism (G1P). Strikingly, the enzyme exhibits dual cosubstrate specificity, however, only in the presence of the activator G1P, $NADP^+$ is the more efficient co-substrate compared to NAD^+ (K_m value NAD^+ 0.4 mM and $NADP^+$ 0.1 mM in presence of 0.1 mM G1P; Lorentzen et al. 2004). No regulation on the transcript level is observed for the *gapN* gene of *T. tenax* under autotrophic and heterotrophic growth conditions, in contrast to the *gap* gene encoding classical GAPDH (Brunner et al. 2001; Zaparty et al. 2008).

The GAPN is a member of the aldehyde dehydrogenase superfamily (PF00171) and the structural basis for the allosteric regulation and substrate specificity of the *T. tenax* GAPN was solved (Lorentzen et al. 2004). Based on sequence comparisons, a similar allosteric regulation was proposed for the enzymes of *Sulfolobus tokodaii*, *S. solfataricus*, *Aeropyrum pernix* and *Pyrococcus furiosus*. The enzyme of *S. solfataricus* has been characterized (Ahmed et al. 2005, Ettema et al. 2007) and as predicted, similar allosteric properties were observed, however, in contrast to the *T. tenax* enzyme, only $NADP^+$ serves as co-substrate for the *S. solfataricus* GAPN (Ettema et al. 2007).

Ferredoxin-dependent GAP oxidoreductase In addition to the classical, anabolic GAPDH and the allosteric, catabolic GAPN, *T. tenax* possesses a homolog of a ferredoxin(Fd)-dependent GAP oxidoreductase (GAPOR;AJ621330). Like the GAPN, GAPOR directly oxidizes GAP yielding 3-phosphoglycerate (3PG) in an irreversible and non-phosphorylating reaction (Fig. 1). In contrast to GAPN, the enzyme uses ferredoxin instead of pyridine nucleotides as co-substrate. Whereas, GAPDH and GAPN of *T. tenax* have been characterized in great detail (Hensel et al. 1987, Brunner et al. 1998, 2001), so far no biochemical information is available for the GAPOR of *T. tenax*. However, the proposed glycolytic function of the *T. tenax* enzyme is supported by analysis of counterparts in its close relative *P. aerophilum* (59% identity; Reher et al. 2007) and *P. furiosus* (37% identity; Mukund and Adams 1995; Van der Oost et al. 1998). The enzyme of *P. aerophilum* exhibits higher activity in crude extracts of maltose grown cells and higher transcript amounts of the GAPOR encoding gene (*gor*) were observed in heterotrophically grown cells of *P. furiosus* compared to peptide-grown cells. For both enzymes no allosteric properties were reported

(Mukund and Adams 1995; Van der Oost et al. 1998; Schut et al. 2003; Reher et al. 2007). For the *gor* gene of *T. tenax* higher transcript levels have been detected under heterotrophic growth conditions on glucose compared to growth on H_2/CO_2 (Zaparty et al. 2008), confirming the supposed catabolic function.

Thus, the major control site of the *T. tenax* EMP pathway is characterized by three GAP converting enzymes: The anabolic enzyme-couple GAPDH and PGK and the catabolic enzymes GAPN and GAPOR. Regulation is performed on protein level by the allosteric GAPN, as well as on transcript level by the inversely regulated genes encoding catabolic GAPOR and the anabolic GAPDH-PGK enzyme pair.

Phosphoenolpyruvate/pyruvate conversion Also the conversion of PEP and pyruvate is controlled by three different enzymes: Pyruvate kinase (PK), PEP synthetase (PEPS) and pyruvate, phosphate dikinase (PPDK), in *T. tenax*.

Pyruvate kinase The glycolytic, energy (ATP)-yielding formation of pyruvate from PEP in *T. tenax* is catalyzed by the PK (Fig. 1, Table 1). The enzyme depends on divalent metal cations (Mg^{2+}, Mn^{2+}) for activity. In contrast to most bacterial and eucaryal PKs, the enzyme possesses reduced regulatory properties and exhibits positive binding cooperativity for PEP (at concentrations above 1 mM) and divalent metal cations. No regulation by allosteric effectors (e.g. F1,6P_2, AMP, G1P, G6P, ribose 5-phosphate, P_i) was observed for the *T. tenax* enzyme as for all other studied archaeal PKs, with the exception of the PK from *Thermoplasma acidophilum* (Potter and Fothergill-Gilmore 1992). For the activation by PEP a physiological role in metabolic thermoadaptation by decreasing the thermolabile PEP pool has been suggested (Schramm et al. 2000).

The PK of *T. tenax* shows moderate sequence similarity (25–40%) to its bacterial and eucaryal counterparts [pyruvate kinase family (PF00224)]. Phylogenetic analysis indicates a dichotome tree topology, which is supposed to trace back to an early gene duplication event accompanied by a phenotypic differentiation. Whereas, cluster I PKs require effectors such as F1,6P_2 or other phosphorylated sugars for activity, PKs of cluster II, including archaeal enzymes, are independent of effectors. For the PK an increased transcript amount and enzyme activity (both about fourfold) were observed under heterotrophic growth conditions compared to autotrophic growth on H_2/CO_2 (Schramm et al. 2000).

PEP synthetase The anabolic PEPS catalyzes the unidirectional ATP-dependent conversion of pyruvate to PEP, AMP and P_i (Fig. 1; Tjaden et al. 2006), thus representing a true anabolic enzyme. No activity in the glycolytic direction has been observed. The enzyme follows classical Michaelis-Menten kinetics and revealed high affinity for its (co-)substrates pyruvate and ATP at 70°C (Table 1). Biochemical observations as well as a mutational approach (PEPS-H406Q) suggested a two-step reaction mechanism via the formation of a phosphoenzyme intermediate (Tjaden et al. 2006). The enzyme exhibits regulatory properties and is significantly inhibited in the presence of 2-oxoglutarate, AMP and ADP (Table 1), suggesting reduced activity at a low energy charge of the cell and under ammonia limitation (Table 1, Fig. 2; Tjaden et al. 2006). In accordance with the solely anabolic function of the enzyme, the encoding gene (*pps*) shows an up-regulation in autotrophically grown cells (Tjaden et al. 2006; Zaparty et al. 2008).

Pyruvate, phosphate dikinase The reversible PPDK catalyzes the interconversion of PEP and pyruvate (Fig. 1). The enzymatic characterization of the *T. tenax* enzyme revealed a clear preference for the glycolytic direction (Table 1, Fig. 2). The enzyme exhibits regulatory properties and is strongly inhibited in its catabolic direction by ATP in an AMP competitive manner (Table 1; Tjaden et al. 2006) supporting its glycolytic function. For the PPDK encoding gene (*ppdk)* no additional regulation on the transcriptional level is observed (Tjaden et al. 2006; Zaparty et al. 2008). Phylogenetic analyses revealed the presence of PEPS in Bacteria and Archaea and of PPDK in all three domains of life. Both enzymes, together with enzyme I of the bacterial phosphotransferase system (PEP-utilizing enzymes) belong to the PPDK/PEP-utilizing families (PF01326/PF00391/PF02896). In depth analysis on sequence level depicted highly conserved sequence patterns, which allow for differentiation between both enzymes (Tjaden et al. 2006). Therefore, the third control point in *T. tenax* is also established by three enzymes: The glycolytic PK, without allosteric properties, but an increased transcript amount under heterotrophic growth conditions, the catabolic PPDK, which is regulated on protein level alone and the anabolic PEPS, which is regulated on protein as well as on transcript level.

In summary, the EMP pathway of *T. tenax* and of other Archaea, e.g. *P. furiosus*, is characterized by no or reduced regulation on protein level of the classical enzymes (e.g. PFK, PK), which play a major regulatory role in the bacterial and eucaryal EMP pathway. In contrast, regulation on gene level seems to play a more important role in the control of the EMP pathway in *T. tenax*. Regulation on protein level is accomplished by the allosteric GAPN as well as PEPS and PPDK, and regulation on gene level is observed for the genes encoding PPi-PFK, FBPA, GAPOR, GAPDH, PGK and PEPS (Fig. 2).

The catabolic ED pathway of *T. tenax*

Recent studies have shown that glucose degradation in most Archaea using the ED pathway seems to proceed via

the so-called branched ED pathway, a modified version of the classical ED pathway known from Bacteria (Fig. 1; Ahmed et al. 2005; Siebers and Schönheit 2005; Kehrer et al. 2007). A conserved gene cluster was identified in several Archaea including *T. tenax*, comprising the genes coding for gluconate dehydratase (*gad*; GAD), 2-keto-3-deoxy-(6-phospho)gluconate aldolase (*kdgA*; KD(P)GA), 2-keto-3-deoxygluconate (KDG) kinase (*kdgK*; KDGK) and, specific for *T. tenax*, a glucoamylase (*gaa*; GAA) (Ahmed et al. 2005). The branched ED pathway is characterized by a semi- and a non-phosphorylative ED branch (Ahmed et al. 2005) and seems to represent an exclusively catabolic pathway. In the common shunt of the pathway glucose is converted to the key intermediate of the pathway KDG via glucose dehydrogenase (GDH) and GAD, omitting the initial phosphorylation to G6P found in the classical ED pathway. Key enzymes of this branched pathway are the bifunctional KD(P)GA (Ahmed et al. 2005), which catalyzes the cleavage of KDG as well as 2-keto-3-deoxy-6-phosphogluconate (KDPG), and the KDGK (Ahmed et al. 2005) as well as the glycerate kinase (GK; Kehrer et al. 2007). The latter two enzymes catalyze the ATP-dependent phosphorylation in the semi- and the non-phosphorylative ED branch, respectively (Fig. 1). In contrast to *S. solfataricus*, where the pathway is promiscuous for glucose and galactose degradation (Lamble et al. 2003, 2005) the pathway seems to be specific for glucose degradation in *T. tenax*. So far, detailed enzymatic information is available for the GDH and the GK of *T. tenax* and some initial information for the GAD, KD(P)GA and KDGK (Siebers et al.1997; Ahmed et al. 2005; Kehrer et al. 2007).

Glucose dehydrogenase The GDH of *T. tenax* catalyzes the pyridine-nucleotide-dependent oxidation of glucose forming gluconate (Siebers et al. 1997). Besides glucose also xylose is accepted as substrate, but with lower affinity. In contrast to the *S. solfataricus* enzyme no significant conversion of galactose was observed. The native enzyme possesses dual co-substrate specificity for NAD^+ and $NADP^+$, however, the 100-fold higher affinity for the latter suggests that $NADP^+$ is the physiological co-substrate. Strikingly, both co-substrates influenced the oligomeric state and activity of the enzyme in vitro. In the absence of $NAD(P)^+$ a multimeric, but inactive enzyme (>600 kDa) and in the presence of cosubstrates the active homodimer (84 kDa) was observed. Initial phylogenetic analyses revealed that the enzyme is a member of the alcohol dehydrogenase (PF08240)/zinc-binding alcohol dehydrogenase family (PF00107).

Gluconate dehydratase The GAD of *T. tenax* catalyzes the dehydration of gluconate yielding KDG (Fig. 1). The encoding *gad* gene was identified by comparative gene context analysis of the archaeal ED genes and the recombinant enzyme was partly analyzed (Ahmed et al. 2005; Table 1). The activity of the *T. tenax* enzyme was confirmed by the protein-dependent formation of KDG. The enzyme revealed substrate specificity for gluconate and in contrast to the *S. solfataricus* enzyme, which is active on both substrates, no activity with galactonate was observed. In agreement with this finding only GAD activity on gluconate was detected in *T. tenax* crude extracts. The novel type of GAD shows no similarity to the classical ED (6-phosphogluconate) dehydratase (EDD), but is a member of the enolase superfamily (mandelate racemase/muconate lactonizing enzyme family (PF02746/PF01188); Ahmed et al. 2005). Interestingly, for the GAD from *S. solfataricus* (SSO3198) regulation by protein modification via phosphorylation/dephosphorylation was reported recently (Kim and Lee 2005). Thus, it is tempting to speculate that regulatory protein modification may also play a role in *T. tenax*.

2-Keto-3-deoxy-(6-phospho)gluconate aldolase The bifunctional KD(P)GA of *T. tenax* catalyzes the reversible cleavage of non-phosphorylated (glyceraldehyde (GA), KDG) as well as phosphorylated (GAP, KDPG) substrates, thus representing a true KD(P)GA (Fig. 1). The protein-dependent formation of KDG from GA and pyruvate and of KDPG from GAP and pyruvate was demonstrated (Ahmed et al. 2005) and therefore the enzyme is a key player in both the non- and semi-phosphorylated ED branch. KD(P)G aldolases show no similarity to the classical ED aldolase (EDA) (Buchanan et al. 1999), but are members of the *N*-acetyl neuraminate lyase (NAL) superfamily (PF00701; Babbit and Gerlt 1997). The crystal structure of the KD(P)GA of *T. tenax* was resolved recently and a combined gaschromatography and mass spectroscopy approach revealed that the *T. tenax* enzyme lacks stereo control and catalyzes the formation of the two C4 epimers KDG and 2-keto-3-deoxygalactonate (KDGal) from GA and pyruvate (Pauluhn et al. 2008). Therefore, like for the *Sulfolobus* enzyme, activity with all four substrates (KDG, KDPG, KDGal and KD(P)Gal) is proposed for the *T. tenax* enzyme. However, since *T. tenax* is not able to grow with galactose as carbon source (Zillig et al. 1981) and the initial enzymes of the pathway (GDH, GAD) exhibit stereocontrol, the role of KD(P)G aldolase promiscuity in *T. tenax* is unclear. A trade-off between required catabolic flexibility needed for the conversion of phosphorylated and non-phosphorylated substrates and stereocontrol has been discussed (Pauluhn et al. 2008).

2-Keto-3-deoxygluconate kinase The KDGK of *T. tenax* phosphorylates KDG in an ATP-dependent reaction yielding KDPG, thus, obviously representing the key enzyme of the semi-phosphorylative ED branch (Fig. 1). The substrate-dependent formation of KDPG was demonstrated (Ahmed et al. 2005; Table 1). The enzyme is a

member of the ribokinase (PfkB) enzyme superfamily (PF00294), which is composed of prokaryotic sequences related to ribokinase, including enzymes like fructokinases, the minor 6-phosphofructokinase of *Escherichia coli*, 1-phosphofructokinase and archaeal ADP-dependent glucokinases and phosphofructokinases.

Glycerate kinase The GK of *T. tenax* catalyzes the ATP-dependent phosphorylation of glycerate yielding 2-phosphoglycerate (2-PG; Fig. 1). The enzyme is specific for glycerate and highest activity is observed with ATP as phosphoryl donor and Mg^{2+} as divalent metal ion. At higher glycerate concentration substrate inhibition was observed and in addition, the product ADP showed an inhibitory effect (competitive inhibition), thus suggesting regulation by the energy charge of the cell (Table 1; Kehrer et al. 2007). Phylogenetic analyses revealed the presence of three distinct glycerate kinase classes (I–III) in extant organisms, which show a separate phylogenetic distribution. The archaeal glycerate kinase is a class II enzyme (MOFRL family, Pf05161) and homologs were identified in all three domains of life (Kehrer et al. 2007).

For all genes specific for the branched ED pathway of *T. tenax*, with the only exception of the GK encoding gene (*garK*), no regulation on the transcript level in response to heterotrophic (glucose) compared to autotrophic (CO_2/H_2) growth conditions is observed (Zaparty et al. 2008). The *garK* gene seems to form an operon with an ORF encoding a gene homolog, which shows similarity to classical 6-phosphogluconate dehydratase [Entner-Doudoroff dehydratase (EDD), *edd;* EC 4.2.1.12) and dihydroxy-acid dehydratase (DHAD, *ilvD*; EC 4.2.1.12]. The respective EDD/DHAD homolog from *S. solfataricus* has been characterized, and significant activity on both substrates (dihydroxyisovalerate, gluconate) was shown (Kim and Lee 2006). The authors suggest a role in the branched chain amino acid synthesis as well as in the branched ED pathway in *S. solfataricus*. However, despite of the functional organization, the observed increased *garK* transcript levels in *T. tenax* grown autotrophically remain unclear (Zaparty et al. 2008).

In summary, the physiological function of the branched ED pathway as well as its regulation is still unclear. In vivo NMR studies confirmed that the pathway is active as minor catabolic route in addition to the EMP pathway under heterotrophic growth on glucose. In addition, ED enzyme activities were demonstrated in crude extract of auto- and heterotrophically grown cells (Selig and Schönheit 1994; Selig et al. 1997; Siebers et al. 1997; Ahmed et al. 2005), which is further supported by the constitutive expression of the ED genes within the transcriptional analysis (CCM microarray; Zaparty et al. 2008). Therefore, the branched ED pathway in *T. tenax* might be induced under different—so far unknown—growth conditions. An additional role of the classical ED pathway is described for some Bacteria like *E. coli*, where it serves as a funnel for sugar acid and polymer degradation (Peekhaus and Conway 1998).

Physiological function of the EMP and the branched ED pathway in *T. tenax*

The coexistence and utilization of two pathways for carbohydrate metabolism, the modified reversible EMP and the catabolic, branched ED pathway, in parallel raises questions about their regulation and physiological function.

From the localization of the *gaa* gene coding for a glucan-1,4-α-glucosidase (glucoamylase) homolog within the ED operon (*kdgA-kdgK-gaa*) of *T. tenax* a central role of the branched ED pathway in the hydrolytic degradation of glycogen (carbon storage compound; König et al. 1982) has been proposed (Ahmed et al. 2004). In contrast to that, the EMP pathway is supposed to be involved in the phosphorolytic degradation of glycogen via glycogen phosphorylase, which has been characterized recently (Dörr 2002; Siebers et al. 2004). The enzyme forms G1P, which is supposed to be converted to G6P via phosphoglucomutase (PGM); a homolog has been identified in the genome of *T. tenax* (TTX_2058; AJ621332).

In addition, also the energy demand of the cell might influence the selection of the different catabolic routes in *T. tenax*. The net energy gain of the EMP pathway is one mol ATP per mol glucose, considering that (a) the enzyme-couple GAPDH-PGK in the catabolic direction is substituted by non-phosphorylating GAPN and/or GAPOR omitting substrate-level phosphorylation, and that (b) *T. tenax* utilizes a reversible PFK, which uses PP_i, which is generally regarded as waste product of the cell, instead of ATP as phosphoryl donor. Glycogen degradation via glycogen phosphorylase gains two mole of ATP per mole G6P. In contrast, no net ATP is generated by the semi- and non-phosphorylative branch of the ED pathway. Therefore, it has previously been suggested that the different glycolytic routes in *T. tenax* may not just display pathway parallelism, but reflect the ability of the cell to respond to the physiological cellular needs (Ahmed et al. 2004). Furthermore, it has been discussed recently that employing GAPOR, GAPN and the non-phosphorylative ED pathway might be favorable for growth at elevated temperatures and thus allow for metabolic thermoadaptation (Brunner et al. 1998, 2001; Walden et al. 2004; Ahmed et al. 2004; Ettema et al. 2007).

The connection between the glycolytic pathways and the TCA, the oxidative decarboxylation of pyruvate to acetyl-CoA, was shown to be catalyzed by a pyruvate:ferredoxin-oxidoreductase (POR) in Archaea, e.g. *P. furiosus* (Blamey and Adams 1993). POR activity could be measured in cell extracts of *T. tenax* (Selig and Schönheit 1994) and three

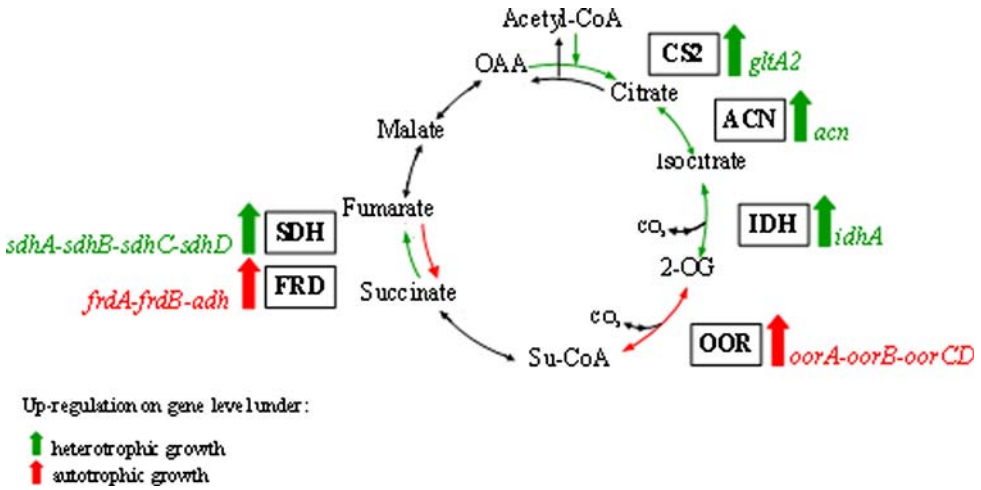


Fig. 3 The reversible TCA cycle of *T. tenax*. The enzymes of the TCA cycle, for which the encoding genes show a regulation on gene level depending on auto- and heterotrophic growth (*red/green arrows*) and their supposed physiological role are indicated. Abbreviations enzymes: *ACN* aconitase, *CS2* citrate synthase 2, *FRD* fumarate reductase, *IDH* isocitrate dehydrogenase, *OOR* 2-oxoglutarate ferredoxin-oxidoreductase, *SDH* succinate dehydrogenase. Abbreviations intermediates: *2-OG* 2-oxoglutarate, *OAA* oxaloacetic acid, *Su-CoA* Succinyl coenzyme A

possible POR candidates were identified (TTX_1757 and TTX_1758; TTX_1455 and TTX_1454; TTX_1785 and 1786) in the genome. Due to the high similarity of ferredoxin-dependent oxidoreductases an unequivocal annotation was difficult.

The reversible TCA cycle

In *T. tenax* under heterotrophic growth conditions a reversible TCA cycle is operative for the complete oxidation of organic compounds to carbon dioxide with elemental sulfur or thiosulfate as electron acceptor (oxidative cycle; Fig. 3). This has been shown by fermentation studies and determination of enzyme activities in crude extracts (Selig and Schönheit 1994). The *T. tenax* genome data suggest that the reductive TCA cycle is used for the fixation of carbon dioxide under autotrophic growth conditions (Fig. 3; Siebers et al. 2004; Zaparty et al. 2008) like it has already been described for the closely related species *T. neutrophilus* (Schäfer et al. 1986; Beh et al. 1993) and *P. islandicum* (Hügler et al. 2003; Hu and Holden 2006).

For almost all enzymes of the reversible TCA cycle encoding gene homologs were identified in the *T. tenax* genome, with the only exception of 2-oxoglutarate dehydrogenase subunit encoding genes (Siebers et al. 2004). However, so far initial enzymatic information is only available for two of the *T. tenax* TCA cycle enzymes citrate synthase 1 and aconitase (Tjaden 2003; Uhrigshardt, unpublished data). Focused transcriptional analysis revealed a substantial, coordinated regulation of the genes coding for TCA cycle enzymes of *T. tenax* in response to heterotrophic and autotrophic growth conditions (glucose vs. CO_2). Therefore, the transcriptional regulation seems to be essential for controlling the catabolic and anabolic carbon flux through the cycle (Zaparty et al. 2008). The oxidative direction of the TCA cycle seems to be triggered by an up-regulation of the genes coding for the reversible aconitase (AJ515539) as well as isocitrate dehydrogenase (AJ621308) and the catabolic citrate synthase 2 (CS2; AJ621309) and succinate dehydrogenase (AJ621266-69). The reductive direction during carbon dioxide fixation is forced by the enhanced transcription of the genes encoding the predicted, anabolic fumarate reductase (AJ621278-79) and one of two candidates coding for 2-oxoglutarate Fd-oxidoreductase (AJ621338-40) (Zaparty et al. 2008). This candidate is supposed to operate in both directions in *T. tenax* (Siebers et al. 2004). However, in order to elucidate the detailed regulation of the TCA cycle, biochemical information about the involved enzymes and their regulatory potential is necessary.

Concluding remarks

In contrast to the catabolic, branched ED pathway of *T. tenax*, which is only subjected to minor regulation on gene level, the reversible EMP variant and the reversible TCA cycle show a highly coordinated regulation of the respective genes under heterotrophic growth on glucose compared to autotrophic growth. Therefore, regulation on gene level seems to play a major role in controlling the carbon flux in *T. tenax* (Zaparty et al. 2008), whereas regulation on the protein level seems to be reduced compared to the known pathways from Bacteria and Eucarya.

The comparison with other Archaea reveals common regulatory sites: In all Archaea studied so far, regulation of the EMP pathway is executed on gene level as well as on protein level. The major control point of the EMP pathway at least in hyperthermophiles, e.g. *T. tenax*, *P. furiosus* or *T. kodakaraensis* is shifted to the level of GAP and is characterized by an allosterically regulated GAPN (Van der Oost et al. 1998, Brunner et al. 2001; Schut et al. 2003; Lorentzen et al. 2004; K. Matsubara, H. Atomi and T. Imanaka, personal communication; Zaparty et al. 2008). An additional control point of the EMP pathway, which is well established in *T. tenax* and for example in *T. kodakaraensis* (Imanaka et al. 2006), is localized at the level of PEP/pyruvate conversion (Schramm et al. 2000; Tjaden et al. 2006).

Interestingly, two CCM enzymes from *S. solfataricus* were identified (GAD and phosphohexomutase), which are subject to regulatory protein phosphorylation (Kim and Lee 2005; Ray et al. 2005). In Archaea, the knowledge about regulation by protein modification is rather scarce. So far, initial studies were performed only in *S. solfataricus*, and eucaryal-like protein kinases and phosphatases as well as few phosphoproteins were described (Kennelly 2003), however, the signal transduction pathways are still unknown. Therefore, suggesting that other unidentified regulatory mechanisms might play an additional role in the regulation of the CCM in Archaea.

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References

Ahmed H, Tjaden B, Hensel R, Siebers B (2004) Embden-Meyerhof-Parnas and Entner-Doudoroff pathways in *Thermoproteus tenax*: metabolic parallelism or specific adaptation? Biochem Soc Trans 32:2–4

Ahmed H, Ettema TJ, Tjaden B, Geerling AC, Van der Oost J, Siebers B (2005) The semi-phosphorylative Entner-Doudoroff pathway in hyperthermophilic Archaea: a re-evaluation. Biochem J 390:529–540

Babbit PC, Gerlt JA (1997) Understanding enzyme superfamilies—chemistry as the fundamental determinant in the evolution of new catalytic activities. J Biol Chem 272:30591–30594

Beh M, Strauss G, Huber R, Stetter KO, Fuchs G. (1993) Enzymes in the reductive citric acid cycle in the autotrophic eubacterium *Aquifex pyrophilus* and in the archaebacterium *Thermoproteus neutrophilus*. Arch Microbiol 160:306–311

Blamey JM, Adams MWW (1993) Purification and characterization of pyruvate ferredoxin oxidoreductase from the hyperthermophilic Archaeon *Pyrococcus furiosus*. Biochim Biophys 1161(1):19–27

Brunner NA, Brinkmann H, Siebers B, Hensel R (1998) NAD^+-dependent glyceraldehyde-3-phosphate dehydrogenase from *Thermoproteus tenax*: the first identified archaeal member of the aldehyde dehydrogenase superfamily is a glycolytic enzyme with unusual regulatory properties. J Biol Chem 273:6149–6156

Brunner NA, Siebers B, Hensel R (2001) Role of two different glyceraldehyde-3-phosphate dehydrogenases in controlling the reversible Embden-Meyerhof-Parnas pathway in *Thermoproteus tenax*: regulation on protein and transcript level. Extremophiles 5:101–109

Buchanan CL, Connaris H, Danson MJ, Reeve CD, Hough DW (1999) An extremely thermostable aldolase from *Sulfolobus solfataricus* with specificity for non-phosphorylated substrates. Biochem J 343:563–570

Dörr C (2002) Diploma thesis. University of Essen, Germany

Dörr C, Zaparty M, Tjaden B, Brinkmann H, Siebers B (2003) The hexokinase of the hyperthermophile *Thermoproteus tenax*: ATP-dependent hexokinases and ADP-dependent glucokinases, two alternatives for glucose phosphorylation in Archaea. J Biol Chem 278:18744–18753

Ettema TJG, Ahmed H, Geerling ACM, Van der Oost J, Siebers B (2007) The non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN) of *Sulfolobus solfataricus*: a key-enzyme of the semi-phosphorylative branch of the Entner-Doudoroff pathway. Extremophiles (epub ahead of print). doi: 10.1007/s00792-007-0082-1

Fischer F, Zillig W, Stetter KO, Schreiber G (1983) Chemolithoautotrophic metabolism of anaerobic extremly thermophilic archaebacteria. Nature 301:511–513

Galperin MY, Aravind L, Koonin EV (2000) Aldolases of the DhnA family: a possible solution to the problem of pentose and hexose biosynthesis in archaea. FEMS Microbiol Lett 183(2):259–264

Hansen T, Schönheit P. (2003) ATP-dependent glucokinase from the hyperthermophilic bacterium *Thermotoga maritima* represents an extremely thermophilic ROK glucokinase with high substrate specificity. FEMS Microbiol Lett 226:405–411

Hansen T, Urbanke C, Schönheit P. (2004a) Bifunctional phosphoglucose/phosphomannose isomerase from the hyperthermophilic Archaeon *Pyrobaculum aerophilum*. Extremophiles 8:507–512

Hansen T, Wendorff D, Schönheit P (2004b) Bifunctional phosphoglucose/phosphomannose isomerases from the Archaea *Aeropyrum pernix* and *Thermoplasma acidophilum* constitute a novel enzyme family within the phosphoglucose isomerase superfamily. J Biol Chem 279:2262–2272

Hensel R, Laumann S, Lang J, Heumann H, Lottspeich F (1987) Characterization of two D-glyceraldehyde-3-phosphate dehydrogenases from the extremely thermophilic arachaebacterium *Thermoproteus tenax*. Eur J Biochem 170:325–333

Hu Y, Holden JF (2006) Citric acid cycle in the hyperthermophilic Archaeon *Pyrobaculum islandicum* grown autotrophically, heterotrophically and mixotrophically with acetate. J Bacteriol 188(12):4350–4355

Hügler M, Huber H, Stetter KO, Fuchs G (2003) Autotrophic CO_2 fixation pathways in Archaea. Arch Microbiol 179:160–173

Imanaka H, Yamatsu A, Fukui T, Atomi H, Imanaka T (2006) Phosphoenolpyruvate synthase plays an essential role for glycolysis in the modified Embden-Meyerhof pathway in *Thermococcus kodakarensis*. Mol Microbiol 61(4):898–909

Jung JH, Lee SB (2006) Identification and characterization of *Thermoplasma acidophilum* glyceraldehydes dehydrogenase: a new class of $NADP^+$-specific aldehyde dehydrogenase. Biochem J 397:131–138

Kardinahl S, Schmidt CL, Hansen T, Anemüller S, Petersen A, Schäfer G (1999) The strict molybdate-dependence of glucose-degradation by the thermoacidophile *Sulfolobus acidocaldarius* reveals the first crenarchaeotic molybdenum containing enzyme—an aldehyde oxidoreductase. Eur J Biochem 260:540–548

Kehrer D, Ahmed H, Brinkman H, Siebers B (2007) The glycerate kinase of the hyperthermophilic Archaeon *T. tenax*: new insights into phylogenetic distribution of physiological role of members of the three different families. BMC Genomics. doi: 10.1186/1471-2164-8-301

Kennelly P (2003) Archaeal protein kinases and protein phosphatases: insights from genomics and biochemistry. Biochem J 370:373–389

Kim S, Lee SB (2005) Identification and characterization of *Sulfolobus solfataricus* D-gluconate dehydratase: a key enzyme in the non-phosphorylated Entner-Doudoroff pathway. Biochem J 387:271–280

Kim S, Lee SB (2006) Catalytic promiscuity in dihydroxy-acid dehydratase from the thermoacidophilic Archaeon *Sulfolobus solfataricus*. J Biochem 139:591–596

König H, Sorko R, Zillig W, Reiter WD (1982) Glycogen in thermoacidophilic archaebacteria of the genera *Sulfolobus*, *Thermoproteus*, *Desulfurococcus* and *Thermococcus*. Arch Microbiol 132:297–303

Koonin EV, Galperin MY (2003) Sequence, evolution, function—computational approaches in comparative genomics. Kluwer, Dordrecht

Lamble HJ, Heyer NJ, Bull SD, Hough DW, Danson MJ (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(36):34066–34072

Lamble HJ, Theodossis A, Milburn CC, Taylor GL, Bull SD, Hough DW, Danson MJ (2005) Promiscuity in the part-phosphorylative Entner-Doudoroff pathway of the Archaeon *Sulfolobus solfataricus*. FEBS Lett 579:6865–6869

Lorentzen E, Pohl E, Zwart P, Stark A, Russell RB, Knura T, Hensel R, Siebers B (2003) Crystal structure of an archaeal class I aldolase and the evolution of (ßa) 8 barrel proteins. J Biol Chem 278(47):47253–47260

Lorentzen E, Hensel R, Knura T, Ahmed H, Pohl E (2004) Structural basis of allosteric regulation and substrate specificity of the non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase from *Thermoproteus tenax*. J Mol Biol 341:815–828

Lorentzen E, Siebers B, Hensel R, Pohl E (2005) The mechanism of the schiff base forming fructose-1, 6-bisphosphate Aldolase: Structural analysis of reaction intermediates. Biochemistry 44(11):4222–4229

Mukund S, Adams MWW (1995) Glyceraldehyde-3-phosphate ferredoxin oxidoreductase, a novel tungsten-containing enzyme with a potential glycolytic role in the hyperthermophilic Archaeon *Pyrococcus furiosus*. J Biol Chem 270(15):8389–8392

Pauluhn A, Ahmed H, Lorentzen E, Buchinger S, Schomburg D, Siebers B, Pohl E (2008) Crystal structure and stereochemical studies of KD(P)G aldolase from *Thermoproteus tenax*. Proteins (epub ahead of print). doi:10.1002/prot.21890

Peekhaus N, Conway T (1998) What's for dinner? Entner-Doudoroff metabolism in *Escherichia coli*. J Bacteriol 180:3495–3502

Potter S, Fothergill-Gilmore LA (1992) Purification and properties of pyruvate kinase from *Thermoplasma acidophilum*. FEMS Microbiol Lett 73:235–239

Rashid N, Imanaka H, Kanai T, Fukui T, Atomi H, Imanaka T (2002) A novel candidate for the true fructose-1,6-bisphosphatase in Archaea. J Biol Chem 277(34):30649–30655

Ray WK, Keith SM, DeSantis AM, Hunt JP, Larson TJ, Helm RF, Kennelly P (2005) A phosphohexomutase from the Archaeon *Sulfolobus solfataricus* is covalently modified by phosphorylation on serine. J Bacteriol 187(12):4270–4275

Reher M, Gebhard S, Schönheit P (2007) Glyceraldehyde-3-phosphate ferredoxin oxidoreductase (GAPOR) and non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN), key enzymes of the respective modified Embden-Meyerhof pathways in the hyperthermophilic crenarchaeota *Pyrobaculum aerophilum* and *Aeropyrum pernix*. FEMS Microbiol Lett 273:196–205

Ronimus RS, Morgan HW (2003) Distribution and phylogenies of enzymes of the Embden-Meyerhof-Parnas pathway from Archaea and hyperthermophilic bacteria support a gluconeogenic origin of metabolism. Archaea 1:199–221

Sakuraba H, Goda S, Oshima T (2004) Unique sugar metabolism and Novel Enzymes of Hyperthermophilic Archaea. Chem Rec 3:281–287

Sato T, Imanaka H, Rashid N, Fukui T, Atomi H, Imanaka T (2004) Genetic evidence identifying the true gluconeogenic fructose-1,6-bisphosphatase in *Thermococcus kodakaraensis* and other hyperthermophiles. J Bacteriol 186(17):5799–5807

Schäfer S, Barkowski C, Fuchs G (1986) Carbon assimilation by the autotrophic thermophilic archaebacterium *Thermoproteus neutrophilus*. Arch Microbiol 146:301–308

Schramm A, Siebers B, Tjaden B, Brinkmann H, Hensel R (2000) Pyruvate kinase of the hyperthermophilic crenarchaeote *Thermoproteus tenax*: physiological role and phylogenetic aspects. J Bacteriol 182(7):2001–2009

Schut GJ, Brehm SD, Datta S, Adams MWW (2003) Whole-genome DNA microarray analysis of a hyperthermophile and an Archaeon: *Pyrococcus furiosus* grown on carbohydrates or peptides. J Bacteriol 185(13):3935–3947

Selig M, Schönheit P (1994) Oxidation of organic-compounds to CO_2 with sulfur or thiosulfate as electron-acceptor in the anaerobic hyperthermophilic Archaea *Thermoproteus tenax* and *Pyrobaculum islandicum* proceeds via the citric-acid cycle. Arch Microbiol 162:286–294

Selig M, Xavier KB, Santos H, Schönheit P (1997) Comparative analysis of Embden-Meyerhof and Entner-Doudoroff glycolytic pathways in hyperthermophilic Archaea and the bacterium *Thermotoga*. Arch Microbiol 167:217–232

Siebers B, Hensel R (1993) Glucose catabolism of the hyperthermophilic Archaeum *Thermoproteus tenax*. FEMS Microbiol Lett 111:1–8

Siebers B, Wendisch VF, Hensel R (1997) Carbohydrate metabolism in *Thermoproteus tenax*: in vivo utilization of the non-phosphorylative Entner-Doudoroff pathway and characterization of its first enzyme, glucose dehydrogenase. Arch Microbiol 168:120–127

Siebers B, Klenk HP, Hensel R (1998) PP_i-dependent phosphofructokinase from *Thermoproteus tenax*, an archaeal descendant of an ancient line in phosphofructokinase evolution. J Bacteriol 180:2137–2143

Siebers B, Brinkmann H, Dörr C, Tjaden B, Lilie H, Van der Oost J, Verhees CH (2001) Archaeal fructose-1,6-bisphosphate aldolases constitute a new family of archaeal type class I aldolase. J Biol Chem 276(31):28710–28718

Siebers B, Tjaden B, Michalke K, Dörr C, Ahmed H, Zaparty M, Gordon P, Sensen C, Zibat A, Klenk HP, Schuster SC, Hensel R (2004) Reconstruction of the central carbohydrate metabolism of *Thermoproteus tenax* by use of genomic and biochemical data. J Bacteriol 186(7):2179–2194

Siebers B, Schönheit P (2005) Unusual pathways and enzymes of central carbohydrate metabolism. Curr Opin Microbiol 8:695–705

Stec B, Yang H, Johnson KA, Chen L, Roberts MF (2000) MJ0109 is an enzyme that is both an inositol monophosphatase and the 'missing' archaeal fructose-1, 6-bisphosphatase. Nat Struct Biol 7:1046–1050

Tjaden B (2003) PhD thesis. University of Essen, Germany

Tjaden B, Plagens A, Dörr C, Siebers B, Hensel R (2006) Phosphoenolpyruvate synthetase and pyruvate phosphate

dikinase of *Thermoproteus tenax*: key pieces in the puzzle of archaeal carbohydrate metabolism. Mol Microbiol 60(2):287–298

Van der Oost J, Schut G, Kengen SWM, Hagen WR, Thomm M, de Vos WM (1998) The ferredoxin-dependent conversion of glyceraldehyde-3-phosphate in the hyperthermophilic Archaeon *Pyrococcus furiosus* represents a novel site of glycolytic regulation. J Biol Chem 273:28149–28154

Van der Oost J, Siebers B (2007) The glycolytic pathways of Archaea: evolution by tinkering. In: Garrett RA, Klenk H-P (eds) Archaea: evolution, physiology and molecular biology, 1st edn. Blackwell, Oxford, pp 247–260

Verhees CH, Kengen SW, Tuininga JE, Schut GJ, Adams MWW, De Vos WM, Van der Oost J (2003) The unique features of glycolytic pathways in Archaea. Biochem J 375:231–246 (erratum in: Biochem J 377:819–822, 2004)

Walden H, Taylor GL, Lorentzen E, Pohl E, Lilie H, Schramm A, Knura T, Stubbe K, Hensel R, Tjaden B (2004) Structure and function of a regulated archaeal triosephosphate isomerase adapted to high temperature. J Mol Biol 342(3):861–875

Zaparty M, Zaigler A, Stamme C, Soppa J, Hensel R, Siebers B (2008) DNA microarray analysis of the central carbohydrate metabolism: glycolytic/gluconeogenic carbon switch in the hyperthermophilic crenarchaeum *Thermoproteus tenax*. J Bacteriol 190(6):2231–2238

Zillig W, Stetter KO, Schäfer W, Janekovic D, Wunderl S, Holz I, Palm P (1981) *Thermoproteales*: a novel type of extremely thermoacidophilic anaerobic archaebacteria isolated from Icelandic solfatares. Zentbl Bakteriol Hyg I Abt Org C 2:205–227